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Review article

A review of Canadian forest vegetation management research and practice

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Abstract – Research and practice in Canadian forest vegetation management was reviewed for the period 1990 to present. Results indicate continued evolution toward a more integrated and ecologically sound program with appropriate focus on key competitors and crop species. Increasing collaboration between academia, government and industry has resulted in > 666 new scientific publications, substantially augmenting the existing knowledge base. The development of (*Chondrostereum purpureum*) as the first biocontrol agent in Canadian forest vegetation management and the use of nutrient-loaded seedlings to enhance establishment success are considered key research highlights. Recent trends in operational practice include a move toward more intensive management on higher quality sites and adoption of innovative approaches (e.g. nutrient loaded seedlings, larger planting stock) and advanced technologies (e.g. electronic guidance in aerial herbicide applications). The lack of long-term growth response data and economic analyses demonstrating positive cost/benefits remain as shortcomings, however continued development of the program will undoubtedly enhance sustainable wood supply and minimize impact on the forest environment.

vegetation / management / Canada / review

Résumé – **Recherche sur la gestion de la végétation forestière au Canada et les pratiques : une revue.** Le présent document passe en revue la recherche sur la gestion de la végétation forestière au Canada et les pratiques à cet égard depuis les années 1990 jusqu'à ce jour. Les résultats de cet examen révèlent une progression continue vers un programme plus intégré et plus respectueux de l'environnement, axé, fort judicieusement sur les principales espèces concurrentes et les espèces du peuplement final. La collaboration accrue du monde universitaire, des gouvernements et de l'industrie s'est traduite par la parution de plus de 666 nouvelles publications scientifiques qui ont considérablement enrichi la base des connaissances actuelles. La mise au point au Canada du *Chondrostereum purpureum* comme premier bioherbicide et l'utilisation de semis gorgés d'éléments nutritifs afin d'accroître le taux de réussite de l'établissement sont considérées comme des grandes percées de la recherche. Parmi les tendances récentes en matière de pratiques opérationnelles figurent le recours à des méthodes de gestion plus intensive dans les stations à indice de qualité plus élevé et l'adoption de méthodes novatrices (p. ex., semis gorgés d'éléments nutritifs) et de techniques de pointe (p. ex., système de guidage électronique des applications aériennes d'herbicide). Même si les données sur le taux de croissance à long terme des semis et les analyses économiques mettant en évidence les effets positifs sur le plan coûts-avantages font encore défaut, l'évolution incessante du programme améliorera sans aucun doute l'approvisionnement durable en bois et réduira au minimum les répercussions sur le milieu forestier.

gestion / végétation / Canada

1. INTRODUCTION

Representing approximately 10% of the global forested landmass, Canada's forests are key elements in biogeochemical cycling and biodiversity [31]. Specifically, our forests are important carbon sinks and provide habitat for an estimated 140 000 species of plants, animals and microorganisms, including 85 species that are considered as forest dependent and at risk of extinction [53]. Forests cover almost one-half of our country and are one of the unique features defining our nation. Based on marked differences in topography, soils, climate and dominant tree species, several distinct forest regions are recognized in Canada (Fig. 1). Many of Canada's forest regions are dominated by softwood species (68%), with mixed-woods (18%) and hardwoods (15%), being most prevalent and economically important in the southern Great Lakes – St. Lawrence, Carolinean and Acadian forest regions.

Unlike many other nations, 94% of Canadian forests are owned by the public [31] and managed on their behalf by provincial and territorial governments. Forest industry is allowed to extract timber resources under a system with similarities to landlord-tenant lease arrangements [93]. In this context, management of the Canadian forest resource may be viewed as an attempt to strike a sustainable balance among a diverse array of economic, environmental, aesthetic, and spiritual values. Erdle [26] recently described the potential conflicts, tradeoffs

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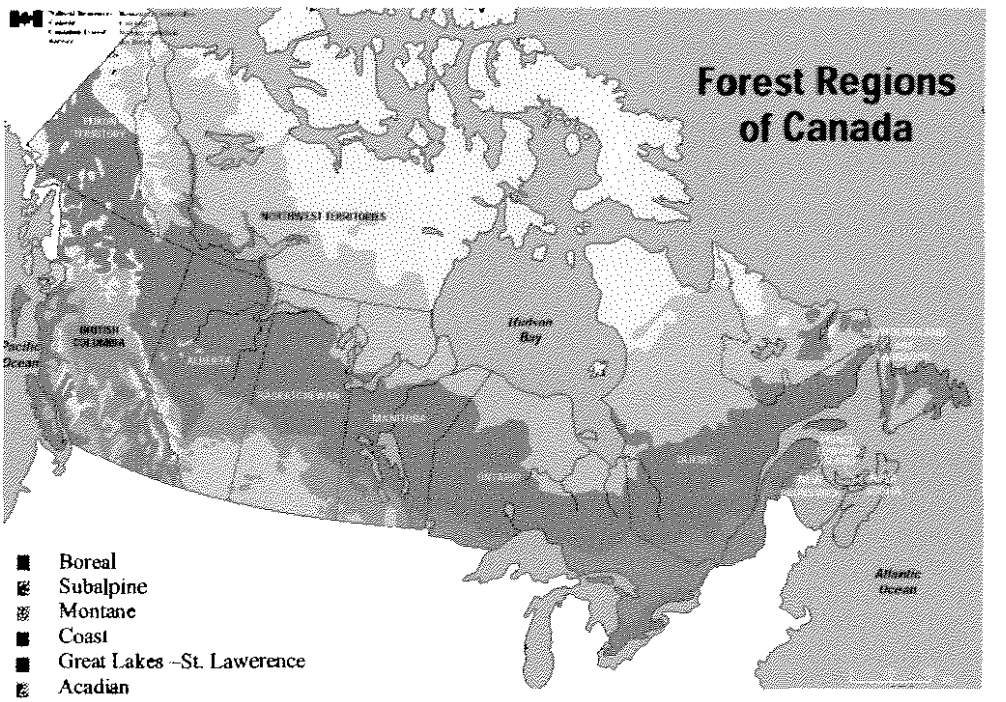


Principal Crop Tree Species

Boreal – White and black spruce, Balsam fir, Jack pine, White Birch, Poplar
Subalpine – Englemann spruce, Lodgepole pine
Montane – Lodgepole pine, Pitch pine, Poplar
Coast – Red cedar, Hemlock, Sitka spruce, Douglas fir
Great Lakes –St. Lawerence – Red and White pine, Beech, Maple, Red oak,
Acadian –Red and Black spruce, Balsam fir, Yellow Birch

Figure 1. Major forest regions and associated principal crop tree species in Canada (a colour version of this figure is available online at http://www.edpsciences.org/afs).

and mitigating strategies associated with managing forested lands for a variety of values. The latter paper clearly reflects the complex and dynamic mixture of policy, politics, emotion and science, which are pervasive in Canadian forest management.

Although management of the Canadian forest resource is inherently complex, a number of fundamental principals apply, including the need for integrated management and sustainable development, management for multiple values across the larger landscape, protection of ecological function and integrity, and protection of sensitive, unique or high value sites. From any perspective – global, national, cultural, environmental or economic – Canadian forests are a critically important renewable resource worthy of our best management efforts.

From an economic viewpoint, commercial timber, pulp, and paper production is central to our national economy. Canada controls a 15 to 30% share of worldwide lumber, pulp and paper commodities and ranks first in net value of exported forest products [68]. In 1999, the total value of Canadian forest products exports reached an all time high of 44.2 billion dollars. The forestry sector also supports approximately 877 000 direct and indirect jobs. These economic benefits are derived from a relatively small proportion (~50%) of the total 253.6 million ha considered as commercially productive in Canada (Fig. 2). On an annual basis, the utilization rate or Annual Allowable Cut (AAC), approximates 1 million ha, equating to ~ 0.2% of the total forested land area.

Canada is blessed with a generally vast and diverse forest resource. However, only a small portion of the land base is commercially viable and directly managed for derivation of economic benefits. This portion is being continuously diminished by demands for urban, agriculture, parks, recreation and other alternate land uses. On such a diminishing commercial forest landbase, the practice of vegetation management is one

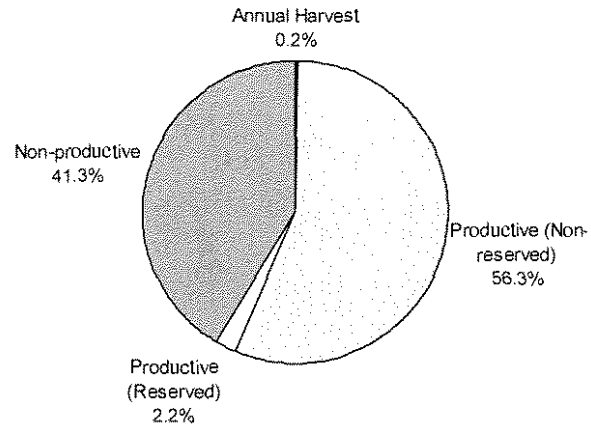


Figure 2. Relative proportion of annual harvest, productive and non-productive forest comprising the total forested land base in Canada.

of the principal means of optimizing productive capacity [91]. Sufficient knowledge of forest ecology, inter- and intra-species competition mechanisms and thresholds, and plant succession allows management of competing vegetation to meet specific silvicultural or wildlife management goals. The intensity of forest management applied to a particular site is based on site-specific prescriptions formulated by professional forest managers and may be classified on a gradient of intensity typically referred to as extensive (natural regeneration only) through basic (including assisted natural and artificial regeneration) to intensive (multiple interventions). The highest level of intensity approaches that used in agricultural production scenarios [3, 19, 25, 52, 90]. In this regard, and in comparison to many other forest producing nations, the current vegetation management program in Canada is generally characterized by single, low-intensity interventions on a small proportion of potential sites, and may be considered as basic management.

Several previous reviews have dealt with vegetation management in Canada to varying degrees [15–17, 18, 27, 78, 81, 93], however many of these were focused on the use and optimization of chemical herbicides. Most recently, Wagner and Colombo [85] published an excellent text documenting the principles and practices of vegetation management and forest regeneration with a particular focus on the province of Ontario. The objective of this paper, is to synthesize information on forest vegetation management at the national level, by reviewing trends and developments in research and practice over the last decade (1990-present).

2. MATERIALS AND METHODS

The Canadian Forest Pest Management (CFPM) database (http://www.glfc.cfs.nrcan.gc.ca/cfpm) and the National Forestry Database Program (NFDP) (http://nfdp.ccfm.org) were used to assess trends in Canadian forest vegetation management research and practice, respectively, from 1990 to present. Both databases are freely and universally accessible via the Internet and both are hosted and maintained by the Canadian Forest Service, Natural Resources Canada. Basic information from these two sources was augmented by information gathered through an informal electronic questionnaire sent to a number of leading researchers, industrial foresters, and academics from across the country. The questionnaire attempted to get a broader perspective on the adequacy of Canadian forest vegetation management research, knowledge and techniques.

At the time when searches were conducted, the CFPM database [75] comprised approximately 11 000 scientific publication records, each including abstracts and an extensive list of data fields facilitating search, selection and sorting functions. For the purposes of this paper, several queries of the database were invoked using the advanced search function to look for specific terms (e.g. "release", "efficacy" or "site preparation") in one or more of the various data fields (e.g. keyword, management technique, abstract, title). All searches were restricted to records originating in Canada, having vegetation management as their primary focus and with publication dates of 1990 or later. Where information pertinent to a particular species or province was sought, appropriate keywords were specified in the name of competing vegetation, crop species fields or province/state fields, respectively.

The NFDP was founded by the Canadian Council of Forest Ministers (CCFM) in 1990 and provides a comprehensive source of statistical data and information describing the nature, extent and change in Canada's forest resources through time. The NFDP also provides information on how these resources are being managed, their economic contribution to Canadian society, and expenditures required to maintain healthy forests. For the purposes of this review, information contained within the "Compendium of Canadian Forestry Statistics" in subsections on forest inventory, silviculture and pest control product use were particularly valuable.

3. RESULTS

3.1. Recent trends in Canadian forest vegetation management practice

Harvesting is clearly the major anthropogenic disturbance influencing the development of most managed forest stands. As such, it is the key factor controlling the type and amount of competing vegetation which subsequently occupies the site. Clear-cutting continues to be the major harvesting method used in Canadian silviculture [19], however use of partial harvesting systems has recently increased in virtually every forest region of the country.

Post-harvest regeneration of Canadian forests has largely been achieved through natural means, with planting or seeding playing a much smaller role (Fig. 3). The cumulative regenerating land base accruing since 1975 on crown lands has been estimated at approximately 16 million hectares in 1998. Trend data [19] suggest that 75 to 80% of the harvested area has been successfully regenerated seven years after cutting and the proportion of the landbase considered as free from non-crop competition has been increasing slowly but consistently (Fig. 4).

In Canada, forest vegetation management activities are largely conducted within a 5-year period post-harvest on areas successfully regenerated by either natural or artificial means, but which require further treatment to achieve silvicultural objectives. Vegetation that rapidly establishes on newly disturbed forest sites often determines whether forest regeneration will be successful [83]. Many of the principal competitor species in Canadian forestry are perennials that reproduce by both seed and asexual means [14] and are highly adapted for rapid establishment and growth in areas with disturbed soils

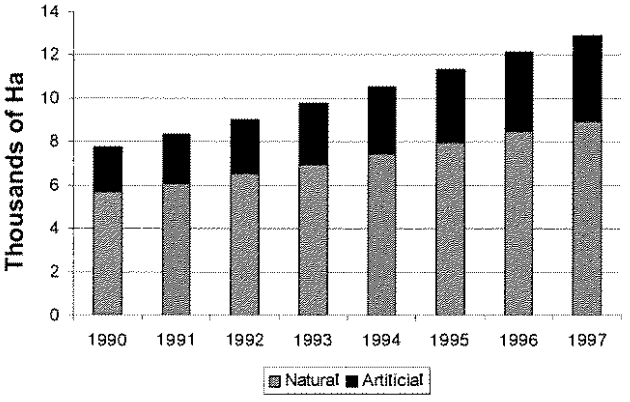


Figure 3. The proportion of Canadian forest lands regenerated using natural and artificial methods (1990–1997).

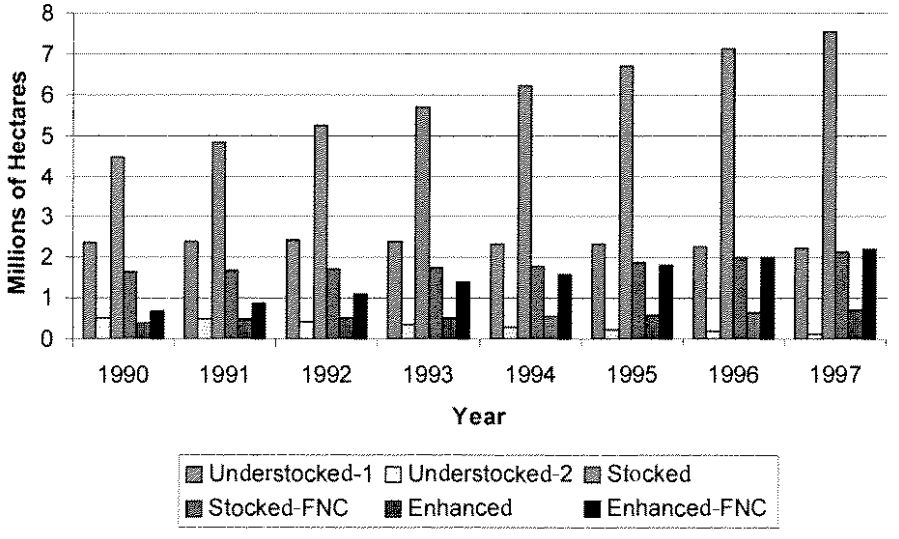
and high light intensity (Tab. I). Often these species grow in complex mixtures of graminaceous, herbaceous and deciduous brush (e.g., *Rubus* spp., *Calamagrostis* sp., *Epilobium* spp.) posing a threat to crop trees and particularly challenging scenarios for foresters who are required to ensure their successful regeneration. A variety of techniques (manual, mechanical, biological, chemical, prescribed burning, etc.) are available to meet these challenges and may be invoked under one of the following strategies:

(1) *Site preparation* – treatment that modifies a site prior to planting, seeding, or natural regeneration and which provides conditions favourable to regeneration establishment [19]. Objectives may include slash alignment or compaction to facilitate planting, and/or the creation of suitable microsites for seed germination and seedling growth.

(2) *Release* – treatment that is applied following regeneration establishment (seeding, planting, or natural regeneration), to free crop trees from vegetative competition. Objectives include the reduction of inter-specific competition and the promotion of diameter growth. [19].

(3) *Pre-commercial thinning* – treatment applied to juvenile stands of either natural or artificial origin to control stand density and composition [19]. Objectives usually include reducing both inter- and intra-specific competition and the promotion of diameter increment and stand quality. In Canada, release and pre-commercial thinning treatments are often grouped and discussed together as "stand tending".

Historical trend data [19] suggest that the total area being site prepared has been decreasing, while the area released has been relatively constant. The most dramatic change has been in the area pre-commercially thinned, which has approximately doubled from 93 thousand ha in 1990 to 184 thousand ha in 2000. Data for the year 2000 show that the total area receiving some type of vegetation management equated to approximately 76% of the total 1 027 222 ha harvested. Similar proportions of the productive forest landbase were treated



Relatively small portions (total < 0.5 million ha) of the harvested area were classified as disturbed but without a timber production objective (non-production) or of unknown status in each year but are not shown here. **Stocked** areas are those where stocking standards have been met, whereas **Understocked** refers to productive area which does not meet stocking standards, either because they require silvicultural treatment to reach stocking objectives (**Understocked-1**), or stocking objectives are expected to be achieved through natural recruitment (**Understocked-2**). **Enhanced** refers to stocked area where density control standards have been met. **Free from non-crop competition (FNC)** refers to stocked or enhanced areas where competition control objectives have been met.

Figure 4. The proportion of harvested forest lands meeting various stocking and competition-free classification standards (1990–1997).

Table I. Key competitor species and control options in Canadian forest vegetation management.

Competitor genus	Example common names	Life cycle/ form	Max height (m)	Modes of reproduction*	Control options (in approximate order of effectiveness)	Practices not recommended
Calamagrostis	Canada blue-joint grass	Perennial grass	1–2	Rh, Se	Hexazinone, glyphosate, winter harvest, partial harvest.	Mechanical site prep., burning, summer harvest.
Epilobium	Fireweed	Perennial herb	< 2	Se, Rs, Rh	Glyphosate, hexazinone, 2,4-D, winter harvest, partial harvest.	Summer harvest, burning, mechanical site prep.
Rubus	Red raspberry, salmonberry	Biennial shrub	2–3	Se, Rs, Ss	Hexazinone, glyphosate, triclopyr, partial harvest.	Cutting, burning, summer harvest.
Alnus	Red, speckled and green Alder	Perennial shrub	3–4	Se, Ss, St	Glyphosate, triclopyr, 2,4-D, *Chondrostereum purpureum*, summer cutting.	Dormant cutting.
Populus	Trempling aspen, balsam poplar	Perennial tree	< 34	Se, Rs, Ss	Glyphosate, triclopyr, summer cutting.	Mechanical site prep., dormant cutting, spring burning.
Salix	Willow	Perennial shrub	1–6	Se, Rs, Ss, St	Glyphosate, triclopyr, summer cutting.	Burning, dormant cutting, mechanical site prep.
Acer	Mountain, striped, red, bigleaf, and sugar maple	Perennial tree	< 35	Se, Ss	Triclopyr, glyphosate, summer cutting.	Dormant cutting.
Betula	White birch	Perennial tree	< 28	Se, Ss	Glyphosate, triclopyr, 2,4-D, *Chondrostereum purpureum*, summer cutting.	Dormant cutting, mechanical site prep.
Prunus	Pin cherry	Perennial shrub	< 5	Se, Ss, Rs	Glyphosate, triclopyr, 2,4-D, *Chondrostereum purpureum*, summer cutting.	Mechanical site prep., burning, summer cutting.
Gaultheria	Salal	Perennial shrub	< 2	Se, Rs	Glyphosate with siloxane surfactant, tryclopyr, burning.	Mechanical site prep., cutting.
Pteridium	Bracken fern	Perennial fern	< 1.5	Se, Rh	Glyphosate, hexazinone, partial harvest.	Mechanical site prep., burning, cutting.
*Cytisus***	Scotch Broom	Perennial shrub	2–3	Se	Triclopyr, glyphosate with siloxane surfactant, burning.	Mechanical site prep., dormant cutting, glyphosate without siloxane.
*Ulex***	Gorse	Perennial shrub	1.5–3	Se	Triclopyr, glyphosate with siloxane surfactant, burning.	Mechanical site prep., glyphosate without siloxane.

* Primary methods of reproduction and spreading post establishment, where: Se = Seed, Rs = Root sucker, Rh = Rhizome, St = Stolons, Ss = Stem or root collar sprouts.
** Recently introduced exotic species.
Sources of information: [14, 21].

by site preparation (306 419 ha) and release (239 521 ha), with a somewhat smaller area (183 863) receiving pre-commercial thinning treatments. Comparatively small areas received other tending treatments (31 480 ha) or scarification (16 923 ha).

Among the major forest producing provinces in Canada, clear differences exist in relative use of these three strategies. Such differences are exemplified by comparative data from 1999 (Fig. 5). In that year, the tending program in the province of Quebec was dominated by pre-commercial thinning, whereas release treatments were employed on relatively greater proportions of the landbase in provinces of British Columbia and Ontario. In New Brunswick, the area released was approximately equivalent to that being pre-commercially thinned, while in Alberta and Saskatchewan, only release strategies were employed.

Throughout the 1990s, site preparation was conducted principally by mechanical methods in all provinces, with relatively small areas treated using prescribed burning, chemical, or other techniques. The dominance of mechanical site preparation techniques is exemplified by national level data for 1999 (Fig. 6). Numerous mechanical site preparation treatments, including screefing, mounding, trenching, mixing, subsoiling, clearing, raking, chopping, and masticating are used in Canada, depending upon site conditions, vegetative species on the site, operational constraints, and economics. Treatments are applied using a wide variety of equipment, including chains, with or without shark-finned barrels, Bräcke scarifiers and mounders, disc trenchers, shear blades, ripper teeth, and drum choppers. Ryans and Sutherland [63] provide a detailed synthesis of treatments, equipment and environmental considerations associated with mechanical site preparation pertinent to Ontario and across Canada generally.

In contrast, the aerial application of chemical herbicides was by far the most frequent technique employed in release

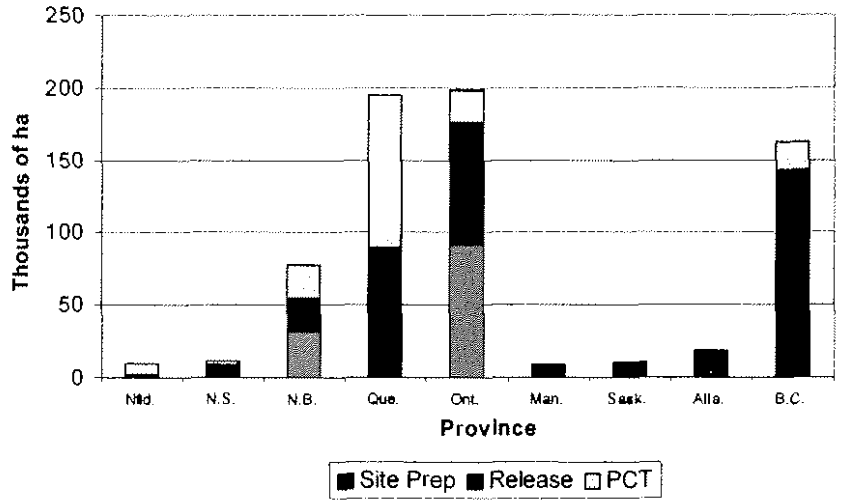


Figure 5. Proportion of forest lands receiving mechanical site preparation, release or pre-commercial thinning treatments by province (1999).

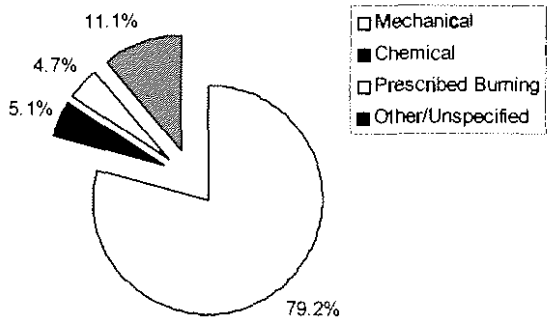


Figure 6. Relative percentage use of various site preparation techniques in Canada.

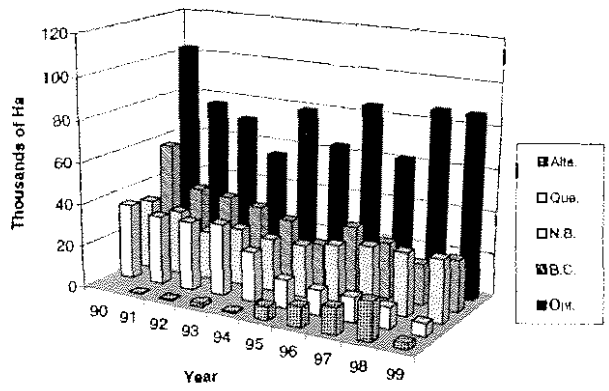


Figure 7. Regenerating forest area treated with chemical herbicides in Canada by year and province.

strategies. Although 5 herbicidal compounds (2,4-D, hexazinone, glyphosate, simazine, and triclopyr) are registered for broadcast use in Canada [19], glyphosate (Vision®) has accounted for over 90% of the total forest herbicide-use market throughout the 1990s [19]. While regionally variable, glyphosate was predominantly aerially applied for release of high-value conifers (e.g., jack pine (*Pinus banksiana* Lamb), black spruce (*Picea mariana* Mill.), and white spruce (*Picea glauca* Moench.) from competing vegetation. Through the last decade, herbicide use rates were relatively constant at about 200 000 ha per year, equivalent to approximately 20% of the area harvested annually, or roughly 40% of the landmass artificially regenerated. The majority (43%) of forest area treated with herbicides occurs in the province of Ontario, while New Brunswick and British Columbia use somewhat lesser amounts (Fig. 7). In the province of Quebec, use of chemical herbicides has dropped precipitously since 1995 in anticipation of a ban on the use of forest herbicides which took effect in that province in the summer of 2001.

A number of alternative techniques for controlling competing vegetation (e.g. livestock grazing, mulches, cover crops and biological control agents) are available or under development, but with the exception of livestock grazing in western Canada [22], none have been widely used in operational practice to date.

3.2. Recent trends in Canadian forest vegetation management research

A search of the CFPM database reveals that 1256 (11%) of the current 11 260 scientific publication records currently within the CFPM database relate directly to forest vegetation management in Canada. Of these, 666 were published since 1990 and the principal focus of Canadian publications since

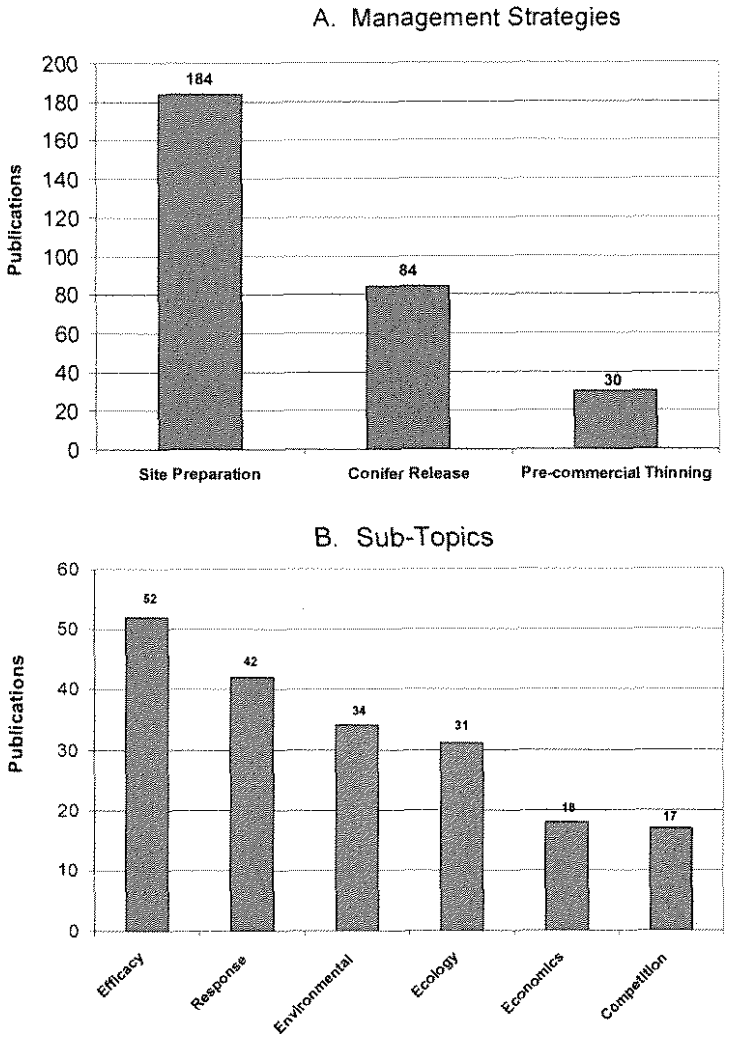


Figure 8. Number of journal publications pertaining to vegetation management in Canada (1990–present) in relation to (A) management strategy or (B) sub topic focus.

that time frame has been on site preparation (62%), with relatively fewer studies on conifer release (28%) or pre-commercial thinning (10%) strategies (Fig. 8a). Research has been relatively evenly distributed across sub-topics of efficacy, crop response and environmental effects, with relatively few studies examining economic aspects (Fig. 8b). Of papers classified as documenting environmental effects of forest vegetation management practices in Canada since 1990, almost all were studies investigating the environmental fate and effects of chemical herbicides [29, 65, 69, 76, 77, 79] and several were generated by a major multidisciplinary study conducted in northwestern Ontario [45, 66, 79]. Very few studies were conducted on the potential environmental effects of other vegetation management techniques.

Canadian research publications reflect the predominance of chemical and mechanical techniques in operational use patterns (Fig. 9). As might be expected, the majority of publications (352/358) relating to chemical methods involved glyphosate (Vision®), with environmental fate and effects assessments and medium-term (~10 years) efficacy studies predominanting. Publications relating to silivicultural techniques were dominated by studies on thinning, prescribed fire, and environmental assessment of alternative harvesting techniques in relation to clearcutting [23, 51]. Publications on other methods, such as biological control, have been few and resulted largely from targeted research programs such as the Vegetation Management Alternatives Program (VMAP) [84] and the related federal BICOVER network initiative [80].

Vegetation management research has been primarily centered on key high quality coniferous crop species groups including fir, pine, cedar and hemlock (Fig. 10a) and targeted at key competitors including particularly *Populus*, *Alnus* and *Rubus* spp. (Fig. 10b). Unfortunately, the vast majority of crop response data resulting from Canadian research is derived from relatively short term (< 10 yr studies), with progressively fewer studies providing data over the (10–24 yr) and long (> 25 term (Fig. 10c). No data derived from observations made over a full rotation cycle is yet available.

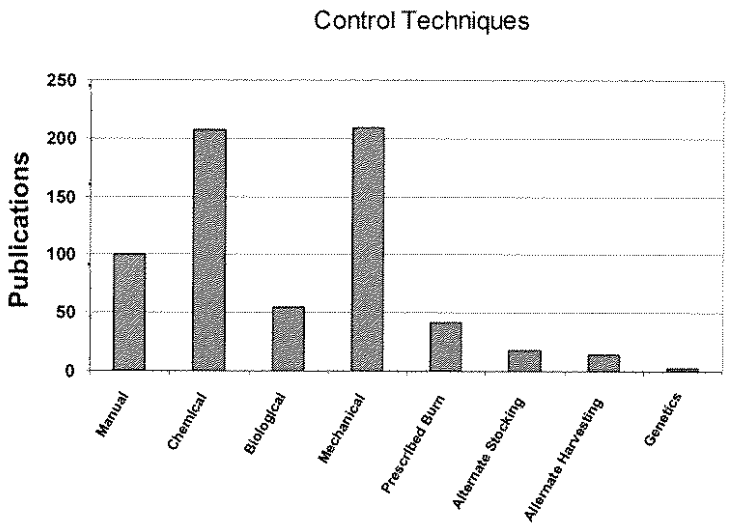


Figure 9. Number of journal publications pertaining to vegetation management in Canada (1990–present) in relation to focus on various control techniques.

Significant positive growth responses (height and diameter) in a variety of conifer crops following various site preparation or release treatments have been demonstrated in both short- [57, 60, 61, 87] and medium-term studies [10, 62, 94]. Although data supporting similar growth effects over the long term are scarce, at least one study [70] demonstrates that positive effects continue through a period of 30 years in white spruce. Other studies illustrate dramatic declines in juvenile conifer dominance with the absence of treatment [9, 60–62]. While these studies are based on relatively short-term data, it is unlikely that such trends will reverse themselves without silvicultural intervention.

Not surprisingly, publications relating to vegetation management research in Canada were largely derived from provinces traditionally known for forest production including, in order, British Columbia, Ontario and Quebec, and the number of papers providing information pertinent to particular forest regions is roughly proportional to the size of each of the major Canadian forest regions, with the vast area of the boreal forest predominating. Studies focused on vegetation management in non-traditional crop-species such as hybrid poplar [73] were not numerous.

4. DISCUSSION

4.1. Current status assessment

Results suggest that over the past decade, important advances in research and practice have been made. Research programs have been appropriately focused on key target and crop species, and have generated an increasing knowledge base supporting the principal operational techniques used in forest vegetation management across the nation. A number of papers directly pertinent to forest vegetation management conditions in Canada have enhanced knowledge in basic sub-disciplines of critical silvics, ecophysiology, and plant autecology [15, 33, 34, 48], as well as competition mechanisms and critical competitive thresholds [7, 67, 71, 82, 87].

Several innovative approaches and techniques for controlling competing vegetation, or off-setting resultant losses in growth of crop trees, have been developed and reported since 1990. These include the application of plant growth regulators for enhanced stocking success and seedling growth in Douglas fir (*Pseudotseuga menziesii* (Mirb.) Franco) [64], nutrient loading of black spruce seedlings to reduce competitive effects [38, 39, 48], and development of *Chondrostereum purpureum* as a biological control agent. An extensive knowledge base relating to this fungal pathogen and its potential use for control of re-sprouting woody competitor species has been generated [30, 35, 40, 59, 88, 89]. This scientific knowledge base has been key to the recent registration of Myco-Tech Paste™ (Myco-Forestis Corp.) as the first commercial biocontrol agent for forestry in Canada [58], as well as a pending application for registration of a second product based on this same fungal organism. Development and registration of this biocontrol agent represents a major breakthrough, particularly in the province of Quebec, where herbicide use in forestry has been banned. However, a limited efficacy spectrum and application technology issues have constrained operational use to date. Recent innovations have purportedly overcome these limitations (B. Ure 2003, personal communication). In contrast, the use of nutrient loaded stock has become a common operational practice in many regions. However, the degree to which other approaches and technologies become incorporated into industrial programs remains to be seen.

Trend data indicating that 75–80% of harvested forest lands have been successfully regenerated and that an increasing proportion of the area is free from non-crop competition (Fig. 4), suggests that operational vegetation management techniques have generally been successful. However, successful regeneration should consider not only stocking levels and competition, but also the need to match species to site. Lack of attention to this aspect has led to substantive changes in species composition in the boreal forests of northern Ontario, where spruce has been replaced by increased hardwood components in many second growth forests [13, 36].

At a finer level of resolution, several vegetation management problems remain unsolved and there is a general tendency toward curative as opposed to preventative approaches.

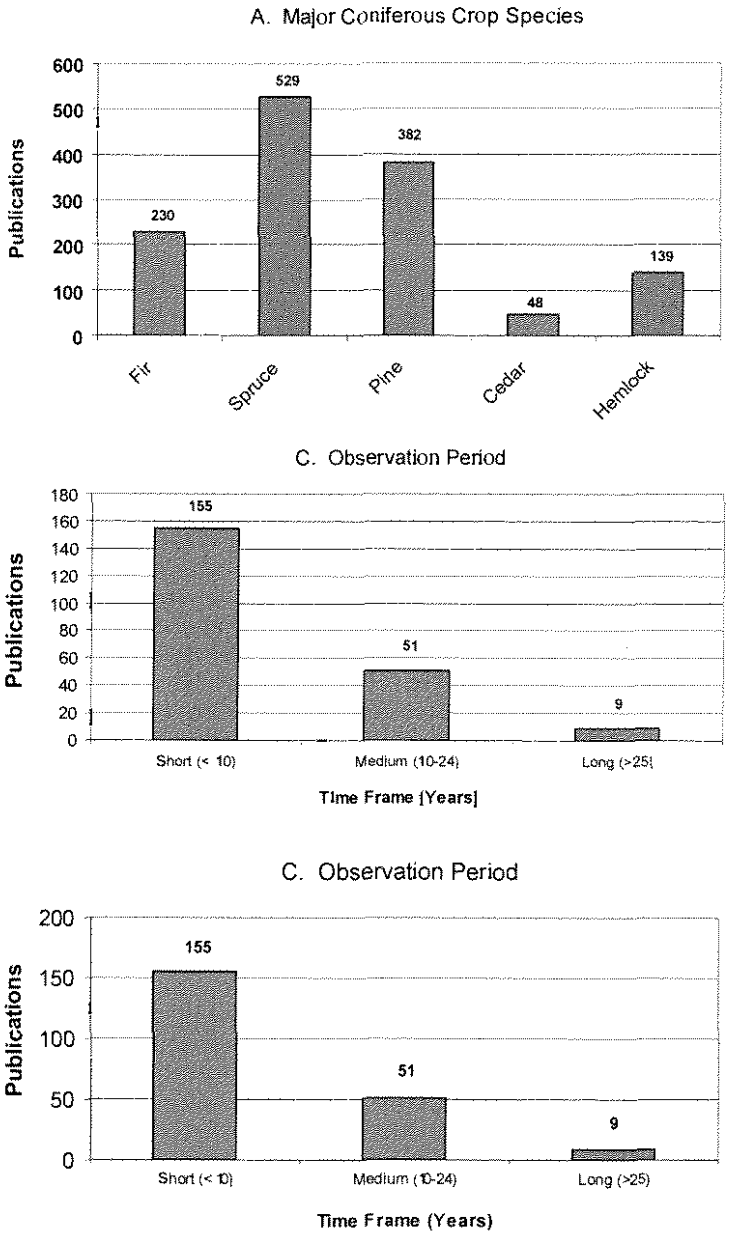


Figure 10. Number of journal publications pertaining to vegetation management in Canada (1990–present) in relation to (A) major crop species, (B) major competitor species or (C) observation period.

In addition, further optimization of many conventional practices may be possible. For example, although the chemical release program is cost-effective and efficacious, it is reliant on essentially one compound (glyphosate). Few alternative herbicides are available and, with the exception of an attempted minor use registration for imazapyr, we know of none that are under development for forestry. Despite many years of herbicide applications for forest vegetation management, there is still much to be learned with regard to optimum timing [5] and the extent and duration of weed control required to meet silvicultural objectives on specific site types [7]. More efficient methods of herbicide application have also been identified as a requirement, particularly for ground-based techniques and in relation to advanced methods of aerial application using electronic guidance systems for optimal control and targeted delivery of the chemical to the site. Practitioners of forest vegetation management often identify a need for changes in legislation to allow for the use of tank mixtures [7] as an efficient means of controlling competing vegetation complexes such as *Rubus/Calamagrostis/Epilobium*, or for use in vegetation management in deciduous crop tree and mixedwood silvicultural systems.

As many of the key competitor species in Canada (Tab. I) resprout vigorously from stems, underground rhizomes, and/or root stocks, use of manual and mechanical techniques often exacerbate competition problems or require repetitive applications which can be cost-prohibitive. In the pursuit of alternatives to chemical herbicides, substantial effort has been expended on increasing our understanding of manual cutting efficacy [4] and optimizing efficacy based on the season and timing of cutting [6].

The potential to minimize post-harvest vegetation problems from developing by employing certain preventative preharvest, harvest, and site-preparation measures has been discussed for years [90]. In practice to date, many of these measures ultimately have little effect on competition-prone sites and, as a result, trends in Canada have leaned away from site preparation and early intervention, with relatively little research and development effort expended to explore preventative approaches such as alternative harvesting methods and larger stock (Fig. 8). Moreover, there is a distinct need for development and demonstration of effective intensive management strategies that integrate a number of techniques, either concomitantly or through time, to maximize growth response and productivity of key high value crop species on quality sites. Wagner et al. [82, 87] and [60–62] have demonstrated that early and sustained intervention to control herbaceous competition yields substantial increases in stem diameter and volume of several coniferous crop species. Jobidon et al. [41] and Thiffault et al. [72] have also demonstrated the benefits of early release from competition and, at least in some cases, a multiplicative effect of vegetation control when combined with planting of larger stock [41]. Although such intensive vegetation management approaches are commonly employed in other countries such as the USA, New Zealand and Australia, vegetation management in Canada has largely been restricted to either a single site preparation or release treatment on any given site, usually focusing on overtopping woody competition. Neither of these approaches would generally be expected to yield maximal crop growth response or economic benefits.

Several independent studies that document significant crop growth response benefits resulting from applications of glyphosate (Vision®) support the continued use of this product in terms of increased growth response in various crops and relative to other vegetation control methods including brushsaw, and triclopyr basal bark treatments in jack pine [61], polyethylene mulch mats in hybrid poplar [74], manual cutting in black spruce [42] and repeated mechanical cutting in Englemann spruce (*Picea engelmannii* Parry ex Engelm.) [21]. However, as is the case for essentially all crop growth response data in Canada, these study results are short-term (< 10 yrs) in nature and may not accurately reflect treatment benefits to be expected at full rotation. In fact, some studies yield conflicting conclusions as to whether such crop growth responses to early vegetation management treatments translate into significant increases in total stand volume at longer time frames (i.e., ½ to full rotation age). Differentiation must now be made between objectives surrounding the maintenance of conifer-dominated ecosystems and those pertaining strictly to fibre production.

In this regard, a concerted effort is necessary to identify, maintain and re-measure previously established research plots and to establish permanent research sites with the potential to address these and other long-term data requirements. While some long-term study sites have been established (e.g. Carnation Creek, Cowichan Lake and MASS study sites in British Columbia [12], Fallingsnow Site in Ontario [45]), the commitment and funding support to these is often tenuous. Moreover, although long-term studies are recognized as having substantial potential for effects on forest management policy (BCMOF 2001), there has been no coordinated strategic planning at the national level to ensure that appropriate sites, representative of the major forest regions in Canada, are selected for comparative, long-term, vegetation management studies. We suggest that multi-disciplinary studies covering larger spatial scales and longer time frames, which include comparative assessments of new approaches against current industry standards and concomitant evaluations of efficacy, crop response economics and environmental effects at higher levels of biological organization (population, stand or ecosystem level), are required to advance science in this area.

Further, detailed economic analyses required to support the need for and benefits of various vegetation management strategies and techniques are scarce in Canada. As noted by [54], benefit-cost analysis is generally considered the most suitable method for evaluation but may yield different results when considered at the stand and forest levels. In their example for a hypothetical jack pine forest in Ontario, an option involving heavy site preparation, planting of container stock, and aerial herbicide application resulted in a benefit-cost ratio of 5.76 and an estimated net present value of 10 055 $/ha, compared to 4 721 $/ha for natural regeneration which was the second highest value among five other management options examined. The paucity of detailed economic analyses is surprising, given the necessary industrial focus on costs and return on investments [2] and undermines our ability to convince skeptical forest managers, forest certification auditors, and the public of the benefits of vegetation management in Canadian forestry.

In summary, while Canadian forest vegetation management research and practice have been generally successful, there are shortcomings including:

(i) reliance on curative chemical and mechanical methods as opposed to more preventative techniques such as the use of alternative stocking or harvesting methods,

(ii) reliance on relatively late release rather than early intervention approaches, including site preparation,

(iii) reliance on a single chemical herbicide (glyphosate),

(iv) a paucity of data clearly demonstrating long-term growth response and economic benefits.

4.2. Key factors influencing the future of vegetation management in Canada

In addition, to the land tenure system [93] and differential provincial management policies, which directly or indirectly influence Canadian forest management generally, there are several other national and international factors that may be postulated to interact and force change in Canadian forest vegetation management over the next decade. These factors include:

Increased use of alternative harvesting techniques – although clearcutting has and will probably remain the predominant

silvicultural system in Canada for the foreseeable future, continued national and international opposition to this practice is beginning to induce change. For example, the Weyerhauser Corporation in British Columbia has committed to discontinue clear-cutting on their coastal forest land holdings in favor of "variable-retention harvesting". In Ontario, increasing use is being made of "careful logging around advanced growth" and similarly in Quebec, "cutting with protection of regeneration and soils" was mandated by the *Forest Act*. An approximate 60% increase in use of this use of careful logging has occurred on the area harvested under even-aged management on Crown lands between 1987 and 1999 [19]. Changes in harvesting practices may be expected to alter the degree of canopy opening, soil disturbance and microclimate – all of which are primary drivers influencing the vegetative community re-occupying a site. Hence, any change to harvesting practices may induce concomitant changes in both the species mixture and intensity of vegetation competing for limiting resources. The few studies which compare succession following alternative harvesting [44, 46, 49], associated vegetation management requirements [32, 47] and crop productivity [43, 47, 50], suggest that in some cases alternative harvesting practices could minimize the intensity of competition and resultant need for vegetation management incursions, at least within some site types.

However, as noted by Dey and MacDonald [24], techniques such as shelterwood harvesting without proper preparation of the seedbed and control of competing vegetation often result in regeneration failures. Recent studies investigating vegetative response to uniform shelterwood harvesting techniques in Alberta demonstrate some reduction in raspberry and poplar competition, but very little impact on *Calamagrostis canadensis* grass. Similarly, we have observed trembling aspen to remain a serious competitor in the understory of Ontario white pine (*Pinus strobus* L.) shelterwood cuts, despite more than 50% crown closure in the overstory. We have also observed some recent "variable retention harvests" that amount to little more than complete removals of the merchantable stems and retention of the low quality stems and less merchantable species. There is neither the operational experience nor scientific understanding available to deal with the vegetation management problems that are likely to ensue from such activities. Moreover, recent studies [37] predict that some alternative harvesting techniques such as group selection cutting may result in increased soil losses over those induced by clearcutting. Such observations emphasize the need for full comparative evaluations of the efficacy, cost-effectiveness and environmental impacts associated with any potential sequence of silvicultural events. Thus, while alternative harvesting may be useful on some sites, it is unlikely to be the "silver bullet" for competing vegetation problems generally and in many cases may simply induce a shift to a different set of competitive interactions and challenges for researchers and practitioners alike.

Commercial and non-commercial use of species historically considered as weeds – may restrict the application of vegetation management techniques in some areas. In particular, new mills or milling policies have been established to take advantage of the widespread and rapid growth of species such as trembling aspen, previously considered strictly as problematic competitors, resulting in reduced regional demand for vegetation competition control. While aspen culture may be important in some areas, particularly for production of oriented strand board products, high quality conifer production will undoubtedly remain as Canada's key international forest product market niche and the maintenance of conifer habitat will always be a priority. Thus, it is critical that programs of research and practice maintain focus on integrated vegetation management techniques for enhancing growth and production of high quality conifer crops.

The introduction of exotic competitors may also provide Canadian vegetation management specialists with new challenges. As with many of our native pioneer "weedy" species, exotic plant species such as scotch broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*) are also well adapted to highly disturbed conditions such as roadsides, providing them a foothold for further expansion in to the Canadian forest landbase. The potential for these species to become serious new competition problems, particularly in Western Canada, has recently been noted [20] and rapid action to curtail expansion is recommended. Adopting expertise available from international research colleagues and practitioners intimately familiar with these problem species is an obvious first step to controlling these and other potential exotic problems.

Increasing segregation and protection of forest lands are expected to continue, further diminishing the forest area available for timber, pulp, and paper production. This process is already well underway. In 1995, approximately 7.6% (roughly 32 million hectares) of Canada's forest land was protected by legislation. Since that time, many provinces have increased the number and size of protected areas. For example, in Ontario, the Ontario Forest Accord resulted in the creation of 378 new parks and protected areas, enhancing previously protected areas by more than 2.4 million hectares. As part of this accord, forest industry, environmentalists and the public have agreed that overall production rates and wood supply to mills will not be constrained [56]. Avoiding a wood supply limitation requires development of truly integrated silvicultural strategies and greater production from less area in less time [3]. In the short term, these needs are expected to be met largely by increased commercial thinning. In the longer term, intensive vegetation management applied to high quality sites may also help to offset wood supply problems and focus efforts on those areas with greatest potential economic benefit [11]. Indirectly, intensive management on these selected smaller portions of the landbase could result in an overall reduction of human intervention and environmental impacts on the broader forest landscape [8] and may be viewed as a positive development from an environmental perspective as well.

Biotechnology research has the potential to modify forest vegetation management in the future, particularly through the development of herbicide-resistant tree species, in a manner parallel to developments in the agricultural sector. However, as for any vegetation control technique or other anthropogenic disturbance to natural systems, use of these biotechnologies carry potential risks for adverse environmental effects [92] and may be considered unacceptable by some Canadian public landowners as has previously been demonstrated for other novel vegetation control technologies [86]. While opposition to such biotechnology in the agricultural sector appears to be lower in North America as compared to Europe [28], we anticipate

that substantial public opposition will continue to constrain practical realization of these potentials in the Canadian forestry sector.

Forest certification is considered to be a powerful indirect economic incentive influencing forest vegetation management practice in Canada. According to a recent status report [1] approximately 95% of Canada's 119 million hectares of managed forest lands have been certified under various 3rd party certification standards. The high degree of forest certification demonstrates a strong industry commitment to sustainable forestry and intent to meet client demands.

Targeted government research programs – Some examples in the last decade include the federal Green Plan, Forest Renewal BC, the Ontario Vegetation Management Alternatives Program, and the Ontario Forest Accord. The latter is in its initial stages and reflects a general trend wherein a greater proportion of the forested landbase is being allocated to parks or conservation areas in an effort to enhance biodiversity, aesthetic and recreational values, while more intensive forestry is conducted on the reduced commercial forest landbase, to ensure sustainable timber, pulp and paper production. The Ontario Forest Accord and related Living Legacy Trust have forged innovative partnerships among governments, environmentalists, communities and resource industries, enhancing investment in vegetation management research and altering the approach to natural resources management generally. Many consider this an excellent model which has already and will continue to accelerate research, development and innovation in vegetation management research and practice as well as forest management generally.

5. CONCLUSIONS

Substantial progress has been made in Canadian forest vegetation management research over the last decade and operational programs focused largely on mechanical site preparation and applications of glyphosate for conifer release have been critical to the successful regeneration of major forest crop species in Canada. Despite these advances and general operational success, previous calls (e.g., FRACC 1992; [80]) to reduce our dependence on mechanical site preparation and release with a single chemical herbicide have not been fully met. Both the research community and industry practitioners are responding to new demands and opportunities associated with a decreasing commercial forest land base, international forest certification, new scientific discoveries, and alternative harvesting practices now coming into vogue. New research initiatives should include a balanced focus on preventative rather than curative strategies, alternatives to mechanical site preparation and chemical release, and development of holistic vegetation management techniques that are consistent and integrated with other silvicultural activities over the entire rotation cycle. Research and development of a full suite of vegetation management techniques and strategies that are demonstrably cost-effective, efficacious and environmentally acceptable to international standards will be important in continued evolution of a knowledge-based, integrated, and sustainable forest management [55], ensuring a continuous supply of quality forest products to Canadian mills with minimal deleterious effects on the multiple values associated with our forest ecosystems.

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Editorial

POTENTIAL EFFECTS OF HERBICIDES ON NATIVE AMPHIBIANS: A HIERARCHICAL APPROACH TO ECOTOXICOLOGY RESEARCH AND RISK ASSESSMENT

In their recent book on ecotoxicology of amphibians and reptiles, Sparling et al. [1] noted that several phenomena, including localized population declines, unusually high malformation rates, endocrine disruption, and loss of biodiversity, have focused scientific research and regulatory attention on amphibians, a relatively understudied class of vertebrate organisms. Although amphibians have long been recognized as a physiologically unique and ecologically important group of vertebrates, it is only within the last decade that a substantial body of knowledge on amphibian ecotoxicology has begun to develop. Determining direct and indirect effects of agrochemicals on amphibian species continues to be identified as a critical research need [1]. Moreover, multiple anthropogenic or natural stress factors often occur concomitantly in ecological systems. Considering multiple stressor interactions in risk and impact assessment is important [2].

Although many amphibian species are strongly associated with forest or woodland habitats and population decline phenomena have been repeatedly observed in relatively pristine environments [3–5], there has been a disproportionate emphasis on agricultural scenarios in amphibian ecotoxicology research. In addition, a number of recent studies [e.g. 6–8] support the general postulate that interactions among multiple stressors, either natural or anthropogenic, may be involved in both amphibian decline and high malformation incidence phenomena.

In an effort to contribute to the amphibian ecotoxicology information base relating to these issues, a 3-year project involving collaboration among researchers at the Canadian Forest Service, the University of Guelph, and Dartmouth College was conceived. A tiered series of investigations was implemented to examine the effects of two common forest-use herbicide formulations on amphibians indigenous to northeastern North America.

Conceptually, the project was based on the view that adequate ecological risk assessment and protection of wetland biota, including amphibians, requires an ecologically based and comprehensive research program having both laboratory and field studies [9]. Potential interactive effects among pH and herbicide stressors were identified as a key focus of the work because pH regimes of forest wetlands in this area vary widely as the result of both natural and anthropogenic acid deposition and the toxic effects of both herbicides chosen for investigation are influenced by pH.

The project was structured to parallel regulatory and risk assessment paradigms extant in both Canada and the USA, progressing in a hierarchical series to include comparative laboratory toxicity tests involving native anuran species as well as *Xenopus laevis* (Tier I), laboratory studies assessing multiple species and multiple stressor interactions (Tier II), in situ enclosure or field mesocosm studies to assess impacts under representative natural forest wetland conditions (Tier III), and chemical and biological monitoring studies conducted under conditions directly relevant to silvicultural uses in northern Ontario, Canada (Tier IV).

The four papers appearing in this journal issue are based on platform presentations made at the Annual Meeting of the Society of Environmental Toxicology and Chemistry in 2001 and are a subset of the overall project. These papers pertain specifically to the assessment of the glyphosate-based formulation Vision®, which dominates silvicultural herbicide use in Canada, and which is identical to the Roundup Original® formulation used extensively in the USA and many other countries worldwide.

In general, experimental protocols of lower tiers of this research project were characterized by relatively greater standardization, experimental control and cross-comparability, while upper-tier studies involved tradeoff of experimental control for increased ecological complexity and environmental relevance. Each tier of study provides unique and valuable data pertaining to potential impacts on native amphibian species, with both confirmatory and discrepant results observed among the various tiers of investigation. In combination, the research effort also touches on a number of broader issues in amphibian ecotoxicology, including the potential to enhance efficiency of standardized laboratory toxicity testing through use of central composite rotatable designs (Tier I); the validity of using *Xenopus laevis* as a surrogate for indigenous amphibians species and the need for appropriate choice of life stages in laboratory toxicity testing (Tier I); the need for consideration of multiple stress and multiple species interactions in ecotoxicological research, risk assessment, and regulation (Tier II); the value of in situ enclosure or mesocosm testing in terms of enhancing ecological relevance and reducing extrapolative error, particularly as these relate to the mitigative effects of natural dissipation and degradation mechanisms (Tier III); and the utility of monitoring studies to define the magnitude and probability of real-world exposures as a requisite of probabilistic risk assessment and in confirming risks postulated from lower tier study results (Tier IV)

We hope that readers will find that this body of work is effective in characterizing the potential risk posed to amphibians as a result of the use of Vision herbicide in forest scenarios, that it exemplifies the value of a hierarchical approach in ecotoxicology research and risk assessment, and that it contributes to advancement in the science of amphibian ecotoxicology.

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COMPARATIVE EFFECTS OF pH AND VISION® HERBICIDE ON TWO LIFE STAGES OF FOUR ANURAN AMPHIBIAN SPECIES

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Abstract—Vision®, a glyphosate-based herbicide containing a 15% (weight:weight) polyethoxylated tallow amine surfactant blend, and the concurrent factor of pH were tested to determine their interactive effects on early life-stage anurans. Ninety-six-hour laboratory static renewal studies, using the embryonic and larval life stages (Gosner 25) of *Rana clamitans*, *R. pipiens*, *Bufo americanus*, and *Xenopus laevis*, were performed under a central composite rotatable design. Mortality and the prevalence of malformations were modeled using generalized linear models with a profile deviance approach for obtaining confidence intervals. There was a significant ($p < 0.05$) interaction of pH with Vision concentration in all eight models, such that the toxicity of Vision was amplified by elevated pH. The surfactant is the major toxic component of Vision and is hypothesized, in this study, to be the source of the pH interaction. Larvae of *B. americanus* and *R. clamitans* were 1.5 to 3.8 times more sensitive than their corresponding embryos, whereas *X. laevis* and *R. pipiens* larvae were 6.8 to 8.9 times more sensitive. At pH values above 7.5, the Vision concentrations expected to kill 50% of the test larvae in 96-h (96-h lethal concentration [LC50]) were predicted to be below the expected environmental concentration (EEC) as calculated by Canadian regulatory authorities. The EEC value represents a worst-case scenario for aerial Vision application and is calculated assuming an application of the maximum label rate (2.1 kg acid equivalents [a.e.]/ha) into a pond 15 cm in depth. The EEC of 1.4 mg a.e./L (4.5 mg/L Vision) was not exceeded by 96-h LC50 values for the embryo test. The larvae of the four species were comparable in sensitivity. Field studies should be completed using the more sensitive larval life stage to test for Vision toxicity at actual environmental concentrations.

Keywords—Vision Amphibians Aquatic toxicity Life-stage sensitivity Generalized linear models

INTRODUCTION

The global phenomenon of localized amphibian population declines [1], a high prevalence of amphibian malformations [2], and the increasing presence of amphibians on threatened or endangered species lists [3] have stimulated research investigating amphibian response to both natural and anthropogenic stressors. In addition to habitat destruction and fragmentation [3], stressors such as enhanced ultraviolet-B [4], pesticides [5], acid deposition [6], disease [7], and parasites [8] have been shown to have significant adverse effects on amphibians. In natural settings, the likelihood of interactive and indirect effects of these stressors on amphibians is unknown.

In Canada, the home range for most native amphibian species include forested landscapes [9]; however, there is a knowledge gap on amphibian ecotoxicological studies in this key component of their range. Herbicides are a major tool used in managing competing vegetation to hasten regeneration of harvested forests. Over the last decade, Vision® (Monsanto Canada, Winnipeg, MB, Canada), a glyphosate-based herbicide, has accounted for more than 80% of this use pattern and has been applied using predominantly aerial techniques [10]. Vegetative buffer zones are often required to mitigate inputs from aerial applications into water bodies that can be identified on large-scale (1:20,000) topographical maps. While buffer zones have proven to be effective for this purpose [11,12], small wetlands that are ubiquitous components of forested landscapes are often not visible either on topographical maps or from the air. These small water bodies are typically not protected by buffer zones, resulting in a substantially higher potential for these systems to receive direct overspray. Small wetlands also show a wide range in physical and chemical variables, including pH. For example, data available for small wetlands in Northern Ontario show a 95th percentile range of pH from 4.5 to 8.5 [13]. Well documented is the inhibition or delay of hatching in amphibian embryos exposed to low pH, where the perivitelline membrane is prevented from expanding with a resultant curling effect of the growing embryo within [14]. Further, lethal concentrations appear to vary for Vision depending on the pH of the media. While the herbicidal ingredient of Vision, glyphosate acid, becomes more toxic to fish and aquatic invertebrates as pH is decreased, Vision becomes more toxic as pH is increased [15]. Amphibians that utilize small forest wetlands for breeding and foraging habitat are potentially adversely affected by both herbicide contamination from off-target deposition and pH stress.

The hazard for amphibian populations exposed to either Vision or pH stress, alone or in combination, is not well understood. Pesticide hazard identification is based on the relation of the expected environmental concentration (EEC) to standard toxicological endpoints such as lethal concentration

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(LC) estimates. In accordance with Canadian regulatory authorities, the EEC was calculated as the maximum concentration of active ingredient predicted to occur in a body of water 15 cm deep if directly oversprayed with the maximum application rate [13,16]. This value is used to predict a worst-case scenario in the assessment of hazard and was used here as a baseline value of environmental concentrations for comparison with laboratory results. The EEC for glyphosate and its surfactant, based on the maximum application rate of Vision at 2.1 kg glyphosate acid equivalents (a.e.) a.e./ha, is 1.4 mg a.e./L and 0.20 mg/L, respectively. Acute toxicity testing of glyphosate and its formulated products using aquatic organisms has been vast [17]. Using the Frog Embryo Teratogenesis Assay—*Xenopus*, Perkins et al. [18] estimated a 96-h LC50 value for the chemically identical formulation, Roundup Original® (Monsanto) of 9.3 mg a.e./L. Using the larvae of four Australian anurans, Mann and Bidwell [19] reported a 48-h LC50 range of 2.9 to 11.6 mg a.e./L. Similarly, Folmar et al. [15] reported a 96-h LC50 range for rainbow trout of 1.4 to 7.6 mg a.e./L at pH 9.5 and 6.5, respectively. In general, toxicity values from the limited amphibian studies reported are similar to those for other aquatic organisms [17,20].

The three native Canadian anurans, *Bufo americanus*, *Rana pipiens*, and *R. clamitans* [9], and the exotic, *Xenopus laevis*, span varying life-history strategies. In Northern Ontario, *B. americanus* breeding sites are generally ephemeral ponds, shallow streams, or temporary ditches where egg laying occurs in April to early May. Metamorphosis is rapid with a larval period of about two months. Both *R. pipiens* and *R. clamitans* seek predominantly permanent water bodies as breeding sites. *Rana pipiens* breeds in late April to early May and *R. clamitans* from May to August. *Rana clamitans* overwinter as larvae and require two summers to metamorphose whereas *R. pipiens* metamorphoses in about three months. The completely aquatic anuran, *X. laevis*, is native to Southern Africa. *Xenopus laevis* is the most widely studied anuran due to the ease of adult maintenance and the opportunities for breeding at any time of the year. Unfortunately, the relevance of this species to Canadian environments for predicting effects on native anurans is unknown.

To further our understanding of how the combination of herbicides and other stressors affect early life-stage amphibians, the first project tier was designed as a laboratory study and formed the basis of comparison for upper-tier studies [13,21]. The objectives of this tier were (1) to further characterize the effects of pH on the toxicity of Vision herbicide to early life-stage anurans; (2) to evaluate potential sensitivity differences between the embryonic and larval stages to treatments of the combination of pH and herbicide concentration; (3) to perform an interspecies comparison of sensitivity using four anuran species, *X. laevis*, *R. pipiens*, *R. clamitans*, and *B. americanus* including an assessment of *X. laevis* as a surrogate; and (4) to compare expected environmental concentrations to estimated toxicity values for the purposes of preliminary hazard identification.

MATERIALS AND METHODS

Test substances and analytical chemistry

Vision herbicide was formulated with a guarantee of 356 g glyphosate a.e./L as the isopropylamine salt containing a 15% (weight:weight) polyethoxylated tallow amine surfactant blend, MON 0818 (Monsanto, lot PIT8903-301F). The concentration of glyphosate and its primary degradation product, aminomethylphosphonic acid, were verified using validated gas chromatographic techniques [13]. Glyphosate acid equivalents were used as a basis of comparison. However, the test product was the formulated product, Vision. To convert to mg/L Vision, the conversion is 1 mg a.e./L = 3.226 mg/L Vision [17].

Culture water

Water used in all breeding tanks, controls, and treatments conformed to the American Society for Testing and Materials guideline for the performance of the Frog Embryo Teratogenesis Assay—*Xenopus* [22]. It was composed of 625 mg NaCl, 96 mg $NaHCO_3$, 30 mg KCl, 15 mg $CaCl_2$, 60 mg $CaSO_4.2H_2O$, and 75 mg $MgSO_4$/L of deionized water. To ensure the maintenance of pH, a 0.01 M phosphate buffer was added. Preliminary embryonic studies, using all species but *B. americanus*, demonstrated that the presence of the buffer did not alter mortality, the prevalence of malformations, or embryo length when compared with treatments containing no buffer (data not shown). All solutions were pH adjusted using 1 N NaOH or 1 N HCl.

Animal care

Xenopus laevis adults were housed at the Hagen Aqualab, University of Guelph (Guelph, ON, Canada). These adults were maintained under flow-through conditions using filtered, irradiated well water at 18°C under a 12:12-h light:dark cycle. A feeding rotation of beef liver and Frog Brittle® (Nasco, Fort Atkinson, WI, USA) was provided biweekly. *Xenopus laevis* mating was stimulated by injection of 600 IU and 800 IU of human chorionic gonadotrophin (Sigma-Aldrich Canada, Oakville, ON, Canada) in the dorsal lymph sac of males and females, respectively. Amplexus, egg laying, and fertilization occurred within 12 h in a 22°C, darkened room. Embryos of *R. pipiens* and *B. americanus* were field collected from uncontaminated sites in Guelph from April to early May in 2001 and 2002. *Rana clamitans* embryos were collected in June and July of 2000 and 2001. Field-collected embryos were held in 22°C culture water at a pH of 7.0 to 7.4 prior to testing. The gelatinous coating of all embryos was removed using a 2% (weight:volume) cysteine solution prepared in culture water and pH adjusted to 8.1 using 1 N NaOH [22]. Three clutches of embryos for *B. americanus*, *X. laevis*, and *R. pipiens* and two clutches of embryos for *R. clamitans*, each from different parents, were pooled within species and randomly allocated to embryo and larval test units to minimize parental effects in the definitive tests.

Test procedure—Embryo test

The frog embryo teratogenesis assay—*Xenopus* [22] is a whole-embryo test inclusive of life stages Gosner 8 to 10 to Gosner 25 [23]. Only normally cleaving embryos at Gosner 8 to 10 were selected for the tests. Each experimental unit consisted of a 60·15-mm plastic petri dish containing 20 embryos in 10 ml of treatment solution. Each treatment solution represented a specific combination of pH and Vision concentration (Fig. 1). Treatment solutions were prepared every 48 h using a 1,000 mg a.e./L Vision stock solution, though the pH of the treatment solutions were tested and adjusted daily between preparations. Treatment and control solutions were renewed every 24 h. The experimental units were incubated at 23 ± 2°C. At 24-h intervals, dead embryos were recorded and removed and the number of embryos that had hatched from the

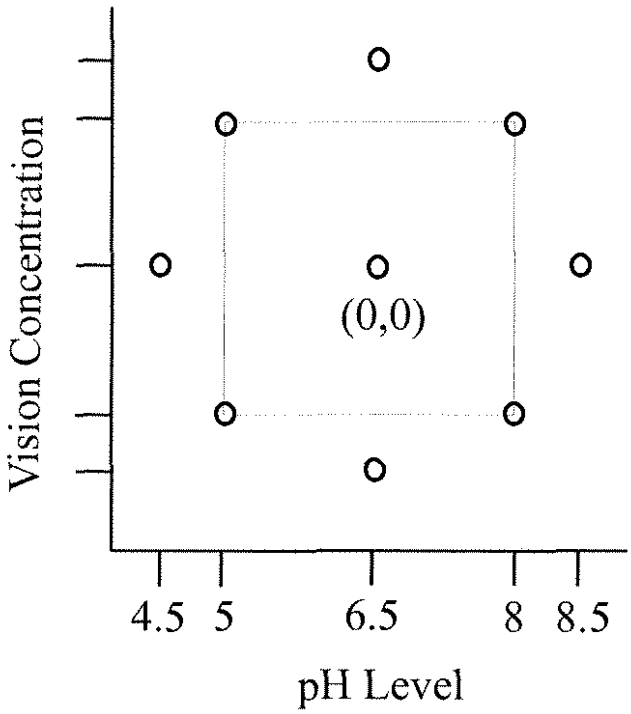

Fig. 1. Central composite rotatable design points (denoted by ○) using nine combinations of pH and Vision® (Monsanto Canada, Winnipeg, MB, Canada) concentration levels graphed on linear scales. Design points were coded based on a central point (0, 0) and eight other points spaced on the circumference of a circle with a radius of 1.41. The inner square represents the graphed surface. The *y*-axis does not contain actual Vision concentrations because definitive tests employed varying concentrations based on results from preliminary tests.

egg capsule was recorded. Prior to hatching, dead embryos were defined as those lacking membrane integrity generally exhibited as large, white embryos. A lack of heartbeat was used as a measure of death posthatch. Tests were terminated when 90% of the controls had reached Gosner stage 25. At this time, surviving embryos were fixed in a 3% formalin solution. By 96 h, 90% of the control *X. laevis* and *B. americanus* embryos had reached Gosner stage 25. Exposures were continued until 5 and 7 d for *R. pipiens* and *R. clamitans*, respectively. The prevalence and type of malformations, scored using the *Atlas of Abnormalities* [24], and total length were recorded following fixation. During the test, pH was monitored and found to deviate no more than 0.5 units from the original pH within 24 h.

Test procedure—Larval test

The American Society for Testing and Materials document for the testing of larval amphibians was used as a guide in the performance of the larval tests [25]. Experimental units consisted of a 2-L aquarium containing 10 healthy Gosner stage 25 larvae in 1 L of treatment solution. Treatment solutions were prepared daily prior to renewal using pH-adjusted culture water and spiked using a 1,000-mg a.e./L Vision stock solution. Exposure continued for 96 h and treatment solutions were renewed every 24 h. The experimental units were held at 22 ± 1°C. For 4 additional d, surviving larvae were kept in pH-maintained culture water containing no herbicide to observe possible latency effects. During this 4-d period, larvae were fed a mixture of ground Tetramin® (Tetra, Blacksburg, VA, USA) and Spirulina® (Hagen Industries, Waverly, NY, USA) flakes ad libitum, the culture water was renewed every 48 h, and there was aeration provided to each experimental unit. Mortality and the presence of gross external malformations were monitored daily. At the end of the test, the length and weight of each surviving larvae were recorded following euthanasia in tricaine methanesulfonate (MS-222; Sigma-Aldrich Canada) and further fixation in 3% formalin. Water-quality variables of dissolved oxygen, ammonia, and temperature were recorded every 48 h and pH was monitored at least every 24 h. In all larval tests, pH deviated no more than 0.4 units from the original pH within 24 h and the dissolved oxygen content did not fall below 80%.

Experimental design

All definitive tests employed a central composite rotatable design to provide a response surface while requiring a minimum of experimental units [26,27]. Variables included pH and Vision concentration. Northern Ontario wetlands were found to be in the pH range of 4.5 to 9.1, with a mean of 7.0 [13]. This provided the impetus for the range and midpoint pH chosen for testing and was subsequently the same for all tests. The pH levels used in the definitive tests were 4.5, 5.0, 6.5, 8.0, and 8.5 (Fig. 1). For definitive tests, the range and midpoint for Vision concentrations were determined based on preliminary dose–response studies at pH 5.5 and pH 7.5 using concentrations ranging from 0.1 to 20 mg a.e./L. The midpoint (0, 0) of the central composite rotatable design was replicated five times. All other design points were replicated once, for a total of 13 experimental units per test (Fig. 1). Each test was replicated twice, totaling 520 embryos and 260 larvae for the embryo and larval data sets, respectively. Two to four replications of the control were used, depending on the availability of animals. The control contained culture water at a pH of 8.0. To determine the pH used in the controls, animals from all four species at both life stages were held in pH-adjusted culture water containing no chemical. Among the five pH levels used, 4.5, 5.0, 6.5, 8.0, and 8.5, a pH of 8.0 offered the least mortality and the greatest animal growth (data not shown). Control mortality during the definitive tests was not used in model generation but was used as a measure of experimental validity.

STATISTICAL ANALYSES

Model generation

Mortality and the prevalence of malformations were fit as generalized linear models with a binomial response distribution and a probit link function. Model generation was accomplished as in [28]. In summary, for mortality, the probit of percent mortality (p) was assumed to be under the influence of both pH (x) and test substance concentration (y). The mortality data from all experimental data sets were fit to the model

$$\text{Probit}(p) = \beta_0 + \beta_1 x + \beta_2 y + \beta_3 x^2 + \beta_4 y^2 + \beta_5 xy \quad (1)$$

where β_0 was the intercept coefficient, β_1 and β_2 were the linear coefficients, β_3 and β_4 were the quadratic coefficients and β_5 was the interaction coefficient term. Model building followed a backward selection approach, where terms in Equation 1 were removed from the full model based on significance. The difference in deviance between successive models was assessed for significance based on the 5% critical value of the chi-square distribution on one degree of freedom, 3.84. When the difference in deviance between a model with p (number of regression coefficients) parameters and another with one

Table 1. Model equations, Pearson's chi-square statistics, model degrees of freedom (*df*), and critical values for determining overdispersion for the toxicity of pH and Vision® (Monsanto Canada, Winnipeg, MB, Canada) concentration to four anuran species at two life stages. These models describe response surfaces for the effect of pH (x) and Vision concentration (y) on the probit of predicted mortality on the normal scale. Models were derived using the general linear model (GLM) function in S-Plus (S-Plus 2000 Professional Release 2, MathSoft, Cambridge, MA, USA)

Species	Life stage[a]	Model[b]	Pearson's χ^2	*df*	Critical value for Pearson's χ^2
Xenopus laevis	Embryo	Mortality = $2.3 - 0.66x - 10.3y + 2.3y^2 + 1.5xy$	25.2	21	32.7
	Larvae	Mortality = $334.9 - 119.8x - 274.8y + 10.1x^2 + 108.4y^2 + 50.7xy$	11.4	20	31.4
Bufo americanus	Embryo	Chi-square: Mortality = $-12.0 + 4.5x - 9.3y - 0.49x^2 + 2.2xy$	44.0	20	31.4
		F-distribution model = chi-square model	NA[c]		NA
	Larvae	Mortality = $17.1 - 6.4x - 18.0y + 0.51x^2 + 11.4y^2 + 3.1xy$	28.6	21	32.7
Rana clamitans	Embryo	Mortality = $-4.5 + 1.1x - 6.6y - 0.16x^2 - 2.1y + 2.2xy$	57.7	20	31.4
		F-distribution model = chi-square model	NA		NA
	Larvae	Mortality = $19.9 - 7.3x - 17.1y + 0.60x^2 + 5.2y^2 + 3.1xy$	22.8	20	31.4
Rana pipiens	Embryo	Mortality = $106.4 - 25.8x - 85.3y + 1.6x^2 + 23.6y^2 + 8.4xy$	11.4	20	31.4
	Larvae	Mortality = $12.0 - 4.8x - 15.4y + 0.43x^2 + 3.7xy$	13.1	21	32.7

[a] Embryo = Gosner 8 to Gosner 25; larvae = Gosner 25.
[b] Mortality = probit of predicted mortality on normal scale; x = pH; y = logarithm (base 10) of Vision concentration in mg acid equivalents/L.
[c] Not applicable.

less parameter was greater that 3.84, the model with $p - 1$ parameters was considered inadequate [29]. Model generation for the prevalence of malformations was equivalent to that for mortality except, prior to model generation, the malformation rate was corrected for the control malformation rates using Abbott's formula [30].

Point estimates and confidence intervals

Solving the fitted model equations for test substance concentration at set pH and probit values resulted in lethal concentration point estimates. Ninety-five percent confidence intervals were obtained by the profile deviance approach [31] using the general linear model (GLM) function in S-Plus (S-Plus 2000 Professional Release 2; MathSoft, Cambridge, MA, USA) [32].

Overdispersion

Models fit to binomial data with residual deviances higher than expected are said to demonstrate overdispersion [31–33]. When the Pearson's chi-square statistic was greater than the 95% point on the appropriate chi-square distribution, the model was considered overdispersed [33]. For overdispersed models, the deviance no longer follows a chi-square distribution and confidence intervals are found using the quasideviance approach. Quasideviance is obtained by dividing the deviance of the fitted model by the estimated scale parameter of the fullest model. The scale parameter is the Pearson's chi-square statistic divided by its degrees of freedom [34]. The profile quasideviance approach to constructing confidence intervals is analogous to the profile deviance, with the exception that the scaled quasideviance follows an approximate F distribution. Thus, the inclusion of parameter values into the confidence interval are compared with the appropriate F distribution [33].

Multiple mean comparisons of length of the surviving embryos and larvae were compared against the control using a Dunnett's test. Correlation analysis for percent growth, relative to the controls, and percent mortality was used in an attempt to link the variables affecting mortality to those contributing to growth inhibition. Response surface graphs were generated in S-Plus and the type 1 error rate was set at 0.05 for all statistical tests.

RESULTS

Control mortality did not exceed 10% (median = 5%; 8 models). The 96-h data sets and the 5-d (*R. pipiens*) or 7-d (*R. clamitans*) data sets for the slower developing embryos generated LC values that were not significantly different. To ease comparison, the 96-h responses were used in model generation resulting in the presentation of 96-h LC10 and LC50 values. Only the *B. americanus* and *R. clamitans* embryo models showed overdispersion based on a Pearson's chi-square statistic greater than its associated critical value (Table 1). These models were considered overdispersed and were subsequently adjusted using the quasideviance approach [33].

The generated models were all second-order polynomials with a significant interaction (xy) term, implying an interactive effect of pH on the toxicity of Vision to both life stages of all anuran species (Table 1). The interaction term was positive, representing an increase in Vision toxicity with a concomitant increase in pH (Table 1). This was evident in a comparison of 96-h LC10 and LC50 values calculated at two representative pH levels, pH 6.0 and pH 7.5 (Table 2), and through an examination of the response surface graphs (Fig. 2). It appeared from Table 2 that *B. americanus* and *R. clamitans* embryo LC10 and LC50 values were not different regardless of pH. At the pH levels 6.0 and 7.5, the pattern was inconsistent with an increase in toxicity with an increase in pH (Table 2). The pattern was reestablished when the LC10 and LC50 values were computed below a pH of 5.5 for both species (see graph B in Fig. 2).

The predicted LC50 estimates from the embryonic models were higher than those obtained from the larval models (Table 2). The embryos of *X. laevis* and *R. pipiens* were between 6.8 to 8.9 times less sensitive to Vision than their corresponding Gosner stage 25 larvae under similar pH conditions. There was not as great a difference between the embryo and larval sensitivities of *B. americanus* and *R. clamitans*, with predicted embryo toxicity estimates being 1.5 to 3.8 times greater than their corresponding larval estimates.

Sensitivity differences between the four species were dependent on the life stage examined. Predicted toxicity estimates for the Gosner stage 25 larvae were similar regardless of pH. At pH 7.5, the LC50 estimates for the embryos were

Table 2. Comparative sensitivity of the embryonic and larval stages (Gosner 25) of four anuran species to Vision® (Monsanto Canada, Winnipeg, MB, Canada) at representative pH levels of 6.0 and 7.5. Based on these lethal concentration (LC) estimates, the embryo models produced values greater than those of the larvae and, in general, Vision was more toxic at pH 7.5 than at pH 6.0. The asterisks denote a point estimate at or below the expected environmental concentration of 1.4 mg acid equivalents (a.e.)/L

Species	Life stage[a]	pH	96-h LC10 (mg a.e./L) (95% confidence interval)	96-h LC50 (mg a.e./L) (95% confidence interval)
Xenopus laevis	Embryo	6.0	6.2 (4.7, 7.4)	15.6 (12.7, 23.0)
		7.5	4.0 (3.1, 4.7)	7.9 (7.2, 8.7)
	Larvae	6.0	1.99 (1.7, 2.0)	2.1 (2.0, 2.7)
		7.5	0.85 (0.55, 0.87)*	0.88 (0.84, 0.92)*
Bufo americanus	Embryo	6.0	2.2 (0, 3.8)	4.8 (4.0, 5.7)
		7.5	4.3 (0, 7.5)	6.4 (5.8, 7.0)
	Larvae	6.0	2.1 (1.8, 3.9)	2.9 (2.3, 10.5)
		7.5	1.2 (1.0, 1.4)*	1.7 (1.5, 1.9)
Rana clamitans	Embryo	6.0	2.6 (0, 6.0)	5.3 (3.9, 9.2)
		7.5	2.8 (2.2, 3.8)	4.1 (3.4, 6.4)
	Larvae	6.0	2.1 (1.7, 2.5)	3.5 (3.0, 4.6)
		7.5	0.89 (0.70, 1.1)*	1.4 (1.2, 1.7)*
Rana pipiens	Embryo	6.0	13.1 (12.8–13.3)	15.1 (14.0–17.5)
		7.5	6.7 (6.3–6.9)	7.5 (7.0–9.0)
	Larvae	6.0	1.1 (1.0–1.3)*	1.8 (1.5–2.2)
		7.5	0.83 (0.71–0.92)*	1.1 (0.96–1.14)*

[a] Embryo = Gosner 8 to Gosner 25; larvae = Gosner 25.

similar. At pH 6.0, LC50 estimates for *X. laevis* and *R. pipiens* embryos were similar, whereas *B. americanus* and *R. clamitans* embryos were similar but about three times more sensitive than *X. laevis* and *R. pipiens*. Thus, at low pH levels, the models of these two groupings diverged.

For the embryo tests, *X. laevis* and *B. americanus* did not show a significant prevalence of malformations in any treatment combination when corrected for the prevalence of control malformations using Abbott's formula [30]. Malformations occurred at levels above the controls for both *R. pipiens* and *R. clamitans* embryos. They were dominated by laterally bent tails in *R. pipiens* embryos and abnormal face, eye, and gut development in *R. clamitans* [24]. The generated malformation model for *R. pipiens* did not allow for the attainment of the effective concentration required to cause malformations in 50% of the test organisms (EC50) because total mortality occurred before a 50% malformation rate could be achieved (model not shown). The model for *R. clamitans* embryos produced EC50 estimates that were not different than the previously estimated LC50 values. The teratogenic index was used as a measure of the teratogenicity potential of the pH/Vision combinations. According to the American Society for Testing and Materials [22], a teratogenic index greater than 1.5 indicates that the test substance is a suspect teratogen. This value, obtained by dividing the LC50 by the EC50, was equal to or less than 1 for *R. clamitans* embryos at both a pH of 6.0 and 7.5, suggesting that the treatments were not substantially teratogenic. Data from concurrent tests using only pH as a variable in the range of pH 4.5 to pH 8.5, showed background levels of malformations that were not pH dependent (data not shown). No visible malformations were evident in the larval test.

Hatching was >95% complete by 48 h for *X. laevis*, 72 h for *R. pipiens* and *B. americanus*, and 96 h for *R. clamitans*. The observation that death rarely occurred prior to hatching was made and supported by the data such that the number of animals hatched at any one observational point always exceeded the number of animals dead. Further, the combined death of all embryo tests was only 2% in the first 24 h, a time when few to no animals had hatched. An exception was that, in three treatment combinations, all 20 unhatched *R. clamitans* embryos were viable at 48 h and dead at 72 h. One treatment combination caused the same result in *X. laevis*, with death at 48 h. These treatment combinations represented those of the highest Vision concentrations and pH and were consistent among test replications. Time to hatching was similar between controls and treatment combinations for *X. laevis*, *B. americanus*, and *R. pipiens*. Delayed hatching of *R. clamitans* occurred at pH 4.5 and pH 5 but was postponed less than 24 h in these experiments.

Length measurements for both embryos and larvae of all species were no less than 61% of the control. The mean length (millimeters) and standard deviation for the controls at the end of the tests are shown in Table 3. *X. laevis*, *R. clamitans*, and *R. pipiens* embryos showed significant growth inhibition where growth was calculated as a percentage relative to the controls (Table 3). Significant growth inhibition did not occur in any other tests. Correlation coefficients were calculated to determine if the factors regulating mortality, pH, and Vision concentration also regulated growth. Strong relationships ($r < -0.70$) were found in four of the eight tests where increases in mortality lead to a decrease in growth (Table 3).

DISCUSSION AND CONCLUSIONS

Single-species laboratory testing was employed in an attempt to further characterize the relationship between pH and chemical concentration, to determine how species and life-stage differences affected sensitivity, and to evaluate the potential hazard of Vision use to early life-stage anurans. Laboratory testing occurred in a highly controlled environment that was particularly advantageous for these comparisons. Relating laboratory toxicity to that observed in the field is of utmost importance for chemical risk assessment with amphibians [35]. The information gained in these laboratory studies was ultimately validated by field studies [21].

The advantages of using multifactorial testing and modeling

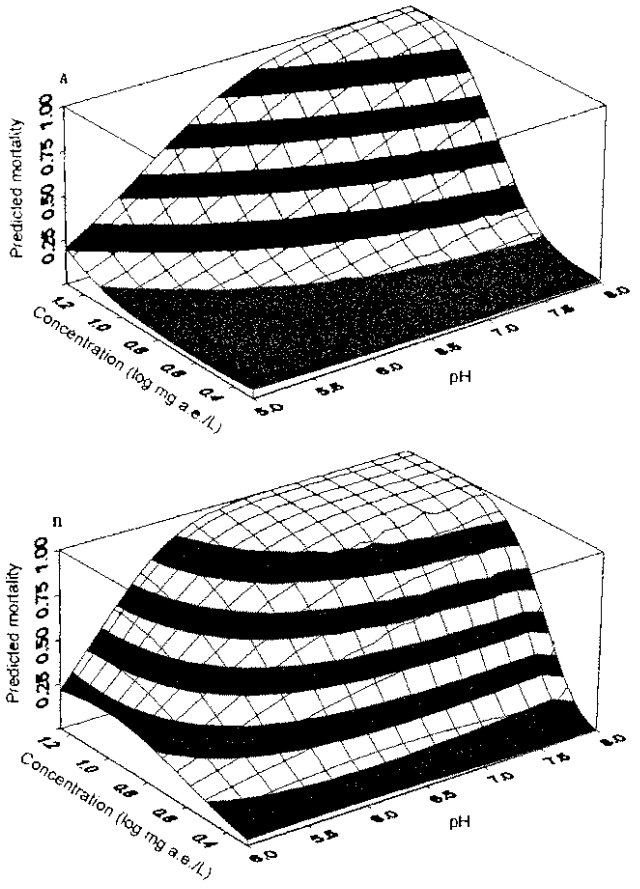

Fig. 2. Combined toxicity of the herbicide Vision® (Monsanto Canada, Winnipeg, MB, Canada) and pH to *Xenopus laevis* (**A**, mortality = $2.3 - 0.66x - 10.3y + 2.3y^2 + 1.5xy$) and *Rana clamitans* (**B**; mortality = $-4.5 + 1.1x - 6.6y - 0.16x^2 - 2.1y + 2.2xy$) embryos. Positive interaction between Vision concentration and pH indicated that Vision toxicity would be predicted to increase with increases in pH. Concentration values are reported as $\log_{10}$ transformed, in glyphosate acid equivalents (a.e.; 1 mg a.e./L = 3.226 mg/L Vision). Generated probit values were transformed to the probability scale for the standard normal distribution. The S-Plus® (S-Plus 2000 Professional Release 2, MathSoft, Cambridge, MA, USA) is capable of calculating predicted values for the generation of a three-dimensional circular graph. However, for illustrative purposes, the graphed portion of the model lies within the square outlined in Figure 1.

include the ability to characterize the extent of the interaction, the ability for prediction based on point-estimate generation at any level of the variables used in the experiments, and/or to predict a worst-case scenario. In this study, we used the generated models for predictive purposes by estimating the toxicity of Vision at two representative pH values. An examination of the 96-h LC10 (8.0 mg a.e./L) and LC50 (9.3 mg a.e./L) estimates for *X. laevis* embryos at a pH of 7.6 to 7.9, by Perkins et al. [18], demonstrated that our estimated values were consistent with the literature. The Mann and Bidwell [19] study estimated 48-h LC50 values in the range of 2.9 to 11.6 mg a.e./L for early larval stages of four Australian anuran species at a pH range of 5.1 to 8. Specific pH ranges for the individual tests were not reported. Nevertheless, an extension of this study to 96 h would have lead to lower LC values than those reported at 48 h. The models generated in this study produced toxicity estimates that were consistent with the current literature [15,18,19].

An increase in Vision toxicity with increased pH was demonstrated in both the embryo and the larval tests. Previous research in this area has demonstrated similar results with Vision, its agricultural counterpart Roundup Original, and their surfactant MON 0818, all showing greater toxicity at elevated pH levels to rainbow trout [15] and *X. laevis* embryos [28]. The similarity in toxic profiles of MON 0818 and Vision [15,18,28] and the relatively low toxicity of the isopropylamine salt of glyphosate [28] demonstrates that the surfactant is the major toxic component of the formulation. Because MON 0818 is a tertiary amine blend with one fatty alkyl group and two polyoxyethylene groups attached to a nitrogen atom, we hypothesize that it is also responsible for the weak base interaction with pH. Gill uptake of many surfactants occurs readily [36] and it is hypothesized that, at high alkalinities, gill accumulation is accelerated due to the high proportion of the nonionized form of MON 0818 (-N) as compared with the ionized form ($-NH^+$). A titration of a 10% and 1% solution of MON 0818 with a 0.1 M and 0.01 M hydrochloric acid solution demonstrated a p*Ka* range for MON 0818 of 6.5 to 7.0 (data not shown). This is tangibly evident in the comparison of lethal concentration estimates such that, when the ionized form predominated (pH 6.0), toxicity was lower than when the nonionized form predominated (pH 7.5). Similarly with rainbow trout and bluegills, the toxicity of Roundup Original was low

Table 3. Length results following the exposure of four anuran species at two life stages to the combination of pH an Vision® (Monsanto Canada, Winnipeg, MB, Canada) concentration. Only animals surviving at the test end were measured. An asterisk denotes significant ($p < 0.05$) growth inhibition, where growth is calculated as a percentage relative to the controls. Correlation coefficients were calculated to determine the relationship between percent mortality and percent growth

Species	Life stage[a]	Mean length (SD) of controls (mm)	Greatest growth inhibition	Correlation coefficient (% mortality: % growth)
Xenopus laevis	Embryo	9.7 (0.52)	83%*	−0.87
	Larvae	11.4 (1.2)	82%	−0.94
Bufo americanus	Embryo	7.8 (1.5)	61%	−0.45
	Larvae	11.3 (0.52)	82%	−0.98
Rana clamitans	Embryo	8.5 (0.32)	86%*	−0.75
	Larvae	15.2 (1.6)	92%	−0.43
Rana pipiens	Embryo	10.6 (0.88)	87%*	−0.13
	Larvae	13.2 (1.6)	68%[b]	−0.65

[a] Embryo = Gosner 8 to Gosner 25; larvae = Gosner 25; SD = standard deviation.
[b] Only one remaining.

at pH 6.5 but higher and stable at pH levels at and above 7.5 [15]. It follows that the major toxic component of Vision is its surfactant and that the surfactant is likely responsible for the weak base interaction with pH. Based on this information, future studies using Vision or Roundup Original may wish to focus on the measurement of the surfactant as opposed to the glyphosate-active ingredient. Currently, however, analytical techniques for MON 0818 are unpublished.

Larval amphibians, based on limited testing, have been found to be more sensitive to chemical contaminants than embryos [37–40], newly hatched embryos at Gosner 18 to 20 [39], or juvenile/adult amphibians [19,40]. In the studies reported herein, the Gosner stage 25 larvae of all four species were more sensitive to the treatments than their embryonic counterparts, albeit in varying ratios. One possible reason for this disparity is a lack or insensitivity of target organs in the embryonic stages compared with Gosner 25, leading to differential exposure times of sensitive target organs in these tests. Another possible reason is the exclusion of the chemical by embryonic membranes. The target organs for Vision and its components are currently unknown in amphibians. However, surfactants have been demonstrated to cause lysis of gill epithelial cells in rainbow trout [41]. Full development of the gills occurs between Gosner 21 to 23 [23]. In these tests, the exposure of functional gilled stages to Vision was shorter for the embryonic experiments (2–3 d depending on species) in comparison with the larval experiments (4 d). As is known that surfactants affect the ability of the gills to maintain osmotic balance, that exposure times of functional gills differed, and the observation that death in the embryo test predominantly occurred following hatching, we concluded that the significant uptake and effects caused by Vision occurred during exposure of those stages containing functional gills. Thus, the lack of functional gills during most of the early embryonic exposure may partly explain the increased tolerance of embryos to Vision as compared with their larval counterparts. In addition to a lack of functional gills, it appeared, in most cases, that the perivitelline membrane offered protection to the embryo, a hypothesis that has been advanced by other researchers [38,39]. It was apparent that, when the concentrations of MON 0818 in the nonionized form were extremely high at elevated pH, death of the entire experimental unit occurred despite the perivitelline membranes of all animals remaining intact. In these cases, any protection offered by the perivitelline membrane must have been overcome, leading to increased accumulation of Vision resulting in death. Life-stage sensitivity differences have implications for animals in the field. For many native Canadian amphibians, the Gosner 25 stage is the longest larval stage in their development. It is greatly protracted in *R. clamitans* and *R. catesbeiana*, where the overwintering stage is Gosner stage 25. Vision herbicide is applied in late spring, consequently overlapping with larval stages of amphibians that lay their eggs in early spring (e.g., *B. americanus*, *R. pipiens*). The herbicide is also sprayed periodically throughout the summer months, overlapping many species larval periods. Life-stage sensitivity differences also have implications for animal field testing. Based on the evidence provided here, further limnocorral [21] and in situ [13] bioassay work included the Gosner 25 stage.

The ease of maintaining and breeding adults and the ease of culturing early life stages of *X. laevis* make this amphibian model a popular laboratory animal. Based on a comparison of LC10 and LC50 estimates, the sensitivities of the four larval anuran species were comparable whereas the embryonic tests showed *X. laevis* and *R. pipiens* embryos three times less sensitive than the *R. clamitans* and *B. americanus* embryos. This study parallels another study using the Release herbicide at varying pH levels [38]. In that study, the larval stages of the same species were equisensitive whereas the *R. pipiens* embryos were slightly less sensitive (1.3–1.8×) than the embryos of the other species. A comparison of species sensitivity based on the endpoint of growth was inconclusive. Three of the four embryo tests and none of the larval tests showed significant growth inhibition. The treatments didn't consistently cause growth inhibition and/or the tests were not long enough to observe such inhibition. Species responded similarly with respect to hatching and malformations. Although the time to hatching was variable, depending on the species, this endpoint was not dependent on either the concentration of Vision used or the pH of the treatment (excluding the minor exception of the 24-h delayed hatching of *R. clamitans* in low pH treatments relative to the controls). For malformations, although the Ranid species were the only ones that showed malformation rates greater than the controls, all species demonstrated that the treatments were not teratogenic. Although using *X. laevis* as a surrogate species for the three natives seems appropriate here, the authors caution against using *X. laevis* as a surrogate in all anuran toxicity tests. Amphibian ecotoxicology contaminant studies, surveyed from 1972 to 1998, constituted only 2.9% of all vertebrate ecotoxicology citations. When based on their relative class size, amphibian studies should be represented by at least 11.4% of the ecotoxicological literature base [42]. The infancy of amphibian ecotoxicology and the relatively few contaminant studies in the literature make it difficult to determine if the biological responses of *X. laevis* are similar to native anuran responses for other chemicals and endpoints.

The importance of determining a worst-case scenario may allow for a more comprehensive approach to hazard identification. For this situation, the worst-case scenario would include a highly alkaline wetland oversprayed with Vision during early larval anuran stages. Laboratory tests, such as those conducted here, justify the need for higher tier testing because, using a hazard quotient analysis to risk characterization (EEC/LC50) [43], three times out of four, the quotient was greater than one when larval toxicity estimates at pH 7.5 were used. Based on this information, we concluded that, at EEC levels, there was an appreciable concern of adverse effects to larval amphibians in neutral to alkaline wetlands. The finding that the mean pH of Northern Ontario wetlands is 7.0 [13] further compounds this concern. To understand the risks associated with Vision in forestry situations, actual environmental concentrations following a spray event must be determined [13,43]. The higher level tiers in this project link exposure assessment and anuran toxicity, leading to an understanding of the risk associated with this herbicide to native anuran larvae in Northern Ontario forests [13,21].

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EFFECTS OF VISION® HERBICIDE ON MORTALITY, AVOIDANCE RESPONSE, AND GROWTH OF AMPHIBIAN LARVAE IN TWO FOREST WETLANDS

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Abstract—The effects of Vision® (glyphosate, 356 mg acid equivalents (a.e.)/L) on mortality, avoidance response, and growth of larval amphibians (*Rana clamitans* and *Rana pipiens*) were investigated using in situ enclosures deployed in two forest wetlands of northern Ontario, Canada. In addition to untreated controls, Vision was applied to yield initial concentrations ranging from 0.29 to 14.3 mg a.e./L (0.94–46.1 mg/L of Vision). Resultant 96-h median lethal concentration (LC50) values ranged from 2.70 to 11.5 mg a.e./L (8.71–37.1 mg/L of Vision) depending on the species or site involved. Substantial mortality and incidences of abnormal avoidance response occurred only at concentrations exceeding the expected environmental concentrations (EEC) (1.43 mg a.e./L, or 4.61 mg/L of Vision) as calculated by Canadian regulatory authorities. The concentration dependence of larval growth rate and maximum size varied depending on site and species. Mean growth rates and maximum sizes exposed to 1.43 mg a.e./L (EEC) treatments were the same or greater than controls. Experimental site and biotic/abiotic factors therein, such as pH and suspended sediments, substantially affected the expression of Vision herbicide toxicity in the amphibian larvae tested. Overall, results suggest that the silvicultural use of Vision herbicide in accordance with the product label and standard Canadian environmental regulations should have negligible adverse effects on sensitive larval life stages of native amphibians.

Keywords—Amphibians Glyphosate Vision Wetlands Ecotoxicology

INTRODUCTION

This article describes the third study of a multitiered investigation of the effect of multiple stressors on larval amphibians native to northern Ontario, Canada. Whereas the previous two studies were based in the laboratory [1,2], this investigation was conducted in situ at two different forested wetlands in northern Ontario.

Global declines in amphibian populations have caused concern in the scientific community [3] and provided the impetus for this study. Numerous physical, chemical, and biological causes for these declines have been postulated [4], and in some instances, interactions of multiple causes have been implicated [5,6]. In the context of forested environments in northern Ontario, Canada, two coincident factors (among many) that may impact amphibian populations are herbicides and pH.

The wetlands present in these forested areas exhibit a wide range of pH [7]. Ambient pH has been found to cause a variety of direct and indirect effects on larval amphibians, which range from mortality and delayed development in acidic environments [8] to interactions with solutes such as metals and dissolved organic carbon [9], which may enhance or mitigate adverse effects induced by pH effects alone. A few studies, including the two preceding studies in this series [1,2] found that Vision® herbicide (Monsanto, Winnipeg, MB, Canada) was more toxic at elevated pH [1,2,10], while the opposite was true for pure glyphosate [10]. The Vision formulation is identical to Roundup Original® (Monsanto).

Herbicides are used in Canadian forestry as a means of vegetation control to promote the regeneration of coniferous forests postharvest by decreasing competition from early successional broadleaf trees and shrubs. In Canada, glyphosate (Vision formulation) dominates the forest herbicide use market, accounting for more than 90% of the market nationally [11], with the majority of applications being made using aerial dispersal techniques. Through the last decade, herbicides have been applied to approximately 200,000 ha of regenerating forest lands per year, equivalent to approximately 20% of the area harvested. The majority (43%) of the treated area occurs in the province of Ontario, with somewhat lesser amounts in New Brunswick and British Columbia, Canada [11]. In the forested regions of these provinces, aquatic ecosystems are ubiquitous and are thus at risk of herbicide contamination via direct overspray, drift, or runoff [12]. The degree of contamination varies with the mechanism of input, with highest inputs resulting from direct overspray [13]. While larger systems are typically buffered by riparian reserves and no-spray buffer zones, this may not be the case for smaller wetlands, which are seldom identified on topographical maps and difficult to see and avoid during aerial spray applications. As a result, small wetlands are particularly at risk of direct overspray. While many of these small wetlands do not contain fish, they are nonetheless critical breeding and foraging habitat for a variety of native amphibians.

Because glyphosate has a plant-specific mode of action, it is expected that the greatest risk in forested wetlands would be to nontarget aquatic vegetation and algae [14,15]. However, reviews of the existing literature suggest that the formulated product Vision is relatively equitoxic to fish, zooplankton, plants, and invertebrates with median lethal concentration

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Table 1. Physical characteristics and water chemistry of study sites. All values were measured within two weeks prior to herbicide application. Water chemistry parameters represent the mean ($n = 3$) of control enclosures, and were measured on the day of herbicide application. Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie (ON, Canada)

Wetland site	Site A	Site B
Location	46°53′20″N, 84°7′45″W	47°02′04″N, 84°23′06″W
Area (ha)	2.0	1.8
Depth (m)	1.0	2.1
Sediments	Flocculent, high carbon	Flocculent, high carbon
Macrophytes (% cover)	30	0
Amphibian presence	*Rana pipiens, Rana clamitans, Rana septentrionalis, Rana sylvatica*	*Rana pipiens, Rana clamitans, Rana septentrionalis, Rana sylvatica, Pseudacris crucifer*
pH	6.4	7.0
Dissolved oxygen (mg/L)	8.02	7.85
Conductivity (μS/cm)	13.7	37.3
Alkalinity (meq/L)	0.055	0.27
Total organic carbon (ppm)	213	104
Total inorganic carbon (ppm)	0.68	3.15
NH_4 (ppm)	0.13	0.07
$NO_2 + NO_3$ (ppm)	0.013	0.017
Total nitrogen (ppm)	2.6	2.8
Total phosphorus (ppm)	0.094	0.066
Suspended sediment (mg/L)	6.23	2.23

(LC50) values within the same order of magnitude [14,16,17]. Several previous articles [10,16,18–20] demonstrated that the surfactant blend (MON 0818) is the principal toxicant in the Vision formulation and that glyphosate alone or formulations without the MON 0818 are markedly less toxic to a variety of aquatic organisms. However, formulations containing a surfactant are required to ensure reliable efficacy on a broad spectrum of competing vegetation species.

In Canada, hazard quotients are often calculated to characterize the risk of toxic effects associated with the use of a particular pesticide. For aquatic organisms, hazard quotients are calculated as the ratio of standard acute toxicity endpoints (e.g., LC50) to expected environmental concentrations (EEC) based on the assumption of full deposition at the maximal label rate into a body of water 15 cm deep [21]. Based on the maximum label rate (2.14 kg/ha) of Vision herbicide in Canadian forest vegetation management, the calculated EEC value is 1.43 mg acid equivalents (a.e.)/L or 4.61 mg/L of the formulated product (Vision). Laboratory toxicity studies [10,14], including the two preceding tiers of study in this series [1,2], have revealed that amphibian larvae, zooplankton, algae, and aquatic macrophytes are sensitive to Vision herbicide at levels equivalent to or below the calculated EEC value, thus indicating the need for higher tier toxicological testing.

Interactions among biotic and abiotic elements in aquatic ecosystems may influence the effects of acidification and the fate of herbicides as well as their direct and indirect effects on amphibian larvae. Single-species and laboratory-based toxicity tests do not adequately simulate such interactions [22].

Aquatic in situ enclosures are a type of mesocosm that allows studies to be conducted under environmental conditions (temperature, pH, dissolved oxygen, etc.) varying naturally over time. In addition, natural degradation (e.g., microbial metabolism) and dissipation (e.g., sorption) mechanisms are fully active. Finally, enclosures contain subsets of coadapted zooplankton and phytoplankton species assemblages, allowing for realistic multitrophic biological interactions [23].

This study investigated the effects of Vision treatment on the mortality, avoidance response, and growth of native larval amphibians, as influenced by experimental site. Larval *Rana pipiens* (northern leopard frog) and *R. clamitans* (green frog) were examined using in situ enclosures deployed in two different forest wetland sites.

MATERIALS AND METHODS

Description of wetland sites

The wetlands chosen for this study were selected as representative of the type of aquatic systems that might receive herbicide inputs as a result of their use in Canadian forest vegetation management practices. Sites A and B were located northeast of Sault Ste. Marie, Ontario, Canada. Their size and physical and chemical characteristics are summarized in Table 1. The study, initiated July 18, 2000 (site A), and July 20, 2000 (site B), was timed to coincide with typical operational application of herbicides for control of competing vegetation in northern Ontario forest regeneration sites. The timing of the study also coincided with the presence of *Rana* larvae at both study sites.

Enclosure design

Twenty-four in situ enclosures (4.2 × 4.8 m) were positioned in an 8 × 3 matrix at each site. Thirteen enclosures were used for this study. Details of enclosure construction have been previously published [12]. In brief, the enclosures were constructed from impervious polyethylene sidewalls suspended from Styrofoam® (Dow Chemical, Midland, MI, USA)-filled wooden floats and were lowered into the bottom sediments, where they were anchored with an iron frame. After deployment, the enclosures were inspected by scuba divers to ensure that the enclosed volume of water was sealed from the rest of the wetland, thereby isolating relatively undisturbed columns of water inclusive of natural species assemblages and sediments, to which treatments could then be applied.

Herbicide treatment levels and application method

Treatments comprised several different concentrations of the herbicide Vision, as well as untreated controls, were randomly assigned to 13 enclosures. A hybrid experimental design [24] with disproportional replication [25] was used in order

Table 2. Vision® (Monsanto, Winnipeg, MB, Canada) test concentrations and number of replicates. See Table 1 for site characteristics and locations

Herbicide concentration		Average concentration[a] (mg a.e./L)		
mg a.e./L	mg Vision®/L	Site A	Site B	Number of replicates
0	0	ND[c]	ND	3
0.29	0.94	0.27	0.28	2
0.72	2.31	0.74	0.79	2
1.43	4.61	1.78	1.07	3
7.15	23.1	9.27	8.08	2
14.3	46.1	18.0	14.1	1

[a] Herbicide concentration measured in enclosures 3 h following application, averaged across replicates. a.e. = glyphosate acid equivalents.
[b] Vision formulated product concentration = acid equivalents × 3.226.
[c] Below analytical detection limit of 0.01 mg a.e./L.

to maximize the number of concentrations tested, increase replication for lower concentration treatments, and allow for direct comparison between control and the 1.43 mg a.e./L (EEC) treatment level. Glyphosate, as the formulated product Vision (glyphosate isopropylamine salt, 356 g a.e./L + MON 0818 surfactant blend 15% by weight) (lot MHVR0662A), was applied at the nominal test concentrations given in Table 2. Throughout this article, test concentrations are reported in units of milligrams of glyphosate acid equivalents per liter (mg a.e./L) to relate most directly to analytical confirmation values and the bulk of the previously reported literature. Conversion to units of formulated product may be easily made by multiplying values in mg a.e./L by a factor of 3.226 [16]. The amount of formulated product required to achieve the desired nominal concentrations was based on enclosure volume: the average depth ($n = 4$) of each enclosure was measured two weeks prior to treatment and used to calculate the volume of each enclosure (4.2 m width × 4.8 m length × depth). Enclosure volumes were approximately 2.02×10^4 L in site A and 4.03×10^4 L in site B. Herbicide treatments consisted of various concentrations of Vision spanning environmentally realistic levels and relevant toxicological endpoints for selected aquatic biota and included the EEC (1.43 mg a.e./L) as calculated for silvicultural use in Canada.

Herbicide applications were made to site A and site B in the early morning (~0600–0800) on July 18 and July 20, 2000, respectively. Vision was applied to the surface of individual enclosures using a backpack sprayer (model 4F, R&D Sprayers; Opelousas, LA, USA), pressurized by CO_2 with a boom pressure of 110 kPa. The sprayer was equipped with a 2-m wand and an open-orifice nozzle to minimize drift. The nozzle was held approximately 30 cm above the surface of the water, and the herbicide was applied in two passes in opposite directions over the entire surface of the enclosure (20.16 m^2). On the morning of application, premeasured amounts of the herbicide formulation were mixed into the 12-L stainless steel spray tanks containing distilled water to yield 2 L of spray solution. During application, 1-m plastic barriers were held around the perimeter of the enclosure being treated, thereby negating drift to surrounding enclosures. The application of herbicide progressed sequentially from lowest to the highest concentration with spray tanks and booms rinsed between applications to minimize any potential residual chemical carryover. Rinsates were added directly to the appropriate enclosures to ensure complete delivery of premeasured formulation volumes.

Amphibian larvae care

Free-swimming *R. pipiens* larvae (Gosner 21–24) [26] were captured at Stokely Creek, (30 km north of Sault Ste. Marie, Ontario). *Rana clamitans* embryos (Gosner 8–12) were collected near Waterloo, Ontario. Larvae of both species were kept in aquaria within an environmental chamber maintained at 19.5°C and 16:8-h light:dark cycle, where they were fed dried *Spirulina* and goldfish flakes ad libitum. Approximately one week prior to herbicide treatment, larvae were acclimated in untreated water collected from the experimental sites. Within a few days of herbicide treatment, larvae were transported to the experimental sites and placed inside mesh cages that were previously deployed within each enclosure. Housing larvae in cages has previously proven to be a successful technique, as repeated assessments of the same individuals can be easily completed [27]. Similar in size and make to those used by Cooke [28], larval enclosure cages were constructed of modified tomato cages (30 × 41 cm) that were slipped into a crinoline mesh sleeve and cinched at both ends, enveloping a volume of 30 to 35 L. The mesh was of a gauge small enough (pore size ~1 mm^2) to prevent larval escape and incursion of predators and large enough to allow the free exchange of water and small particulates. Two cages were deployed within each enclosure, each cage containing 10 to 12 randomly assigned larvae (Gosner 25 at time of herbicide application) of either *R. pipiens* or *R. clamitans*. This number of larvae were used so that overcrowding would not impact on larval growth. Larvae fed on periphytic algae, which developed on the cage mesh during the acclimation period and thereafter as their primary food source, and no artificial feeding supplement was applied.

Effects assessment and statistical analysis

For all statistical analyses, a Type I error rate (α) of 0.05 was used. Assessment of larvae was conducted the day of treatment (0), and 1, 4, 7, 11, 14, 21, 28, 42, 56, and 77 d after treatment (DAT). Larvae were scooped out of the cages using small minnow nets and placed in glass or enamel baking pans containing approximately 2 cm of water from the respective enclosure.

Mortality. Larval mortality was recorded and any dead larvae were removed. Larvae were considered dead if they did not respond to repeated prodding and removal from water. Analysis of mortality at 96 h postherbicide application followed the protocol detailed by Edginton and others [1,20], using the General Linear Model function in S-Plus® (S-Plus 2000 Professional Release 2; Mathsoft, Cambridge, MA, USA). In brief, the probit of percent mortality was fit as a generalized linear model using a Type I analysis of deviance approach. Data from the various combinations of site and species were fit to the model

$$p = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 \quad (1)$$

where p was the probit of percent mortality, x was $\log_{10}$ transformed glyphosate concentration, and β_0, β_1, β_2, and β_3 were the intercept, linear, quadratic, and cubic coefficients, respectively. Terms in Equation 1 were sequentially added to the model, and any significant difference in deviance between successive models was assessed based on the 5% critical value of the chi-square distribution with 1 *df* (3.84). Lethal concentration point estimates were derived by solving for concentration at set probit values. Calculation of 95% confidence intervals about these point estimates were obtained using a profile deviance approach. Where the dose–response curve was

steep relative to the spread of tested concentrations, maximum likelihood-based calculation of point estimates and confidence intervals was not possible; in these instances, point estimates and confidence intervals were derived using the asymptotic likelihood ratio [29]. Models were considered to be overdispersed when residual deviances were higher than expected (Pearson's chi-square goodness of fit > 95% on the chi-square distribution) and thus did not follow a chi-square distribution. In these instances, models and confidence intervals were generated using quasideviance, which follows an F distribution.

Using the General Linear Model procedure of SAS® (Ver 8.01; SAS Institute, Cary, NC, USA), a variance analysis was used to compute 96-h mortality least-squares means for each combination of site and species. Preplanned comparisons were conducted to determine significant differences between mean mortality observed in untreated controls and in replicate enclosures treated at the 1.43 mg a.e./L (EEC) level. Assumptions of the variance analyses were tested by examining the normality of residuals using the Shapiro–Wilk's test and visual inspection of error homogeneity using plots of residuals against predicted values and herbicide concentration. The presence of outliers was tested using Lund's test of studentized residuals [30]. Herbicide concentration was $\log_{10}$ transformed to meet assumptions of the analysis.

Avoidance response. Avoidance response was assessed by gently prodding individual larvae and gauging their response as normal (larva swims away immediately) or abnormal (delayed or no response, impaired swimming ability). Within hours of herbicide treatment (DAT 0), the avoidance response of surviving larvae was assessed. For each combination of site and species, the proportion of surviving larvae displaying an abnormal avoidance response was subjected to a chi-square-based linear trend analysis [31], using BMDP Statistical Software (SPSS, Chicago, IL, USA) to determine if it was related to herbicide concentration. Data were pooled across replicate enclosures. Midrange concentrations (0.29 and 0.72 mg a.e./L) and the two highest concentrations (7.15 and 14.3 mg a.e./L) were combined to maximize fit to the chi-square distribution. Combining these concentrations resulted in an averaging of proportion abnormal response. In addition, a one-tailed Fisher's exact test was used to compare proportion of abnormal avoidance response in controls with that from treatment at 1.43 mg a.e./L (EEC).

Growth. Larval size was measured using digital image analysis. A Nikon® Coolpix 950 digital camera (Tokyo, Japan) was used to capture images of larvae in the pans, as well as a 1-cm reference line, at a resolution of 640 × 480 pixels. After observations had been made, larvae were returned to their respective cages. Larval length (body + tail) was later measured from the digital image using Scion Image for Windows computer software (Release Beta 4.0.2© Scion 2000, Frederick, MD, USA). This digital measurement technique was validated by capturing images of manually premeasured larvae (as well as a 1-cm reference line) and measuring the larvae in the digital image. Measurements derived digitally were very similar to those derived manually. This methodology allowed for a large number of larvae to be measured while minimizing handling stress for the larvae as well as time taken for measurements to be completed in the field. In addition, these digital measurement techniques were believed to provide more accurate and consistent estimates of size than would be derived by manual measurement in the field, and have the advantage of archival retention for remeasurement or other a posteriori assessments.

Lengths were plotted graphically (by site, species, and enclosure) against time to examine growth trends. Because most *R. clamitans* larvae reached their growth plateau at DAT 56, whereas most *R. pipiens* larvae reached their peak size at DAT 21, analysis of body length data was restricted to periods between 0 to 21 and 0 to 56 d after treatment for *R. pipiens* and *R. clamitans*, respectively. Maximum size was determined by taking the mean larval length (by enclosure) at DAT 21 for *R. pipiens* and DAT 56 for *R. clamitans*. To determine larval growth rate, the length versus time relationship for each site, species, and enclosure was subjected to a regression analysis with partitioning into linear and quadratic components; the estimate of the linear (slope) parameter was used as larval growth rate. In order to meet assumptions of normality and homogeneity of variance, larval lengths were natural logarithm (ln) transformed, and resultant growth rates are given as ln mm/day.

Using the general linear model procedure within SAS, variance of maximum size and growth rate for each species were partitioned into site, concentration, and site × concentration interaction. Assumptions of the analysis were tested as stated earlier. Least-squares means were computed for each combination of site and species. Preplanned comparisons were conducted to determine significant differences in maximum size and mean growth rate observed in untreated controls versus enclosures treated at the 1.43 mg a.e./L (EEC) level. Orthogonal regression/lack-of-fit partition of site, concentration, and site × concentration into linear and quadratic components was used to account for all treatment variation. Parameters of prediction equations were determined based on the results of the orthogonal regression/lack-of-fit partition. Where terms from the regression model were significantly different from background variation, parameters for prediction equations were obtained using the solution option. Relationships of maximum size and growth rate with herbicide concentration were deemed to be significant if the linear parameter estimates (slopes) were significantly different from zero.

Glyphosate residues. Aquatic residues of glyphosate and its primary metabolite, aminomethylphosphonic acid, were determined in each enclosure on the day of treatment and in selected enclosures to confirm nominal concentrations and track environmental fate and persistence throughout the course of the study. The analytical method employed is described elsewhere [32]. Data confirming initial aqueous concentrations in each enclosure are provided here whereas full environmental fate, including residues in biofilms, water, and bottom sediments will be detailed in a separate article.

RESULTS

Aqueous concentrations of glyphosate measured on the day of herbicide treatment were within 20% of nominal test concentrations (Table 2), thus analysis of herbicide effects were based on nominal concentrations. Deviations from nominal concentrations were likely due to ballooning effects of the enclosure liner, inaccuracies in depth measurements, and changes in water level between time of measurement and time of application, all of which could affect enclosure volume. The time taken for 50% of the nominal concentration to dissipate (median dissipation time) in enclosures treated at EEC levels (1.43 mg a.e./L) were markedly different, at 4.2 and 26.4 d in sites A and B, respectively. This difference is postulated to be

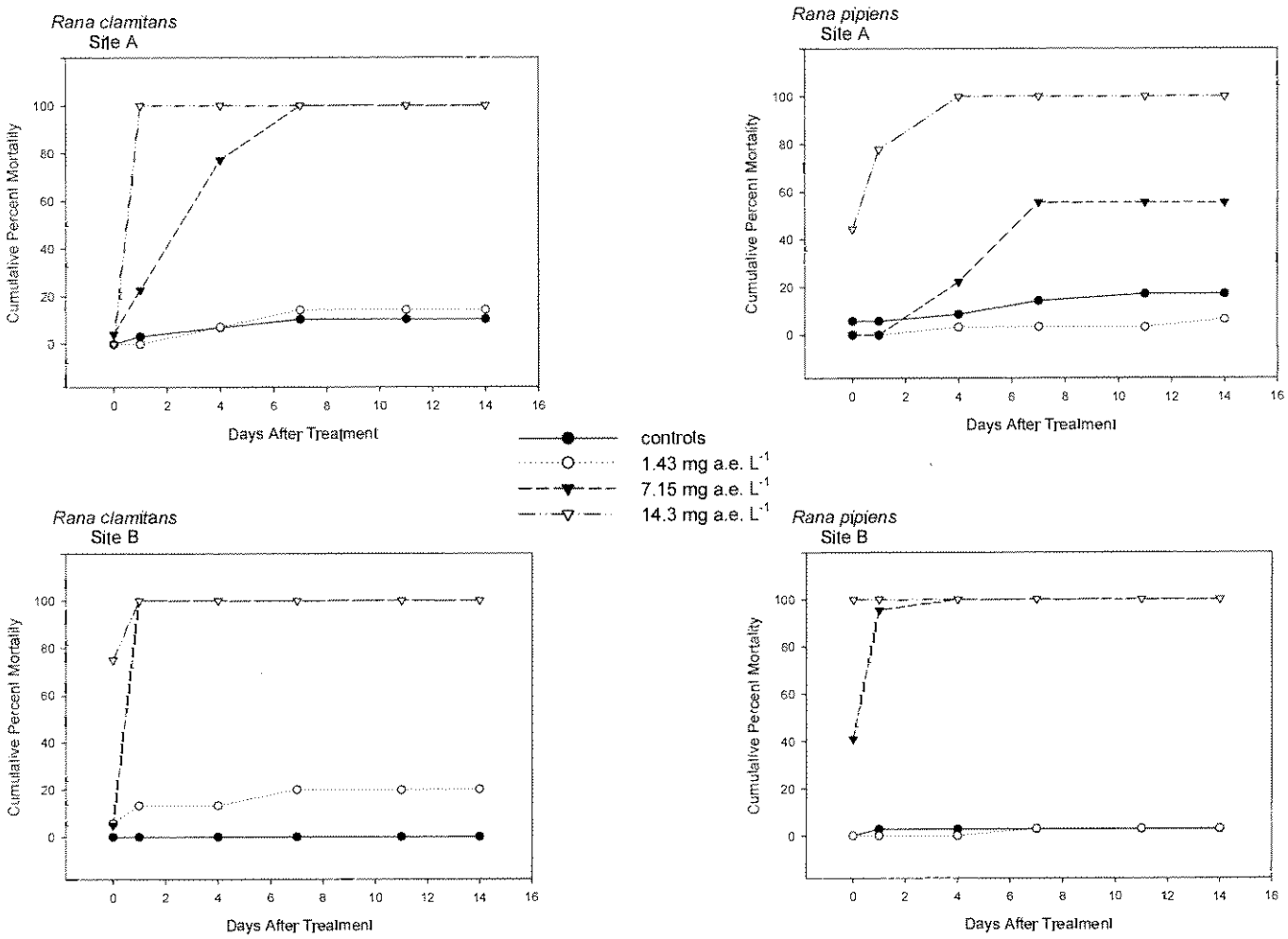

Fig. 1. The progression of larval mortality through time for several Vision® (Monsanto, Winnipeg, MB, Canada) treatment concentrations. Individual points represent cumulative percent mortality pooled across replicate enclosures. Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. Site A: 46°53′20″N, 84°7′45″W; Site B: 47°02′04″N, 84°23′06″W. a.e. = glyphosate acid equivalents.

the combined result of greater sorption (suspended sediments, biofilms, and macrophytes) and enhanced microbial degradation in the more eutrophic site (site A).

Mortality of larvae in control treatments at 96 h ranged between 0 and 10%. Control-corrected larval mortality was used in the determination of lethal concentration estimates. With the exception of *R. pipiens* at site A, complete mortality was observed at treatment concentrations exceeding 1.43 mg a.e./L (EEC) within one week of treatment, with most of the mortality occurring within the first 96 h (Fig. 1). Larvae in site B appeared to succumb more quickly to the toxic effects of herbicide concentration than in site A. Models generated for *R. clamitans* mortality were first order, whereas those generated for *R. pipiens* were third order (Table 3, Fig. 2). Lethal concentration point estimates at 10% and 50% mortality (LC10 and LC50) are presented in Table 4. Least-squares means comparison revealed that control mortality was not different from treatment at 1.43 mg a.e./L (EEC) for all combinations of site and species.

Proportion of abnormal avoidance response in the control treatment was less than 10%. Proportion of abnormal avoidance response within hours of treatment showed an increasing linear trend with concentration for both species in site B only ($p < 0.05$) (Fig. 3). Results of the one-tailed Fisher's exact test revealed that there were no significant differences in abnormal avoidance response between control larvae and larvae exposed to 1.43 mg a.e./L (EEC) in all instances at DAT 0.

Larval size (length) steadily increased with time until it reached a plateau (*R. clamitans*) or maximum, after which larval size decreased (*R. pipiens*). As site accounted for a significant amount of variation in growth rate and maximum size in both species ($p < 0.0001$), it was necessary to use separate prediction equations for each site to determine the relationship of growth rate and maximum size with herbicide concentration. Growth rates and maximum sizes were consistently smaller in site A than in site B (Fig. 4). Predictive equations based on the relation of growth rate or maximum size to herbicide concentration alone typically explained less than 58% of the variation in the data and in five cases were statistically insignificant (Table 5). Least-squares means comparisons for both sites revealed that larvae exposed to the 1.43 mg a.e./L (EEC) treatment level showed either greater (*R. clamitans*) or equivalent (*R. pipiens*) growth rates as compared with controls. Maximum larval size for both species following exposure at the 1.43 mg a.e./L (EEC) treatment level were either significantly greater than or equivalent to those in controls within both experimental sites.

Table 3. Model equations, Pearson's chi-square statistics, and critical values for determining overdispersion for the toxicity of Vision® (Monsanto, Winnipeg, MB, Canada) to *Rana pipiens* and *Rana clamitans* at each experimental site 96 h after treatment. Models describe the relationship between herbicide concentration and mortality and were derived using the general linear model function in S-Plus® (S-Plus 2000 Professional Release 2; MathSoft, Cambridge, MA, USA). Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. Site A: 46°53′20″N, 84°7′45″W; Site B: 47°02′04″N, 84°23′06″W

Site	Species	Model	Pearson's χ^2	Critical value for Pearson's χ^2
A	*Rana clamitans*	Mortality[a] = 2.90 + 3.30(log$_{10}$conen.[b])	6.52	15.5
	Rana pipiens	Mortality = 3.52 − 1.95(log$_{10}$conen.) − 0.27(log$_{10}$conen.)^2 + 3.23(log$_{10}$conen.)^3	5.82	12.6
B	*R. clamitans*	Mortality = 3.44 + 3.63(log$_{10}$conen.)	7.60	15.5
	R. pipiens	Mortality = −1.11 + 3.88(log$_{10}$conen.) + 17.23(log$_{10}$conen.)^2 − 12.62(log$_{10}$conen.)^3	0.69	12.6

[a] Mortality = probit of predicted mortality on normal scale.
[b] Logarithm (base 10) of Vision concentration in milligrams glyphosate acid equivalents per liter.

DISCUSSION

Mortality

In this experiment, cumulative mortality through time was clearly concentration dependent, and responses of larvae to treatment at 1.43 mg a.e./L (EEC) appears to be similar to that of controls with the exception of *R. clamitans* in site B (Fig. 1). Larval mortality at 96 h was not different between controls and treatment at 1.43 mg a.e./L (EEC) but was affected by Vision treatment in a concentration-dependent manner. Examination of LC10 and LC50 estimates revealed that toxicity appears to be dependent on experimental site and species tested. Vision toxicity to both species was generally greater in site B than in site A. *Rana clamitans* seemed to be more sensitive than *R. pipiens* in both sites. The somewhat wide 95% confidence limits about the LC10 and LC50 point estimates are indicative of larval population variability compounded with a relatively small sample size.

Differential sensitivity of these two species is not consistent with results from tier I studies [1]. The differential species sensitivity in this study may be the result of differences in larval size at the time of treatment, whereas in the laboratory toxicity study [1], *R. clamitans* and *R. pipiens* larvae were similar in size (mean ± standard deviation, 15.2 mm ± 1.6 and 13.2 mm ± 1.6, respectively). We postulate that the decreased sensitivity of *R. pipiens* in this study could be explained by differences in size at the time of treatment. A *t* test and examination of mean body lengths indicate that *R. pipiens* larvae (mean, 14.5 mm ± 2.39) were significantly larger than *R. clamitans* larvae (9.49 mm ± 1.49). Smaller larvae may be more susceptible to the direct toxic effects of herbicide treatment because the toxicant concentration required to reach the critical body burden would be less for a smaller organism and because the larger surface area-to-volume ratio would enhance toxicant uptake. The extent and timing of the toxic response (Fig. 1 shows a delayed response in site A) can also be affected by experimental site through interactions that can occur among the active ingredient; surfactant blend; the physical, chemical, and biological characteristics of the aqueous environment; and biological tissues of the affected organism, which in turn affect fate, persistence, and bioavailability.

Aquatic dissipation of glyphosate depends on local conditions and is therefore site specific [16]. Most biotic and abiotic characteristics differed between experimental sites (Table 1). Differential dissipation is not attributable to temperature because it was not significantly different between sites (Student's $t = 0.26$; $p = 0.79$; $n = 456$). Site A had a greater amount of suspended sediments than site B, which could decrease glyphosate and surfactant blend toxicity through adsorption. Glyphosate and MON 0818 surfactant blend adsorb rapidly and strongly to soils, sediments, and suspended particulate, limiting mobility and bioavailability [16]. Indeed, aqueous concentrations of glyphosate dissipated much more quickly in site A than in site B, thereby reducing toxicity through decreased aqueous exposure. However, sorption of glyphosate and surfactant blend onto suspended particulate and biofilms could lead to added exposure via ingestion [2], through which it may take a longer time to reach toxic body burdens. The experimental sites also differed in pH and alkalinity. Glyphosate dissipation was found to be slow in alkaline waters [33], which concurs with the slower dissipation observed in site B. At elevated pH, gill uptake of the surfactant blend may be enhanced, which could lead to toxic body burdens [1]. Chen and others [2] made the observation that Vision-treated *R. pipiens* larvae died sooner at pH 7.5 than at pH 5.5, which concurs with the relative timing of toxic effects in this study. In previous studies, the toxicity of Vision or Roundup Original to amphibians [1,2,20] and fish [10] was influenced by pH and was more toxic at pH 7.5 than at pH 6.5.

The LC50 estimates as determined in this study are well within the range of previously reported values for amphibians or fish larvae exposed to chemically equivalent glyphosate herbicide formulations (Fig. 5). Thus, the larval amphibians used in this study do not appear to be unusually sensitive to the toxic effects of this glyphosate formulation. Despite the equisensitivity with fish for this herbicide, the inclusion of larval amphibians in ecotoxicological investigations should continue, especially with in situ studies. Differences between fish and larval amphibians in physiology (e.g., metamorphosis) and habitat selection can result in differential exposures to toxicants.

All LC50 value estimates derived in this study were greater than both the calculated EEC value (1.43 mg a.e./L) and the upper 99% confidence limits on exposure concentrations determined by monitoring oversprayed wetlands under operational spraying scenarios [32]. The LC10 estimates derived from *R. clamitans* larvae were very close to the EEC of 1.43 mg a.e./L. However, as shown in Figure 6, the range of LC10 values estimated for amphibian larvae in either lab or field studies also consistently exceed the range in residues observed in field monitoring studies.

Avoidance response

Herbicide concentration affected larval avoidance response in a dose-dependent manner in site B only ($p < 0.05$). Failure to detect a linear trend in site A at DAT 0 ($p > 0.05$) is likely the result of elevated abnormal avoidance response in control

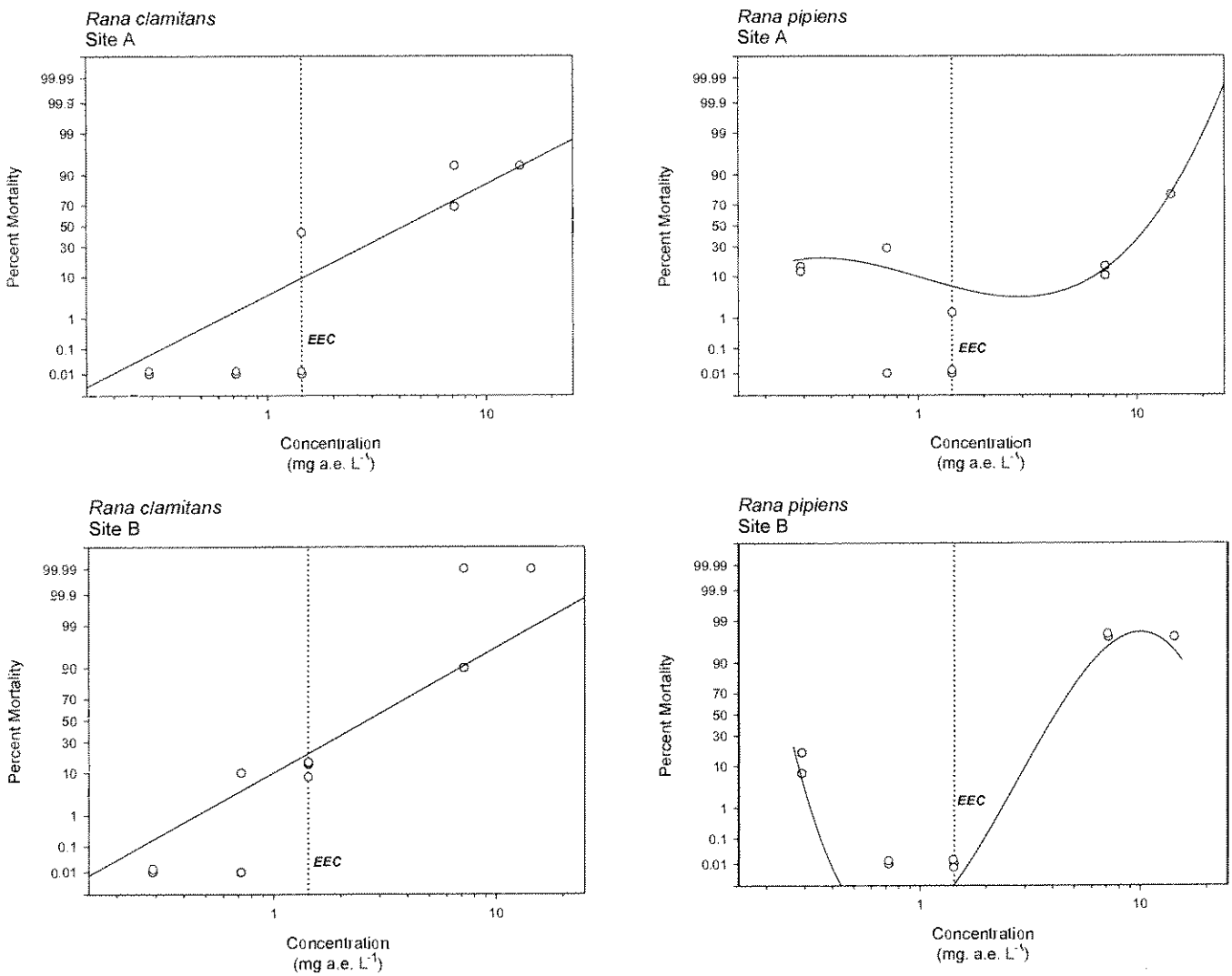

Fig. 2. Scatterplots and predicted models relating larval mortality at 96 h posttreatment to Vision® (Monsanto, Winnipeg, MB, Canada) concentration. Individual points represent control-corrected percent mortality within each enclosure (0% changed to 0.01% and 100% changed to 99.99% to facilitate graphing). Lines represent predicted models derived from Type I analysis of deviance using the general linear model function in S-Plus® (S-Plus 2000 Professional Release 2; Mathsoft, Cambridge, MA, USA). Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. Site A: 46°53′20″N, 84°7′45″W; Site B: 47°02′04″N, 84°23′06″W. EEC = expected environmental concentration (1.43 mg a.e./L); a.e. = glyphosate acid equivalents.

Table 4. Toxicity of Vision® (Monsanto, Winnipeg, MB, Canada) to *Rana clamitans* and *Rana pipiens* larvae. Ninety-six-hour lethal concentration (LC) point estimates at 10 and 50% mortality were estimated from predictive models derived from analysis of deviance. Ninety-five percent confidence limits about point estimates are given in parentheses. Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. Site A: 46°53′20″N, 84°7′45″W; Site B: 47°02′04″N, 84°23′06″W

Site	Species	LC10 (mg a.e.[a]/L)	LC10 (mg Vision/L)	LC50 (mg a.e./L)	LC50 (mg Vision/L)
A	*Rana clamitans*	1.78 (0.99, 2.86)	5.74 (3.19, 9.23)	4.34 (3.05, 6.02)	14.0 (9.84, 19.4)
	Rana pipiens	7.31 (3.83, 9.54)	23.6 (12.4, 30.8)	11.47 (9.50, 14.5[b])	37.0 (30.6, 46.8[b])
B	*R. clamitans*	1.20[c] (0.84, 1.60)	3.87 (2.71, 5.16)	2.70 (2.06, 3.67)	8.71 (6.65, 11.8)
	R. pipiens	3.26 (1.66, 3.61)	10.5 (5.36, 11.6)	4.25 (2.45, 7.10)	13.7 (7.90, 22.9[b])

[a] a.e. = glyphosate acid equivalents.
[b] Above range of concentrations tested.
[c] Below expected environmental concentration (EEC) of 1.43 mg a.e./L.

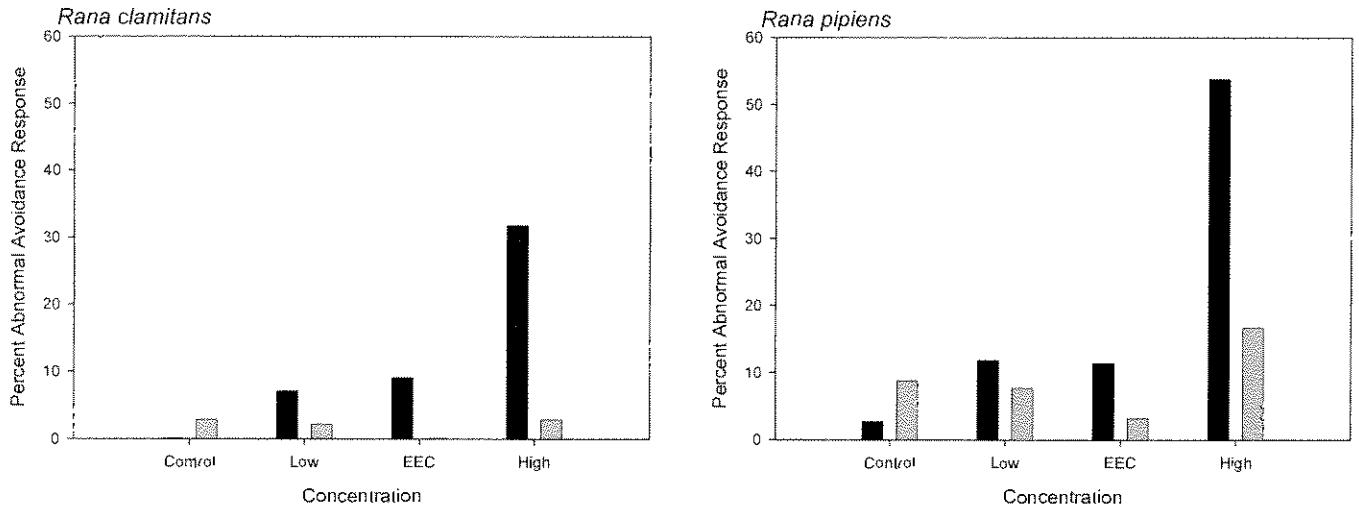

Fig. 3. Percent abnormal avoidance response, as related to Visiun® (Monsanto, Winnipeg, MB, Canada) concentration. Individual bars represent percent abnormal avoidance response pooled across replicate enclosures. Concentration categories are controls; low, 0.29 + 0.72 mg glyphosate acid equivalents (a.e.)/L; expected environmental concentration (EEC), 1.43 mg a.e./L; high, 7.15 + 14.3 mg a.e./L. Open bars = Site A (46°53′20″N, 84°7′45″W); solid bars = Site B (47°02′04″N, 84°23′06″W). Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada.

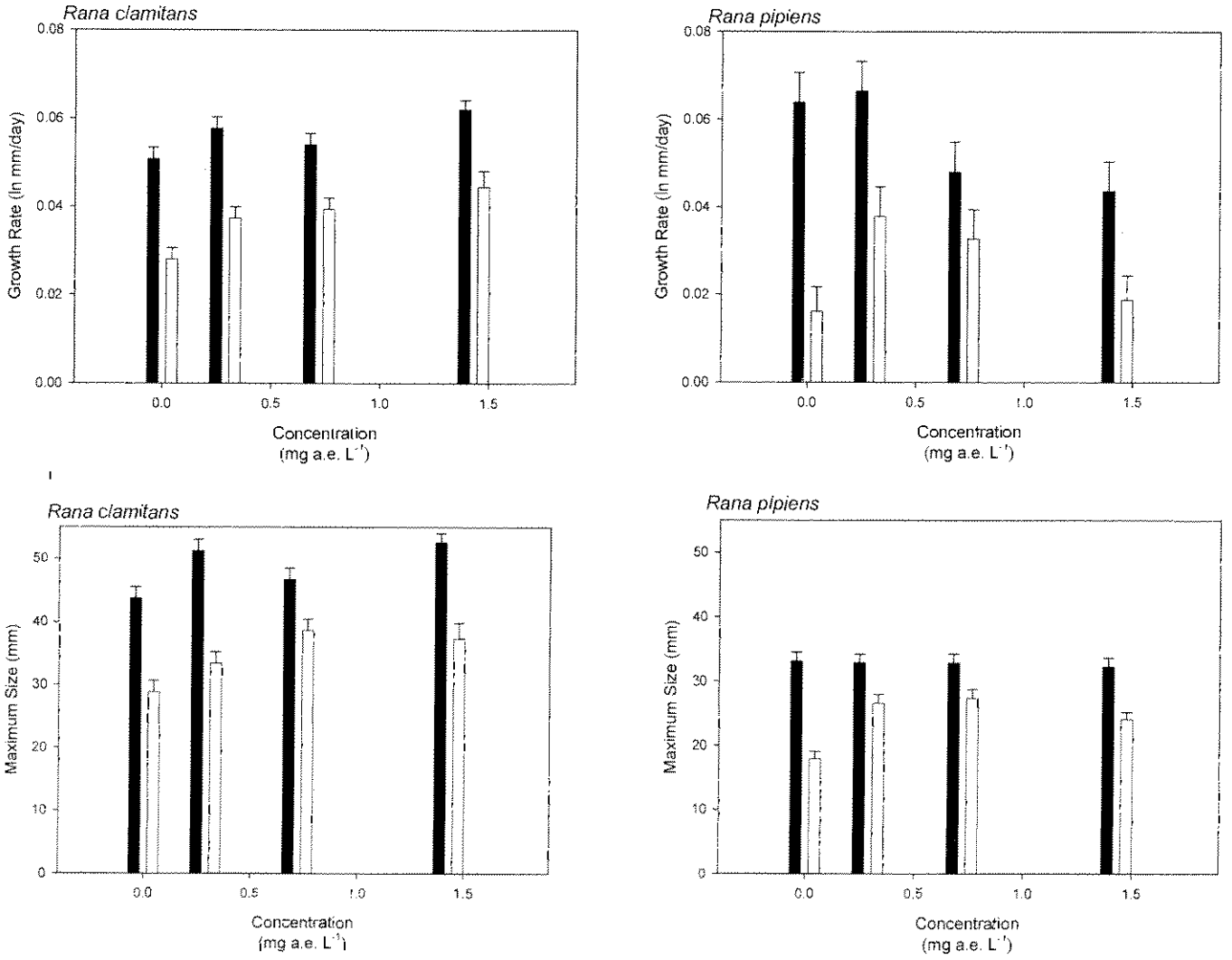

Fig. 4. Larval growth rate and maximum size, as related to Visiun® (Monsanto, Winnipeg, MB, Canada) concentration. Bars represent least-squares means of larval growth rates derived from individual enclosures. Error bars represent standard error of least-squares means. Open bars = Site A (46°53′20″ N, 84°7′45″W); closed bars = Site B (47°02′04″N, 84°23′06″W). Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. a.e. = glyphosate acid equivalents.

Table 5. Model equations describing the relationship of larval growth parameters to Vision® (Monsanto, Winnipeg, MB, Canada) concentration, derived from analysis of variance and linear regression using the general linear model function in SAS® (Ver 8.01; SAS Institute, Cary, NC, USA). Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. Site A: 46°53′20″N, 84°7′45″W; Site B: 47°02′04″N, 84°23′06″W

Site	Species	Model	r^{2a}	p^b
A	*Rana clamitans*	Rate[c] = 0.0309 + 0.0108(concn.[d])	0.55	0.0563[e]
	Rana pipiens	Rate = 0.0184 + 0.0530(concn.) − 0.0371(concn.^{2})	0.56	0.0277[e], 0.0216[f]
B	*R. clamitans*	Rate = 0.0522 + 0.0065(concn.)	0.71	0.0045[e]
	R. pipiens	Rate = 0.0656 − 0.0166(concn.)	0.47	0.0615[e]
A	*R. clamitans*	Max[g] = 30.8 + 6.78(concn.)	0.57	0.0500[e]
	R. pipiens	Max = 18.6 + 24.6(concn.) − 14.6(concn.^{2})	0.73	0.0040[e], 0.0070[f]
B	*R. clamitans*	Max = 45.8 + 4.54(concn.)	0.41	0.0641[e]
	R. pipiens	Max = 33.1 − 0.586(concn.)	0.17	0.3079[e]

[a] Coefficient of determination.
[b] Type I error probability of linear or quadratic coefficient being different from zero.
[c] Growth rate (in mm/day).
[d] Vision concentration (mg a.e./L).
[e] Linear coefficient.
[f] Quadratic coefficient.
[g] Maximum size.

larvae, combined with the slower response of larvae in site A to the sublethal toxic effects of herbicide treatment. Although data shown in Figure 3 suggest a trend in proportion of abnormal avoidance response for both species in Site B, differences among means were not statistically significant when tested using Fisher's exact test. Elevated levels of abnormal avoidance response and mortality at concentrations higher than 1.43 mg a.e./L (EEC) indicate that the majority of sublethal and lethal effects of Vision occur at concentrations exceeding 1.43 mg a.e./L (EEC). Inspection of avoidance response and mortality data taken at DAT 1 and 4 from site A show greater instances of abnormal avoidance response as well as increased mortality relative to DAT 0 (data not shown), whereas the data from site B show nearly complete mortality (no survivors with which to assess avoidance response) at concentrations exceeding 1.43 mg a.e./L (EEC) at DAT 1. Abnormal avoidance response may render amphibian larvae more susceptible to predation ([34] and references therein). As mentioned earlier, the differences in the nature of the aqueous environment between sites A and B may explain the differences in timing and extent of sublethal effects between the two experimental sites.

Very few studies have investigated the effect of glyphosate on avoidance response of larval amphibians. Exposure of *R. clamitans* larvae to 4 mg active ingredient/L glyphosate (equivalent to 3 mg a.e./L [16]) (Roundup Original formulation) caused abnormal avoidance responses in 5 to 42% of the exposed larvae; larvae remained unaffected at lesser concentrations [34].

Larval growth

The difference in the nature of the growth curves as well as timing of the occurrence of maximum size between the two species were not unexpected and are attributable to differences in their life histories. *Rana pipiens* metamorphose in the same season its eggs are laid, and total lengths eventually decrease due to tail resorption during metamorphosis. On the other hand,

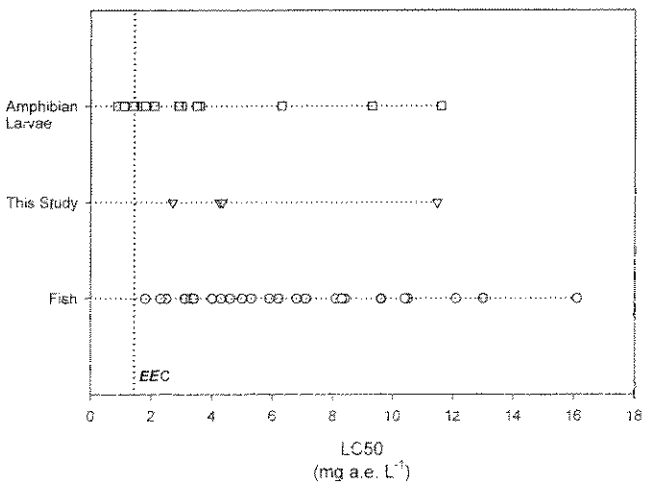

Fig. 5. Comparative toxicity of Vision® (Monsanto, Winnipeg, MB, Canada) or Roundup Original® (Monsanto) to amphibian larvae and fish. Individual points represent 48- and 96-h median lethal concentration (LC50) values derived from this and other studies [1,10,16,18,19]. EEC = expected environmental concentration (1.43 mg a.e./L); a.e. = glyphosate acid equivalents. □, amphibians; ○, fish; ▽, this study.

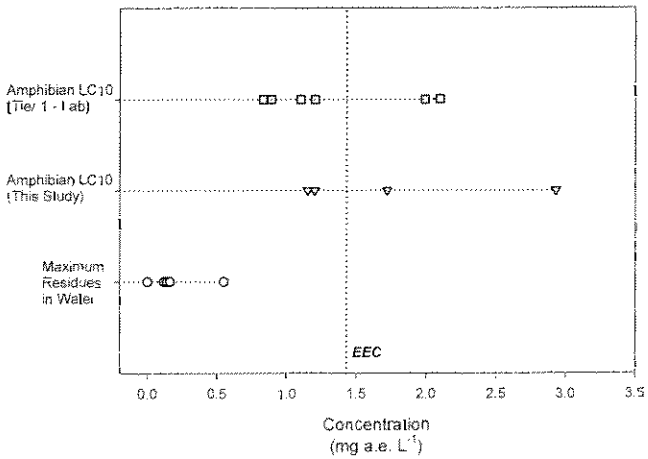

Fig. 6. Comparison of larval amphibian LC10 values to potential Vision® (Monsanto, Winnipeg, MB, Canada) or Roundup Original® (Monsanto) exposure concentrations. Ten percent lethal concentration (LC10) values were taken from tier 1 [1] (□) and this study (▽). Aquatic residues represent the maximum concentrations of glyphosate detected following silvicultural application of Vision [13,32–35,39–41] (○). EEC = expected environmental concentration (1.43 mg a.e./L); a.e. = glyphosate acid equivalents.

R. clamitans overwinter as larvae and do not metamorphose until the following summer.

This investigation consistently revealed the significant effect of experimental site on growth rate and maximum size, with values being lower in site A. Although temperature has a significant effect on larval amphibian growth [35], the effect of experimental site on larval growth patterns is not attributable to differences in water temperature. Water temperatures measured throughout the study revealed that mean water temperatures (±standard error) for sites A and B, respectively, were 20.26 ± 0.13°C and 20.30 ± 0.12°C, and no significant difference between sites was detected (Student's $t = 0.26$; $p = 0.79$; $n = 456$). However, the growth rate—concentration trends for each species appear to be similar across sites (Fig. 4) and is reflected in the lack of significant site × concentration interaction ($p > 0.05$). The maximum size × concentration interaction is similar across sites in *R. clamitans* ($p = 0.12$); however, it is different for *R. pipiens* ($p = 0.01$) (Fig. 4). Based on the low coefficients of determination of the growth versus concentration models determined in this study (Table 5), the growth patterns of these larval amphibians may have been affected by other environmental variables besides herbicide concentration, such as food availability [35]. Least-squares means comparison also revealed that growth rates and maximum sizes of larvae treated at 1.43 mg a.e./L (EEC) levels were similar to or greater than those of controls. These results confirm those of previous laboratory studies [1,18] that failed to demonstrate significant growth effects for amphibian larvae following exposures to Vision at environmentally relevant concentrations.

Larval growth rate and size at metamorphosis are related to food quality [30]. Physical, chemical, and biological characteristics of the experimental sites may undoubtedly influence the quality and quantity of food available to the larvae, and interactions of these parameters with herbicide treatment could manifest in the growth patterns of larval amphibians living in herbicide-exposed aquatic ecosystems. In this study, levels of dissolved oxygen decreased in the days following herbicide application and recovered soon thereafter, suggesting a pronounced, but temporary, dose-dependent functional response of primary producers in the system. Vision treatments also induced site-dependent changes in phytoplankton community composition. However, despite the structural and functional responses observed, transient but substantial stimulatory effects on phytoplankton abundance and periphyton biomass were generally observed (B.E. Wojtaszek, University of Guelph, Guelph, ON, Canada, unpublished data). Anuran larvae are primarily herbivorous, grazing on algae [37]. Algae have been shown to have a wide range of sensitivities to glyphosate; hence, exposure of aquatic ecosystems to glyphosate can alter algal species richness, biodiversity, and primary productivity [15,38]. Consequent herbicide-induced proliferation of algal species highly acceptable to the larval diet may explain our observations of greater growth at moderate exposure levels as compared with controls. Similar positive growth effects have been observed for other anuran species exposed to moderate concentrations of diquat or dichlobenil [28].

CONCLUSION

The nature of the relationship between herbicide concentration and larval mortality, avoidance response, growth rate, and maximum size appears to be influenced by species as well as experimental site and the physical, chemical, and biological characteristics therein. The significant effect of pH on Vision toxicity seen in the two previous tiers of study is reflected as a site effect in this study. Lethal concentration point estimates derived here are somewhat higher than those derived in tier I laboratory toxicity studies [1]. The results of this in situ enclosure study provide no evidence to conclude that environmentally relevant concentrations of Vision cause significant mortality, abnormal avoidance, or reduced growth in native larval amphibians used in this study. Conclusions of this study are consistent with those of two broader risk assessments [16,17], which conclude that terrestrial or overwater uses of Roundup Original or Vision formulations of glyphosate pose only minimal risks to aquatic organisms including amphibians.

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CHEMICAL AND BIOMONITORING TO ASSESS POTENTIAL ACUTE EFFECTS OF VISION® HERBICIDE ON NATIVE AMPHIBIAN LARVAE IN FOREST WETLANDS

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Abstract—In conjunction with operational forest herbicide spray programs in Ontario, Canada, chemical and biological monitoring studies were conducted in 51 different wetlands to quantify the probability and magnitude of contamination by a glyphosate herbicide formulation (Vision®). Wetlands were classified as oversprayed, adjacent, or buffered in relation to the operational target spray blocks. Results show that vegetated buffers significantly mitigated against exposure and thus potential for acute effects. Aqueous concentrations of glyphosate in buffered wetlands were below analytical limits of quantitation (0.02 mg acid equivalent [a.e.]/L) in 14 of 16 cases, with mean concentration (0.03 ± 0.02 mg a.e./L) significantly ($p < 0.05$) less than that of either adjacent (0.18 ± 0.06 mg a.e./L) or oversprayed wetlands (0.33 ± 0.11 mg a.e./L). Biomonitoring with caged amphibian larvae showed no significant differences among mean mortality (48 h) of either *Rana pipiens* ($p = 0.194$) or *Rana clamitans* larvae ($p = 0.129$) exposed in situ to Vision® under these various wetland conditions. Percent mortality was not significantly ($p = 0.05$) correlated with exposure concentrations for either amphibian species tested. Results suggest that exposures typically occurring in forest wetlands are insufficient to induce significant acute mortality in native amphibian larvae.

Keywords—Glyphosate Vision Amphibians Wetlands Ecotoxicology

INTRODUCTION

Herbicides are valuable tools for maximizing timber production of forest lands [1]. The benefit in productive capacity is realized through reallocation of light, water, nutrients, and space resources away from competing vegetation and toward commercially valuable crop tree species. As detailed in the National Forestry Database Program (http://nfdp.cfs.nrcan.gc.ca), an average of 175,000 ha, or approximately 18% of the total harvested forest area, are treated with herbicides annually in Canada. Throughout the 1990s, the majority (average 45%) of this treated area occurred in Ontario. During the last decade, glyphosate (Vision®, Winnipeg, MB, Canada) dominated the forest-use market both nationally and in the province of Ontario, accounting, on average, for more than 90% of the total area receiving herbicide treatments. Due to relative cost effectiveness versus other techniques and also to the remote location, difficult access and rugged terrain characterizing many regeneration sites, aerial dispersal is often the preferred method of chemical application. Loss of aerial herbicide application in Ontario would result in an approximate 40% reduction in forest productivity on this land base and an overall fourfold increase in tending costs [2].

Rational use of herbicides for forest vegetation management requires an assessment of both benefits and risks. One important, but often overlooked, environmental risk pertains to potential deleterious effects in small wetlands that are ubiquitous in many forest landscapes. Unlike larger fish-bearing aquatic systems (lakes, ponds, rivers, and streams), there are no requirements to protect small wetlands with no-spray buffer zones. Additionally, small wetlands are difficult to observe from the air and may be more difficult to avoid during aerial application of herbicides. Thus, small wetlands occurring within the target site are likely to be directly oversprayed, resulting in relatively higher potential exposures and effects for constituent biota as compared with those in adjacent or buffered wetlands. Many of these small wetlands constitute prime breeding and foraging habitat for frog species, particularly northern leopard (*Rana pipiens*), green (*Rana clamitans*), wood (*Rana sylvatica*), and mink (*Rana septentrionalis*) frogs as well as the American toad (*Bufo americanus*). Further, the timing of aerial herbicide applications in northern Ontario (July–September) may be coincident with early larval stages of development for some species. Several researchers have conducted laboratory studies that demonstrate that native amphibians [3–5], particularly in early larval stages [3,4], are susceptible to herbicide intoxication. The majority of available toxicological data on amphibians is derived from short-term, single-species, laboratory-test protocols. While such data are valuable, particularly in the comparative context, many scientists have questioned the extrapolative inference and ecological relevance of such studies and have called for the addition of more ecologically pertinent studies in the risk assessment process [6–9]. More specifically, Kapustka and coworkers [10] have called for greater reliance on monitoring data to test risk predictions and reduce uncertainty particularly in relation to real-world exposure regimes.

Consistent with both of these views, we undertook a comprehensive, tiered approach in an attempt to characterize the risk to native amphibian larvae resulting from potential exposure to the herbicide glyphosate (Vision). Results of studies comprising Tiers I through III of the research program are

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Table 1. Mean and range for various parameters characterizing wetlands monitored in this study[a]

Characteristic	Adjacent (n = 11)	Buffered (n = 16)	Oversprayed (n = 24)	Untreated controls (n = 8)
Area (m^2)	341; 12–998	296; 1–1,440	337; 7–936	132; 4–455
Depth (m)	0.44; 0.17–0.75	0.49; 0.6–2.0	0.34; 0.15–0.80	0.36; 0.15–0.70
Volume (m^3)	1,011; 27–2,545	1,789; 1–12,750	876; 11–3,780	282; 12–683
Dissolved oxygen (mg/L)	5.8; 0.9–8.5	5.1; 0.2–7.9	6.1; 2.0–15.0	7.5; 3.3–12.1
Temperature (°C)	22; 17–27	21; 15–26	21; 15–29	23; 17–28
Conductivity (μS/cm)	150; 11–425	123; 2–452	72; 10–400	NA
Macrophyte cover (%)	20; 0–70	29; 0–90	39; 0–95	37; 0–80
pH	7.3; 5.3–8.9	6.9; 4.8–8.6	6.8; 4.5–9.1	7.9; 7.2–9.0

[a] All values are means followed by maxima and minima.

described in preceding articles in this series [11–13]. In this article, we describe an operational monitoring study comprising Tier IV of the overall research program. The objectives of this study were to enhance our quantitative understanding of real-world exposure regimes for native larval amphibians and other biota in small wetland ecosystems typical of those that may be contaminated by Vision during use for forest vegetation management and directly monitor for acute mortality in caged native larval amphibians exposed in situ in small wetlands oversprayed, adjacent, or buffered in relation to spray blocks receiving aerial applications of the herbicide Vision.

MATERIALS AND METHODS

Site characterization and experimental design

Over a period of three application seasons (1999–2001), chemical and biological monitoring studies were conducted on a variety of spray blocks targeted to receive aerial herbicide treatments for release of conifer crop trees from competing vegetation. Spray blocks were representative of jack pine (*Pinus banksiana*) and black spruce (*Picea mariana*) regeneration sites in the boreal forest region of northwestern and northeastern Ontario. The herbicide applied in all operational monitoring studies was the commercial formulation Vision, which is identical to the product Roundup Original® (Monsanto, Winnipeg, MB, Canada) and which contains the isopropylamine salt of glyphosate and the polyethoxylated tallowamine surfactant also known as MON0818 (15% by volume). Throughout this article, exposure concentrations are expressed in terms of mg acid equivalents (a.e.)/L of glyphosate. Using acid equivalent concentrations facilitates comparisons with other studies, which may use a variety of different formulations. The reporting units do not imply that effects are solely the result of exposure to the free acid form of glyphosate, rather biological responses are the result of exposures to the whole formulated product. Concentration units of mg a.e./L of glyphosate as used throughout this article may be converted to mg/L of Vision using a multiplication factor of 3.23 [14].

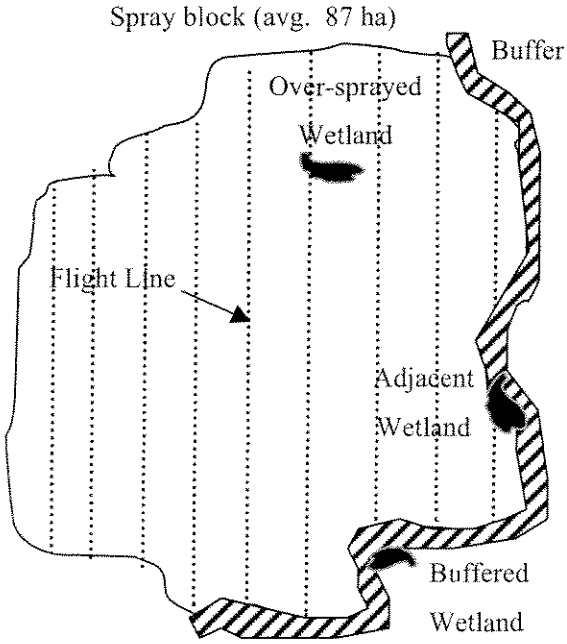

Fig. 1. Schematic representation of the experimental design for operational monitoring.

All spray blocks were treated according to normal operational procedures and following standard aerial application guidelines. Herbicide applications were made using either rotary-wing (Bell Jet Ranger helicopter; Bell Helicopter Textron, Fort Worth, TX, USA) or fixed wing aircraft (Grumman Ag-Cat; Schweizer Aircraft, Elmira, NY, USA) fitted with conventional boom and nozzle (D8-46 or D6-45) dispersal systems. A number of application variables, including chemical rate, block size, aircraft, buffer size, wind speed and direction, release height, and time of application were recorded for each spray session. Operational monitoring studies were conducted on a total of 19 different spray blocks targeted to receive aerial applications of the herbicide Vision. Monitoring took place over the course of three field seasons (1999–2001) with herbicide treatments applied during the month of August in each year. The average size of spray blocks receiving herbicide treatments was 83 ha (range 43–133 ha). The nominal rate of chemical application to these blocks ranged from 1.07 to the maximum label rate for conifer release of 2.14 kg a.e./ha, with an overall average of 1.92 kg a.e./ha. During spray sessions, winds were light to variable with wind speeds <6 km/h and typically ranging from 0 to 3 km/h. Depending on site conditions and aircraft employed, release heights ranged from 10 to 20 m above ground level. Summary data characterizing the various wetlands (total of 59 inclusive of 8 untreated control wetlands) monitored in this study are provided in Table 1.

On each site, small wetlands were selected in the area expected to be oversprayed, in the area immediately adjacent to the overspray zone, and in an area separated from the treatment zone by a vegetated buffer ranging in size from 30 to 60 m. Chosen wetlands were classified as oversprayed, adjacent, or buffered, respectively. A schematic representation of the general experimental design is shown in Figure 1. The exact location of each wetland was determined using a Garmin 12 Map (Garmin, Olathe, KS, USA) hand-held global positioning system. Each wetland was characterized by recording size,

average depth, and species of macrophytes present. Water chemistry parameters, including pH, temperature, dissolved oxygen, and conductivity, were determined using portable monitoring devices (YSI, Yellow Springs, OH, USA) and Hydrolab minisond (Loveland, CO, USA), respectively.

Chemical monitoring

Within each designated wetland, water samples were obtained both prior (~24 h) and subsequent (within 6 h) to herbicide applications. Water samples (~800 ml) were obtained by immersing a 1-L high-density polyethylene plastic bottle through the top 20 cm of the water column in an open area of the wetland. Sample bottles were capped, labeled, and stored on ice in dark coolers while on site and then transferred to frozen storage within 18 h postsampling. Glyphosate and its primary degradation product, aminomethylphosphonic acid (AMPA), were determined using a validated gas chromatographic technique employing nitrogen-phosphorous detection. After thawing, water samples were thoroughly mixed and a known volume aliquot (typically 100 ml) was withdrawn for analysis. A known amount of glufosinate ammonium was added as an internal standard to each sample aliquot and the samples were then rotary evaporated to dryness (water bath, 60°C). Subsequently, the flask was held in an oven at 135°C for 3 min to ensure complete dryness. Glacial acetic acid (3 ml) and trimethylorthoacetate (6 ml) were added sequentially to each flask, and the reaction mixture was heated under reflux at 110 to 135°C for a period of 3 h. The derivatization reaction converts amino, hydroxyl, and carboxyl functional groups to volatile, less polar acetyl or methyl derivates amenable to gas chromatographic analysis. Excess derivatization reagent was removed from samples by repetitive rotary evaporation in ethyl acetate (3 × 15 ml) and final samples were brought to a constant volume of 10 ml in ethyl acetate. Chromatographic analyses were performed on a gas chromotograph (HP5890 Series II; Agilent Technologies, Mississanga, ON, Canada) equipped with an autosampler set to deliver 3-μl aliquots via splitless injection to a DB17 capillary column (15 m × 0.53 mm; 1.0 μM film thickness) (J&W Scientific, Folsom, CA, USA). Under the temperature-gradient conditions employed, chromatographic retention times for AMPA, glyphosate, and glufosinate derivatives were 6.09, 7.99, and 8.92 min, respectively. Concentrations of glyphosate and AMPA were calculated based on relative peak area response of the nitrogen-phosphorous detector in comparison with the internal standard (glufosinate) using Chemstation software (Agilent Technologies) and reported in units of mg a.e./L in the original aqueous sample. Using this method, limits of detection and limits of quantitation for both glyphosate and AMPA were conservatively set at 0.01 and 0.02 mg a.e./L, respectively.

Biomonitoring

Prior to initiation of the monitoring study, green frog (*R. clamitans*) and leopard frog (*R. pipiens*) egg masses were collected from relatively pristine forest wetland sites near Sault Ste. Marie, Ontario. Egg masses were reared under controlled conditions in an environmental chamber as detailed by Wojtaszek et al. [13] until larvae reached Gosner stage 25 and taxonomic identifications were confirmed by microscopic analysis [15]. As sufficient numbers of green frog larvae were not available in the first year, only leopard frog larvae were used for biomonitoring in 1999. Larvae were transported to field sites at ambient temperature in insulated coolers with aeration. Prior to deployment in the field, larvae were fed dried *Spirulina* and goldfish flakes ad libitum. At each spray block, groups of five individual larvae were placed into separate Ziploc® plastic bags and shuttled to chosen wetland locations, where they were transferred into separate cages. The cages used for the in situ exposures were constructed of a rectangular metal frame (21.5 cm × 11.5 cm × 11.5 cm) enclosed with crinolin mesh (mesh size ~ 1 mm²). The mesh was gathered at each end and held tightly closed using elastic hair bands. In near-shore areas not occluded by riparian or aquatic macrophyte vegetation, cages were deployed at a depth of 10 to 20 cm below the water surface. Cages were held in place with metal pig-tail-tree pins inserted through the elastic closures and anchored into the bottom sediments. Frog larvae were allowed to acclimatize to the specific wetland conditions (typically for 18–24 h) and checked immediately prior to herbicide treatment. Following field deployment, larvae fed on periphytic algae that colonized the crinoline mesh material without any artificial diet supplement. In a few instances, larvae escaped through, or were killed by entrapment in, mesh folds at cage ends. In the summer of 2000, persistent inclement weather prevented aerial herbicide applications to three of the spray blocks designated for monitoring. Mean mortality levels for leopard and green frog larvae in untreated control wetlands (n = 8) were 5.0 and 11.4%, respectively, suggesting that green frog larvae were inherently more susceptible to stress associated with transport, handling, and in situ deployment.

During the pretreatment check, dead or missing larvae were replaced with new individuals if necessary to ensure that five larvae were exposed in each case. Larval condition was observed and recorded periodically at approximately 6, 24, 48, and 96 h following herbicide applications. Larvae that responded vigorously to prodding were considered alive, while those not responding to repetitive prodding were considered dead. Larvae that had obviously become entrapped and perished in mesh folds posttreatment were excluded from the mortality data.

Statistical analysis

Percent mortality at 48 h posttreatment was calculated as the key response variable. Testing for temporal trends in mortality rates demonstrated no significant differences (p = 0.05) in five of six cases, with only a marginal difference (p = 0.04) being detected for leopard frog larvae in buffered systems. As such, data for each larval test species were pooled across years and mean mortality rates were calculated for each wetland classification (oversprayed, adjacent, or buffered). All statistical analyses were performed using standard statistical software (SigmaPlot, Version 7.0, and SigmaStat, Version 2.03, SPSS, Chicago, IL, USA) [16]. Glyphosate aqueous concentration and percent mortality data for both green and leopard frog larvae did not conform to standard assumptions of normality or variance homogeneity and hence were subjected to a Kruskal–Wallis one-way analysis of variance on ranks to determine significant differences among the three wetland types. Where a significant difference (p = 0.05) was detected by analysis of variance, Dunn's method for all pairwise comparisons was employed to isolate specific differences significant at the p = 0.05 level. As quantifiable glyphosate concentrations within adjacent and oversprayed wetlands spanned a wide range of values, regression analysis was also used in an effort to detect significant trends in percent mortality for both test species in relation to glyphosate exposure concen-

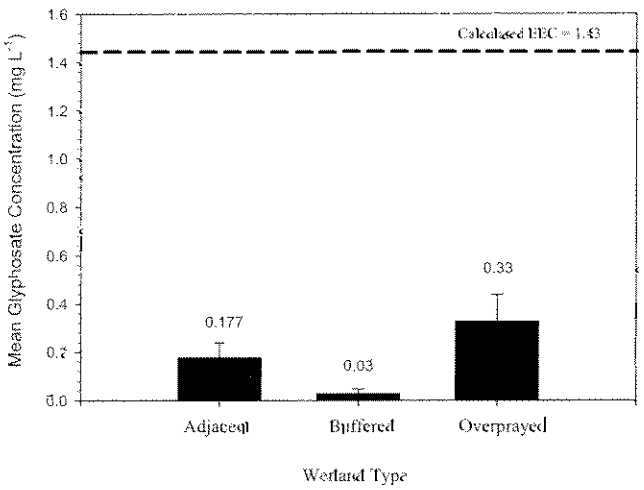

Fig. 2. Mean (±standard error) aqueous concentrations of glyphosate in oversprayed, adjacent, and buffered wetlands following operational aerial application of the herbicide (Vision®, Monsanto, Winnipeg, MB, Canada) in relation to expected environmental concentrations (EEC) as calculated by Canadian regulatory authorities assuming full deposition at the maximum label rate into a water body 15 cm in depth.

tration. Pearson product moment correlation analyses were used to detect potential relations between wetland characteristics and observed aqueous glyphosate concentrations.

RESULTS

Chemical monitoring

Analysis of water samples taken immediately prior to herbicide applications showed no glyphosate residues above analytical limits of quantitation (0.02 mg a.e./L) in any wetland. Results of analyses conducted on water samples taken within 6 h posttreatment showed variable aqueous concentrations of glyphosate among wetlands. Of the 16 cases for buffered wetlands, all but two showed aqueous glyphosate concentrations less than or equal to analytical limits of detection (0.01 mg a.e./L). The exceptional cases had measured concentrations of 0.05 and 0.31 mg a.e./L. The mean glyphosate concentration (0.03 mg a.e./L) in water samples taken from buffered wetlands was significantly ($p < 0.05$) lower than those taken from adjacent or oversprayed wetlands (Fig. 2).

Quantifiable levels of glyphosate were observed in approximately 45% of the adjacent wetlands (5/11 cases) and in 83% of oversprayed wetlands (20/24 cases). Although the mean concentration (0.18 mg a.e./L) in adjacent wetlands was approximately half that of oversprayed wetlands (0.33 mg a.e./L), this difference was not statistically significant ($p = 0.05$) because of the high variation in residue levels within each wetland classification. With respect to oversprayed wetlands, aqueous glyphosate concentrations ranged from below limits of detection (0.01 mg a.e./L) to a maximum of 1.95 mg a.e./L, with a mean concentration of 0.33 mg a.e./L. The observed mean concentration was well below the expected environmental concentration (EEC) value of 1.43 mg a.e./L. A portion of this differential can be explained by differences in average actual water depth of 34 cm (Table 1) as compared with the assumed value of 15 cm used in calculating EEC. The remainder is attributable to the fact that only a portion of the released spray cloud actually deposits at ground level, as discussed below. In adjacent wetlands, minimum residue levels were below limits of detection (0.01 mg a.e./L) and exhibited

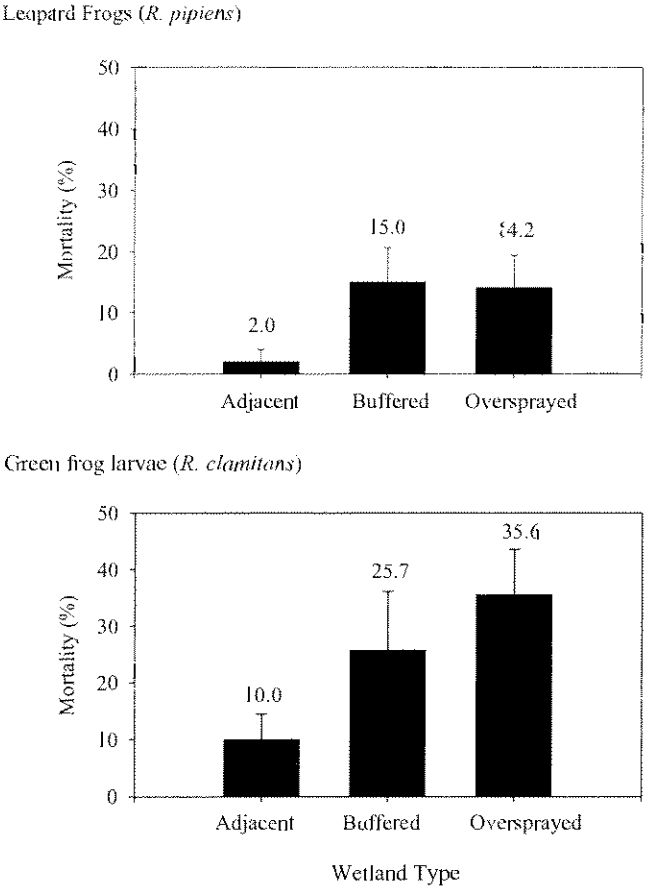

Fig. 3. Mortality in Gosner stage 25 larval leopard (*Rana pipiens*) and green (*Rana clamitans*) frogs following in situ exposure in various forest wetlands.

a relatively lower maximal value of 0.74 mg a.e./L. In all cases, only trace levels (<0.015 mg a.e./L) of the degradation product AMPA were observed.

Biological monitoring

Results of in situ biological monitoring using either caged green or leopard frog larvae are shown in Figure 3. Overall mean mortality rates among leopard frog larvae were less than 15% in all wetland types. No significant differences ($p = 0.194$) in mean mortality rates were observed for leopard frog larvae exposed to markedly different glyphosate concentrations under adjacent, buffered, or oversprayed wetland scenarios. Although green frog larvae showed somewhat higher mean mortality rates of 10, 26, and 36% in adjacent, buffered, and oversprayed wetlands, respectively, these differences were not statistically significant ($p = 0.129$) nor were they correlated to trends in mean glyphosate residue levels as described above. In an attempt to further assess the potential for concentration dependence, mortality rates for each larval species were regressed against observed glyphosate concentration for all cases where quantifiable residues were observed in adjacent and oversprayed wetlands. Neither regression showed statistically significant slopes ($p > 0.05$).

DISCUSSION

The ultimate fate of aerially released spray clouds is controlled by a variety of application and meteorological and microsite variables. Among numerous application variables, re-

lease height, spraying speed, wind direction, and wind speed are considered as the most important factors [17]. In oversprayed wetland scenarios, variation in aqueous concentrations results from a number of different factors, including potential skips and overlapping of spray swaths during chemical application, differential interception by surrounding vegetation, and micrometeorological effects on the depositing spray cloud, degree of occlusion of the water surface by aquatic macrophytes, as well as total surface and volume of the wetland. For adjacent and buffered wetlands, additional variation may be induced by relative upwind or downwind position with respect to the spray block and the degree of interception by vegetation or other objects between the spray block edge and the receiving wetland. We emphasize here that both the mean and upper 99% confidence limits in glyphosate concentrations, including those directly oversprayed wetlands, were substantially below the calculated EEC value as used by Canadian regulators. This differential results from representative small wetlands in northern Ontario being typically deeper than the assumed 15 cm depth used in calculating the EEC and by the fact that only a portion of the aerially released spray cloud deposits at ground level. Previous studies [18] demonstrate that, under typical scenarios, the proportion of herbicide penetrating to the ground layer on target may be relatively low (~12%) and that substantial proportions are impinged within the vegetation canopy as required to result in acceptable silvicultural efficacy. Thus, full deposition to the surface of forest wetlands is atypical and the calculated EEC may generally be considered as an overestimate of aqueous concentrations, even in directly oversprayed forest wetlands. Given the number, range, and interaction among variables controlling deposition in forest wetland environments, assessment of potential risks to native amphibians as well as other species requires determination of both the probability of exposure and the range in magnitude of exposures that may occur in real-world scenarios.

Probabilistic assessment of exposure

The low probability of detecting glyphosate concentrations in buffered wetlands as observed in this study is consistent with several previous studies [19–22] and generally supports the concept of buffers as protective of sensitive biota in aquatic ecosystems. In this study, buffer zones were typically vegetated with mature standing timber and a mixture of smaller brush and herbaceous riparian vegetation near the wetland monitoring sites. In comparison with conventional buffers of 60 to 100 m width, we used smaller widths (typically 30 m) and in some cases barren roadbeds as buffers. In both cases where quantifiable concentrations of glyphosate in buffered wetlands were observed, the wetlands were immediately adjacent to roads where the lack of vegetation resulted in minimum potential to intercept drifting spray clouds. Neither case was characterized by unusual wind speeds or release heights.

Overall, the upper 99% confidence limit on mean glyphosate concentrations observed in buffered wetlands (0.09 mg a.e./L) is approximately one order of magnitude below the lowest 96-h lethal concentration for 10% of the population (LC10) value (0.85 mg a.e./L) reported for most sensitive life stages of amphibian larvae exposed to Vision in either laboratory [11] or field experiments [13] and well below no-effect concentrations for growth or abnormal avoidance response as observed in field studies [13]. This relation suggests that typical aerial applications of Vision herbicide pose essentially no risk to native amphibian larvae in buffered wetlands.

The relatively greater frequency and magnitude of glyphosate contamination observed in adjacent and oversprayed wetlands supports the postulate of greater risk for biota in these systems. However, 99% confidence-limit values of 0.39 mg a.e./L and 0.55 mg a.e./L calculated for glyphosate concentrations in adjacent and oversprayed wetlands still provide a margin of safety relative to the lowest LC10 value reported for amphibian larvae [11] and well below concentrations (1.43 mg/L) showing no effect on sublethal parameters such as growth or avoidance response [13]. Thus, exposure concentrations as observed in this monitoring study are considered insufficient to generate significant acute mortality, impaired growth, or abnormal avoidance response in sensitive early larval development stages of *R. pipiens* or *R. clamitans*.

In situ biomonitoring for acute lethality in R. pipiens *and* R. clamitans *larvae*

The postulate of no acute lethal effects derived from probabilistic assessment of exposure as presented above is supported by the lack of significant differences in mean morality rates of caged amphibian larvae *R. pipiens* or *R. clamitans* exposed in buffered, adjacent, or oversprayed wetlands. The case for lack of effect on *R. pipiens* is particularly strong given that mean mortality rates were below 15% in all wetland types and trends were inversely proportional to glyphosate exposure concentration. Substantial differences in mean mortality for *R. clamitans* were observed with highest mortality in oversprayed wetlands. However, mean mortality values were not significantly different ($p > 0.05$), nor correlated with substantially different mean aqueous concentrations observed among the three wetland types. In addition, regression analyses provided no evidence to conclude a concentration-dependent relationship between mortality rates and quantifiable exposure concentrations for either larval test species.

Although naturally variable, environmental conditions among the three wetland types were generally similar, with mean pH values approximating neutrality. Based on the significant interaction between pH and Vision toxicity as reported in earlier tiers of this study [11,12], pH levels in monitored wetlands would, on average, be expected to exacerbate Vision-induced mortality in larval amphibians. In several wetlands, dissolved oxygen concentrations were very low (<4 mg a.e./L), adding the potential for concurrent anoxia stress for amphibian larvae in some cases.

CONCLUSION

Tier I laboratory studies [11] reported 96-h LC10 and LC50 estimates ranging from 0.85 to 3.5 mg a.e./L for early larval stages (Gosner 25) of *R. clamitans* and *R. pipiens* exposed to Vision. It was also reported that larvae were more sensitive than embryos and that the larval life stages of the four amphibian species tested were equisensitive to Vision effects.

Comparison of Tier I test results [11] to toxicity values published in the literature [14,23,24] for the chemically equivalent formulated products Vision or Roundup Original show that larval amphibians are among the most sensitive of freshwater aquatic species tested (Fig. 4). For example, the lowest 96-h LC50 value reported for amphibians (*X. laevis*; 0.85 mg a.e./L) [11] is actually less than the lowest value reported for freshwater fish (*Onchorhyncus mykiss*; 1.3 mg a.e./L) [25]. Given the herbicidal mode of action for these products, it is not surprising that both algae and aquatic macrophytes are also quite sensitive to Vision or Roundup Original. The lowest

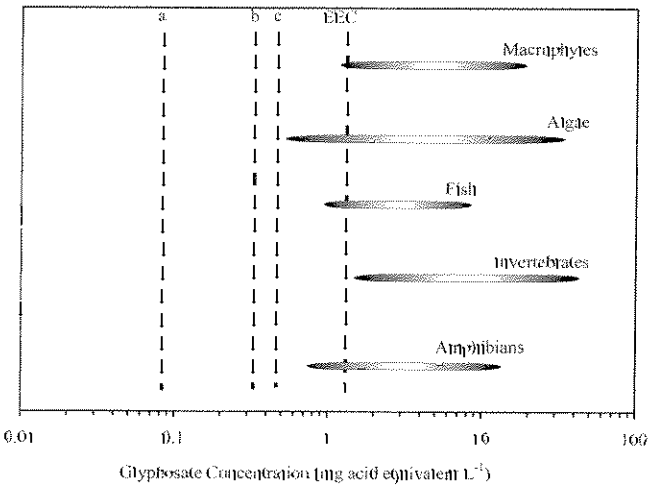

Fig. 4. The range of published toxicity values (median lethal concentration or median effective concentration) for various freshwater taxa in relation to the upper 99% confidence limits for concentrations observed in buffered (a), adjacent (b), or oversprayed wetlands (c) and worst-case expected environmental concentrations (EEC) as calculated by Canadian regulatory authorities assuming full deposition at the maximum label rate into a water body 15 cm in depth. (All literature data are for organisms exposed to the formulated product Vision®, Monsanto, Winnipeg, MB, Canada; or its equivalent Round-up Original® [Monsanto]; concentrations are expressed in terms of glyphosate mg acid equivalents/L to facilitate comparison with actual measured concentrations in wetlands.)

reported median effective concentration (EC50) values following 14-d exposures to these formulated products are 0.65 mg a.e./L for *S. capricornutum* [26] and 1.6 mg a.e./L for *Myriophyllum spp.* [27,28].

Tier II studies [12] confirmed the interaction of pH and Vision toxicity in *R. pipiens* larvae and showed parallel effects for zooplankton population response parameters, suggesting that the pH–Vision interaction is of general ecological significance. In addition, Tier II studies demonstrated that Vision effects on zooplankton reproduction could also be exacerbated by food deprivation stress. The standard laboratory toxicity endpoints as determined in Tier II studies and 1 overlap or approximate the EEC of 1.4 mg a.e./L as calculated by regulatory officials in Canada (Fig. 4), suggesting little to no margin of safety and necessitating, at minimum, further testing under more environmentally realistic scenarios. Moreover, both laboratory studies support the postulate of multiple-stress interaction and suggest the potential that toxicity of Vision may be enhanced under environmentally realistic conditions of high pH or where amphibian larvae are exposed to other concomitant stressors such as food deprivation.

Results of Tier III in situ enclosure studies [13], while similar to the laboratory results in some respects, provided evidence to refute both the postulate of significant risk of mortality and multiple stress effects under real-world exposure scenarios. Enclosure studies conducted in two different wetlands showed most 96-h LC10 and LC50 values consistently higher than those derived from laboratory studies and above-calculated EEC levels. Although a trend of greater mortality and sublethal effect (growth and abnormal avoidance response) was observed in the higher pH wetland environment (paralleling laboratory results), the site effect was masked by natural variation and not statistically significant. Moderated effects relative to laboratory predictions may be attributed to natural dissipation (e.g., sorption) and degradation (microbial breakdown, photolysis) processes that attenuate the exposure concentration in natural environments. Based on Tier III in situ enclosure studies, it was concluded that typical silvicultural applications of Vision should not generate significant mortality in native amphibian larvae. This conclusion was strongly supported by both chemical and biological monitoring results from Tier IV operational monitoring studies as reported herein, which show no statistically significant differences in mean mortality among larvae differentially exposed in oversprayed, adjacent, and buffered wetlands. Results of the operational monitoring study are consistent with concentration–response relations from both Tier I and III studies because 99% confidence limit for real-world exposure concentrations in all wetland cases were below both estimated LC50 and LC10 values (Fig. 4). The general equisensitivity among a variety of amphibian larval species exposed to Vision [11,24] suggests that this conclusion may be conservatively extrapolated to other anuran species.

Overall, results of this tiered research program confirm that amphibian larvae are particularly sensitive to Vision herbicide and that these effects may be exacerbated by high pH or concomitant exposure with other environmental stressors (e.g., food deprivation). Although results from laboratory studies were very useful in the comparative sense and in understanding mechanisms of interaction, they tended to overestimate effects as observed under natural exposure scenarios. For nonpersistent compounds such as Vision, static renewal tests tend to overestimate toxicity compared with natural exposures, where dissipation and degradation may reduce exposure. For example, in Tier III studies, we estimated site-dependent reductions in aqueous glyphosate concentrations ranging from 30 to 40% over the acute 96-h exposure period. Unfortunately, parallel data on surfactant fate is not available. As evidenced in this comprehensive tiered research program, combining laboratory toxicity data with unrealistic estimates of environmental concentration may lead to prediction of significant risk to native amphibian larvae that conflicts with results of field studies using either in situ enclosure or operational monitoring techniques.

As a general conclusion, results of this tiered research program indicate that aerial applications of the herbicide Vision, as typically conducted in northern Ontario, do not pose a significant risk of acute effects to the most sensitive aquatic life stages of native amphibians in forest wetland environments. This conclusion is consistent with comprehensive probabilistic risk assessments [14,29] and the general literature documenting the ecotoxicological safety of this herbicide.

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Persistence, Movement, and Degradation of Glyphosate in Selected Canadian Boreal Forest Soils

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Persistence, mobility, and degradation studies of glyphosate, *N*-(phosphonomethyl)glycine, under actual field conditions of boreal forest soils of Ontario, were undertaken after spraying Roundup at the rate of 2 kg of active ingredient (AI)/ha. Soils at three depths were collected and analyzed for residues of glyphosate and its metabolite (aminomethyl)phosphonic acid. Glyphosate was found to remain consistently to a level below 50% of the highest residue values observed beyond 24 days. More than 95% of the total herbicide residue was found in the upper organic layer at any time. There was no evidence of lateral movement of the glyphosate either in runoff water or through subsurface flow. In general, concentrations of the metabolite (aminomethyl)phosphonic acid were very low.

The herbicide glyphosate (GLYPH), *N*-(phosphonomethyl)glycine (Monsanto's Roundup), is an environmentally safe broad-spectrum herbicide having a potential for use in silvicultural programs such as site preparation, conifer release, and nursery stock production. This herbicide has been recommended for use in agricultural as well as forestry substrates in Ontario. Studies on the behavior of glyphosate in or on soil have been reported (Torstensson, 1982; Stark, 1982; Salazar and Appleby, 1982; Torstensson and Stark, 1979, 1981; Rueppel et al., 1977; Torstensson and Aamisepp, 1977; Sprankle et al., 1975a,b). However, inadequate data exist as to its behavior under boreal forest conditions in Ontario, and hence this study was undertaken.

EXPERIMENTAL SECTION

Reagents. Glyphosate (98%) and (aminomethyl)-phosphonic acid (AMPA) (94%) were supplied by Monsanto Chemical Co. Trifluoroacetic anhydride and trifluoroethanol were purchased from Aldrich Chemical Co. Anhydrous sodium sulfate was heated at 140 °C overnight prior to use. All organic solvents used were pesticide grade (Caledon Laboratories, Georgetown, Ontario, Canada).

Location and Experimental Design. One sand site for persistence and leaching studies and one clay site for mobility study were selected. The sand site was part of a recently planted jack pine plantation that also contained the occasional blueberry plants. The clay site was in an open cutover covered by weeds and the occasional remnant of the original forest, namely white birch, black spruce, and poplar. The sand and clay sites were located in Harker (48°30′ N, 79° W) and Lamplugh (48°35′ N, 79° W) townships, respectively, about 40 km east of Matheson in the district of Cochrane, Ontario. Each site (20 m × 20 m) was divided into five replicate strips separated by buffer zones (1 m × 20 m). Each strip (2 m × 20 m) was further subdivided into 10 squares (2 m × 2 m) as sampling plots.

Site Preparation. All dead wood, live brush, and as much vegetation as possible were manually removed from the site with minimal disturbance of the duff layer (5–10 cm in depth). For the mobility study, dead wood and other

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Table I. Characteristics of the Study Soils[a]

	soil[c] fraction	clay[b] mobility site	sand[b] persistence site
pH	O	4.48	3.50
	M	4.43	3.73
% clay	O	0.00	0.00
	M	87.60	5.60
% silt	O	0.00	0.00
	M	12.40	14.00
% sand	O	0.00	0.00
	M	0.00	80.40
% organic matter	O	29.47	39.70
	M	0.00	0.76
CEC, mequiv/100 g	O	21.40	18.50
	M	3.80	5.30
% moisture content	O	8.26	7.71
	M	0.85	0.56
field capacity	O	137.43	24.47
	M	38.94	9.24

[a] Soil classification (Jotcham, 1985): clay of the Ryland series, Orthic Humic Gleysol type; sand of the Abitibi series, Orthic Humo-Ferric Podzol type. [b] Soil texture. [c] Key: O = organic (5–10 cm); M = mineral (20–25 cm in depth of the total 30-cm soil core).

Table II. Chemical Application to the Sand Site

	persistence site		
rep	vol appl, mL	rate appl, kg/ha	rate appl from deposit sheets,[a] kg/ha
A	1360	2.550	1.734
B	1230	2.300	1.595
C	1100	2.060	1.607
D	1120	2.100	1.445
E	955	1.790	1.362
av	1153	2.160	1.549

[a] An average of the three replicate deposit sheets per application zone.

Table III. Chemical Application to the Clay Site

rep	spray strip
vol appl, mL	1430
rate appl, kg/ha	2.680
rate appl from deposit sheets,[a] kg/ha	1.789

[a] An average of three replicate deposit sheets.

matter thought to have a potential for runoff channeling were removed from the site, and an application strip at the top of the slope was cleared as above. A back-hoe was used to prepare a trench at the bottom of the slope for the collection of the runoff water.

Soil. The characteristics of the typical boreal forest soil of Ontario are given in Table I.

Chemical Application. Chemical application were made on June 20, 1984, to the zone of application for the mobility site and on July 19, 1984, to the replicate strips in the persistence site. Glyphosate, *N*-(phosphonomethyl)glycine, was applied as an aqueous solution of Roundup (35.6% AI) with a pestex backpack sprayer (boom length 2 m, number of nozzles 4, nozzle type TEE Jet AL 8004) using compressed air (200 kPa) as a propellent. An application rate of 2 kg (AI)/ha was targetted, and actual rates were determined by the use of deposit sheets as well as by the reservoir volumes before and after spraying (Tables II and III). Deposit sheets prepared from 20 cm × 20 cm glass plates wrapped in aluminum foil were placed in each application strip. Immediately after application the foil sheets were unwrapped (thereby quantitatively trapping the deposit), labeled, and frozen until analyzed.

Sampling. Soil cores were taken from sampling plots with use of a random number table. Samples were also collected at 3, 6, 9, and 12 m downslope from the chemical application zone in case of mobility site. A soil auger (length 54 cm, diameter 10 cm) was driven to the depth of 32 cm with a sledge hammer. The bottom 2 cm of the core was discarded and the adjacent 15 cm mineral soil (M2, 15–30 cm) collected. The remainder of the core was divided into organic and mineral (M1, organic to 15 cm) layers and collected separately. The sections were bagged, weighed, and stored at −20 °C. The sampling schedule was 0, 2, 7, 14, 28, 43, 78, 125, 365, 721, and 792 days and 0, 1, 6, 14, 49, 96, 335, 691, and 762 days for the mobility and persistence sites, respectively. Water samples of 1 L were collected from the trench and stored at −20 °C.

Weather. Field weather stations were used to monitor rainfall and temperature. On examination of the rainfall data produced from these, it was discovered that they had intermittently malfunctioned and as a result these data were discarded. However, weather data were obtained from the Ministry of Natural Resources (MNR) district weather station in Kirkland Lake, Ontario, which is approximately 40 km southwest from the study sites. It was felt that these data from the MNR are the second best source, which will provide a reasonable approximation of the climatic conditions during the experimental period. These weather data were used to indicate that the year 1984 was climatically normal compared to those in past years (Table IV).

Soil Preparation. Frozen soil cores were allowed to thaw at 25 °C, air-dried, homogenized in a heavy-duty stainless steel blender, and sieved through a 10-mm-mesh brass sieve (Feng and Klassen, 1986). The final moisture content was 5–7%.

Extraction and Cleanup. An analytical methodology has been established by our laboratory for the isolation and quantification of glyphosate and its metabolite AMPA and has been reported earlier (Roy and Konar, 1988). To a subsample (5 g) of the finely ground, homogenized soil in a 250-mL screw-cap bottle was added concentrated phosphoric acid (0.5 mL). The bottle was capped and shaken manually for 2 min. Deionized water (100 mL) was

Table IV. Monthly Rainfall and Temperature Data for the Period 1975 (May–September) to 1984 (May–September)

month	1984	1983	1982	1981	1980	1979	1978	1977	1976	1975
					Rainfall, mm					
May	54.8	118.4	46.5	46.6	41.0	80.5	55.9	19.9	69.4	101.6
June	128.5	70.4	69.5	94.1	49.4	151.0	143.2	118.2	71.3	83.9
July	93.7	55.2	71.7	46.3	44.8	96.4	141.8	54.1	95.8	49.0
Aug	88.5	64.0	68.5	64.2	79.8	94.1	103.0	71.9	40.2	50.3
Sept	41.0	38.3	85.0	79.0	111.2	92.0	62.1	58.8	156.4	103.9
					Temperature, °C					
May	10.1	10.1	17.7	12.1	14.9	14.4	19.6	17.0	12.1	17.6
June	17.4	19.3	16.0	18.0	16.1	19.0	16.6	16.9	22.2	16.1
July	21.0	21.5	20.9	23.4	21.7	21.6	20.2	21.1	20.2	22.0
Aug	20.4	20.9	15.0	21.0	20.9	17.3	19.0	17.3	20.5	20.2
Sept	13.6	15.9	12.4	11.7	11.8	13.7	12.6	13.7	12.8	11.0

added followed by the addition of chloroform (50 mL) and the resulting slurry quantitatively transferred to a small domestic blender. After blending for 2 min, the solution was filtered under suction, the extract transferred to a separatory funnel, and the residue rinsed twice with water (2 × 40 mL) and chloroform (50 mL). The aqueous fraction was washed first with hexane (50 mL) and then with ethyl acetate (50 mL). Both hexane and ethyl acetate washings were discarded. Darco (G-60) charcoal (1 g) was added to the aqueous fraction and filtered under suction. The filtrate was concentrated to ~5 mL in vacuo at 60 °C and filtered through a Millipore filter (0.45 μm, Millipore, Waters). After filtration, the pH was adjusted to 0.5 with phosphoric acid and the sample was evaporated to dryness in vacuo at 60 °C. The dried samples were stored under vacuum in a desiccator containing phosphorus pentoxide.

Derivatization. The derivatization reactions utilized in this procedure are based on those described by Deyrup et al. (1985). The flask containing the residues of GLYPH and AMPA from the previous extraction was equipped with a Claisen condenser and an anhydrous calcium chloride guard tube. A gentle stream of dry nitrogen was passed through the system. Trifluoroacetic anhydride (2 mL) followed by trifluoroethanol (1 mL) were added. The mixture was then refluxed for 90 min in an oil bath at 80 °C. The excess reagents were removed by a gentle stream of nitrogen at 40 °C. The derivatives were cooled in an ice–water bath, water (5 mL) was added, and the contents were transferred to a 125-mL separatory funnel with water (5 mL) and then chloroform (60 mL) as rinse. After the mixture was shaken for ~1 min, the chloroform layer was collected and the aqueous layer was extracted two more times with chloroform (2 × 60 mL). The combined chloroform extract was dried over anhydrous sodium sulfate (2-cm bed) followed by the removal of the solvent in a vacuum rotary evaporator at 60 °C. The residue was dissolved in ethyl acetate and injected into the gas chromatograph. The derivatized sample was stable for at least 2 weeks.

Runoff water samples were allowed to thaw at 4 °C. They were then evaporated to 100 mL and extracted according to the method described above.

The deposit sheets were also thawed at 4 °C and the contents eluted with water, concentrated to 100 mL, and extracted as above.

Gas Chromatographic Analysis. The gas chromatographic analysis was conducted on a Shimadzu GC-9A gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a nitrogen–phosphorus detector. The chromatographic column was Ultra-bond 20SE on 80/100-mesh support (Ultra Scientific, Hope, RI): 1.8-m glass; 3-mm i.d. The operating parameters of the gas chromatographs were as follows: detector temperature, 250 °C; column temperature, 150 °C; injector temperature, 250 °C; gas flow rate, nitrogen 50 mL/min (ultra high purity), hydrogen 4 mL/min (pure), and air 175 mL/min (high purity). Samples were sandwiched between two injections of the same standard. A detector fluctuation of ±10% was considered acceptable, and a linear range between 50% and 200% of the average of standards was used in the quantification. Residue concentrations were quantified by the comparison of peak areas to the average peak area of standards run before and after each sample. A Shimadzu C-R3A data processor was used for quantification.

Fortification. Aliquots of prespray residue free soil were placed in a 250-mL screw-cap bottle. Appropriate aliquots of previously prepared solutions containing both GLYPH and AMPA were added to provide fortification levels of 1, 0.48, and 0.096 ppm for GLYPH and 0.5, 0.18, and 0.035 ppm for AMPA, respectively. The bottles were capped, manually shaken to ensure thorough mixing, and stored at −20 °C for 24 h to simulate residue sample storage conditions. The fortification of water was performed in the same manner.

Table V. Residue Values of Glyphosate and Its Metabolite AMPA from the Sand Persistence Site

days post-spray	soil layer[a]	glyphosate residue, μg ± SD	AMPA, μg ± SD
0	O	707.3 ± 61.4	ND
	M1	ND	ND
	M2	ND	ND
1	O	1289.2 ± 208.6	ND
	M1	ND	ND
	M2	ND	ND
6	O	1005.2 ± 102.3	59.9 ± 3.6
	M1	ND	ND
	M2	ND	ND
14	O	741.2 ± 121.9	89.7 ± 49.6
	M1	58.6 ± 48.6	ND
	M2	ND	ND
49	O	214.2 ± 112.3	23.4 ± 7.1
	M1	ND	ND
	M2	ND	ND
96	O	75.4 ± 7.0	52.1 ± 5.5
	M1	ND	ND
	M2	ND	ND
335	O	20.4 ± 6.0	11.8 ± 3.8
	M1	ND	ND
	M2	ND	ND
691	O	ND	ND
	M1	ND	ND
	M2	ND	ND
762	O	ND	ND
	M1	ND	ND
	M2	ND	ND

[a] Key: O = organic; M1 = organic to 15 cm; M2 = 15–30 cm; ND = not detectable; limit of detection 0.05 and 0.01 ppm (μg/g) for glyphosate and AMPA, respectively.

RESULTS AND DISCUSSION

Recovery Efficiency. Recoveries for GLYPH from fortified soil and water samples were as follows: 77.8 ± 8.6% in organic matter; 52.1 ± 1.9% in the clay; 47.7 ± 2.3% in the sand; 94.2 ± 3.6% in water. Similarly recoveries for AMPA were 65.0 ± 4.5% in organic matter, 50.1 ± 1.3% in the clay, 42.6 ± 3.1% in the sand, and 91.1 ± 5.4% in water.

Persistence. In the case of the sand persistence site, the time required for dissipation of GLYPH to less than 50% of the highest residue values observed was 24 days. After 78 days postspray, these values were reduced to below 10% (Table V). Throughout the observation period GLYPH was found to remain in the organic layer. A general trend of dissipation of GLYPH residues with time is evident. The results showed that the recovery of GLYPH at 0 time was lower than expected. No adequate explanation for this could be found. However, two important reasonings could be considered for this high variability: (i) Immediately after spraying the chemical reaching the ground was of unilayer in thickness and sufficient time had not been allowed for its translocation within the soil. In this situation the collection of the soil cores could have resulted in a loss of the herbicide via sorption to plastic bags or instruments used. (ii) Certain vegetation cover still remaining on the ground prevented the total spray from reaching the ground.

Leaching. It was evident from the results of the persistence site that the average GLYPH residue in the upper organic layer was always more than 95% of the total present in the soil core at any time (Table VI). Results

Table VI. Mean Percentage Distribution of Glyphosate in Layers[a] from the Sand Persistence Site

days postspray	organic layer, %	organic 15 cm (M1), %	15–30 cm (M2), %
0	100.00	0.00	0.00
1	100.00	0.00	0.00
6	100.00	0.00	0.00
14	95.16	4.84	0.00
49	100.00	0.00	0.00
96	100.00	0.00	0.00
335	100.00	0.00	0.00
691	–[b]	–	–
762	–	–	–

[a] Calculated as the basis of total amount in the cores at each sample time. [b] Not determined because no detectable amounts of glyphosate were found from the analyzed samples for those two sampling dates.

Table VII. Glyphosate Residue Values (μg) from the Clay Mobility Site[a]

days postspray	distance, m				
	0	3	6	9	12
0	1337.9	ND	ND	ND	ND
2	918.4	ND	ND	ND	ND
7	1185.2	ND	ND	ND	ND
14	273.0	ND	ND	ND	ND
28	1059.5	ND	ND	ND	ND
43	373.0	ND	ND	ND	ND
78	162.4	ND	ND	ND	ND
125	NA	ND	ND	ND	ND
365	17.1	ND	ND	ND	ND
721	ND	ND	ND	ND	ND
792	ND	ND	ND	ND	ND

[a] Only the top strip was sprayed. Key: ND = not detectable; NA = not available; limit of detection = 0.05 ppm (μg/g).

also indicate that GLYPH has a very limited potential to leach vertically through the soil column under the conditions of the study. Overall the study shows that no detectable residue of GLYPH or AMPA could be found either in the organic to 15 cm or 15–30 cm levels; thus this chemical can be considered as essentially nonleachable under the conditions of the experiment.

Mobility. On the basis of the results of this study on the clay mobility site, there was no evidence of lateral movement of the GLYPH down the 8° slope in either runoff water or through subsurface flow as no GLYPH in the quantifiable range (0.1 ppm) could be detected either at a distance 3 m from the top of the application zone or in the runoff water collected in the trench (Table VII).

Metabolite AMPA. The overall trend of metabolite AMPA formation showed that within the observation period as GLYPH concentration decreased, concentration of metabolite AMPA increased and then decreased, indicating that it is a nonpersistent metabolite. The maximum amount of metabolite AMPA formation was 10.24% with respect to GLYPH concentration. In general, the concentrations of metabolite AMPA in these sites were very low in comparison with GLYPH (Table V).

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Uptake and persistence of the herbicide glyphosate (Vision®) in fruit of wild blueberry and red raspberry

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Field studies on the uptake and persistence of glyphosate (*N*-(phosphonomethyl)glycine) on wild blueberry (*Vaccinium myrtilloides* Michx.) and red raspberry (*Rubus strigosus* Michx.) under boreal forest (Matheson, Ont.) conditions were undertaken. Uptake studies indicated that less than 10% of glyphosate penetrated the fruit in the first 9 h postapplication. Results of the persistence studies showed a gradual decline in residue levels with time. Times to 50% dissipation for glyphosate residue as determined by curvilinear regression analyses were <20 days (95% confidence limit of 8–26 days) and <13 days (95% confidence limit of 6–14 days) for blueberry and raspberry fruit, respectively. Initial residue levels dissipated to approximately 4 and 6% after 61 and 33 days for the blueberry and raspberry, respectively. Results also showed that at no time during the study period did glyphosate levels in either substrate dissipate to below the maximum permissible residue level (0.01 ppm) as established by Health and Welfare Canada.

ROY, D. N., KONAR, S. K., BANERJEE, S., CHARLES, D. A., THOMPSON, D. G., et PRASAD, R. 1989. Uptake and persistence of the herbicide glyphosate (Vision®) in fruit of wild blueberry and red raspberry. Can. J. For. Res. **19** : 842–847.

Des essais au champ ont porté sur l'assimilation et la persistance du glyphosage (*N*-(phosphonométhyle)glycine) sur la Myrtille sauvage (*Vaccinium myrtilloides* Michx.) et la Framboise rouge (*Rubus strigosus* Michx.) en forêt boréale (Matheson, Ontario). Les études d'assimilation ont montré que moins de 10% du glyphosate pénétrait les fruits durant la période de 9 h suivant l'application. Les résultats des études sur la persistance ont montré un déclin graduel des niveaux de résidus avec le temps. La période provoquant une dissipation à 50% des résidus de glyphosate, telle que déterminée au moyen d'analyses de régression curvilinéraire, a été <20 jours (95% de probabilité de 8 à 26 jours) et <13 jours (95% de probabilité de 6 à 14 jours) pour la myrtille et la framboise, respectivement. Les niveaux initiaux de résidus se sont dissipés à environ 4 et 6% au bout de 61 et de 33 jours pour la myrtille et le framboisier, respectivement. Les résultats ont aussi montré qu'en aucun moment durant la période sous étude le niveau de glyphosate ne s'est dissipé sous le niveau de résidu maximal permis (0,01 ppm) dans l'un ou l'autre substrat tel qu'établi par Santé et Bien-être social Canada.

[Traduit par la revue]

Introduction

Glyphosate (Vision®) (formerly Roundup®) is a broad spectrum herbicide and widely used in silviculture for the control of competing vegetation. Two common competing species in the Ontario boreal forest are wild blueberry (*Vaccinium myrtilloides* Michx.) and red raspberry (*Rubus strigosus* Michx.). Chemical applications of glyphosate are typically made in mid- to late-summer when wild fruit are fully ripe. Although treated areas are posted, contaminated fruit may be consumed by various wildlife species and picked by humans for personal consumption or commercial sale. Data pertaining to the levels of glyphosate residue to be expected in berries following herbicide applications and the persistence of such residues in ripe, edible berries under conditions typical of the boreal forest ecosystem of Canada are lacking. Without a thorough understanding of these aspects, assessment of the environmental hazard associated with contaminated fruit is questionable. The purpose of this work was to establish an analytical method to determine glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), residues from treated fruit of wild blueberry red raspberry and to determine the uptake and persiste of glyphosate into the fruit of those berries under f conditions.

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Materials and methods

Site selection and preparation

Two sites were selected in the district of Cochra Ontario, for these studies. The blueberry site was loca 40 km east of Matheson, Ontario, and 15 km south Highway 101 in Harker Township (48°30′N, 79°W) n Ghost Mountain. The raspberry site was located Lamplugh Township (48°35′N, 79°W), 40 km east Matheson and 17 km north of Highway 101 and Gh Mountain. Because these sites differ in their physical natu their site preparation will be discussed separately.

Blueberry site

Six plots (2 × 20 m), separated by buffer zones with minimum width of 5 m, were selected and designated Hark A–F. Criteria for this selection were the abundan of berry crop and a minimum amount of other vegetatio namely small jack pine trees. The site was prepared

TABLE 1. Assessment of deposit following glyphosate application to the blueberry site

	Volume applied (L/ha)	Rate applied (kg/ha)	Rate applied from Petri dish[a] Mean ± SD (kg/ha)	%CV
Hark A	433.75	2.316	2.290 ± 0.144	6.3
Hark B	388.75	2.075	1.660 ± 0.069	4.2
Hark C	453.75	2.423	1.919 ± 0.136	7.1
Hark D	458.75	2.449	1.905 ± 0.119	6.2
Hark E	391.25	2.089	2.657 ± 0.151	5.7
Hark F	316.25	1.688	1.420 ± 0.072	5.1
Average	407.08	2.173 ± 0.287 (13.2)[b]	1.975 ± 0.443	22.4

[a]An average of the three replicate Petri dishes per application zone.
[b]% of carrier volume is given in parentheses.

TABLE 2. Assessment of deposit following glyphosate application to the raspberry site

	Volume applied (L/ha)	Rate applied (kg/ha)	Rate applied from Petri dish[a] Mean ± SD (kg/ha)	%CV
Lamp A	441.25	2.356	2.100 ± 0.120	5.7
Lamp B	266.25	1.420	1.065 ± 0.052	4.9
Lamp C	348.75	1.860	1.780 ± 0.064	3.6
Lamp D	466.25	2.480	2.720 ± 0.133	4.9
Lamp E	373.75	1.990	1.587 ± 0.043	2.7
Lamp F	378.75	2.020	1.446 ± 0.077	5.3
Average	379.17	2.021 ± 0.377 (18.6)[b]	1.783 ± 0.574	32.2

[a]An average of the three replicate Petri dishes per application zone.
[b]% of carrier volume is given in parentheses.

moving logs and cutting small trees to ensure unobstructed ıemical application. Small trees were cut back to within ın of the ground wherever necessary. Glass Petri dishes 53.8 cm^2) were placed within the plots on wooden posts approximately the height of the blueberry plants to deter- ine the actual amount of chemical deposited on the berries. lants from an adjacent but unsprayed area were selected a check control.

Raspberry site

Six plots (2 × 20 m), separated by buffer zones with a inimum width of 5 m, were selected and designated as amp A–F. Criteria for the selection were the presence of ines at least 2 years old and the abundance of berry crop. reparation of the sampling plot consisted only of the ıtting back of any tall weed or sapling cover to the height f the raspberry growth. Glass Petri dishes (153.8 cm^2) ere again placed within the plots on wooden posts at)proximately the height of the raspberry plants to deter- ine the actual amount of chemical deposited on the berries. lants from an adjacent but unsprayed area were selected a check control. All plots were marked with red and white)lored posts, indicating that the site was an experimental ea, with berry picking prohibited.

hemical application

Chemical applications were made on August 8, 1985, at :00 to replicate strips in both the blueberry and raspberry es. Glyphosate, N-(phosphonomethyl)glycine, was applied [illegible] active ingredient (a.i.)) with a Pestex® backpack sprayer (boom length, 2 m; number of nozzles, four; nozzle type, Tee Jet AL 8004; capacity of reservoir, 2000 mL) using compressed air (200 kPa) as a propellant. The height of raspberry plants at the time of application was 1.2 m above the ground, and the boom was held ~30 cm above the raspberry canopy. Carrier volume rate was 500 L/ha. Because of the limitations of the delivery rates and speed of the hand-operated sprayer, a relatively large volume rate (500 L/ha instead of 35 L/ha) of spray was perforce employed.

The target chemical application rate was 2 kg a.i. per hectare. Actual application rates were determined using glass Petri dishes (153.8 cm^2) as artificial deposit collectors. Replicate Petri dishes were placed inside the experimental strips at the height of the plants. Immediately after application, the Petri dishes were collected, labelled, and frozen until analysis. Sprayer reservoir volumes were monitored before and after spraying of each strip, which allowed calculation of rate of application. All volume measurements were made using a 2-L graduated cylinder. Application rates for both the sites are given in Tables 1 and 2.

Sampling

Samples of ripe berries were collected at 0 and 9 h and at 1, 2, 13, 20, 33, and 61 days from the date of spraying. Samples selected at random along the length of the plot were collected using disposable surgical gloves to prevent contamination. Fresh weights of samples were determined using a triple beam balance immediately following collection. The

TABLE 3. Recovery efficiency for glyphosate and AMPA from blueberries and red raspberries

	Fortification ppm (μg/g)		% recovery ± SD (%CV)	
	Glyphosate	AMPA	Glyphosate	AMPA
Blueberries	1.06	0.41	57.52 ± 2.09 (3.64)	108.56 ± 6.29 (5.79)
	0.11	0.04	102.46 ± 4.69 (4.58)	91.03 ± 3.18 (3.49)
	0.05	0.02	51.58 ± 3.86 (7.48)	100.58 ± 5.19 (5.16)
Raspberries	1.06	0.41	62.91 ± 2.95 (4.69)	98.79 ± 10.69 (10.83)
	0.11	0.04	72.89 ± 4.43 (6.07)	103.89 ± 3.28 (3.16)
	0.05	0.02	97.87 ± 7.30 (7.46)	85.06 ± 3.52 (4.14)

NOTE: There were 3 and 4 samples per concentration for blueberries and raspberries, respectively. Limits of detection for glyphosate and AMPA were 0.025 and 0.01 ppm, respectively.

samples collected between 0–9 h were placed in preweighed collanders (diameter, 11.5 cm). The collanders were supported over plastic funnels and their contents washed copiously with distilled water that was collected in plastic bottles. The washings together with washed fruit were stored frozen until analysis. These samples were used for the uptake study. At the same time, unwashed berries were collected for use in the persistence study. Fruit from an adjacent but unsprayed area were sampled as a control. Samples were brought to the laboratory in Styrofoam containers packed with ice. On arrival at the laboratory, samples were frozen at −20°C until analysis.

Weather

Weather conditions were monitored during the 7 days immediately postspray. There was no rainfall during the first 3 days, whereas 6 mm fell on the 4th day and 7 mm on the 6th day. During this period, the temperature averaged 21°C ± 6°C. Unfortunately the rain periods fell during a nonsampling period and therefore no attempt to correlate weather to residue decay could be made. This is a factor, however, in negating any initial loss of herbicide by rain in the uptake study, as no rain fell during the time of that study.

Analytical methodology

A method has been standardized for the quantitative extraction and analysis of glyphosate and its metabolite AMPA from berries. This method involves water extraction, removal of pigments by charcoal treatment followed by column chromatography using cation exchange resin for removal of sugar, and a single-step derivatization reaction.

Extraction and cleanup

A representative berry sample (10 g) was placed in a Waring blender and homogenized with a mixture of water and chloroform (100:50 v/v). The resulting slurry was filtered on a Buchner funnel and the filtrate quantitatively transferred to a 500-mL separatory funnel using several small volumes of water as rinse. After stabilization, the phases were allowed to separate and the chloroform layer was discarded. The aqueous fraction was partitioned with *n*-hexane (50 mL) and ethyl acetate (50 mL), and the organic fractions were discarded. The aqueous fraction was concentrated to ~30 mL and treated with charcoal (0.8 g, Darco

evaporator at 60°C and the pH adjusted to 2.1 wit HCl. This was further concentrated to 4 mL and a to a Dowex 50W-X8 (H^+ form) cation exchange c (2.2 × 6.5 cm) equilibrated in pH-2.1 water (Guinivar 1982). The first fraction (21 mL), which contain sugars, was discarded and the eluent changed to dei water (pH 7.0). The next fraction (225 mL), whic tained the herbicide and metabolite, was collected an centrated in a vacuum rotary evaporator at 60°C fo by evaporation to dryness under nitrogen. The dried sa were stored overnight under vaccum (5 mm Hg) desiccator that contained phosphorous pentoxide.

The contents of the Petri dishes for artificial depos lector analyses were thawed, eluted with water (200 concentrated to 100 mL, and extracted as described e Berry washings taken in the field were concentrat 100 mL and extracted as described earlier.

Derivatization

The derivatization reaction utilized in this proced based on that described by Deyrup et al. (1985). The containing the residue of glyphosate and AMPA fro previous extraction was equipped with a Claisen cond and anhydrous calcium chloride guard tube, and a stream of dry nitrogen was passed through the sy Trifluoroacetic anhydride (TFAA) (4 mL) followe trifluoroethanol (TFE) (2 mL) were added. The mixtu then refluxed for 90 min in an oil bath at 80°C. The reagents were removed by a gentle stream of nitrog 50°C, and the residue was dissolved in ethyl acetat analyzed by gas chromatography. Confirmation of the tity of the volatile derivatives produced by these rea was established by electron impact as well as chemical io tion mass spectroscopy.

Gas chromatographic analysis

The gas chromatographic analysis was conducted Shimadzu GC-9A gas chromatograph equipped with a detector and with a 1.8-m column of Ultra-bond 2 80/100, with nitrogen (50 mL/min) as the carrier Column temperature was 150°C.

Field samples were injected alternately with external dards. Using a Shimadzu C-R3A data processor, re concentrations were quantified by the comparison of

TABLE 4. Uptake of glyphosate in blueberry and raspberry fruit immediately after spray application

	Hours postspray	Wt. (g)	Total μg in the washings		Total μg in washed berries		ppm in the washings (μg/g)		ppm in the washed berries (μg/g)	
			Glyphosphate	AMPA	Glyphosphate	AMPA	Glyphosphate	AMPA	Glyphosphate	AMPA
Blueberry	0	39.8	323.9	ND	ND	ND	8.14	ND	ND	ND
	0	45.0	373.5	ND	ND	ND	8.30	ND	ND	ND
	0	33.4	265.5	ND	ND	ND	7.95	ND	ND	ND
	9	42.3	269.5	ND	54.6	ND	6.37	ND	1.29	ND
	9	51.7	381.6	ND	47.4	ND	7.38	ND	0.92	ND
	9	39.9	278.7	ND	41.5	ND	6.98	ND	1.04	ND
Raspberry	0	37.4	768.9	ND	ND	ND	20.56	ND	ND	ND
	0	32.9	649.5	ND	ND	ND	19.74	ND	ND	ND
	0	43.7	881.4	ND	ND	ND	20.17	ND	ND	ND
	9	40.2	735.3	ND	78.4	ND	18.29	ND	1.95	ND
	9	43.4	760.8	ND	72.5	ND	17.53	ND	1.67	ND
	9	38.7	664.9	ND	72.8	ND	17.18	ND	1.88	ND

ND, not detectable; limits of detection for glyphosphate and AMPA were 0.025 and 0.01 ppm, respectively.

range of the detector was 0.05 to 10 ng for glyphosate and 0.01 to 30 ng for AMPA. Limits of detection for glyphosate and AMPA from this method were 0.025 and 0.01 ppm, respectively, for both blueberry and raspberry.

Analytical method validation

Validation studies were undertaken to determine the recovery efficiency and precision of the analytical technique and to detect a correlation between recovery efficiency and residue concentration. For validation studies, blank berry samples (10 g) collected from the field sites were placed in screw-cap bottles, macerated with a stirring rod, and fortified using a mixed standard of glyphosate and AMPA (70.4 and 40.5 ppm, respectively) at the levels listed in Table 3. Validation test samples were manually shaken and equilibrated for a period of 48 h to ensure adequate adsorption of the chemical to the substrate.

Quality assurance program

A quality assurance program involving two fortified check samples processed concurrently with batches of field samples was employed to check day-to-day variability and recovery efficiency of the method. Quality assurance samples were fortified at various concentrations that approximated expected residue levels in the field samples and were processed and analyzed as for field sample analyses.

Statistical analysis

An analysis of variance was conducted from the data obtained from blueberry and raspberry sites to determine if results were statistically significant. Polynomial regression analysis was also performed on data, and the line of best fit was determined. The mathematical model for these regression analyses are represented by

$$\text{Residue} = a + (b_1 \times \text{days}) + (b_2 \times \text{days}^2)$$

where

Residue = residue value from blueberry and raspberry samples after treatment in mg/kg.

The above regressions were fitted to the data by the least squares method, SAS (SAS Institute Inc. 1985). The 95% confidence limits for the regression lines were also plotted, and the regression equations were used to calculate times for 50% dissipation (DT_{50}).

Results and discussion

Analytical method validation results

The results of the recovery data (Table 3) indicate that the recovery of glyphosate and AMPA from blueberry and raspberry was concentration dependent. This concentration dependence was reproducible over several subsequent trials, but no adequate explanation for this phenomenon could be found. These results negated the use of a universal preanalysis correction factor. Therefore, residue concentrations reported were corrected for analytical efficiency based on mean recovery determined from two quality assurance samples that were analyzed concurrently with batches of five field samples. The recovery efficiencies of glyphosate and AMPA from water were 90.23 and 85.25%, respectively (Roy et al. 1987).

Deposit distribution

Deposit data are presented in Tables 1 and 2. Overall results showed that the estimates of initial glyphosate deposits calculated mathematically from measured sprayer volumes agree closely with empirical residue data as determined by residue analyses conducted on artificial deposit collectors (Petri dishes).

Uptake study

The data from this study (Table 4) indicate that the berries of each species absorb the herbicide at different rates. For blueberry, the figure was 13.63 ± 2.94%, whereas for raspberry only 9.39 ± 0.62% had been taken up 9 h after spray of glyphosate. These uptake data were calculated from the analyses of berry washings and washed berries at 0 and 9 h postspray.

TABLE 5. Residue levels of glyphosate and AMPA in blueberry and red raspberry collected from boreal forests of Ontario

	Days postspray[a]	Glyphosate (mg/kg)		AMPA (mg/kg)	
		Mean ± SD	%CV	Mean ± SD	%CV
Blueberry	0	7.94 ± 0.678	8.54	ND	—
	1	6.60 ± 0.708	10.73	ND	—
	2	5.66 ± 1.210	21.38	0.055 ± 0.017	30.91
	13	3.73 ± 0.535	14.34	0.051 ± 0.009	17.65
	20	2.50 ± 0.524	20.96	0.031 ± 0.010	32.26
	33	1.23 ± 0.248	20.16	ND	—
	61	0.19 ± 0.035	18.13	ND	—
Raspberry	0	19.49 ± 2.110	10.83	ND	—
	1	18.25 ± 2.570	14.08	ND	—
	2	17.12 ± 0.990	5.78	0.102 ± 0.024	23.53
	13	5.55 ± 0.880	15.86	0.089 ± 0.031	34.83
	20	3.39 ± 0.420	12.39	0.033 ± 0.008	24.24
	33	1.22 ± 0.122	10.00	0.024 ± 0.004	16.67
	61	NA	—	NA	—

NOTE: Data corrected for glyphosate and AMPA recovery efficiencies. ND, not detectable; limits of detection for glyphosate and AMPA were 0.025 and 0.01 ppm, respectively. NA, not available; no berries on the plantation.
[a]Zero days postspray was August 8, 1985.

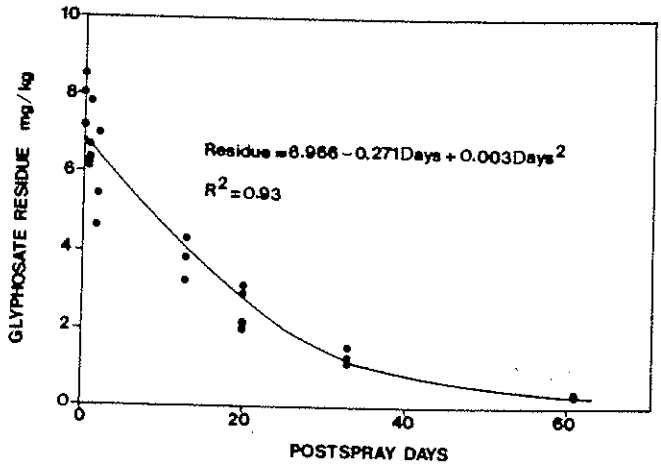

FIG. 1. Dissipation in glyphosate residue from blueberry site.

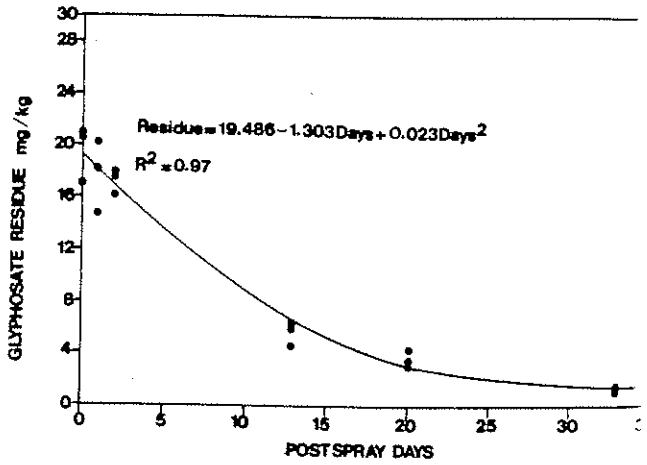

FIG. 2. Dissipation in glyphosate residue from raspberry si

Actual numerical values for mean glyphosate and AMPA concentrations in the two fruit types at specific sampling times are presented in Table 5. Initial mean residue values in blueberry (7.94 ppm) were lower than those for raspberry (19.49 ppm). It is important to note that application equipment and spray parameters as used in this study are different from those of typical operational chemical applications for glyphosate, and thus the initial residue data should not be considered as residue levels that would be expected from operational spraying of glyphosate. However, the data do clearly indicate declining residue levels of glyphosate with time (Figs. 1 and 2). Transient increases in AMPA levels suggest that microbial and (or) metabolic degradation actually takes place in or on the fruit (Table 5). Curvilinear regression analyses for glyphosate residues with time were characterized by excellent R^2 values (0.92 and 0.96, respectively) and highly significant F values for slopes ($P > F = 0.0001$). From the regression analyses, DT_{50} values were estimated as <20 days (95% confidence limit 6–14 days) for blueberry and raspberry fruit, respectiv Under the conditions of this study, residues remained ab detectable levels and above the maximum permissible resi level (0.01 ppm) as established by the Health and Welf Canada, Food and Drug Regulation (1980).

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Fate of Glyphosate in a Canadian Forest Watershed. 1. Aquatic Residues and Off-Target Deposit Assessment

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Glyphosate and AMPA residues in oversprayed and buffered streams were monitored following application of ROUNDUP (2.0 kg/ha) to 45 ha of a coastal British Columbia watershed. Maximum glyphosate residues (stream water, 162 μg/L; sediments, 6.80 μg/g dry mass; suspended sediments, <0.03 μg/L) were observed in two intentionally oversprayed tributaries, dissipating to <1 μg/L within 96 h postapplication. Buffered streams were characterized by very low glyphosate residue levels (2.4–3.2 μg/L in streamwater). Results of the off-target deposit assessment indicated <0.1% of applied glyphosate at 8 m from the spray boundary. Increases in residue levels were observed in relation to the first storm event postapplication. Ratios of maximum stream water concentrations of glyphosate observed in buffered and oversprayed tributaries relative to literature toxicity values indicated a substantial margin of safety under either operational or worst case scenarios.

Glyphosate [*N*-(phosphonomethyl)glycine], marketed under the trade names ROUNDUP or VISION (Monsanto Corp., St. Louis, MO), has been registered for use in site preparation and conifer release programs in Canada since 1984 (Malik and Vanden Born, 1986). The behavior of glyphosate in aquatic systems has been investigated in the United States and elsewhere (Comes et al., 1976; Rueppel et al., 1977; Edwards et al., 1980; Ghassemi et al., 1981; Norris et al., 1983; Newton et al., 1984; Wan, 1988). However, only the latter two reports are pertinent to the environmental fate of glyphosate in a coastal watershed ecosystem. In the province of British Columbia, Canada, coastal watersheds are used extensively for timber production. In such areas, the steep-sloped terrain, high annual rainfall (>2000 mm), and proximity of treatment areas to salmon spawning streams combine to approximate a worst case scenario with respect to potential for aquatic impacts following silvicultural chemical applications. As a result, current regulatory guidelines in British Columbia require the establishment of a 10-m pesticide-free zone with appropriate buffers, usually 100 m, to protect aquatic ecosystems. These guidelines restrict silvicultural treatment of the highly productive forest lands that border rivers and streams in coastal regions. In 1984, as a component of the continuing investigations on the effects of forestry practices on native fish populations in the Carnation Creek watershed (Hartman, 1982), a study was initiated to investigate the environmental fate of glyphosate in major aquatic and terrestrial compartments of the ecosystem, following a silvicultural application of ROUNDUP herbicide. Components of the environmental fate research program relating to aquatic residues and off-target deposit assessment are described in this, the first of a two-part series. The specific objectives of this portion of the environmental fate research were (1) to monitor residue levels and dissipation rates of glyphosate and AMPA in water, stream-bottom sediment, and suspended sediment of both buffered and oversprayed streams and (2) to assess the off-target deposit of glyphosate following an aerial spray application and evaluate the effectiveness of a 10-m vegetation zone in protecting forest streams from contamination resulting from off-target deposit.

MATERIALS AND METHODS

Site Description. The study site was located in the Carnation Creek watershed on the west coast of Vancouver Island, British Columbia (45°50′ N, 125°2′ W), approximately 200 km northwest of Victoria (Figure 1). The 10-km² watershed is within a coastal hemlock and cedar ecozone (Krajina, 1969) and is characterized by annual precipitation ranging from 2500 to 3800 mm, occurring mainly from October through March (Hetherington, 1982).

Following clear-cutting in 1975, the study area was site-prepared and planted in 1976 (Dryburg, 1982). Seedlings were soon dominated by salmonberry (*Rubus spectabilis* Pursh) and red alder (*Alnus rubra* Bong.) (King and Oswald, 1982). In September 1984, salmonberry and alder ranged in height from 1.5 to 2.5 m and 7 to 10 m, respectively.

The main stream of Carnation Creek meanders through an alluvial floodplain and is fed by a number of permanent and ephemeral tributaries. The main stream and side channels support populations of coho (*Oncorhynchus kisutch* Walbum) and chum (*Oncorhynchus keta* Walbum) salmon. The four tributaries involved in this study have confluences with the main stream channel at 750, 1450, 1600 and 2200 m (C-Creek) upstream from the Carnation Creek estuary. The locations of spray blocks relative to the streams and main creek channel are shown in Figure 1. Tributaries 750, 1450, and C are ephemeral and in 1984 started flowing with the first seasonal rainfall (35 mm), which occurred on September 4.

Tributary 750, a small, ephemeral stream, occluded by riparian vegetation for most of its length was intentionally oversprayed in this study. At the time of application, it was slow flowing (0.001 m³/s); however, owing to its steep drainage pattern, flow rates increased markedly during storm events. Tributary 1600 received direct chemical application for about 600 m of its 800-m length. At the time of application the stream was fast flowing (0.02 m³/s) and contained pools ranging from 0.5 to 1 m in depth. Stream banks were covered with salmonberry and alder vegetation, but occlusion of the stream channel was less than that for tributary 750.

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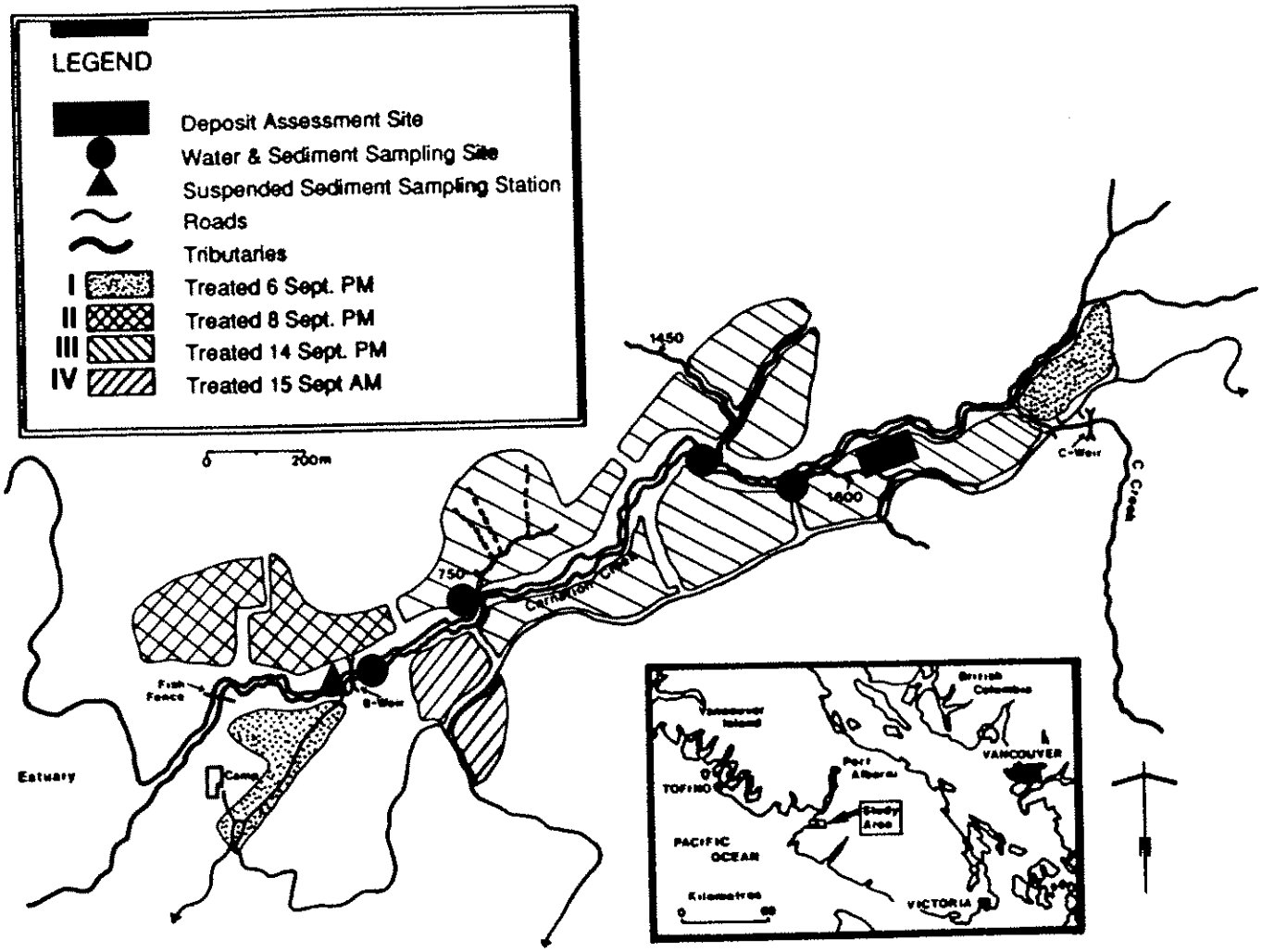

Figure 1. Location of the Carnation Creek watershed study area, aquatic and off-target deposit sampling sites.

Tributary 1450 was selected to represent a worst case situation for operational spraying. This tributary consisted of a network of branching stream channels, midportions of which either flowed underground or were covered with dense 2-m-high salmonberry. The stream boundary of these sections could not be clearly identified from the air. About two-thirds of its length (600–800 m) was adjacent to treatment area III (Figure 1). Stream flow at the time of application was 0.01 m^3/s.

A weather station and a broadcast weir (B-weir) were established near the mouth of Carnation Creek to monitor precipitation and stream discharge throughout the study period.

Herbicide Application. ROUNDUP (isopropylamine salt of glyphosate) was applied in September 1984 at 2 kg of acid equivalent (ae)/ha utilizing a Bell-47 helicopter equipped with a MICROFOIL (Union Carbide Inc., Ambler, PA) boom and 1.5-mm hayrake nozzles, calibrated to deliver 258 L/ha at an airspeed of 40 km/h and flying at a height of 6–18 m above the canopy (Reynolds et al., 1989). The main channel of Carnation Creek, as well as tributaries 1450 and C, were buffered with a 10-m untreated vegetation zone. Tributaries 750 and 1600 were intentionally oversprayed. A total of 45 ha was sprayed on four different days (Figure 1); the specific times, dates, and conditions associated with each application are presented in Table I.

Aquatic Residue Sampling. Sampling stations for the three tributaries were located about 5 m from the confluence with the main channel of Carnation Creek. The sampling station for Carnation Creek was located at B-Weir, 500 m upstream from the estuary and immediately below the major treatment area (Figure 1). At each station, intensive sampling of the stream water was conducted for the first 96 h postapplication. Intensive sampling was achieved by taking integrated stream water samples (IS) (six samples of 150 mL at 10-min intervals to yield a final sample (900 mL) integrating streamwater residues over the 1-h period of collection) during the initial period and single grab samples of 900 mL thereafter; the frequency of intensive streamwater sample collection is provided in Table II.

Table I. Meteorological Conditions for Chemical Applications at Carnation Creek Watershed (Modified from Reynolds et al. (1989))

block[a]	date[b]	time[c]	area[d]	temp[e]	WS[f]	skies[g]	rain[h]
I	6	1900–2005	5.6	15	<7	OC, I	nil
II	8	1416–1940	8.5		7–10	OC	nil
III	14	1430–1931	24.2	21	5–11	OC, I	nil
IV	15	1041–1101	3.4	14	<5	S	1345

[a] Treatment block numbers (refer to Figure 1). [b] Date of application for Sept 1984. [c] Time of application, 2400-h clock (beginend). [d] Total area (ha) of spray block treated. [e] Air temperature (°C). [f] Wind speed (km/h). Wind direction was east to west for all applications. [g] General cloud cover during chemical application: OC = overcast; I = intermittent sun; S = sunny. [h] Time of initiation of rainfall subsequent to application.

Storm event sampling was initiated when the instantaneous discharge of Carnation Creek at B-weir reached a threshold level of 7 m^3/s. Poststorm sampling commenced when the discharge decreased to below the same threshold. The frequency of sample collection and correlation with storm events is indicated in Table II. In conjunction with major storm events, water samples were collected at B-weir and at tributaries 750, 1450, and 1600. Suspended sediment samples (20 L of water filtered through Whatman No. 114 filters contained in a 15-cm Buchner funnel) collected during each storm event were taken at B-weir only.

Long-term water and bottom sediment point samples were collected on a biweekly schedule at B-weir and from tributaries 750, 1600, and C; frequency of long-term sampling is indicated in Table II. Long-term water samples were obtained as

Table II. Sampling Schedules for the Carnation Creek Watershed Study

sampling site	schedule[a]	time postapplication
Intensive Stream Water Sampling (Time, h)		
Carnation Creek	IS	0 1 2 3 4 5 6 7 8
	PS	11 14 20 29 30 31 32 33 48 72 96
tributary 750	IS	0 1 2 3
	PS	6 9 15 27 36 48 72 96
tributary 1450	IS	0 1 2 3 4
	PS	7 10 16 22 48 72 96
tributary 1600	IS	0 1 2 3 4 5 6 7
	PS	10 166 28 30 32 34 48 72 96
Storm Event Sampling for All Substrates (Time, Days)		
major storm events[b]		23 25 49 59 66 84 91 150
all sites		23 24 25 27 30 33 40 47 49 51 53 57 59 60 62 66 67 69 73 80 84 85 87 150 151 153 157 164 171
Long-Term Stream Water and Bottom Sediment Sampling (Time, Days)		
all sites		196 210 224 238 252 263 280 297 311 326 750 339 355 364

[a] Key: IS = integrated sample, 6 × 150 mL taken at 10-min intervals; PS = point sample (1 × 900 mL). See Figure 1 for exact location of sampling sites. [b] Major storm events designated as those resulting in a stream discharge of 7.0 m^3/s or greater at B-weir of Carnation Creek.

indicated for PS samples in the intensive monitoring period. Bottom sediments were collected using a wide-mouth plastic bottle (250 mL) to scoop samples from areas containing fine sediments within the sample area. Large organic debris were excluded from the sample, and excess water was decanted from the sample prior to closure. Samples of all matrices were cooled immediately following collection and frozen within several hours of collection. Samples were maintained in a frozen state during transport and subsequent storage until extracted and analyzed.

Assessment of Off-Target Deposit. The off-target deposit study area (100 × 20 m) bordered the southern bank of Carnation Creek and was located approximately 2000 m upstream of the estuary (Figure 1). The area included a vegetation buffer strip 10 m wide by 100 m long, divided roughly in half relative to the two dominant vegetation types (salmonberry and red alder). Within each vegetation zone, three transects 20 m long and oriented at right angles to the stream bank were established at 20-m intervals. Off-target deposit (the amount of chemical impinging on surfaces outside the intended zone of application) was assessed with deposit collection plates placed at 5-m intervals along each transect (Figure 2). In open areas, deposit collection plates (400 cm^2) constructed of aluminum foil sheets on corrugated cardboard were placed 20 cm above either ground or water level. In areas with vegetation, collectors were set at levels roughly corresponding to the height of salmonberry canopy (170 cm). The actual swath edge (Figure 2), defined as the line demarcating the zone of 100% injury to salmonberry, was determined by assessing phytotoxicity 10 months postapplication. The distances of the deposit collectors to the actual swath edge were remeasured and used for subsequent interpolation of glyphosate deposit at various distances from the target area.

Residue Analysis. Samples of ROUNDUP formulation (nominally 356 g of AI/L) were collected prior to mixing for application and kept at ambient temperature. Quadruplicate 1-mL aliquots were serially diluted (105×) with KH_2PO_4 buffer solution (mobile phase). The diluents were filtered with Millipore filter units (Millex HV, 0.45 μm) and subjected to HPLC analysis for glyphosate and AMPA. Tank-mix samples (400 mL) collected immediately prior to application on each day of treatment were stored frozen until analysis. Duplicate 1-mL aliquots were diluted (103×) in buffer solution, filtered, and quantified by an HPLC–vis technique as described by Thompson et al. (1989).

Residues of glyphosate and AMPA were extracted from the deposit collector sheets with use of an intensive rinsing/shaking and sonicating procedure with 0.1 N HCl as the extraction solvent. The extracts were then subjected to cation- and anion-exchange column cleanup (Monsanto, 1985a) prior to quantification by HPLC analysis. The limits of detection (LOD) observed for deposit collectors equated to 2.5×10^{-6} kg/ha AI. Limits of quantification (LOQ) were established as 1.25×10^{-4} kg/ha AI.

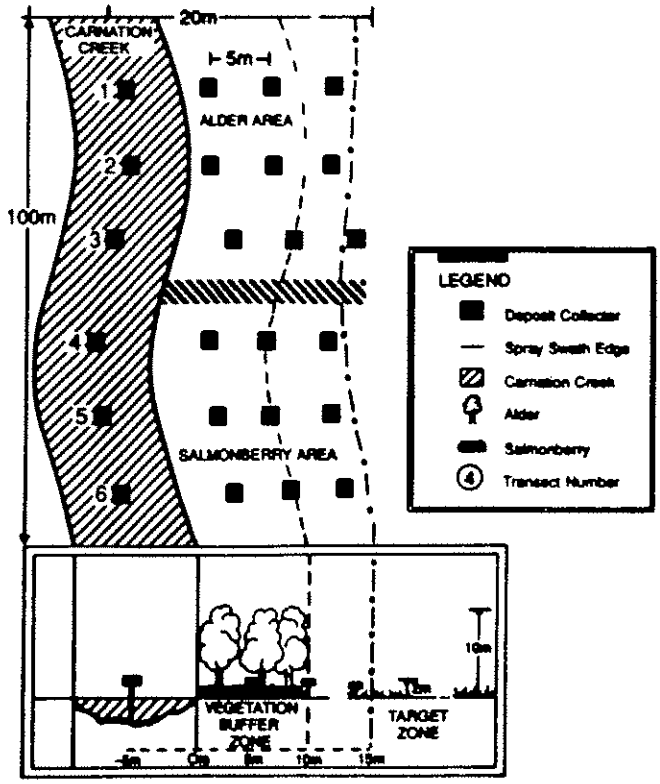

Figure 2. Experimental layout for the off-target deposit assessment.

Frozen water samples were thawed, acidified with HCl to pH 2, and filtered through a Millipore filtering apparatus (0.45-μm HA disk filters), prior to cation- and anion-exchange column cleanup. For water samples, the extraction and cleanup procedures of Cowell et al. (1986) were used with slight modification to provide samples for HPLC analysis. The procedure was altered by elution of the cation-exchange column with 6.5 N HCl and recovery of the final sample in mobile-phase solution. A preliminary validation for determination of glyphosate and AMPA from water indicated quantitative recovery with good precision (Table III). On the basis of these results, residues in water samples were not corrected for losses in the analytical method.

Samples of bottom sediments were air-dried, and aliquots (20-g air-dry mass) were extracted, cleaned up, and analyzed, with residue levels reported as micrograms per gram of dry mass. Suspended sediment samples including filter papers were weighed, homogenized, and extracted, with residue results calculated as micrograms per liter, based on 20 L of filtered water. For both bottom and suspended sediment samples, extraction, cleanup, and analyses were conducted following the method of Thompson et al. (1989).

Throughout the course of analytical determinations for bottom and suspended sediment samples, a quality control (QC) program was conducted. Blank field samples fortified with varying levels of glyphosate and AMPA were processed and analyzed daily in conjunction with field samples. Results of the QC program are presented as an indication of the accuracy and precision of the method (Table III). The QC data were used to correct the field sample data for recovery efficiency of the analytical method. For the purposes of this study, limits of detection were defined as concentrations yielding a signal at the retention time of interest equivalent to twice the value of the noise generated by a field blank (2 × S:N). Limits of quantification were established based upon the lowest concentrations in fortified quality control samples with coefficients of variation <15% (Table III).

Statistical Analysis. Residue data from stream water, bot-

Table III. Quality Control Data for Analytical Methods

substrate type (amt)	spike level,[a] μg	N[b]	analyte	mean ± SE (CV, %)	LOD, ppb	LOQ, ppb
water (900 mL)	1.0–40.0	25	GLYPH	98.4 ± 7.6 (7.8)	0.1	1.0
	0.25–10.0		AMPA	86.4 ± 6.3 (7.3)	0.025	0.25
bottom sediment (20 g)	1.0–16.0	36	GLYPH	79.7 ± 6.3 (7.9)	30	50
	0.25–4.0		AMPA	64.0 ± 10.1 (15.4)	10	12.5
suspended sediment (20 L of water)	0.50–4.0	16	GLYPH	65.5 ± 9.1 (13.9)	0.003	0.03
	0.13–1.0		AMPA	54.3 ± 8.5 (15.7)	0.001	0.01

[a] Spike level = mass of glyphosate added per quantity of substrate. [b] N = total number of samples analyzed.

tom sediment, and suspended sediment analyses were plotted on arithmetic scales, and the time required for residues to dissipate below limits of detection was interpolated directly from the graphs.

Total residues (glyphosate plus AMPA as glyphosate equivalent) on the off-target deposit plates were converted to kilograms of AI per hectare rates. Log-transformed off-target deposit residue data were subjected to linear regression analysis with the equation $\log Y = o + b(\log X)$, where X = distance off-target (m) and Y = rate (kg/ha) of glyphosate deposited. Statistical differences between regression lines were determined by means of t-tests, comparing slopes (b) and elevations (o) of the lines as described by Zar (1984). Regression equations were used to interpolate distances at which deposit estimates were equal to 10, 1, and 0.1% of full deposit in the target area.

RESULTS AND DISCUSSION

Formulation and Tank-Mix Analyses. Analyses of the ROUNDUP formulation used in this study indicated an active ingredient (glyphosate) content of 363 g/L ± 2% CV. This value is approximately 2% in excess of label concentration (356 g of ae/L). No AMPA was detected in the formulation, which had been stored at ambient temperature (20 °C) for 3 months.

Analyses of tank mix samples showed concentrations of 7889 and 58 μg/L of glyphosate and AMPA, respectively, in the tank mix used for application to part of treatment area III. AMPA was found in only one of four tank-mix samples and not in the formulation samples. Detection of AMPA in this tank mix was attributed to a 6% (v/v) contamination of the tank mix with a mixture used 6 days earlier to spray areas adjacent to the Carnation Creek watershed.

Initial Stream Water Residues in Oversprayed Tributaries. Initial residues of glyphosate in the two directly oversprayed tributaries (750 and 1600) are presented in Figure 3. The maximum stream water residue (162 μg/L) was observed in tributary 1600, 2 h postapplication. In contrast, initial residues in tributary 750 were very low (<1.5 μg/L). The differences in initial residue response in the two oversprayed tributaries were attributed to differences in the degree of occlusion by riparian vegetation and general flow characteristics, as described previously.

In both oversprayed tributaries, glyphosate concentrations rose dramatically (100-fold) in response to the first rainfall event 27 h postapplication and then decreased rapidly, falling below detectable levels (LOD = 0.1 μg/L) within 96 h postapplication. The magnitude of the initial concentrations and rate of residue dissipation in tributary 1600 were comparable to those observed by Newton et al. (1984), who found maximum stream water concentrations of 270 μg/L following an aerial application of glyphosate at a rate of 3.3 kg/ha. Wan (1988) recently reported much lower initial levels (23 μg/L) in a stream unprotected by buffer zones. The increase in glyphosate stream water residues following the first rainfall event is consistent with previous research (Edwards et al., 1980; Newton et al., 1984; Wan, 1988) and may result from several sources of input including mobilization of residues

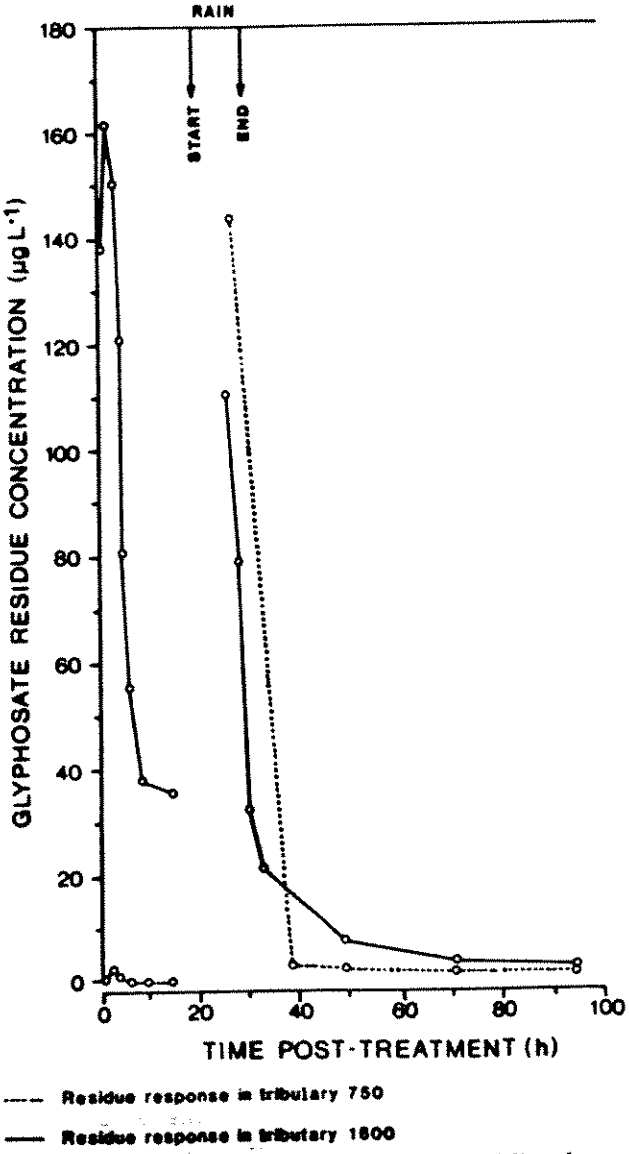

Figure 3. Glyphosate residues in stream water of directly oversprayed tributaries 750 and 1600 of the Carnation Creek watershed.

in ephemeral stream channels feeding the tributary, washoff of unabsorbed residues from overhanging vegetation, surface runoff, and subsurface flow. Concentrations of AMPA found in both tributaries 750 and 1600 were approximately 2% of concurrent glyphosate concentrations.

Initial Stream Water Residues in Buffered Tributaries. No quantifiable residues (LOQ = 1.0 μg/L) of glyphosate were found in tributaries buffered by a 10-m vegetation zone (C-Creek and 1450), with the exception of three samples from tributary 1450, which contained extremely low but quantifiable glyphosate residues. The maximum residue observed in this case was 2.47 μg/L at 10 h postapplication; the residue response observed in this tributary was attributed to unintentional overspray of the stream channel.

Stream water samples from Carnation Creek were

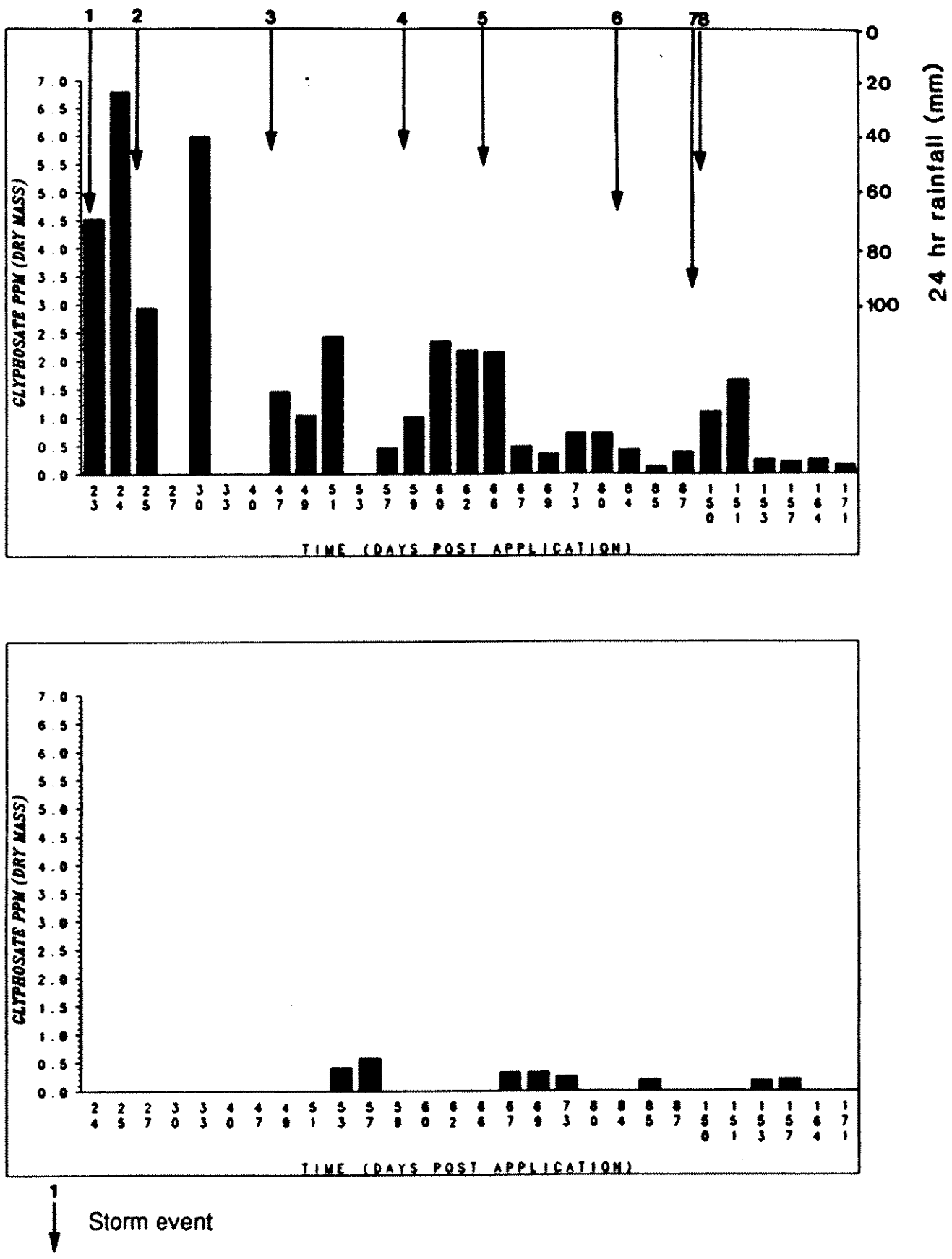

Figure 4. Glyphosate residues in bottom sediments of directly oversprayed tributaries 750 and 1600 of the Carnation Creek watershed.

obtained at B-weir and thus reflected residue inputs from the entire watershed. Initial samples were taken in association with all chemical applications (treatment areas I–IV, Figure 1). Only samples collected following application to treatment area III showed quantifiable glyphosate concentrations, with residue levels <1.5 μg/L. An increase in stream water residues (maximum 3.2 μg/L) in Carnation Creek subsequent to the first rainfall event was attributed primarily to mobilization of residues associated with oversprayed tributaries 750 and 1600 as discussed previously.

In general, these results corroborate other research indi-

cating rapid dissipation of glyphosate in both lentic surface waters (Legris et al., 1985) and lotic systems (Comes et al., 1976; Norris et al., 1983; Legris et al., 1985) as the result of degradation, dilution, adsorption to organic substrates, or uptake by biota.

These results are also consistent with the observations of other workers (Kimmins, 1975; Newton et al., 1984; Wan, 1988; Edwards et al., 1980), who have previously reported that the first rainfall after treatment generates the highest residue concentrations in stream water and runoff water.

Storm Event and Long-Term Stream Water Residues. During the study, seven rainfall events resulting in mainstream flows above 3 m^3/s were identified as major storms (Table II). Stream flows observed in Carnation Creek for these storm events were 13.1, 8.3, 10.0, 5.3, 3.0, 4.0, and 4.4 m^3/s, with cumulative rainfall for the period being 1490 mm. A total of 120 water samples were collected from the main channel and three tributaries (750, 1600, and C). Although trace levels of glyphosate (<1 $\mu g/L$) were occasionally detected in the main channel and two oversprayed tributaries, no quantifiable residues (<1 $\mu g/L$) of glyphosate or AMPA were found in any stream water samples associated with storm events. Similarly, biweekly samples taken from the main stream and tributaries 750, 1600, and C during the long-term monitoring period (196–364 days) after treatment contained no detectable residues (LOD = 0.1 $\mu g/L$).

Residues in Bottom and Suspended Sediments. A total of 120 samples of stream bottom sediments were collected in conjunction with storm events. No quantifiable amounts of glyphosate or AMPA were found in buffered tributary 1450 or the main channel during the storm event monitoring period.

The highest residue levels observed in the aquatic compartments of the ecosystem were associated with bottom sediments of the oversprayed tributaries (1600 and 750), indicating that bottom sediments were the major sink for glyphosate residues. Peak concentrations of glyphosate in bottom sediments (6.80 and 6.34 $\mu g/g$ dry mass) occurred in tributary 1600. Concentrations of glyphosate in bottom sediments of tributary 750 were substantially lower (0.44 and 0.58 $\mu g/g$ dry mass). In both cases, peak glyphosate concentrations in bottom sediments appeared following major storm events (Figure 4). In both tributaries, glyphosate and AMPA residues in bottom sediments persisted throughout the storm event monitoring period.

We suggest that differences observed in glyphosate residues associated with bottom sediments of the two oversprayed tributaries may be interpreted on the basis of stream flow dynamics. In meandering streams with slow flow rates, as is the case for tributary 1600, deposition of fine-textured organic sediments is common. In contrast, fast-flowing streams such as tributary 750 inherently have little sediment deposition and are regularly flushed out during storm events; bottom sediments that accumulate are typically coarser in texture, resulting in lower surface area and sorptive capacity for chemical residues.

Analyses of 49 bottom sediment samples taken between 196 and 364 days showed no quantifiable residues in sediments of the Carnation Creek main channel or tributary 1450. However, quantifiable residues (0.14–1.92 $\mu g/g$ dry mass) were observed in bottom sediments of directly sprayed tributaries 750 and 1600. Glyphosate residues in bottom sediments were persistent in comparison to stream water residues but declined over time so that glyphosate concentrations were less than 0.2 $\mu g/g$ dry mass by the end of the long-term monitoring period.

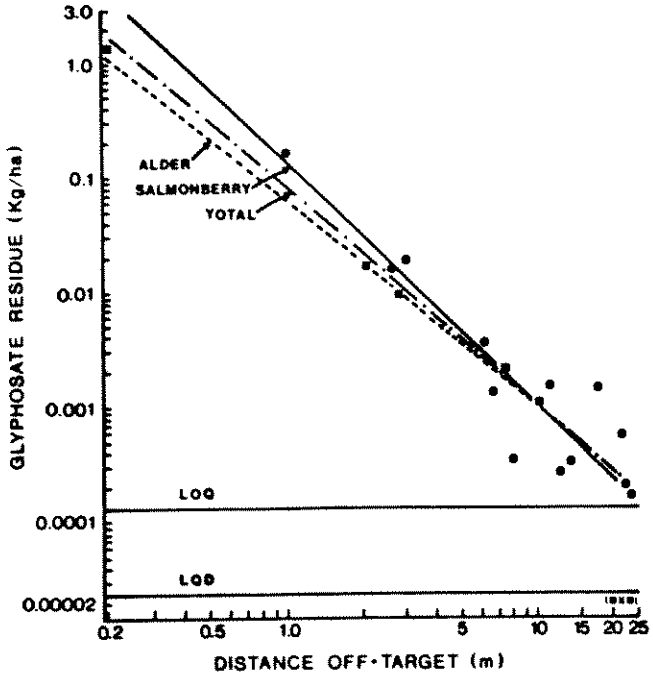

Figure 5. Off-target deposit of glyphosate at the Carnation Creek watershed.

A total of 29 suspended sediment samples were collected at B-weir during the seven storm events that occurred during the study. Quantifiable glyphosate residues were found in only four of the 29 samples collected. The highest concentrations detected in suspended sediments (0.060 $\mu g/L$) were observed 23 days postapplication, following the first major storm event.

Off-Target Deposit Assessment. The highest deposit rate observed (1.882 kg/ha) was found 2.9 m within the target area and equated to 6% less than the nominal application rate for the study. The lowest quantifiable deposits off-target (0.001 55 and 0.000 176 kg/ha) were found 17.2 and 23.1 m off-target for the salmonberry and alder areas, respectively. Statistical analysis of the deposit data indicated a significant ($P < 0.05$) linear regression of log-transformed data with coefficients of determination (r^2) values of 0.89 and 0.94 for the alder and salmonberry areas with slopes of −1.82 and −2.13, respectively. Based on a lack of significant differences between slopes ($t = 1.09$, df = 15) and elevations ($t = -0.178$, df = 16) for the two regression lines, data were pooled to allow calculation of a general extinction rate (Figure 5). The regression of pooled data was characterized by an r^2 value of 0.91 and a slope of −1.05. On the basis of regression of pooled data and a calculated extinction rate equivalent to the slope of this line (−1.05 kg ha^{-1} m^{-1}), the distances from the spray boundary at which 10%, 1%, and 0.1% of the full deposit were interpolated as 0.7, 2.2, and 7.4 m, respectively (Figure 5). The regression equations derived from off-target deposit measurement data indicate that vegetation buffer zones of 10 m should effectively eliminate chemical drift into streams under the specific application, meteorological, and physical conditions of the Carnation Creek site. This hypothesis was borne out by the general lack of glyphosate residues in stream water of buffered tributaries C-Creek and 1450.

Relationship of Environmental Residues to Aquatic Toxicity Data. A relatively large database currently exists with respect to the toxicity of ROUNDUP and/or glyphosate to aquatic biota, the investigation conducted by

Table IV. Toxicity and Calculated Relative Safety Factor Values for ROUNDUP and Glyphosate in Freshwater Aquatic Organisms

organism[a]	LC_{50},[a] mg/L	RSF[b] 1450	RSF[b] CC	reference
	ROUNDUP			
D. magna	25.5	3188	2383	Servizi et al., 1987
O. nerka	26.7	3338	2496	Servizi et al., 1987
P. promelas	2.4 (a)	300	224	Folmar et al., 1979
G. pseudolimnaeus	43	5375	4019	Folmar et al., 1979
L. macrochirus	6.4 (a)	800	598	Folmar et al., 1979
S. gairdneri	8.3 (a)	1038	776	Folmar et al., 1979
S. gairdneri (eggs)	46.0 (a)	5750	4299	Folmar et al., 1979
S. gairdneri (sac-fry)	11.0 (a)	1375	1028	Folmar et al., 1979
S. gairdneri (swim-up fry)	2.4 (a)	300	224	Folmar et al., 1979
S. gairdneri (fingerling)	1.3	163	122	Folmar et al., 1979
S. gairdneri (fingerling)	2.2 (a)	275	206	Folmar et al., 1979
S. gairdneri	32.4	4050	3028	Wan et al., 1988
S. gairdneri	54.8	6850	5121	Hilderbrand et al., 1982
O. kisutch	42.3	5288	3953	Wan et al., 1988
	Glyphosate			
L. macrochirus	24	10000	7500	USDA, 1981
L. macrochirus	150 (a)	62500	46875	Folmar et al., 1979
S. gairdneri	140 (a)	58333	43750	Folmar et al., 1979
S. gairdneri	86	35833	26875	Monsanto, 1985c
P. promelas	97 (a)	40417	30313	Folmar et al., 1979
I. punctatus	130	54166	40625	Folmar et al., 1979

[a] LC_{50} values are for 96-h exposure periods except those values denoted by (a) for which exposure periods are 24 h. [b] Relative safety factor calculated as the ratio of reported toxicity value to the maximum concentration observed in tributary 1450 (0.0024 mg/L glyphosate; ROUNDUP equivalence, 0.008 mg/L) and the main channel of Carnation Creek (CC) (0.0032 mg/L; ROUNDUP equivalence, 0.0107 mg/L). ROUNDUP equivalence calculated based on guarantee of 356 g of acid/L of ROUNDUP formulation with a specific gravity of 1.17 (Monsanto, 1985a). [c] Genus names: *D* = *Daphnia*, *O* = *Oncorhyncus*, *G* = *Gammarus*, *L* = *Lepomis*, *S* = *Salmo*, *P* = *Pimephales*, *I* = *Ictalurus*.

Folmer et al. (1979) being the most comprehensive (Table IV). These data indicate that ROUNDUP is substantially more toxic than technical glyphosate and that toxicity was greatest in sac-fry and early swim-up life stages. Duration of exposure (24–96 h) generally resulted in only marginal effects on LC_{50} values.

In Canada, the use of pesticides in forest management requires establishment of buffer zones (ranging from 60 to 100 m) to reduce or eliminate pesticide input into aquatic systems. Risk assessments for aquatic organisms are based on the relationship between the expected environmental concentration (EEC) calculated from a worst case scenario of direct application of the pesticide at the maximum rate of application to a body of water 0.5 m deep. The EEC is then compared to data derived from standard laboratory toxicity protocols (i.e., 96-h LC_{50} values), in order to determine the margin of safety. We contend that this approach may vastly overestimate the true risk under natural stream conditions, because organisms in lotic systems are typically not exposed to continuous static concentrations for periods of 96 h and because buffer zones required around aquatic habitats effectively eliminate inputs of glyphosate to stream systems.

Our experimental research results, as well as monitoring of operational spray programs (Wan et al., 1988; Eremko, 1986; Gluns, 1989), consistently indicate that establishment of appropriate buffers effectively eliminates stream contamination resulting from off-target deposits of glyphosate. Infrequently, very low residues have been observed in protected streams, and these are generally associated with storm events occurring soon after chemical application. In an effort to estimate realistic margins of safety for organisms in buffered streams, relative safety factors (RSF) have been calculated as the ratio of toxicity end points presented in Table IV to maximum residues observed in buffered streams of the Carnation Creek watershed study. As such, the RSF values may still substantially overestimate risk owing to differences in mode and duration of exposure as discussed previously. The maximum stream water concentration of glyphosate in buffered tributary 1450 was 0.024 mg/L, while that of the main channel of Carnation Creek was 0.0032 mg/L. On the basis of glyphosate acid content of 365 g of acid/L in ROUNDUP formulation with a specific gravity of 1.17 (Monsanto, 1985a), the ROUNDUP equivalences for these maximum observed concentrations are 0.008 and 0.0107 mg/L, respectively. The lowest RSF values calculated in this manner were 122 for ROUNDUP and 7500 for glyphosate. This analysis suggests that residue levels, as typically observed under actual operational conditions, pose essentially no risk to aquatic organisms in terms of acute toxicity.

Stream water residues observed in oversprayed tributaries in this study exhibit a dual-pulse pattern of exposure that may be considered typical for glyphosate/ROUNDUP. The pulse exposures result from an initial direct input due to overspray and a subsequent input associated with mobilization of terrestrial residues with the first storm event following application. The data clearly show (Figure 3) that exposures to peak concentrations of glyphosate or ROUNDUP would be in the order of a few hours; other researchers have observed similar patterns in lotic systems (Wan et al., 1988; Newton et al., 1984). Realistic estimates of risk to aquatic organisms exposed in this manner are difficult, owing to the general lack of toxicity testing conducted with pulse modes of exposure. Folmer and co-workers (1979) conducted tests designed to simulate actual field exposures in which sac-fry of rainbow trout was exposed to ROUNDUP for 6 h. Results of these tests indicated that statistically significant reduction in survival occurred at concentrations of 5 mg/L. On the basis of this value and the maximum concentration of glyphosate observed in the two oversprayed tributaries of the Carnation Creek study (tributary 1600 peak concentration of glyphosate, 0.162 mg/L; ROUNDUP equivalence, 0.540 mg/L), the calculated RSF values for a worst case scenario of direct overspray would be 31 and 9.26 for glyphosate and ROUNDUP, respectively. This assessment would suggest that even under worst case conditions of direct overspray residues of ROUNDUP or glyphosate would not be sufficient to elicit a significant toxic response in aquatic organisms. The highest concentrations observed in oversprayed tributaries were also well below sublethal, no-effect levels (2.78 mg/L) for coho salmon smolt osmoregulation or growth as reported by Mitchell et al. (1987).

Maximum glyphosate residues in bottom sediments were 6.80 μg/g dry mass, and these residues were relatively persistent compared to stream water residues. However, glyphosate residues are known to sorb strongly to organic matter (K_{oc} = 30 000), and its sorption to natural organic substrates is well documented (Sprankle et al., 1975; Wan, 1988). Thus, we suggest that glyphosate residues in bottom sediments are unlikely to be biologically available. A paucity of information on the toxicity of bound sediment residues of glyphosate precludes assessment of potential impacts relating to residues in this compartment of the aquatic ecosystem.

CONCLUSIONS

Glyphosate residues in the aquatic compartments of the watershed were primarily associated with bottom sediments and were more persistent and greater in magnitude compared to stream water residues. These results suggest that the bottom sediments act as a primary sink for glyphosate residues, however, based on the documented tendency of glyphosate to sorb strongly to soils, suggest that such residues are tightly bound and not biologically available. Suspended sediments did not represent a major mechanism for export of aquatic residues from the treated watershed. The stream water residue data confirm the results of the off-target deposit assessment and indicate that, under the conditions of this study, 10-m vegetation buffer zones effectively eliminated direct chemical inputs into protected streams. The low magnitude and transient nature of glyphosate/ROUNDUP residues observed in stream water result in margins of safety ranging from 10 for directly oversprayed streams to >100 for buffered streams.

In summary, the results of the aquatic environmental fate research conducted at Carnation Creek are consistent with previous studies and suggest that, even under worst case conditions of direct overspray, chemical concentrations would be insufficient to result in a significant toxic impact to aquatic organisms. The results support the continued use of glyphosate as environmentally acceptable chemical herbicide for use in forest renewal programs.

ACKNOWLEDGMENT

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Fate of Glyphosate in a Canadian Forest Watershed. 2. Persistence in Foliage and Soils

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Residues of glyphosate [*N*-(phosphonomethyl)glycine] and the metabolite (aminomethyl)phosphonic acid (AMPA) were monitored in foliage, leaf litter, and soils following aerial application of ROUNDUP herbicide (nominal rate 2.0 kg/ha AI) to the Carnation Creek watershed of Vancouver Island, British Columbia. Glyphosate deposit was variable, ranging from 1.85 to 2.52 kg/ha AI, depending upon location within the watershed. Foliar residues in red alder and salmonberry were 261.0 and 447.6 μg/g, respectively, indicating good impingement on the target foliage. Leaf litter residues, which averaged 12.5 μg/g for red alder and 19.2 μg/g for salmonberry initially, declined to less than 1 μg/g within 45 days postapplication (DT_{50} < 14 days). In soils, glyphosate and AMPA residues were retained primarily in the upper organic layers of the profile, with >90% of total glyphosate residue in the 0–15-cm layer. Distribution data for both glyphosate and AMPA suggested strong adsorption and a low propensity for leaching. Glyphosate soil residues dissipated as a function of time with an estimated DT_{50} of 45–60 days. After 360 days, total soil residues of glyphosate were 6–18% of initial levels.

The use of glyphosate (ROUNDUP, VISION (Monsanto Corp., St. Louis, MO)) in Canadian silvicultural management has been increasing steadily since its federal registration in 1984. The environmental fate of glyphosate in soils has been investigated primarily in relation to agricultural use patterns and environmental conditions of the United States (Rueppel et al., 1977; Edwards et al., 1980; Hance 1976; Sprankle et al., 1975; Muller et al., 1981; Moshier and Penner, 1978). The database pertinent to forest use patterns is more limited, although Newton et al. (1984) and Torstensson and Stark (1981) have reported on the fate and behavior of glyphosate in forest soils of the United States and Sweden, respectively. The fate of glyphosate in Canadian forest ecosystems has not been widely studied, and reports have been primarily restricted to government documents (Dotsie et al., 1988; Legris and Couture, 1988). Information pertaining specifically to the environmental fate of glyphosate in soils of Canadian western coastal watersheds is lacking. Similarly, although a relatively large amount of information is available with respect to the fate of glyphosate in agricultural crops or weed species, little information pertaining to the behavior of glyphosate residues in forest brush species is available. Newton et al. (1984) monitored the persistence of glyphosate in red alder and other forest hardwood species. Lund-Hoie (1985a) reported on the uptake, distribution, and metabolism of this herbicide in spruce and later in two brush species—ash and birch (Lund-Hoie, 1985b). Most recently, the persistence of glyphosate residues in foliage (raspberry, grasses, balsam fir, red maple) and in litter (white spruce, red maple) has been reported (Freedman et al., 1988).

In the coastal area of British Columbia, climatic conditions frequently include autumn and winter rainstorms: annual rainfall often exceeds 2000 mm. Winter temperatures are cool, with snowpack occurring in the upper reaches. Under such conditions, watersheds with areas of high water table, seasonally saturated soils, and frequent surface runoff events following major storms are common. Soil profiles in the flood plain of coastal forest watersheds are often highly stratified into organic rich (30% or greater organic matter content) upper horizons,

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Table I. Carnation Creek Watershed Flood Plain Soil Characteristics[a]

depth, cm	OM, %	CEC	N, %	P, ppm	K, %	pH	sand, %	silt, %	clay, %
				Stations 1–3					
0–5	30.39	45.85	0.60	17.05	0.03	4.94	55.85	23.70	20.46
5–15	19.47	51.33	0.50	15.18	0.02	4.55	55.79	23.73	20.48
15–35	9.28	26.37	0.21	1.47	0.01	5.28	62.01	17.50	20.49
				Stations 7–9					
0–5	30.90	50.47	0.12	54.04	0.03	4.49	62.02	24.99	12.99
5–15	14.88	48.13	0.84	10.55	0.02	4.20	54.68	24.90	20.42
15–35	10.21	27.44	0.07	1.03	0.01	4.65	65.80	16.23	17.97

[a] Key: OM = organic matter; CEC = cation-exchange capacity.

underlain by coarse-textured mineral soils, low in organic matter. Given that climatic, hydrological, and physical characteristics of coastal forest ecosystems are unique, that residue mobility and persistence may be affected by such factors, and that no information was available on the behavior of glyphosate in a coastal rainforest ecosystem, a research program was initiated to provide data relevant to the fate of the herbicide glyphosate (ROUNDUP) in a coastal British Columbia watershed.

The specific objectives of this portion of the study were (a) to assess the uniformity of glyphosate deposition following a rotary-wing, aerial application of ROUNDUP with a MICROFOIL (Union Carbide Inc., Ambler, PA) boom, in a coastal British Columbia watershed, (b) to determine foliar residue levels in the major target species and subsequently monitor the persistence of associated litter residues, (c) to monitor the persistence of glyphosate and its major degradation product, AMPA, in both well-drained and seasonally flooded soils over a 1-year period following aerial application, and (d) to determine the leaching potential of glyphosate and AMPA in soil systems typical of a coastal British Columbia watershed.

MATERIALS AND METHODS

Site Description. A general description of the Carnation Creek watershed study site is given in part 1 (Feng et al., 1990). The 10-km^2 study site is typical of coastal watersheds in British Columbia and is characterized by a narrow valley bottom with steep slopes rising to 700 m in elevation. Vegetation in the valley bottom is dominated by salmonberry (*Rubus spectabilis*) and red alder (*Alnus rubra* Bong.) (King and Oswald, 1982).

Soils on the slopes are shallow, coarse-textured, and highly organic (Oswald, 1973). Soils of the alluvial flood plain are highly stratified into organic surface layers underlain by gravel, bedrock, or silty clay deposits depending on location within the watershed. Characteristics of soils from sampling sites used in this study are presented in Table I.

Annual precipitation in the watershed ranges from 210 to 480 cm, falling predominantly as rain in October and March. A detailed description of the watershed hydrology is available in a previous publication (Hetherington, 1989).

Site Preparation. Three soil sampling sites were chosen within the watershed as indicated in Figure 1. Sites in the upper (stations 1–3) and lower (stations 7–9) reaches of the watershed were selected to monitor persistence and leaching in well-drained soils. The middle watershed soil site (stations 4–6) was established in a low-lying area known to be seasonally flooded. Water table levels and degree of soil satration for the various sampling locations and dates are presented elsewhere (Hetherington, 1989). At each site, three 5 × 5 m areas were cleared by removing the plant canopy, large debris, and slash. The area was then subdivided to provide three replicate sampling areas or stations.

At each of the soil sampling sites, a total of eight deposit collectors constructed of aluminum foil supported on corrugated cardboard squares (surface area 400 cm^2) were placed around the periphery of the cleared area. The deposit collectors (approximately 10 cm above ground level) were used to estimate the initial residue deposited on the soil surface at each site and allow assessment of the deposit uniformity throughout the watershed.

Foliage and leaf litter sampling stations were established in areas dominated by salmonberry and red alder, respectively (Figure 1).

Herbicide Application. ROUNDUP (isopropylamine salt of glyphosate) was applied at a nominal rate of 2 kg/ha AI and spray volume of 252 L/ha. Applications were made over a 10-day period to 11 individual spray blocks (Figure 1), with use of a Bell-47 helicopter and a MICROFOIL boom equipped with 1.5-mm-i.d. hayrake nozzles. Details of the herbicide applications, including meteorological conditions at time of spraying, have been reported by Reynolds et al. (1989).

Sampling Methodology. Deposit sampling sheets were collected immediately following chemical application, packaged, stored, and shipped for analyses.

Fresh foliage was collected immediately following treatment by felling a representative tree (red alder) or by clipping brush (salmonberry). Individual leaves were then harvested to provide a composite sample for determination of initial foliar residues in each species.

A total of 20 m^2 of nylon mesh was placed under individual trees at approximately 0.5 m above the ground surface to trap leaf litter. Leaf senescence and defoliation began in mid-September 1984. Leaf litter samples were collected from nylon mesh traps between 1 Oct and 30 Nov. Samples of leaf litter were collected biweekly generally and on a weekly schedule during the peak leaf fall period.

Soil samples were obtained from each sampling station with use of a Campbell soil coring device. Soil cores of 10-cm diameter and 30-cm depth were obtained and subsequently divided into three sections corresponding to 0–5, 5–15, and 15–30 cm soil layers, respectively, with each layer independently analyzed for chemical residue. For samples taken after 150 days, a fourth layer corresponding to a depth of 30–35 cm was obtained. Soil samples were collected on a temporal schedule as shown in Figure 3. Details of sampling methodologies for all substrates are reported by Feng and Klassen (1986).

Residue Analysis. Samples of the ROUNDUP formulation (356 g of AI/L), tank mixes, and deposit collectors were obtained and analyzed as described in part 1 (Feng et al., 1990). Whole samples of foliage and leaf litter were air-dried and macerated by passage through a Hobart chopper. Finely chopped particles were mixed in large, inflated, plastic bags to provide a homogeneous sample. From the homogenized samples, two subsamples (5 g) were taken and used for determination of oven-dry weight and residue content, respectively. Results were corrected for recovery efficiency of the analytical method (Table II) and reported as micrograms per gram of oven-dried leaf tissue.

Soil samples obtained from the Carnation Creek study site were immediately frozen, shipped, and stored under cold (−10 °C), dark conditions until analysis. Subsamples (5 g) of air-dried and homogenized soils were extracted. Results were corrected for recovery efficiency of the analytical method (Table II) and reported as micrograms per gram of air-dry mass. The analytical procedures, including HPLC specifications utilized

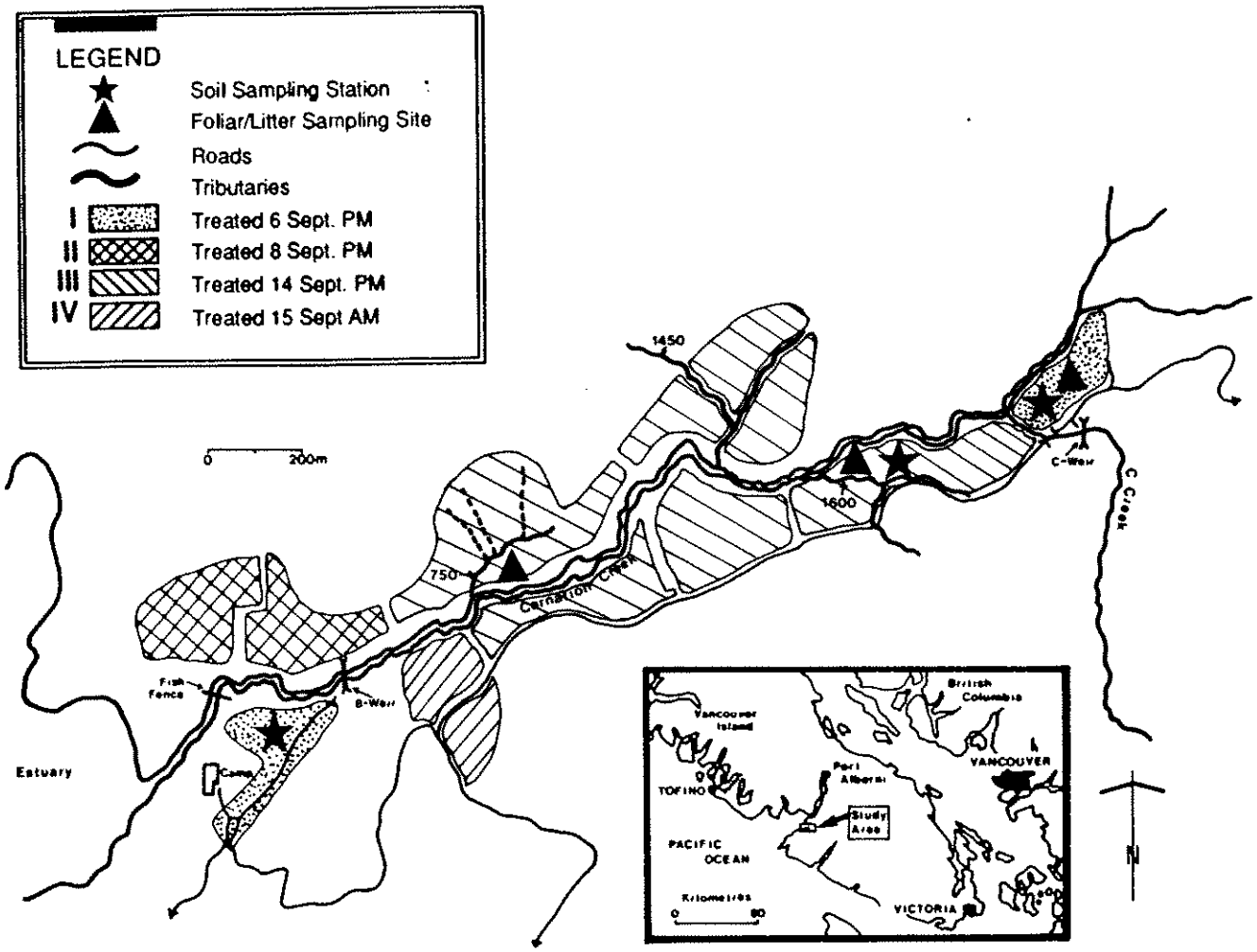

Figure 1. Location of the Carnation Creek watershed study area and terrestrial sampling locations.

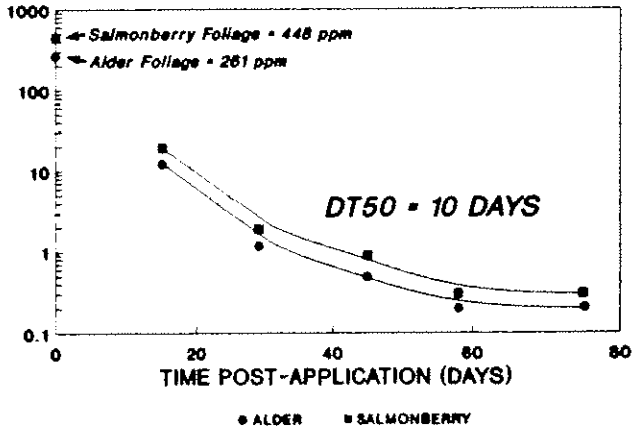

Figure 2. Dissipation of glyphosate residues in leaf litter of the Carnation Creek watershed.

for leaf litter, foliage, and soil sample analyses are detailed in Thompson et al. (1989).

Throughout the course of analytical determinations a quality control (QC) program was conducted. Blank field samples fortified with varying levels of glyphosate and AMPA were processed and analyzed daily in conjunction with field samples. Results of the QC program are presented as an indication of the accuracy and precision of the method (Table II). The QC data were subsequently used to correct the field sample data for recovery efficiency of the analytical method.

Statistical Analysis. Glyphosate residues recovered from artificial deposit collectors or initial soil samples were used to estimate initial deposit rates. Application rate estimates (kg/ha) were calculated by multiplying residues ($\mu g/cm^2$) on deposit collectors by a conversion factor of 0.10. Mean deposit rates calculated for each of the three sampling locations were subjected to a one-way analysis of variance (ANOVA), with 2 degrees of freedom. The Bonferroni (BON) multiple comparison procedure was used to elucidate which deposit rates were significantly different in the case of the ANOVA F value being significant at $P < 0.05$.

Leaf litter residues (micrograms per gram dry mass) were regressed against the log transform of time postapplication by simple linear regression analyses. A significant F value ($P < 0.10$) from the regression ANOVA was interpreted as evidence of a significant decline of litter residues with time, and a coefficient of determination (R^2) greater than 0.75 was considered acceptable in terms of using the regression equation to estimate time to 50% and 90% dissipation (DT_{50} and DT_{90}, respectively). These values were also estimated by graphical interpolation of untransformed data for comparative purposes.

Soil residues (micrograms per gram dry mass) as determined from subsamples in each individual layer were multiplied by the dry mass of the corresponding individual layer and totaled to yield soil residue data in terms of micrograms per layer. Total soil residues (total micrograms per 30-cm core) were then calculated for each sampling station and time and grouped according to location to derive mean soil residues for each watershed location at each sampling time. Mean total soil residue data were subjected to ANOVA and regression analyses similar to that described for litter residues.

RESULTS AND DISCUSSION

Formulation and Tank-Mix Analyses. Results of the ROUNDUP formulation and tank-mix analyses are presented in part 1 of this series (Feng et al., 1990).

Initial Deposit at Soil Sampling Sites. Deposit estimates calculated from artificial deposit collector resi-

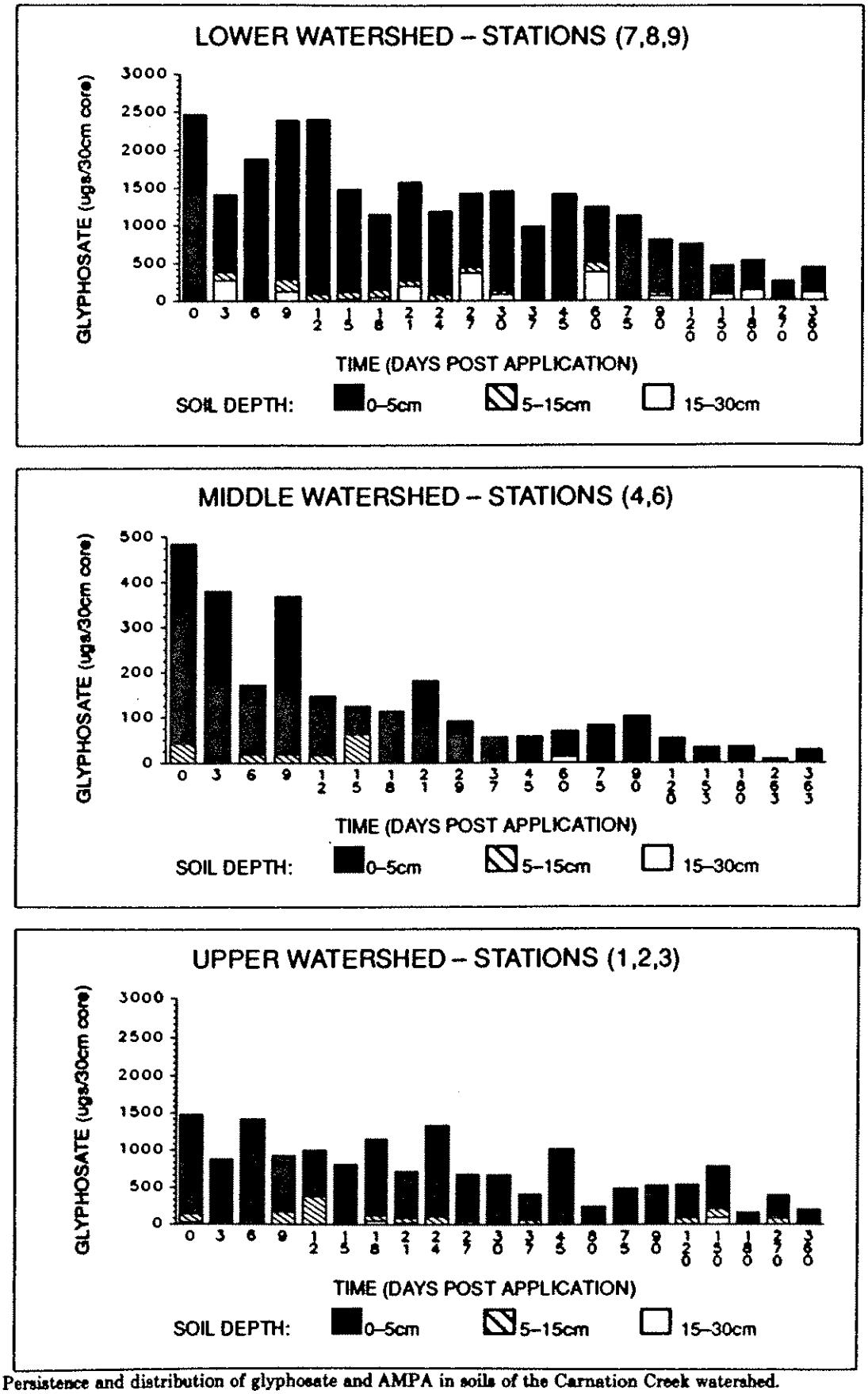

Figure 3. Persistence and distribution of glyphosate and AMPA in soils of the Carnation Creek watershed.

dues were consistently higher than those resulting from initial soil residues (Table III). Mean deposit rates derived from artificial collectors at the upper, middle, and lower watershed locations were statistically different from one another as determined by ANOVA and the BON multiple comparison procedure ($P < 0.05$). The lack of statistical differences in similar comparisons based on initial soil residues is an artifact of the high variability in these residue samples and the lower number of replicates used. High variability in initial soil residues result-

Table II. Validation Data for Analytical Methods[a]

substrate level	fortificn	N	analyte	% recovery mean ± sd (CV, %)	LOD	LOQ
humus	0.1–6.0	132	GLYPH	77.7 ± 8.9 (11.56)	0.05	0.10
soils	0.025–1.5	132	AMPA	67.7 ± 10.9 (16.09)	0.01	0.03
mineral	0.1–6.0	30	GLYPH	73.5 ± 7.7 (10.53)	0.02	0.05
soils	0.025–1.5	30	AMPA	58.2 ± 10.9 (12.77)	0.02	0.05
leaf	0.5–5.0	4	GLYPH	84.4 ± 5.3 (6.25)	0.10	0.30
litter (s)	0.1–1.0	4	AMPA	54.7 ± 1.7 (3.21)	0.03	0.08
leaf	0.5–5.0	4	GLYPH	81.3 ± 7.7 (9.47)	0.10	0.30
litter (a)	0.1–1.0	4	AMPA	60.9 ± 12.2 (20.03)	0.03	0.08

[a] All values presented in terms of ppm = μg/g dry mass (for solids). LOD = limits of detection = detector response equivalent to 2 × S:N ratio. LOD and LOQ values for deposit collectors equate to 2.5×10^{-6} and 1.25×10^{-4} kg/ha, respectively.

Table III. Comparison of Nominal and Empirical Estimates of Initial Glyphosate Deposition at Carnation Creek Watershed

sampling location	treatment area	nom rate[a]	init deposition rate est, kg/ha [mean ± SD (% CV)] deposit collector	soil residue
stations 1–3 (upper watershed)	I	2.0	2.54 ± 0.32 (13) A	1.72 ± 0.31 (18)
stations 4–6 (midwatershed)	III	2.1	1.62 ± 0.24 (15) B	0.60 ± 0.09 (16)
stations 7–9 (lower watershed)	I	2.0	3.42 ± 0.46 (14) C	3.23 ± 2.30 (71)
overall av		2.0	2.52 ± 0.90 (36)	1.85 ± 1.32 (71)

[a] Nominal rates as reported by Reynolds (1988)9 Values based on total residue (glyphosate plus AMPA) with $n = 8$ for deposit collectors and $n = 3$ for soil residues. Deposit collector values followed by different letters are significantly different ($P < 0.05$) as determined by LSD and Bonferroni multiple comparison procedures.

ing from aerial applications is commonly observed (Newton et al., 1984) and may be related to a number of variables. Potential factors affecting ground deposit variability include site variables (air turbulence within microsites, differential interception of the spray cloud by surrounding vegetation), application variables (differences in release height, dispersal system function, orientation as related to wind direction, degree of overlap, swath displacement and/or drift), and meteorological variables (windspeed, wind direction, air temperature, boundary layer stability). One anomalous data value in the initial soil residue data (station 8, 5.67 kg/ha) resulted in an extremely high mean deposit estimate for the lower watershed location. The artificial deposit collector data also showed unusually high residues, indicating that the high deposit rate may be attributed to application error (i.e., swath overlap or nozzle malfunction).

Initial levels of AMPA were less than 2% of corresponding glyphosate residues for all deposit collector samples but varied between 4 and 13% for initial deposit as determined from soil sample residues. The higher levels of AMPA in 0-time soil samples as compared with deposit collector samples may be indicative of slight degradation of glyphosate during the 2-year storage period for soil samples.

Overall average deposit within the watershed ranged from a high estimate of 2.52 based on artificial deposit collector data and 1.85 kg/ha based on initial soil residue data (Table III), indicating an overall application rate close to the nominal rate of 2.0–2.1 kg/ha AI.

Foliar and Leaf Litter Residues. Results of the foliage sample analyses indicated that an effective impingement of glyphosate occurred on both target species, resulting in initial foliar residues of glyphosate ranging from 261 μg/g for red alder to 448 μg/g for salmonberry. Initial foliar residues observed in this study are in agreement with those of Newton et al. (1984), who observed residues ranging from 89 to 489 μg/g in various target foliage samples including red alder and salmonberry, but contrast with results of Freedman et al. (1988) who reported glyphosate residues in red maple foliage of 5.1 mg/g. The apparent discrepancy in these results may be related to differences in mean leaf area and corresponding impingement of glyphosate on the leaf surface and/or differences in methods of sampling. Residues of AMPA were less than 2% of coincident glyphosate residues for both foliage types.

Leaf senescence and defoliation began in mid-September 1984, and the first leaf litter samples were collected 15 days postapplication. Initial leaf litter residues (12.5 and 19.2 μg/g dry mass) were approximately 10-fold lower than initial foliar residues (261 and 448 μg/g dry mass) observed in fresh foliage of alder and salmonberry, respectively, on the day of application. Such a disparity in foliar and leaf litter residues may result from a variety of dissipation mechanisms acting on the foliar residues including uptake, translocation, and metabolism within the plant, washoff resulting from the rainfall event (39 mm), which occurred 23 h postapplication, and/or microbial degradation on the leaf surface. Lund-Hoie (1985b) reported that only 20% of the glyphosate initially applied was absorbed in ash and birch foliage and that decomposition of the absorbed chemical was slow (35% in 2 months). Newton et al. (1984) observed a similar rapid dissipation of glyphosate residues in hardwood foliage following a rainfall of 12 h after application. In combination, these results lend support to the hypothesis that washoff with rainfall is a major mechanism of dissipation for foliar residues where rainfall occurs shortly after application. Further research is required on binding and subsequent washoff of glyphosate from treated leaf surfaces.

Glyphosate residues in leaf litter declined as a logarithmic function of time postapplication in both alder ($F = 8.97$, $P = 0.06$; $R^2 = 0.75$) and salmonberry ($F = 9.2$, $P = 0.06$; $R^2 = 0.76$). Based on regression analysis, DT_{50} and DT_{90} values for both alder and salmonberry litter residues were 10 and 32–35 days, respectively. Similarly, time to 50% dissipation based on graphical interpolation was estimated as 8 days for alder and 9 days for salmonberry (Figure 2). AMPA residues in leaf litter also declined with time and were at or below limits of detection within 29 days postapplication. The estimated DT_{50} value for glyphosate residue in leaf litter is comparable to the value of 14 days determined by Newton et al. (1984).

Leaching Potential. Soil core samples were divided into separate layers according to depth [0–5 cm (organic), 5–15 cm (organic), 15–30 cm (organic and mineral)], and each layer was analyzed separately to allow determinetion of the distribution of glyphosate and AMPA residues within the soil profile. At sampling dates beyond 150 days postapplication, a fourth layer [30–35 cm (mineral)] was obtained and analyzed. Results of these analyses are presented as histograms of mean glyphosate concentration from replicate sample stations according to location within the watershed (Figure 3). Quantifiable residues of glyphosate were found in only 1 of the 32 samples from the 30–35-cm layer; the residue level in this layer (0.46 ppm) was equivalent to 11.5% of the total found in the core. Analysis of residue distribution data indicated that in general (156/168 core samples) >90%

of glyphosate residue occurred in the 0–15-cm organic layer. Two anomalous values (glyphosate residue levels in the 15–30-cm layer exceeding 10% of the core total) were observed in the soil residue distribution data, both occurring in the lower watershed location (day 60, 30.28%, day 360, 27.6%). No differences in leaching potential between the seasonally flooded and well-drained soil sites were apparent. AMPA residue distribution was similar to that of glyphosate, being retained primarily in the upper 0–15-cm layer.

Distribution of glyphosate and AMPA within the soil cores indicates that neither chemical is susceptible to leaching. These findings are consistent with the general literature (Helling, 1971; Sprankle et al., 1975; Rueppel et al., 1977; Damanakis, 1976; Edwards et al., 1980; Roy et al., 1989) and support the conclusion of Torstensson (1985), who stated that glyphosate is practically immobile in soil. Research conducted on the absorption of glyphosate to soils or soil fractions (i.e., mineral components) suggests that glyphosate is bound to soils through the phosphonic acid moiety and that sorption is positively correlated with clay content, cation-exchange capacity, and unoccupied phosphate sorption capacity (Sprankle et al., 1975; Hance, 1976; Glass, 1986). Carnation Creek soils are characterized by a relatively high cation-exchange capacity, clay content, and organic matter content, especially in the upper 15-cm layers (Table I). Thus, we conclude that the lack of vertical mobility exhibited by glyphosate in these soils is due to strong adsorption to cation saturated clays and/or organic matter in the upper soil horizons.

Persistence. The mean total residue of glyphosate (total micrograms per 30-cm core) at each sampling date is represented by the height of the histogram bars in Figure 3. Although total residues of glyphosate clearly declined with time in all locations and ANOVA F values for regression of linear and semilog-transformed data were significant ($P < 0.05$), poor regression coefficients ($0.17 < r^2 > 0.46$) prohibited the use of these models for estimating DT_{50} and DT_{90} values or establishing confidence limits for these endpoints. In addition, no statistically valid comparisons could be made with respect to persistence of glyphosate or AMPA in seasonally flooded soils and well-drained soils.

Graphical analysis of glyphosate residue data indicated that glyphosate residues were consistently less than 50% of initial residues 45–60 days postapplication at all locations, and thus a conservative estimate of DT_{50} for glyphosate was established as 45–60 days. Although quantifiable residues of glyphosate remained after 360 days, residue levels were low, equating to 6–18% of initial glyphosate residue in terms of total micrograms in the 30-cm core. Residues in the organic 0–5-cm layer declined from high levels of 8.16–39.80 ppm initially to 0.25–2.89 ppm after 360 days. AMPA residues levels were low at time 0 (4–13% of glyphosate residues) and followed a pattern of transient increase and decline at all sampling locations. The maximum AMPA residue was observed in the upper organic layer of samples from the upper watershed location on day 18 (mean (n = 3) 9.185 ppm) and equated to less than 40% of initial glyphosate residues. After 360 days AMPA residue levels had declined to 6–27% of initial glyphosate residues. Torstensson (1985) summarizes a number of investigations providing conclusive proof that the degradation of glyphosate in soils occurs primarily via cometabolism by soil microbes. The general decline in glyphosate residues over time at all three sampling locations, coupled with a transient increase in AMPA residues, suggests that microbial degradation is also the major mechanism of dissipation for glyphosate in these soils.

The middle watershed sampling location is low-lying and seasonally flooded. At the time of application and for some time thereafter, surface water was actually moving over portions of the lower lying stations at this location (Hetherington, 1989). In fact, no samples were taken from station 5 due to complete flooding of this sampling location. As a result, initial soil residues of glyphosate in the middle watershed location were very low compared to the upper and lower watershed locations (note differences of scale in Figure 3), probably owing to interception and off-site movement of the chemical with runoff water.

The persistence estimate of 45–60 days resulting from this study is well within the range of 50% degradation times determined for various forest soils studied in the laboratory (Torstensson and Stark, 1981). The DT_{50} estimate is also in good agreement with that of Newton et al. (1984), who reported a value of 40.2 days for glyphosate in a western coastal soil of Oregon, but somewhat longer than that of Roy et al. (1989) who reported a 50% dissipation time of 24 days in a boreal forest soil of Ontario, Canada. Torstensson (1985) noted that the variable rate of degradation of glyphosate cannot be correlated to a single soil factor but reflects the general microbial activity of the soils, which in turn is affected by a multiplicity of environmental factors. The agreement of our data with previously published data indicates that none of the environmental factors in existence at Carnation Creek resulted in excessive persistence of glyphosate.

CONCLUSIONS

Although the overall chemical application to the watershed, empirically estimated as 1.85–2.52 kg/ha, was close to the nominal rate of 2.0–2.1 kg/ha, both methods of deposit estimation (artificial collectors and initial soil residues) indicated highly variable deposit between sampling locations and suggest that aerial applications, as conducted in this study, may result in nonuniform chemical deposit on target. Such differences in chemical deposition are of questionable practical importance in terms of efficacy and may be attributed to day to day meteorological and/or operational variability.

Although initial impingement of the active ingredient on target foliage was high, resultant leaf litter residues were 10-fold lower and dissipated rapidly, thus representing an insignificant, transient source of residue input to other ecosystem compartments. Initial soil residues of glyphosate in well-drained sites were high 31.42–39.80 μg/g, while in a seasonally flooded location initial soil residues were lower (8.16 μg/g) owing to interception of the chemical by surface water present on-site at the time of application. Little evidence of leaching was observed in either well-drained or seasonally flooded soils. Glyphosate soil residues were nonpersistent, dissipating to 13–18% of initial levels within 360 days postapplication, with an estimated time to 50% dissipation of 45–60 days. The behavior of glyphosate soil residues, as observed in this study, is in agreement with the general literature indicating that glyphosate is nonmobile in and relatively nonpersistent in soils.

Given the low acute toxicity to terrestrial organisms ranging from microorganisms to mammalian species (Eijsackers, 1985; Grossbard, 1985; Atkinson, 1985; Sullivan, 1985; Fletcher and Freedman, 1986; Torstensson and Stark, 1978; Muller et al., 1981), its lack of potential to bioaccumulate (Newton et al., 1984), and its environmen-

tal behavior as observed in this and other studies (Newton et al., 1984; Roy et al., 1989), a significant toxic impact resulting from soil or leaf litter residues would be highly unlikely.

In combination, the aquatic and terrestrial environmental fate research programs conducted at the Carnation Creek watershed support the continued registration and use of the glyphosate as a biodegradable, nonpersistent, and nonmobile chemical herbicide for silvicultural management.

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Off-Target Glyphosate Deposits from Aerial Silvicultural Applications under Various Meteorological Conditions

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Abstract: Aerial spray applications of the herbicide glyphosate were made over a forest canopy under various meteorological conditions. A 'Thru Valve Boom' dispersal system carried by a Cessna 188 fixed-wing aircraft flying at 49 m s^{-1} was used to generate an aqueous spray cloud with a volume median diameter of 150 μm. Glyphosate deposits from multiple overlaid crosswind line sources released at 10 m above ground level were measured on ground sheets and artificial foliage at downwind distances between 50 and 400 m. Trials were conducted in stable, neutral and unstable atmospheric boundary layers with average wind speeds between 2·2 and 5·7 m s^{-1} and vertical intensities of turbulence between 0·07 and 0·16. Linear regression lines fitted to logarithmically transformed measurements and downwind distances (x) gave statistically significant correlation coefficients ($P = 0{\cdot}01$), and were compared by ANOVA. Glyphosate deposits on ground sheets and artificial foliage were attenuated at rates inversely proportional to x to the power 1·7–4·3. Regression line comparison showed that, in general, deposits on ground sheets decreased with increasing wind speed and intensity of turbulence, and some statistically significant differences were found in slopes and elevations of regression lines from different trials. However, deposits at the 50-m station increased with wind speed due to the large-drop cloud component. Regression line comparison for deposits on artificial foliage showed that, in general, they were highest in the intermediate wind speed–neutral stability case and similar in the high wind speed–unstable and low wind speed–stable boundary layers, although deposits at the 50-m station also increased with wind speed.

1 INTRODUCTION

Herbicides are an efficacious and cost-effective tool for controlling competing vegetation and are widely used in forestry and agriculture, often in aerial sprays. However, it is recognized that such applications cause off-target deposits resulting in unwanted environmental contamination and wasted herbicide.[1] The meteorological conditions during spray cloud dispersal affect its fate,[2,3] i.e. the proportions of drops deposited on- and off-target, and also the environmental impact caused by herbicide spray applications. To prevent excessive environmental impact in nearby sensitive areas, a minimum distance of approach or buffer zone is maintained for aerial silvicultural pesticide applications.

Regulations governing aerial herbicide spray applications for forestry typically specify an upper wind-speed limit above which spraying must cease. In Canadian forestry the upper wind-speed limit for glyphosate applications is 2·2 m s^{-1} at spray release height. This restriction is intended to minimise environmental contamination caused by the spray, and arises solely from consideration of the large-drop behaviour, i.e. drop diameters greater than 150 μm. These drops have terminal velocities greater than 0·5 m s^{-1}, and sedimentation is the dominant process in bringing them down from the spray release height to ground level; consequently the horizontal distance between release and deposition increases with wind speed. Early morning and late evening are favoured times for aerial herbiciding because of the diurnal wind speed reduction that results from a stable boundary layer.[4] In Canadian forestry this

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provides a typical spray window of two hours or less. When taken together with other meteorological restrictions, e.g. rainfall, this restriction on application time can significantly increase the cost and reduce the timeliness of herbicide spraying, especially when large areas await treatment.

Earlier research suggests that the spray cloud near the ground is more quickly attenuated with distance in an unstable boundary layer than in a stable layer, and with increasing wind speed.[5,6] This implies that off-target deposits may also be reduced under these conditions. In the present investigation, herbicide spray dispersal was studied in atmospheric boundary layers having a range of wind speeds, stabilities and intensities of turbulence to determine which conditions caused the highest off-target herbicide deposits downwind of the application, and would therefore be expected to cause greatest environmental impact. These data may also be used in estimating the environmental impact from such applications,[1] and in the evaluation of spray dispersal models.

2 EXPERIMENTAL METHODS

2.1 Spray applications and materials

Four similar applications were made during July 1986 using an application method representative of those used for aerial herbiciding in forestry. This method employed a 'Thru Valve Boom'® dispersal system (TVB, Waldrum Specialties Inc., Amber, PA) mounted on a Cessna 188 fixed-wing aircraft flown at 10 m above ground level (agl) at an airspeed of 49 m s^{-1}. The TVB was 9·2 m in length and comprised 62 '045' burr nozzles placed 0·15 m apart along a streamlined boom. The tank mix was a 28 g kg^{-1} aqueous solution of the herbicide glyphosate prepared from 'Roundup'® (480 g glyphosate-mono(isopropylammonium) $litre^{-1} \equiv 356$ g glyphosate $litre^{-1}$; Monsanto, St Louis, MO), and applied at a total flow rate of 5·5 litres s^{-1}, providing volume and active ingredient application rates of 75 litres ha^{-1} and 2·1 kg a.i. ha^{-1} with a 15-m swath width. The drop-size spectrum of the cloud at release had diameters of 80, 150 and 280 μm at the 10th, 50th and 90th volume percentiles.[7] In each trial, ten swaths 350 m in length were overlaid along a crosswind track, to average out natural deposit fluctuations caused by atmospheric diffusion. For the range of downwind distance, wind direction and crosswind intensities of turbulence employed these swaths provided deposits that represented an infinite line source.

2.2 Site and canopy

The site was nearly flat and part of a naturally regenerating forest clear-cut covered with a plant canopy 1–2 m in height, and was situated near Lowther, Ontario (49°16′N, 83°17′W). The most common on-site tree species were speckled alder (*Alnus rugosa* (DuRoi) Spreng.), black spruce (*Picea mariana*), various species of willow (*Salix*), and trembling aspen (*Populus tremuloides* Michx.). The upwind fetch was nearly flat with a sparse forest canopy between 2 and 13 m in height. The aerodynamic canopy roughness upwind of and on the site was typical of areas in which herbicides are applied for forestry, and hence the structure of the atmospheric boundary layer and resulting effects on spray-cloud dispersal were also typical.

2.3 Meteorological measurements

On-site measurements were made to characterise conditions during the applications. The wind-speed vector was measured at 2 and 10 m agl with bivane-mounted propellor anemometers (VectorVane, Meteorology Research Inc., Altadena, CA). Air temperatures were measured at the same heights with thermistors (YSI 44018, Yellow Springs Instrument Co., Yellow Springs, OH) shaded to prevent radiative heating, and relative humidity was also measured at 2 m agl with a capacitance hygrometer (Heath Co., Benton Harbour, MI). Electrical signals from the meteorological instruments were sampled at 1 Hz and recorded on a data acquisition system (Isaac 91, Cyborg, Newton, MA) during a 23·3 min period commencing with the spray application. Meteorological conditions during the spray applications are summarised in Table 1. Richardson number was calculated according to Thom.[8] Average wind directions during the trials were within 22° of the perpendicular bisector to the swaths, and downwind distance and line source strength were adjusted for off-axis average wind directions.

2.4 Off-target deposit measurements

Sampling stations were set up at downwind distances of 50, 100, 150, 200, 300 and 400 m from the crosswind track, along its perpendicular bisector. This range of distance falls outside the swath (on-target) region which extends to typically 25–30 m, depending on the wind speed. Glyphosate deposits on imitation water and foliar surfaces were sampled at each station.

Glyphosate deposits were measured on polyethylene ground sheets (0·15 × 1 m) pegged on the ground over larger polyethylene sheets (2 × 2 m) in cleared areas. The aerodynamic form, orientation and position of these collectors were similar to those of the surfaces of standing natural water bodies, and spray deposition and deposits were therefore assumed to be representative of these surfaces. Two collectors were placed at the three sampling stations closest to the spray line, and at the remaining stations four were used. Glyphosate deposit

TABLE 1
Meteorological Conditions During Spraying

Trial number	*1*	*2*	*3*	*4*
Time spraying commenced	10:45	20:57	10:05	20:31
Wind speed ($m\ s^{-1}$)				
(avg ± SD) 10 m agl	5·7 (±1·3)	2·2 (±0·4)	5·2 (±1·1)	3·2 (±0·7)
2 m agl	3·2 (±1·1)	0·3 (±0·2)	—	1·7 (±0·6)
Vertical intensity of turbulence[a] at 10 m (avg)	0·16	0·07	0·14	0·12
Average temperature (°C) at 10 m agl	14·2	14·6	14·2	20·3
Average temperature difference (°C)				
T(10) − T(2)	−0·9	3·0	−1·1	0·1
Stability of lowest 10 m of atmosphere	Unstable	Stable	Unstable	Near-neutral
Relative humidity (%)	75	60	69	61
Richardson number	−0·04	0·2	—	0·01

[a] Standard deviation of vertical wind speed divided by average downwind wind speed.

was also measured on artificial foliage clusters composed of polyethylene discs (o.d. 4 cm) on wire supports. The disc size was representative of deciduous foliage to provide similar airflow patterns and drop impaction efficiencies to those obtained with natural leaves having little or no pubescence. The length and flexibility of the wire supports allowed the discs to move in the wind like deciduous foliage. At the three sampling stations closest to the spray line two clusters were placed just above the canopy at 2 m agl, with a 0·5-m crosswind separation; at the remaining stations four clusters were deployed. These collectors provided a worst case for assessing environmental impact because they were not sheltered by canopy, and would therefore receive relatively high deposits. All collectors were exposed for up to 20 min after the spray application was completed to allow the cloud to be advected beyond the sampling stations, and then collected and stored at −10°C until glyphosate quantification. Observed spray deposits on collectors were insufficient to cause run-off.

2.5 Glyphosate quantification

Glyphosate deposits were washed from the polyethylene sheets with aqueous ammonium hydroxide (0·15 M), and these rinsings were then cleaned-up and concentrated using anion- and cation-exchange columns and mixed with the mobile stage required for high performance liquid chromatography, i.e. potassium dihydrogen phosphate buffer solution (0·005 M; pH 1·9). The polyethylene discs were washed with hydrochloric acid (0·1 M) by mechanical agitation at 180 excursions min^{-1}. The rinsings were then concentrated by evaporation, filtered (Gelman Acro LC 13, 0·45 μm pore size) and mixed with the mobile phase. The more elaborate sample preparation procedure required for the sheets was due to surface contaminants. Residues of glyphosate and its principal metabolite (aminomethylphosphonic acid; AMPA) were quantified by high performance liquid chromatography.[9] The chromatograph was fitted with a variable wavelength UV/vis detector and a ninhydrin post-column reactor, and the peak heights of detector response at 570 nm were interpreted by comparison with analytical standards injected after every second sample. The limit of quantification (signal to noise ratio 2:1) for glyphosate was 500 ng. Glyphosate recovery efficiency was determined by measuring residues recovered from collectors on which a known amount had been deposited; residue measurements were corrected with recovery efficiencies of 86 and 92% for polyethylene sheets and discs respectively. AMPA was less than 2% of the glyphosate residues. Residues from similar collectors exposed at a sampling station during individual trials were pooled.

2.6 Statistical analysis

A linear regression line was fitted to the logarithms of measured deposit (J, ng cm^{-2}) and downwind distance (x, m). The form of the linear regression line was therefore,

$$\log J = \log A + B \log x \qquad (1)$$

where A and B are constant for a particular data set. The slopes and elevations of the various regression lines were compared by analysis of variance (ANOVA).[10]

3 RESULTS

3.1 Glyphosate deposits on ground sheets

Measurements of glyphosate deposits on ground sheets at various downwind distances are presented in Fig. 1, with regression lines. Up to 100-fold differences were observed between deposits at similar downwind distances under different meteorological conditions indicating widely different potential for environmental impact, and deposits on ground sheets ranged between 2% and 0·0001% of the glyphosate application rate (2·1 kg ha^{-1}). Regression coefficients (B), intercepts (log A) and correlation coefficients (r) are summarised in Table 2; values of the latter exceeded those at the 0·01 probability level, indicating that the measurements conformed well with eqn (1).

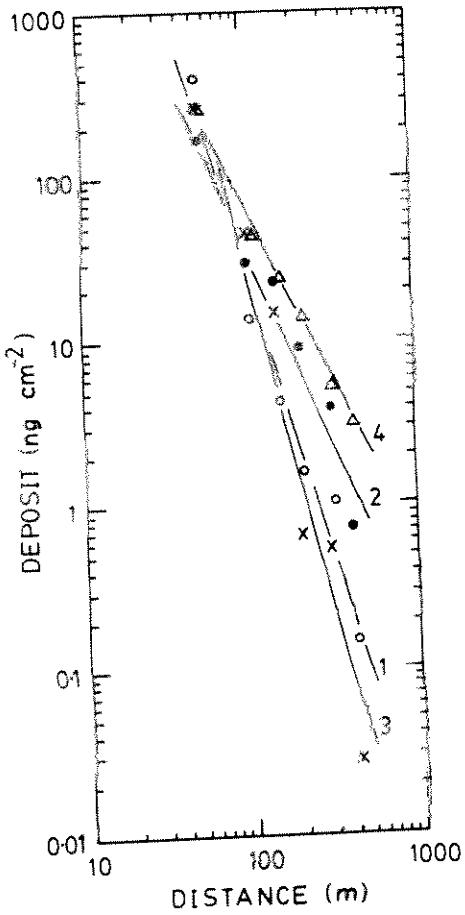

Fig. 1. Glyphosate deposits on ground sheets at various downwind distances from a single swath with a glyphosate application rate of 2·1 kg ha^{-1}, with regression lines. (○) Trial 1, (●) trial 2, (×) trial 3, (△) trial 4.

3.2 Glyphosate deposits on polyethylene discs

Measured glyphosate deposits on polyethylene discs are presented in Fig. 2, with regression lines. Up to 50-fold differences were observed between deposits on discs at similar downwind distances under different meteorological conditions, and glyphosate deposits ranged between 1% and 0·0005% of the application rate. Correlation coefficients exceeded those at the 0·05 probability level in all trials (Table 2), indicating that the measurements also conformed well with eqn (1).

4 DISCUSSION

4.1 Glyphosate deposits on ground sheets

The comparison of the regression lines for measurements from trials 1 and 2 (Table 3) showed that both the slopes and the elevations of the regression lines were significantly different ($P = 0·05$), with faster attenuation and lower elevation in conditions of high wind speed and intensity of turbulence (trial 1). For trials 1 and 3, both of which were carried out in conditions of high wind speed and intensity of turbulence, neither the slopes nor the elevations were significantly different, as expected. The comparison for trials 1 and 4 showed that both the slopes and the elevations of the regression lines were

TABLE 2
Regression Coefficients, Intercepts and Correlation Coefficients for Linear Regression Lines (Ground Sheet = G, Disc = D)

		Trial			
		1	*2*	*3*	*4*
Regression coefficient[a]	G	−3·5 (±0·3)	−2·4 (±0·3)	−4·3 (±0·6)	−2·1 (±0·07)
	D	−2·6 (±0·3)	−2·4 (±0·3)	−3·6 (±0·5)	−1·7 (±0·2)
Intercept (log A)	G	19·34	14·77	23·17	13·82
	D	15·44	13·94	20·24	12·04
Correlation coefficient[b]	G	−0·984a	−0·974b	−0·964b	−0·998a
	D	−0·972b	−0·970b	−0·962b	−0·968b

[a] B (±SD).
[b] a and b indicate that the correlation coefficient was significant at $P = 0·001$ and $P = 0·01$ levels respectively.

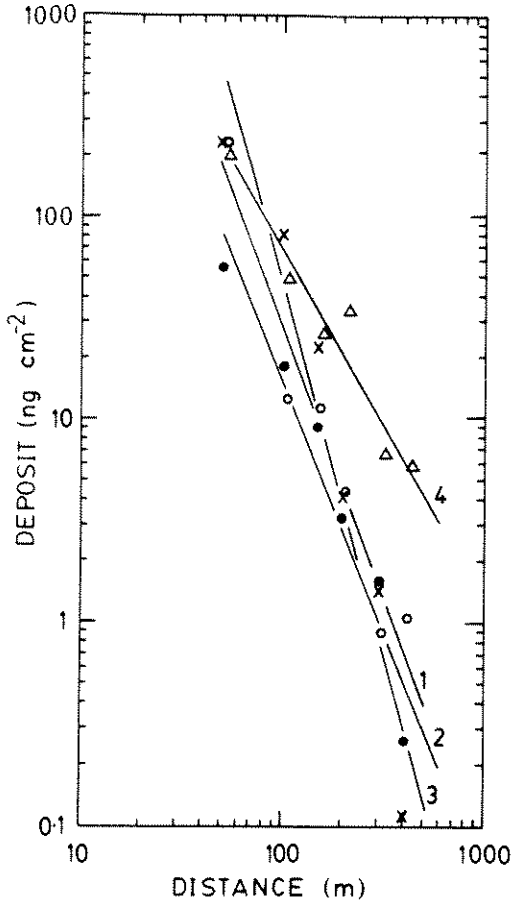

Fig. 2. Glyphosate deposits on polyethylene discs at various downwind distances from a single swath with a glyphosate application rate of 2·1 kg ha^{-1}, with regression lines. (○) Trial 1, (●) trial 2, (×) trial 3, (△) trial 4.

significantly different ($P = 0{\cdot}01$), with faster attenuation and lower elevation in trial 1. Thus, glyphosate deposits on ground sheets were greater with an intermediate wind speed and intensity of turbulence than with a high wind speed and intensity of turbulence. For trials 2 and 3 only the slopes were significantly different ($P = 0{\cdot}05$), with faster attenuation in trial 3. This slope difference is consistent with the comparison between results from trials 1 and 2, as expected. The comparison for trials 2 and 4 indicated that the slopes were not significantly different, but the elevations were ($P = 0{\cdot}01$), with a greater elevation in trial 4. Thus, deposits on ground sheets were greater in intermediate wind speed and intensity of turbulence conditions than with a low wind speed and intensity of turbulence. In trials 3 and 4 both the slopes and the elevations were significantly different ($P = 0{\cdot}01$), with faster attenuation and lower elevation in trial 3. This result is consistent with the comparison between trials 1 and 4.

The trend in glyphosate deposits on ground sheets at the 50-m station differed from that shown by the regression lines, in that deposits tended to increase with wind speed. This result can be predicted from the ballistic behaviour of large drops, i.e. those with sedimentation velocities much greater than the standard deviation of vertical wind speed (drop diameters > 150 μm). An increase in wind speed implies that the flight time to the 50-m station is reduced, consequently fewer large drops have time to fall out of the cloud. This is demonstrated by the calculated upper dropsize limits in the cloud at the 50-m station, derived from the cloud flight time and drop fallspeed; in trials 1–4 they were 240, 100, 220, and 150 μm respectively.

Glyphosate deposits found on ground sheets beyond 100 m were lowest in those trials carried out in high wind speeds and an unstable boundary layer (1 & 3), as compared to those from trials carried out in intermediate wind speed, near-neutrally stable and low wind speed, stable conditions (2 & 4). This can be explained by the increased rate of cloud spread associated with increased intensity of turbulence,[11] which would lead to reduced spray cloud concentrations and deposits on ground sheets beyond 100 m. Increased intensity of turbulence, which occurs in the transition from stable to unstable conditions, is also accompanied by an increase in wind speed due to faster downward momentum transfer.[8,11]

TABLE 3
F-values from Comparison of Regression Lines by ANOVA
(Ground Sheet Data = G, Disc Data = D)

Trial numbers	*F-values for slope comparison*		*F-values for elevation comparison*		*DF*
	G	*D*	*G*	*D*	
1 & 2	6·37	0·196	9·78	2·74	1/8
1 & 3	1·46	2·46	0·535	10^{-5}	1/8
1 & 4	17·8	5·81	53·4	22·7	1/8
2 & 3	8·23	3·66	$2{\cdot}10^{-7}$	$2{\cdot}10^{-7}$	1/8
2 & 4	1·22	3·82	12·0	51·8	1/8
3 & 4	13·5	11·4	22·9	13·2	1/8

4.2 Glyphosate deposits on polyethylene discs

The comparison of regression lines for trials 1 and 2 (Table 3), carried out in a high wind speed and intensity of turbulence and a low wind speed and intensity of turbulence respectively, indicated that neither the slopes nor the elevations were significantly different ($P = 0{\cdot}05$). The comparison between trials 1 and 3 that were both carried out in high wind speeds and intensities of turbulence indicated that neither the slopes nor the elevations were significantly different ($P = 0{\cdot}05$). The comparison for trials 1 and 4, with high and intermediate wind speeds and intensities of turbulence respectively, showed the attenuation rate was significantly faster ($P = 0{\cdot}05$) and the elevation was significantly lower in trial 1 ($P = 0{\cdot}01$). The comparison for trials 2 and 3 indicated that neither the slopes nor the elevations were significantly different, consistent with the comparison between trials 1 and 2, as expected. The comparison between trials 2 and 4 with low wind speed and intensity of turbulence and intermediate wind speed and intensity of turbulence conditions respectively, showed that the slopes were not significantly different but the elevation was higher in trial 4 ($P = 0{\cdot}01$). The comparison between trials 3 and 4 showed the attenuation rate was significantly faster, and the elevation was lower in trial 3 ($P = 0{\cdot}01$), consistent with the comparison between trials 1 and 4.

In summary, deposits on discs were similar in trials 1, 2 and 3 in high wind speed and intensity of turbulence and low wind speed and intensity of turbulence conditions, but generally higher in trial 4 during conditions of intermediate wind speed and intensity of turbulence. The trend observed in glyphosate deposits measured at the 50-m station differs from that in the regression lines in that deposits increased with wind speed, as for deposits on ground sheets. This result is presumably also caused by the different large-drop proportions caused by the different cloud flight times (see Section 4.1).

4.3 Comparison of deposits on sheets and discs

For each trial a comparison was made of the regression lines fitted to measurements of glyphosate deposit on ground sheets (Fig. 1) and polyethylene discs (Fig. 2). No statistically significant differences (Table 4) were found between the slopes or the elevations of the regression lines ($P = 0{\cdot}05$) despite the differences in collector size, position and orientation. This similarity was probably caused in part by the small vertical separation of the collector types (2 m) and the very open canopy, which would have led to similar nearby spray-cloud concentrations, although the different size and location of the collectors would have led to different wind speeds and collection efficiencies for the airborne drops.

4.4 Drop evaporation

Although the drop-size spectra released in these trials were similar, the inter-trial differences in air temperature and relative humidity caused differences in drop-size spectra after evaporation, and also in drop fall-speeds and impaction efficiencies. Residual drop sizes at various times after generation have been calculated to assess the effect of the unequal evaporation rates. The evaporation rate of a water drop in the atmosphere depends on its size and on the local air temperature and relative humidity. The diameter of a drop at any time after generation may be calculated from its initial diameter and these meteorological variables.[12] Table 5 shows calculated water drop sizes at various times after generation under the air temperature and relative humidity conditions experi-

TABLE 4
F-values from Comparison of Regression Lines for Deposits on Ground Sheets and Discs

Trial numbers	*F-values for slope comparison*	*F-values for elevation comparison*	*DF*
1	3·62	$7{\cdot}10^{-6}$	1/8
2	0·0021	$3{\cdot}10^{-7}$	1/8
3	2·01	$1{\cdot}10^{-7}$	1/8
4	2·92	$5{\cdot}10^{-6}$	1/8

TABLE 5
Calculated Residual Water Drop-Sizes at Various Times after Generation

Time after generation (s)	*Drop diameter (μm)*					
	Trial					
	1	*4*	*1*	*4*	*1*	*4*
0	80	80	150	150	280	280
30	69	60	145	140	277	275
100	34	16	131	115	270	263
200	16	16	110	61	260	244

enced during trials 1 and 4, assuming that the initial diameters were those at the 10th, 50th and 90th volume percentiles, and a lower drop-size limit resulting from the low-volatility surfactant. The tabulated values provide a good approximation to the residual drop sizes obtained with the present tank mix, which comprised over 950 ml litre^{-1} water and 10 ml litre^{-1} of surfactant. Of the four trials, the meteorological conditions during trial 1 provided the slowest evaporation, i.e. lowest air temperature and highest relative humidity, while the conditions during trial 4 provided the fastest evaporation. However, these results show that, over the range of downwind distances used, only small between-trial differences in airborne drop-size spectra were caused by the unequal evaporation rates. The effects of unequal air temperatures and relative humidities on the airborne drop-size spectrum were therefore not thought to be of primary importance in causing the observed differences in off-target deposits.

5 CONCLUSIONS

These results show that the potential for environmental impact of off-target deposits is greatly affected by the meteorological conditions during herbicide applications. Off-target deposits beyond 100 m downwind of a typical aerial herbicide application for forestry were unaffected or reduced by increased wind speed or intensity of turbulence. This implies that off-target deposits beyond 100 m from release are not reduced by setting an upper wind-speed limit, and that by using a buffer-zone width adequate to prevent excessive off-target deposits caused by large-drop drift (50–100 m) forestry herbicides could be applied in a wider range of wind speeds ($> 2{\cdot}2$ m s^{-1}) than that currently used without causing increased environmental impact in sensitive areas. This would provide a substantially wider time window for herbicide operations and make them more timely and efficient, and therefore less costly.

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Initial deposits and persistence of forest herbicide residues in sugar maple (*Acer saccharum*) foliage

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Initial deposition and subsequent fate of herbicide residues in sugar maple (*Acer saccharum* Marsh.) foliage were quantified following applications of three different formulations of glyphosate (VISION®, TOUCHDOWN®, MON14420) and one formulation of triclopyr ester (RELEASE®) in a comparative field study. Maximum initial residues were 529, 773, 777, and 1630 mg of acid equivalent per kilogram dry mass, respectively. Initial foliar residues were dependent upon application rate (r^2 = 0.63 to 0.87) and increased by a similar factor (233 to 313 mg·kg^{-1}) for each kilogram per hectare applied, irrespective of formulation type. Foliar residues dissipated following a negative exponential pattern with time, rates of which varied with initial concentration. Mean times to 50% dissipation were 2 days for all glyphosate formulations, 1.5 days for triclopyr ester, and 4 days for triclopyr acid. Mean times to 90% dissipation were <16 days for glyphosate formulations, 9 days for triclopyr ester, and 33 days for triclopyr acid. Multivariate analyses of intercept and rate parameter estimates indicated significant (p = 0.02) differences in dissipation patterns among treatments. Orthogonal contrasts confirmed a priori hypotheses that glyphosate residue dissipation was independent of the salt formulation applied, and that triclopyr ester dissipated faster than either glyphosate (p = 0.004) or triclopyr acid residues (p = 0.07). Results are considered in terms of the exposure and resultant potential toxicity to forest songbirds inhabiting or foraging in treated hardwood canopies.

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Le dépôt initial et le sort ultérieur des résidus d'herbicide dans le feuillage d'érable à sucre (*Acer saccharum* Marsh.) ont été mesurés après l'application de trois formulations différentes de glyphosate (VISION®, TOUCHDOWN®, MON14420) et une formulation d'ester de triclopyr (RELEASE®) dans le cadre d'un essai comparatif au champ. Les résidus initiaux maximaux étaient respectivement de 529, 773, 777 et 1630 mg d'équivalent acide par kilogramme de masse sèche. Les résidus foliaires initiaux dépendaient de la dose appliquée (r^2 = 0,63 à 0,87) et augmentaient de façon semblable (233 à 313 mg·kg^{-1}) pour chaque kilogramme par hectare appliqué, peu importe la formulation. Les résidus foliaires se sont dissipés avec le temps selon une courbe exponentielle négative, le taux de décroissance étant fonction de la concentration initiale. Les temps moyens pour atteindre 50% de dissipation étaient de 2 jours pour toutes les formulations de glyphosate, 1,5 jours pour l'ester de triclopyr et 4 jours pour l'acide de triclopyr. Les temps moyens requis pour obtenir 90% de dissipation étaient inférieurs à 16 jours pour les formulations de glyphosate, 9 jours pour l'ester de triclopyr et 33 jours pour l'acide de triclopyr. Des analyses multivariées des estimés de l'ordonnée à l'origine et du taux de décroissance ont révélé des différences significatives (p = 0,02) dans les patrons de dissipation entre les traitements. Des contrastes orthogonaux ont confirmé les hypothèses a priori voulant que la dissipation des résidus du glyphosate soit indépendante de la formulation et que ceux d'ester de triclopyr disparaissent plus rapidement que ceux de glyphosate (p = 0,004) ou d'acide de triclopyr (p = 0,07). Les résultats sont examinés quant à l'exposition et la toxicité potentielle en résultant pour les passereaux chanteurs forestiers habitant ou se nourrissant dans les couverts feuillus traités.

[Traduit par la Rédaction]

Introduction

Conifers grow most rapidly, and to their greatest sizes, when site resources are fully available (Newton et al. 1992). A variety of grass, forb, and brush species may impair successful conifer regeneration through interspecific competition for light, nutrients, and moisture (Walstad and Kuch 1987). Application of synthetic herbicides continues to be a primary means of managing competing vegetation. Based on area treated, the isopropylamine (IPA) salt of glyphosate, marketed under the trade name VISION®[2] (Monsanto Canada Inc.), accounts for approximately 81% of all herbicide applications in Canadian forestry (Campbell 1990). Although the IPA salt is the only formulation of glyphosate currently registered for such use, experimental formulations such as MON14420 (Monsanto Canada Inc.) and TOUCHDOWN® (ICI-Chipman Canada Inc.), which involve different counterions (Fig. 1) to yield the monoammonium (MAS) and

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[2]Mention of trade names is for information only and does not imply endorsement or disapproval by the authors.

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trimethyl-sulfonium (TMS) salts, respectively (Table 1), have been under development (Pitt et al. 1993). Franz (1974, as cited in Grossbard and Atkinson 1985), demonstrated the similarity in herbicidal effectiveness of glyphosate acid and many of its soluble salts, indicating that the counterion generally influences solubility in the formulation, but not overall biological activity.

Brecke and Duke (1980) demonstrated that glyphosate penetrates plant cuticles within 4-h, but is only slowly taken up by mesophyll cells. Caseley and Coupland (1985) suggest that glyphosate uptake is a biphasic process with initial rapid penetration, followed by slower symplastic uptake, the duration of both steps being dependent on a number of factors, including species, age, environmental conditions, concentration of glyphosate, and concentration of the surfactant. Green et al. (1992) demonstrated that foliar absorption of glyphosate was slow in both red maple (*Acer rubrum* L.) and white oak (*Quercus alba* L.), with only 37 to 38% absorbed over a period of 7 days, and no significant increase in absorption thereafter. Similarly, D'Anieri et al. (1990) reported 42% foliar absorption in red maple over 14 days. Only the former authors suggested that differential foliar absorption played an important role in hardwood tolerance. However, both studies indicated that differential symplastic translocation was an important factor in observed tolerance of red maple. Recent studies by Leung and Webster (1993) also indicate that uptake into foliage of trembling aspen (*Populus tremuloides* Michx.) is slow, with 62% of initial foliar deposits recoverable in leaf washes at 36 h post-treatment.

Owing to weak efficacy of glyphosate on various maple species (*Acer* spp.) (Moore 1985; D'Anieri et al. 1990; Pitt et al. 1992), Canadian forest herbicide research has focused on evaluation and development of compounds with a complimentary activity spectrum. In this regard, RELEASE® (DowElanco Canada Inc.), which contains triclopyr formulated as the butoxyethyl ester (BEE) (Fig. 1), has received the greatest attention, since previous studies in the United States (Newton and Knight 1981; Moore 1985) document its efficacy on glyphosate-tolerant species, including maples.

Bentson (1990) reviewed the fate of xenobiotics in foliar pesticide deposits, noting that the major dissipative processes involved are wash-off by rain, penetration (and translocation), volatilization, and photodegradation. Bentson and Norris (1991) investigated the influence of temperature, illumination, and time on the disposition of triclopyr residues in Pacific madrone (*Arbutus menziesii* Pursh) and giant chinkapin (*Castanopsis chrysophylla* Dougl.) following application of triclopyr BEE. These studies showed an exponential increase in loss of BEE with increased temperature, and significant losses in foliar deposits via photolysis. In both cases, the effects were species specific, indicating that degradative losses were more important in species with less penetrable surfaces, while uptake and translocation were the dominant processes when penetration of the leaf cuticle was facile. Bentson (1990) concluded that most laboratory-generated information relating to herbicide–foliar interaction is unrepresentative of actual behaviour of the substance in the field.

A limited number of field studies document the fate of foliar residues of glyphosate and triclopyr under typical forest regeneration conditions (Feng and Thompson 1990; Fontaine 1990; Newton et al. 1984, 1990). However, none pertain to conditions typical of the Acadian Forest Region in Canada or to maple species that are key competitors in this region. Further, none of the completed studies examine potential differences in behaviour of the various salts of glyphosate or the fate of BEE and acid forms of triclopyr in hardwood foliage.

Ghassemi et al. (1982) correctly stated that field experiments conducted to develop efficacy data provide excellent "piggyback" opportunities for cost-effective collection of environmental data which is needed for accurate assessment of potential environmental effects of herbicides. In consideration of these aspects, a comparative field study was initiated under typical regeneration conditions of the Acadian Forest Region to investigate the efficacy and environmental fate of three different salt formulations (IPA, MAS, TMS) of glyphosate, as well as triclopyr BEE and acid. In addition to addressing scientific data gaps identified above, the study was designed to provide "bridging data" to demonstrate the expected similarities in efficacy and environmental behaviour of generic glyphosate products. Results relating to the comparative efficacy of these materials have been reported previously (Pitt et al. 1993). In this paper, we report on the initial herbicide residues and persistence in foliage of *Acer saccharum* Marsh. and draw relationships among the chemical fate, efficacy, and environmental toxicology associated with these compounds.

Materials and methods

Site description, experimental design, and chemical treatment

Detailed descriptions of the study site and experimental design are provided in Pitt et al. (1993). The chosen site, 16 ha in size, was located approximately 30 km north of Fredericton, New Brunswick. The site had been full-tree harvested in 1986 and replanted with black spruce (*Picea mariana* (Mill.) B.S.P.) container stock in 1987. Subsequently, the site was inundated by a variety of competitive shrub species, among which sugar maple predominated.

In July 1989, the site was divided into 66 plots measuring 40 × 25 m (0.1 ha) and separated by 10-m vegetative buffers (Fig. 2). The experimental design was a randomized complete block with subsampling, and pretreatment crown volume index (CVI) was used as the blocking variable. The blocks were categorized as low (1110 to 2890 $m^3 \cdot ha^{-1}$), medium (3170 to 4180 $m^3 \cdot ha^{-1}$) or high (4260 to 7250 $m^3 \cdot ha^{-1}$) CVI. Experimental treatments took the form of five rates for each of four herbicide formulations, as well as one untreated control (Table 1). The highest treatment rate tested for each compound was equivalent to the maximum label rate proposed for use in Canadian forestry.

Chemical treatments were applied using three CO_2 pressurized backpack sprayers (model 4F, R&D Sprayers Inc., Opelousas, LA 70570) designed for herbicide applications to brush. The sprayers were equipped with a single 1/4 KLC-9 nozzle oriented at 0° (straight up) and were held at 4.5 m above ground level by an extended aluminum boom. All sprayers were calibrated to a boom pressure of 186 kPa, yielding a drop spectrum with a relatively large volume median diameter of 1089 μm and an application volume of 4.32 $L \cdot min^{-1}$. With a metronome-controlled walking speed of 0.76 $m \cdot s^{-1}$ and a swath width of 9.5 m at 2 m above ground level, the resultant spray volume application rate was 100 $L \cdot ha^{-1}$. Chemical treatments were applied to the low, medium, and high CVI blocks on the mornings of September 4, 5, and 6, 1989, respectively. Immediately prior to application, premeasured water and herbicide volumes were thoroughly mixed in 12-L plastic containers, and the entire mixture was decanted into the stainless steel sprayer tank. Prior to the treatment of each block, sprayers were randomly assigned to individual

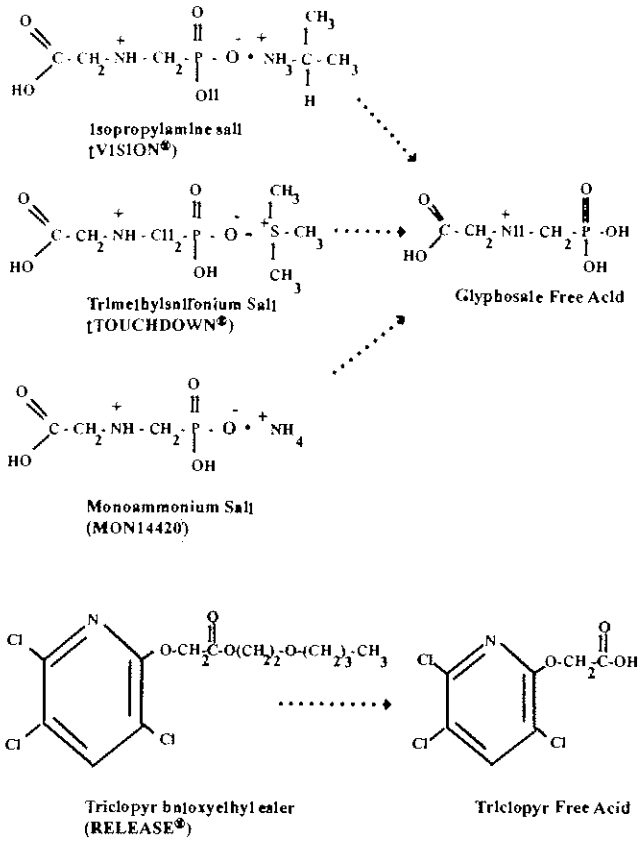

FIG. 1. Chemical structure of formulated and active ingredients of herbicide products VISION®, MON14420, TOUCHDOWN®, and RELEASE®.

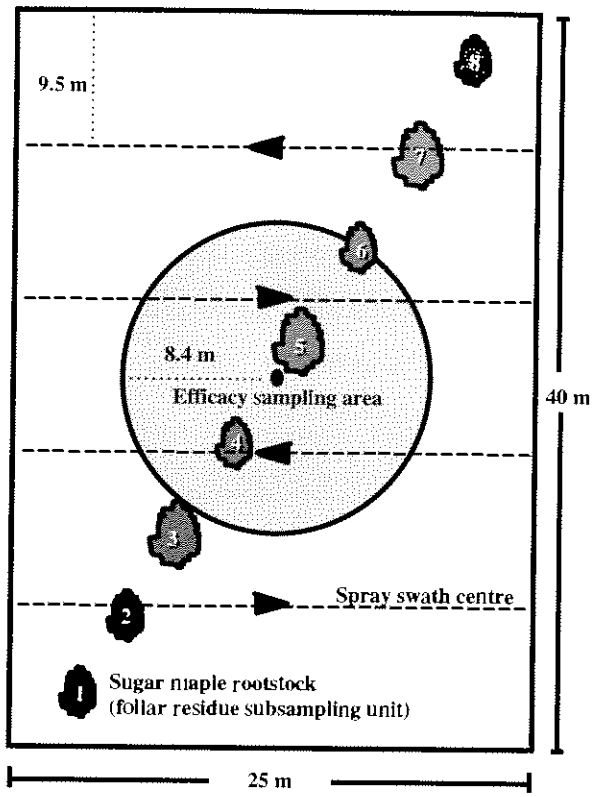

FIG. 2. Experimental design layout for 1 of 66 experimental units showing the environmental fate and efficacy subsampling locations relative to spray swath centres.

herbicide formulations and applications were made in sequence of increasing herbicide rate to minimize cross-contamination errors. Applications were made to each plot using a total of four passes along premarked track spacing (9.5 m) to span the plot width (Fig. 2).

Immediately following application to each plot, residual volumes were decanted from the spray tank, measured, and recorded. At the same time, a 10-mL subsample was taken from each tank mix using a graduated pipet and transferred to a labeled glass dram vial (50 mL) with screw-cap closure. Tank-mix samples were stored and transported back to the laboratory on ice and frozen within 14 h of application. The active ingredient concentrations of residual spray mix volumes were analytically determined and used to calculate the rates of chemical applied to each plot.

Meteorological monitoring

A variety of meteorological parameters were monitored during herbicide applications to characterize conditions at the time of treatment. Wind speed and direction were measured at 4.5 m above ground using a cup anemometer (threshold speed 1.2 $km \cdot h^{-1}$) and wind vane (Heathkit Digital Weather Computer model ID4001, Heath Co., Benton Harbor, Mich.). Instantaneous values were recorded manually at 5-min intervals and averaged over the period of application. Air temperatures were monitored using shaded thermocouples set at 1.5 and 4.5 m above ground, and relative humidity was measured using a wet-dry bulb psychrometer (Campbell Scientific Inc., Logan, Utah) at 4.5 m above ground. During periods of herbicide application, a visual estimate of leaf wetness was made.

Continuous monitoring of meteorological conditions pertinent to foliar residue persistence was restricted to air temperature and relative humidity using the same equipment and methodology as described above. In addition, rainfall was measured throughout the experimental period using a tipping-bucket rain gauge (model RG 2501, Campbell Scientific Inc., Logan, Utah) set to provide contact closure for every 1 mm of rainfall. Air temperature, relative humidity, and rainfall were monitored at 5-min intervals and recorded as 24-h averages using a CR21X data logger (Campbell Scientific Inc., Logan, Utah).

During the three morning spray sessions (06:00–08:00 of September 4, 5, 6, 1989) wind speed monitored at spray release height did not exceed 4.6 $km \cdot h^{-1}$. Ocular estimates of percent foliar surface moisture indicated that dew was present on the foliage at the beginning of each session, with values at 06:30 of 90, 100, and 75% remaining throughout the spray period but declining slowly to values of 50, 30, and 30% foliar surface moisture, respectively, by 08:30. Average daily temperature and relative humidity during the days of application ranged from 17 to 19°C, and 81 to 86%, respectively. No rainfall occurred from September 2 to 14, resulting in a minimum rain-free period for all treatments of 168 h postapplication. The first rainfall event occurred on September 14, 1989, with a minimal total precipitation of 0.254 mm; subsequently, major rain events (>0.5 mm) germane to foliar residue behaviour occurred on September 15 (1.0 mm), 17 (2.54 mm), 20 (5.59 mm), 23 (1.8 mm), 24 (2.29 mm), and 26 (3.05 mm).

Foliar residue sampling

On each plot, a diagonal transect was established from which eight individual rootstocks of sugar maple were selected and tagged for subsequent foliar residue sampling (Fig. 2). Foliar sampling involved harvesting 15 exterior leaves circumferentially and in equal proportion from upper, middle, and lower sections of the crowns of tagged individuals. The harvested foliage was combined to form a single pooled sample for each plot, frozen within 6 h of sampling, and stored frozen (−18°C) pending analysis.

TABLE 1. Nomenclature, composition, and nominal application rates of herbicide treatments tested

Trade name	Formulated ingredient*	Active ingredient†	Nominal application rates ($kg \cdot ha^{-1}$ of a.i.) 1	2	3	4	5
VISION®	Glyphosate IPA ($480\ g \cdot L^{-1}$)	Glyphosate acid ($356\ g \cdot L^{-1}$)	0.25	0.72	1.19	1.67	2.14
TOUCHDOWN®	Glyphosate TMS ($480\ g \cdot L^{-1}$)	Glyphosate acid ($330\ g \cdot L^{-1}$)	0.23	0.67	1.11	1.54	1.98*
MON14420	Glyphosate MAS (74.4% w/w)	Glyphosate acid (68% w/w)	0.25	0.72	1.19	1.67	2.14
RELEASE®	Triclopyr BEE ($667\ g \cdot L^{-1}$)	Triclopyr acid ($480\ g \cdot L^{-1}$)	0.40	1.26	2.12	2.98	3.84

NOTE: Application rates for TOUCHDOWN® are equivalent to those of VISION® and MON14420 in terms of the amount of formulated ingredient (salt content) applied.

*IPA, isopropylamine salt; TMS, trimethyl-sulfonium salt; MAS, monoammonium salt; BEE, butoxyethyl ester.

†The guaranteed concentrations are given in parentheses.

Initially (i.e., within 6 h postapplication), foliar samples were taken from all treated plots and used for estimating foliar impingement, mean on-target deposit, and variability, as well as for correlation with the calculated application rate. Subsequent to the day of application, foliar samples were collected from plots treated at the highest application rate only, on a schedule of 1, 3, 7, 14, 21, 28, 35, and 42 days post-treatment. The resulting data were used to assess dissipation patterns and persistence of foliar residues until 90% leaf drop had occurred (approximately 42 days after initiation of the experiment). Time to 50% (DT_{50}) and 90% (DT_{90}) dissipation were calculated as statistical end points characterizing the persistence of herbicide residues in sugar maple foliage. Separate samples (five leaves per individual rootstock) were obtained, pressed, and dried to allow subsequent image analysis and calculation of mean ($n = 40$) leaf surface area of sugar maple foliage sampled from each plot. Leaf area estimates allowed initial deposits on sugar maple foliage to be additionally expressed in terms of unit area of the impinging surface ($\mu g \cdot cm^{-2}$).

Analytical methodology

Tank-mix subsamples and foliar residues associated with the three glyphosate formulations (VISION®, TOUCHDOWN®, MON14420) were analyzed using high-performance liquid chromatography and visible wavelength detection (HPLC–VIS) following the basic technique described by Thompson et al. (1990). A capillary gas – liquid chromatographic technique with electron capture detection (GLC–ECD) based on that described by Thompson et al. (1991) was used in quantifying triclopyr concentrations in tank-mix samples and foliar residue levels. Triclopyr ester and acid concentrations in tank-mix samples were determined with ester values converted to acid equivalence using a factor based on the molecular weight ratio of the two compounds (ester–acid, 1.39:1). Critical specifications for the analytical methods used are reported in Table 2.

Frozen tank-mix samples were thawed at room temperature and thoroughly mixed prior to obtaining a subsample (1 mL) for dilution and analysis. For glyphosate analyses, subsamples were diluted (1:100 mL) in distilled water and then serially with appropriate volumes of the mobile phase buffer, to bring the final injected aliquot (100 µL) into the linear range of the HPLC detector. For triclopyr tank-mix analyses, subsamples were diluted in acetone and then serially into appropriate volumes of isooctane prior to injection of a 1-µL aliquot to the GLC–ECD system.

Prior to initiation of foliar residue analyses, analytical methods to be employed were validated by fortifying four replicate matrix blanks with three concentrations of each herbicide. Following an approximate 18-h equilibration period under cool, dark conditions, fortified samples were extracted and analyzed to determine the mean analytical recovery efficiency and precision (coefficient of variation) as well as chromatographic resolution, estimated limits of quantification (LOQ), and detection (LOD).

Field samples of foliage were defrosted, macerated, and homogenized using a Hobart chopper (Hobart Mfg. Co. Ltd., Don Mills, Ont.) and divided to provide one subsample (5 g) for percent moisture analysis and a second subsample (5 g) for extraction and residue analysis. Foliar residues of glyphosate were repetitively extracted (three times) using an aqueous base solution (total 200 mL of 0.5 M NH_4OH) and blended in an inverted mason jar fitted to a common household blender (Osterizer Galaxie 14, Sunbeam Corp., Toronto, Ont.). Following centrifugation and filtration, supernatant extracts were pooled and further purified using ion-exchange chromatography as described by Thompson et al. (1990). Triclopyr residues (10 g macerated foliage per subsample) were extracted by repetitive shaking (2 × 15 min) in 45 mL of acetone–hexane (2:1). Extract supernatants were pooled and brought to a constant volume of 100 mL, from which an exact volume aliquot was obtained, diluted in isooctane, and injected directly for quantitation of BEE residues.

A second aliquot was partitioned from an acidic aqueous phase (pH < 2.5) to diethyl ether (2 × 20 mL). Ether extracts were combined and evaporated to near dryness with methanol as a keeper. Following evaporation, concentrates were quantitatively transferred to screw-cap tubes, brought to a final volume of 10 mL methanol, and methylated by reacting with 1 mL of BF_3 in 14% methanol (product 27419, BDH Inc., Toronto, Ont.) at 95°C for 1 h. Postmethylation, samples were diluted with distilled water and partitioned to hexane (3 × 10 mL). Hexane extracts were dried by passing through Na_2SO_4, concentrated to 2 mL by evaporation under nitrogen, and cleaned up by eluting concentrates loaded onto Florisil microcolumns (5 cm bed height) with hexane – ethyl acetate (90:10, 8 mL). Final samples of triclopyr methyl ester were prepared by evaporating eluates to near dryness under N_2, with isooctane as a keeper, and diluting concentrates to a constant volume of 10 mL in isooctane. The total mass of analyte in foliar samples was used in calculating foliar residues of each herbicide and expressed as mass of active ingredient per unit dry mass of sugar maple foliage ($mg \cdot kg^{-1}$).

Throughout the period of field-sample analyses, quality control was monitored by incorporating two blank samples fortified at varying concentrations in each batch of six to eight field samples processed. Validation tests conducted prior to the experiment (Table 3) and quality-control data generated through a check sample program conducted concurrently with analyses of field samples confirm the accuracy and precision of analytical methods used in this experiment. Validation tests demonstrated mean recovery efficiency ranging from 76.87 to 95.75% and excellent precision with coefficients of variation (CV) less than 10% for all formulations and fortification rates tested. Analysis

TABLE 2. Specifications of analytical methodology used in quantifying glyphosate and triclopyr residues in tank mixes and sugar maple foliage

Specification	Glyphosate method	Triclopyr method
Chromatographic	HPLC	GLC
System	Varian 5560	Varian Vista 6000
Detector	UV-200 @ 570 nm	^{63}Ni electron capture
Autosampler	Varian 8085	Varian 8000
Injection technique	na	Hot on-column
Final sample volume	10 mL mobile phase	10 mL isooctane
Injection volume	100 μL	2 μL
Column type	Antinex A-9 (10 cm × 4.6 mm i.d.)	DB-5 0.25-μm film (30 m × 0.25 μm i.d.)
Mobile phase	0.005 KH_2PO_4 (pH 1.9) with 4% methanol (0.55 mL·min^{-1})	Nitrogen (1 + 29 mL·min^{-1})
Operating temperatures		
Injector	na	200°C
Column	50°C	85–250°C @ 30°C·min^{-1}
Detector	na	325°C
Postcolumn reactor	135°C	na
Derivatization	Ninhydrin postcolumn flow (0.5 mL·min^{-1}) coil (284 cm × 0.02 cm i.d.), Varian model V reactor	Methylation @ 95°C (14% BF_3 in methanol)
Retention time	7.1 min	9.04 min
Detector linearity	100–2500 ng injected	10–40 pg injected
Limit of detection	0.10 μg·g^{-1}	0.02 μg·g^{-1}
Validated limits of quantification	0.5 μg·g^{-1}	0.1 μg·g^{-1}

of variance (ANOVA) provided no evidence of significant ($p > 0.05$) differences in recovery efficiency between rates within treatments or between chemical formulations. Similarly, results of the quality control check sample program indicated recovery efficiency ranging from 79.66 to 87.07%, with mean precision over the entire period of analysis of less than 12% for all herbicide formulations. Mean recoveries taken from the quality-control data for each chemical formulation were used in deriving a factor (100/mean) to correct reported foliar residue concentrations for analytical recovery efficiency. The correction factors employed were 1.26, 1.29, and 1.25 for glyphosate residues from VISION®, TOUCHDOWN®, and MON14420 treatments, respectively, and 1.15 for triclopyr acid residues generated by RELEASE® treatments. Recovery of triclopyr BEE was essentially quantitative and thus did not require application of a correction factor.

Statistical methodology

Analytical quality-control data were calculated and reported in terms of percent recovery efficiency relative to known fortification levels. Means ($n = 3$) and coefficients of variation were calculated for each fortification level – analyte combination tested. Data were subjected to ANOVA to detect significant differences in mean recovery efficiency relative to different fortification levels for a given herbicide.

The application rate applied to each plot was calculated as follows:

[1] $CR = k_1(TMC) \times k_2(VRA)$

where

CR is calculated rate (kg·ha^{-1})

TMC is tank-mix concentration (g·L^{-1})

VRA is volume rate applied per 0.1-ha plot as determined from the subformula (12 − RV), where RV is the tank-mix residual volume measured in litres

k_1 is a constant factor of 10^{-3} required to convert TMC in grams per litre to concentration expressed in kilograms per litre

k_2 is a constant factor of 10 required to convert VRA in litres per 0.1 ha to litres per hectare

The mean ($n = 3$), standard deviation (SD), and CV for each treatment were calculated to verify nominal application rates. Calculated rates for each plot were used as the independent variable in simple regression analysis with initial foliar residue concentrations (mg·kg^{-1}). The coefficients of determination derived from these regressions allowed assessment of the degree of variation in foliar deposits, which could be accounted for by the calculated rate alone, and by deduction, the amount of variation associated with other factors.

Following preliminary examination of scatterplots, dissipation of foliar residues of each herbicide was determined by nonlinear regression analysis using a quasi-Newton procedure for least-squares estimation (StatSoft Inc. 1986). A consistent inverse relationship between initial foliar residues and CVI was observed in the residue data for day 0, suggesting a substantive block effect. Therefore, dissipation of foliar herbicide residues (total μg) was modeled by block, using an exponential decline function of the form

[2] $Y = \alpha e^{(\beta X)} + \varepsilon$

where

Y is mass of active ingredient per unit dry mass of sugar maple foliage (total μg)

X is time (days postapplication)

e is euler 2.712

α is the parameter for the intercept

β is the parameter for the rate of change of Y vs. X

ε represents random errors assumed to be normally distributed with mean 0 and constant variance

Nonlinear regression equations derived for block–treatment combinations were examined for significance of the parameter estimates ($p < 0.05$). Models were accepted if residual distributions and residuals plotted against predicted values complied

TABLE 3. Validation data for analytical methods used in quantifying glyphosate and triclopyr residues in sugar maple foliage

Analyte	Fortification concentration (mg·kg dry mass^{-1})*	Recovery efficiency (%)		
		Mean (n = 3)	SD	CV
VISION®, glyphosate	4.272	82.44	2.55	3.10
	1.956	94.79	5.64	5.95
	0.427	79.30	1.94	2.45
TOUCHDOWN®, glyphosate	3.96	78.93	7.79	9.87
	1.815	79.75	2.62	3.29
	0.396	76.87	4.41	5.74
MON14420, glyphosate	3.264	92.34	5.83	6.32
	1.795	80.16	1.42	1.77
	0.489	78.68	2.38	3.02
RELEASE®, triclopyr	5.0	95.75	8.67	9.05
	0.5	84.19	7.80	9.27
	0.1	86.32	7.48	8.67

*For glyphosate formulations, test rates were established to equate to expected field levels on a surface-area basis and biased high to confirm that high analyte concentrations do not exceed ion-exchange resin capacities. Previous research has documented the quantitative capability of the technique at low concentrations (i.e., 0.1 mg·kg dry mass^{-1}) in hardwood foliage (Thompson et al. 1989).

TABLE 4. Nominal and mean calculated application rates relative to initial foliar deposits and residues observed for each herbicide treatment level tested

Chemical	Rate (kg·ha^{-1})*				Error† (%)	Deposit (μg·cm^{-2})			Residue (mg·kg^{-1})		
	Nominal	Calculated	SD	CV		Mean	SD	CV	Mean	SD	CV
VISION®	2.134	2.10	0.06	2.90	−3.07	36.21	3.87	10.68	499.08	33.20	6.65
	1.663	1.67	0.09	5.12	0.70	32.71	8.28	25.32	495.11	116.50	23.53
	1.192	1.15	0.14	12.17	−4.20	21.53	7.36	34.19	287.39	130.32	45.35
	0.721	0.70	0.02	2.47	−2.10	10.37	1.85	17.84	128.07	2.62	2.05
	0.25	0.25	0.03	12.00	0.00	4.67	1.93	41.34	57.78	28.70	49.68
TOUCHDOWN®	1.98	1.83	0.12	6.76	−15.3	36.58	16.9	46.44	508.83	232.78	45.75
	1.543	1.52	0.13	8.58	−2.63	18.69	1.04	5.56	280.14	19.15	6.83
	1.107	1.06	0.10	9.13	−4.37	15.65	3.25	20.75	225.28	10.58	4.69
	0.671	0.64	0.09	13.36	−3.43	9.46	3.66	38.76	147.73	52.46	35.51
	0.235	0.23	0.02	6.74	−0.83	4.45	1.60	35.99	71.25	18.02	25.28
MON14420	2.134	2.18	0.15	6.93	4.60	45.08	6.87	15.24	624.06	134.38	21.53
	1.663	1.63	0.07	4.17	−2.97	37.55	3.84	10.22	465.65	93.33	20.04
	1.192	1.21	0.11	8.71	1.80	31.74	8.71	27.46	428.05	58.87	13.75
	0.721	0.73	0.06	8.33	1.23	21.99	3.44	15.64	294.93	16.54	5.61
	0.25	0.26	0.02	5.80	1.33	4.06	2.51	61.89	55.90	32.70	58.49
RELEASE®	3.48	4.90	0.46	9.35	142.0	126.6	69.17	54.65	1204.7	476.95	39.59
	2.98	3.25	0.16	4.97	27.3	39.84	9.11	22.86	456.61	142.22	31.15
	2.12	2.13	0.21	9.79	0.67	48.50	21.9	45.22	546.46	196.27	35.92
	1.26	0.89	0.46	51.37	−37.3	31.79	7.04	22.15	302.92	74.60	24.63
	0.4	0.32	0.09	28.65	−8.3	9.14	3.59	39.32	104.38	37.72	36.13

*Calculated rates are based on residual tank-mix volumes and active ingredient concentrations.
†Difference between calculated and nominal rates.

with standard regression assumptions (Neter et al. 1985) and the model accounted for more than 80% of the variation in the data. The parameter estimates (a and b) derived from the exponential decline models were used as primary data in a multivariate analysis (MANOVA), wherein both rate and intercept parameter estimates were tested simultaneously to elucidate differences among rates of decline for the various herbicides tested (Meredith and Stehman 1991). Orthogonal contrasts were employed to examine the a priori hypotheses that foliar residue dissipation patterns were equivalent across the three glyphosate and triclopyr ester formulations tested. An analogous multivariate procedure was employed to examine statistical differences associated with dissipation of ester and acid forms of triclopyr specifically. The latter test is, in effect, a multivariate paired t-test (acid vs. ester) with three replications (low, medium, and high CVI blocks).

TABLE 5. Regression statistics for application of model [2] to foliar herbicide residue dissipation following high rate applications to sugar maple, and predicted times to 50% (DT_{50}) and 90% (DT_{90}) dissipation

Treatment	Block*	Proportion of variance†	a	SE	p	b	SE	p	DT_{50}	DT_{90}
TOUCHDOWN®	L	0.98	3 875.72	216.59	0.000	−0.189	0.026	0.000	3.5	24.2
	M	0.97	2 084.12	130.49	0.000	−0.321	0.026	0.001	2.1	14.3
	H	0.95	1 706.16	140.46	0.000	−0.480	0.138	0.013	1.4	9.6
VISION®	L	0.99	2 673.03	66.79	0.000	−0.229	0.017	0.000	3.0	20.0
	M	0.97	2 571.84	202.800	0.000	−0.426	0.085	0.000	1.6	10.8
	H	0.99	2 355.41	103.45	0.000	−0.533	0.078	0.001	1.3	8.6
MON14420	L	0.99	3 897.62	102.06	0.000	−0.243	0.015	0.000	2.8	18.9
	M	0.96	2 812.83	214.89	0.000	−0.291	0.060	0.003	2.3	15.7
	H	0.98	2 678.49	130.82	0.000	−0.318	0.041	0.000	2.1	14.4
RELEASE®										
Ester	L	0.99	14 820.33	593.21	0.000	−0.492	0.060	0.001	1.4	9.4
	M	0.96	11 571.95	800.85	0.000	−0.416	0.082	0.002	1.7	11.1
	H	0.98	5 990.00	290.64	0.000	−0.651	0.118	0.002	1.1	7.1
Acid	L	0.90	10 381.93	1683.35	0.000	−0.252	0.098	0.093	2.6	18.2
	M	0.90	7 047.88	1146.36	0.004	−0.092	0.032	0.184	5.7	48.2
	H	0.83	4 108.67	846.39	0.007	−0.255	0.123	0.229	ns‡	ns‡

NOTE: Model [2] is the exponential model $Y = ae^{(bX)} + \varepsilon$, with parameters as defined in the text.
*Based on pretreatment crown volume index categories: low (L), medium (M), or high (H).
†Proportion of variance accounted for by the nonlinear regression model (uncorrected regression sums of squares divided by uncorrected total sums of squares).
‡Nonsignificant b parameter, resulting in inability to estimate end points.

Finally, logarithmic transforms of the nonlinear regression equations were used to derive estimates for DT_{50} and DT_{90} end points by substituting 0.50 Y_0 and 0.1 Y_0 into each equation and solving for X, a technique referred to as "inverse regression" by Draper and Smith (1981).

Results and Discussion

Nominal and calculated application rates

A comparison of nominal and mean calculated application rates (latter based on measured residual volumes and active ingredient concentrations in tank mixes applied to each plot) are provided in Table 4. Only minor differences (i.e., within ±10%) in calculated and nominal rates of application were observed in the majority of cases, with observed differences primarily related to variable volume application rates. A known source of error in individual plot applications occurred on June 9, 1989, during application of TOUCHDOWN®, when a partial blockage of the spray nozzle resulted in a mean calculated application rate 15% lower than expected (Table 4). In general, analyses of active ingredient concentrations confirmed the accuracy of tank-mix preparations. As exceptions, concentrations of triclopyr in three of five RELEASE® tank mixes varied substantially from expected, resulting in the greatest deviation in the calculated application rates as compared with nominal values (Table 4). During this and other field experiments, we have observed general instability of the RELEASE® formulation. As a result, agitation is employed to maintain a homogeneous emulsion during application of this product. We postulate that the differences shown in Table 5 are sampling artifacts resulting from the formulation instability and length of time that residual mixtures may have been left standing following application and prior to sampling. This hypothesis was supported by subsequent "in-house" laboratory tests and by the fact that no errors of similar magnitude were observed for any of the glyphosate tank-mix analyses.

Initial foliar deposits

Initial deposits on sugar maple foliage, both in terms of residue concentration ($mg \cdot kg^{-1}$) and in terms of deposits per unit area of leaf surface ($\mu g \cdot cm^{-2}$) are summarized in Table 4. The overall average surface area for sugar maple leaves (n = 2440) was 71 cm^2 (CV = 13%). Considerable variation in mean (n = 3) foliar residues for the various treatment rates was observed, with coefficients of variation ranging from 2 to 58%. The overall variability observed for this study (avg. CV = 27%) is lower than that observed for aerial forestry applications (Newton et al. 1990; Thompson et al. 1992), probably reflecting the combined effects of low release height, low wind speed, low proportion of small drops in the spray cloud (10% quantile <618 μm), and high humidity, which would provide optimal on-target deposit in this study.

An inverse relationship between initial deposit and CVI was consistent across all treatments and suggests that differential "shading" or "foliar impingement surface potential" among the blocks was a factor in the observed variation of mean initial deposits. This relationship is reflected in proportionally higher Y-intercept and initial elevations in dissipation curves associated with the low crown volume block as compared with the medium and high blocks (Figs. 3 and 4). This effect is consistent with the more open nature of the canopy in areas with low crown volumes and the resultant increased probability of spray cloud impinging on the sampled maple clumps from any vector. In contrast, treatments in the high crown volume block have lower residues initially, since there is a higher probability of "shading" effects of non-sampled hardwood clumps within the brush canopy, a greater total surface area for impingement of the spray cloud, and a greater probability that sampled leaves were "sheltered" from full impingement of the spray cloud. The inverse relationship between impingement and CVI is contributory to

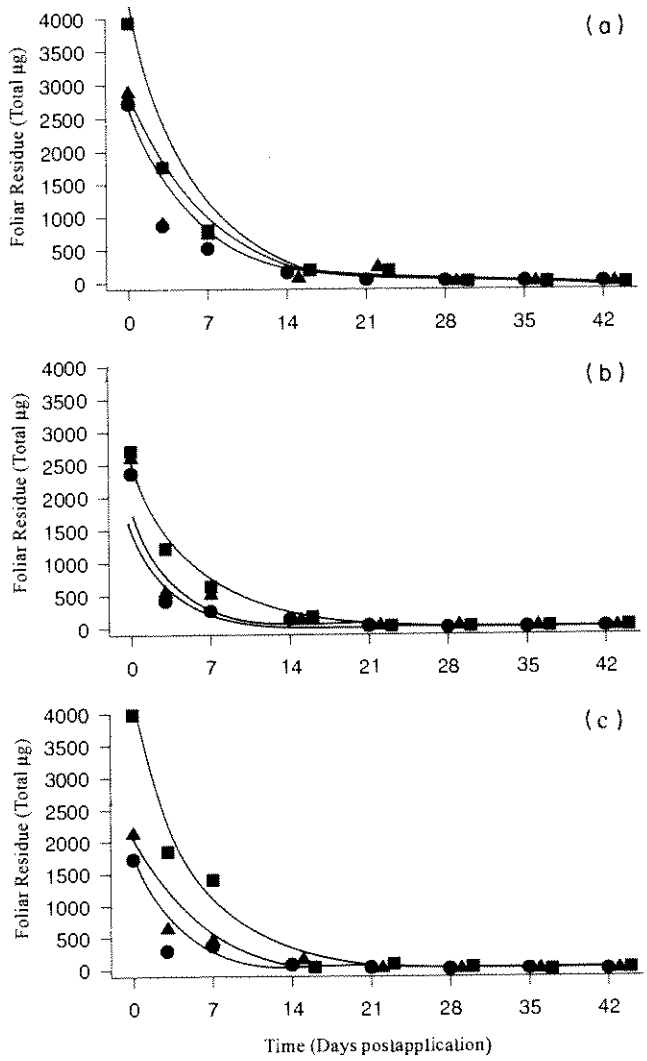

FIG. 3. Dissipation of glyphosate foliar residues as modeled by fitting exponential decline function [2] to data for (*a*) MON14420, (*b*) VISION®, and (*c*) TOUCHDOWN® treatments by block (low (■), medium (▲), and high (●) crown volume index). Validated limits of quantitation equate to 2.0 µg, total, for all glyphosate formulations.

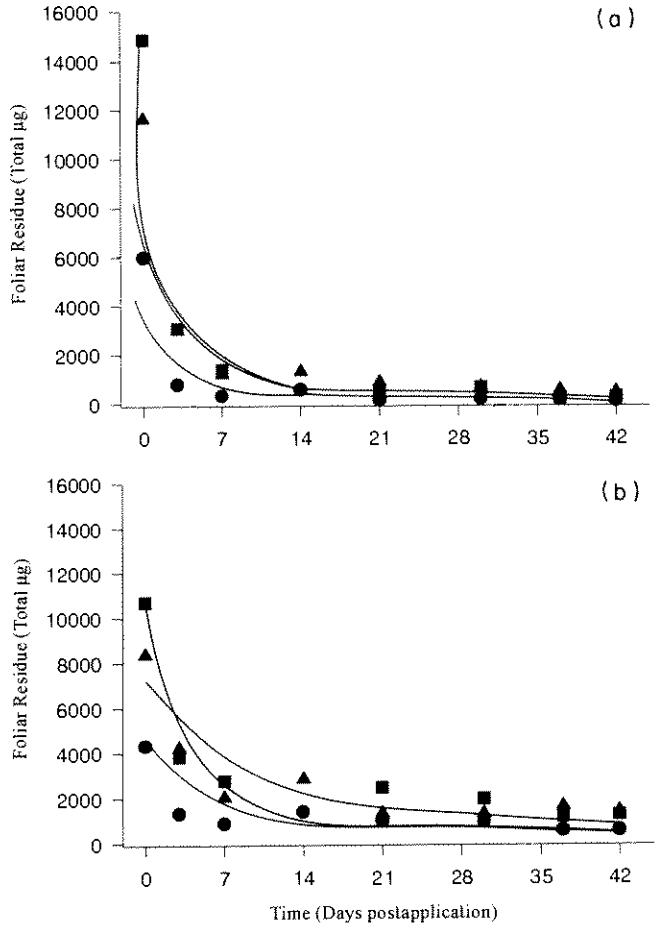

FIG. 4. Dissipation of (*a*) triclopyr ester and (*b*) triclopyr acid foliar residues as modeled by fitting the exponential decline function [2] to data by block (low (■), medium (▲), and high (●) crown volume index), respectively. Validated limits of quantitation equate to 0.5 µg, total, for both triclopyr ester and triclopyr acid.

the large proportion of variation accounted for by block effects as observed in the multivariate analyses (Table 6).

The maximum individual foliar concentrations observed in this study were 529, 773, 777, and 1630 mg·kg^{-1} for VISION®, TOUCHDOWN®, MON14420, and RELEASE®, respectively. High concentrations observed for release treatments are a function of the higher maximum label rate for this compound. As expected, linear regression analysis conducted separately for each herbicide formulation (df =1,14) confirmed that initial foliar residues were highly correlated with rates of application. Coefficients of determination (r^2 values) for VISION®, TOUCHDOWN®, MON14420, and RELEASE® treatments indicated that the calculated rate of application alone accounted for 87, 64, 79, and 63% of the variability in initial foliar residues, respectively. Poorer correlations observed for TOUCHDOWN® and RELEASE® reflect the operational problems encountered as discussed above. Regression coefficients indicated that foliar residues increased by a factor ranging from 233 to 313 mg·kg^{-1} per unit application rate (kg·ha^{-1}), irrespective of the herbicide treatment. This relation provides a basis for approximating expected foliar residue levels when calculated treatment rates are known. Initial foliar residue levels observed in this study are similar to values estimated in relation to nominal application rates by other workers. For example, glyphosate residue levels (131 to 224 mg·kg^{-1} per kg·ha^{-1}) were reported by Feng and Thompson (1990) in studies on red alder (*Alnus rubra* Bong.) and salmonberry, similar values (78 to 148 mg·kg^{-1} per kg·ha-$^{-1}$) were found by Newton et al. (1984) in red alder – bitter cherry following aerial applications. Although our estimated initial deposits of triclopyr are similar to those reported by Fontaine (1990) for trembling aspen (134 to 147 mg·kg^{-1} per kg·ha^{-1}), they are substantially higher than initial residues of triclopyr in crowns of tanoak (*Lithocarpus densiflorus*) (39 to 41 mg·kg^{-1} per kg·ha^{-1}), as estimated by Newton et al. (1990). Differential mass per unit surface area of foliage may be responsible for the observed differences among these various hardwood species.

Foliar residue dissipation

Dissipation of foliar residues was monitored for all herbicides in the high-rate treatments only, with seven sampling events over a period from 0 to 42 days postapplication (time of 90% leafdrop). The exponential decline function

TABLE 6. Summary statistics for two separate multivariate analyses applied to dissipation coefficients derived from exponential decline models fit to residues of glyphosate and triclopyr herbicides in sugar maple foliage

Source of variation	Wilks' λ	*F* value	*p* value
Treatments	0.0727	4.52	0.0180
VISION® vs. MON14420	0.6235	1.51	0.3069
VISION® vs. TOUCHDOWN®	0.8361	0.49	0.6392
All glyphosate vs. triclopyr ester	0.1135	19.52	0.0043
Blocks	0.2105	2.95	0.0754
Treatments (triclopyr acid vs. ester)	0.0053	93.16	0.0731
Blocks	0.0021	10.54	0.0886

NOTE: Overall treatment effects are partitioned into a priori contrasts of interest. While a formal test for differences among blocks does not exist, *F* and *p* values are provided to assess the effectiveness of blocking.

($Y = \alpha e^{(\beta X)}$) consistently provided the best fit for glyphosate and triclopyr foliar dissipation, generally accounting for >90% of the variation in response. In all cases, the parameter estimate *a*, which relates to the *Y*-intercept, or initial residue level, was significant ($p < 0.01$) and accurately reflected the observed inverse relationship between initial residue levels and crown volume in the brush canopy. Similarly, parameter estimate *b*, which relates to the dissipation rate constant, was significant ($p < 0.01$) in all cases except that for triclopyr acid residues in the high CVI block. A clear trend of more rapid dissipation in blocks characterized by high crown volume was also evidenced by differences in *b* parameter estimates (Table 5).

Estimates for the time to 50 and 90% dissipation were calculated based on the exponential decline models and are provided in Table 5. Although these persistence estimates reflected block trends in *Y*-intercepts and dissipation coefficients, the practical similarity in these end points was the most striking result. Mean DT_{50} values and standard deviations for the various glyphosate formulations tested were 2.4 ± 1.1, 2.0 ± 0.9, and 2.4 ± 0.4 days, for TOUCHDOWN®, VISION®, and MON14420, respectively. Similarly, estimated mean DT_{90} values were 16.0 ± 7.5, 13.2 ± 6.1, and 16.4 ± 2.3 days for the respective formulations. The DT_{50} values estimated for all glyphosate formulations in this study are substantially less than the 14-d half-life estimate provided by Newton et al. (1984) for glyphosate IPA salt in midcrown foliage of red alder and bitter cherry, as well as the 26.6-day value reported for shrubs (vine maple (*Acer circinatum* Pursh) – salmonberry).

Similarly, the DT_{50} values for triclopyr ester (avg. 1.5 days) and acid (avg. 4 days) residues in foliage as calculated in our study are substantially less than half-life values calculated by Newton et al. (1990) for total triclopyr residues in tanoak crown (DT_{50} = 74 days) or browse-layer (DT_{50} = 291 days) foliage. Given differences in site, plant species, meteorological conditions, application methods, and kinetic models applied, causation for the substantial differences in persistence estimates for triclopyr cannot be determined. However, we are in general agreement with Newton et al. (1990), who noted that first-order decay functions, which assume a constant rate throughout the period of observation, introduce serious bias by underestimating the initial rate of decay. Nonlinear regression techniques provide more accurate estimates of persistence, since the analysis directly addresses the inherent curvilinearity commonly observed in residue dissipation. Typical residue dissipation patterns are "biphasic" in nature, with an initial rapid decline phase followed by a slower phase in which low-level residues persist for lengthy periods. Such a pattern, perhaps more effectively modeled by segmented-regression techniques, may reflect the increasing effects of physicochemical processes over time, such as increased binding or conjugation, which may in turn reduce bioavailability as required for microbial degradation or metabolism in planta, and thus increase longevity of low-level residues.

Graphical presentation of the foliar residue data over time (Fig. 3) highlights the similarity in curvilinear dissipation patterns for the glyphosate treatments (VISION®, TOUCHDOWN®, and MON14420). A more rapid dissipation of triclopyr ester was observed, and the rate of ester decline was greater than that of the acid form of triclopyr (Fig. 4). Foliar residue levels were minimal and similar for all treatments within 24 days postapplication, irrespective of formulation or block (Figs. 3 and 4). In contrast, triclopyr acid residues in the medium CVI block appeared to persist for a somewhat longer period as compared with the low and high CVI blocks. Results demonstrate very rapid dissipation of both glyphosate and triclopyr ester residues in sugar maple foliage. Similarities and differences in dissipation patterns were confirmed through multivariate analyses and related orthogonal contrasts (Table 6), which show that the significance of the overall treatment effect ($p = 0.02$) is due to the highly significant difference ($p = 0.004$) between triclopyr ester and glyphosate dissipation patterns, rather than differences between VISION® and MON14420 ($p = 0.31$) or VISION® and TOUCHDOWN® ($p = 0.64$). Similarly, a separate multivariate paired *t*-test conducted on triclopyr residues verifies that dissipation of triclopyr acid is significantly slower ($p = 0.07$) than that for triclopyr ester.

The major dissipation mechanisms acting on foliar residues of herbicides are wash-off of poorly bound residues with rain or dew, volatilization, photolytic degradation, and uptake–translocation to other plant parts (Bentson 1990). Since no rainfall was recorded on this site for a minimum of 168 h postapplication, wash off was not a factor in this study. Glyphosate and its salts, as well as triclopyr acid, have exceedingly low vapour pressures ($<10^{-5}$ mm Hg), whereas the butoxyethyl ester of triclopyr has a relatively higher vapour pressure. Thus, while volatilization losses are not a consideration for glyphosate formulations or for triclopyr acid, such losses do represent a possible mechanism for dissipation of triclopyr ester from foliar surfaces. Bentson (1990) noted that the relative importance of volatilization

losses from foliar surfaces is poorly understood and involves complex interactions among the pesticide, the foliar surface, and atmospheric microsite conditions. Therefore, predictions based on physicochemical properties alone are unreliable estimators of real-world volatilization losses. However, under the cool, moist, and low-wind conditions germane to this experiment, we would expect volatilization losses of triclopyr ester to be minimized. This is particularly true if triclopyr ester penetration into maple foliage is essentially complete within the first 14 h postapplication, as was observed by Bentson and Norris (1991) in outdoor studies with Pacific madrone and chinkapin leaves. Photolytic degradation as a mechanism of foliar residue dissipation has not been well studied for either herbicide. Comparison of aqueous photolytic rates reported in the literature (Lund-Hoie and Friestad 1986; McCall et al. 1988; Woodburn et al. 1993) suggests that photolytic degradation is relatively less important for glyphosate than for triclopyr. Bentson and Norris (1991) concluded that in situ photodegradation may be a significant pathway of triclopyr foliar residue dissipation when other processes are inactive. Thus, the rapid dissipation of triclopyr ester observed here could be attributed to rapid foliar uptake and subsequent conversion to the acid form in planta, and (or) to the photolytic–hydrolytic degradation of deposits remaining on the leaf surface. Results of previous studies on the uptake and translocation of triclopyr in tanoak (King and Radosevich 1979; Bentson and Norris 1991) indicate that these processes may be inhibited under conditions in which the herbicide is applied to older leaves, or mature, less physiologically active plants or under cool conditions, all of which are factors characterizing the application scenario in this study. The rapid ester dissipation, combined with relatively lengthy persistence of triclopyr acid residue in the foliage, appears to support the postulate of poor uptake and translocation of triclopyr and provides a plausible explanation for the relatively poor efficacy of RELEASE® compared with glyphosate treatments (Pitt et al. 1993) under the conditions of this study. Contrary to expectations based on previous studies with red maple (Green et al. 1992; D'Anieri et al. 1990), these factors do not appear to have impaired uptake and translocation of the various glyphosate formulations to the point where efficacy was reduced.

Biological significance of foliar residues

Foliar herbicide residues have biological significance in terms of phytotoxicity to the target plant, potential toxicity to animals inhabiting or foraging in the canopy, and as a source of secondary inputs to other environmental compartments. The degree to which these effects are elicited is a function of the magnitude and duration of exposure as well as the inherent toxicity of the compound. Results of this experiment indicate that initially foliar residues approximating 233–313 $mg \cdot kg^{-1}$ are generated for each $kg \cdot ha^{-1}$ of herbicide applied. Although these values may be used as a general guideline for expected levels under operational scenarios, the inherent variability in such deposits must be considered. For example, the maximum foliar residues observed in this study were 777 $mg \cdot kg^{-1}$ for glyphosate and 1630 $mg \cdot kg^{-1}$ for triclopyr acid, with the difference clearly reflecting the proportionally higher maximal label rate for triclopyr (3.84 kg a.e.$\cdot ha^{-1}$).

The initial foliar residue levels were highly phytotoxic to target brush species, as evidenced by the degree of efficacy (i.e., ~21% reduction in total woody crown area) observed for all rates above one-quarter of the maximum label rate (Pitt et al. 1993). The high degree of efficacy is beneficial in terms of maximizing conifer growth but also leads to potential indirect effects on terrestrial wildlife through habitat alteration. Since herbicides are specifically designed to affect plants rather than animals, the potential for such indirect impacts are generally considered to be of greater biological significance than direct toxic effects and hence receive significant scientific attention (e.g., Sullivan 1990; Sullivan and Sullivan 1982; Lautenschlager 1993; Freedman et al. 1993). Bird species that inhabit and (or) forage within hardwood canopies may be considered as the wildlife group at highest risk of toxic effects, since they may be exposed both directly and indirectly to foliar residues as generated by typical herbicide applications in conifer-release programs.

In Canada and the United States, quotient methods are used in estimating the risk to wildlife that may result from exposure during or following pesticide applications. This approach involves extrapolation of exposure and effects on relatively few test species under highly controlled laboratory conditions involving single exposure mechanisms, to field scenarios involving multiple exposure routes, multiple species, and highly variable fate and persistence of residues as dictated by widely varying environmental conditions. The risk estimation process for wild birds is even further complicated by a poor understanding of the relative importance of the potential exposure pathways (oral, dermal, preening, inhalation) that may be involved (Driver et al. 1991). Notwithstanding these limitations, foliar residues quantified in this experiment may be used as a first approximation of maximal levels and duration of exposure and compared with relevant toxicity values available in the scientific literature. The comparisons assume an approximate consumption rate of 23% of body weight per day as estimated from laboratory studies on zebra finch (Holmes et al. 1994) and equivalence of residue concentrations in foliage to that in vegetative food sources (seeds, berries) used by many birds. Inhalation exposure is considered insignificant in this case as very little of the spray cloud applied (volume median diameter = 1068 μm; 10% quantile < 618 μm) involved droplets considered to be respirable (<10 μm). Given these assumptions and a worst-case scenario of maximal foliar residue levels for glyphosate and triclopyr as noted above, resultant dose level estimates (179 and 375 mg·kg body $wt.^{-1}$, respectively) are substantially lower than the lowest acute oral toxicity values reported by Sassman et al. (1984) for bird species tested in the laboratory (LD_{50} (glyphosate) = 4640 mg·kg body $wt.^{-1}$ for mallard ducks and bobwhite quail, and LD_{50} (triclopyr acid) = 1698 mg·kg body $wt.^{-1}$ for mallard ducks).

Results from this study show that dissipation of glyphosate and triclopyr acid foliar residues is essentially complete by 24 and 48 days postapplication, respectively. Using mean intercept and decline rate values from the exponential regression equations detailed in Table 5, daily concentrations were predicted and averaged to estimate time-weighted average foliar residue concentrations (mean foliar dry mass of 6.5 g) over an 8-day period of exposure. The calculated values for glyphosate resulting from applications of VISION®, TOUCHDOWN®, and MON14420 were 116, 106, and 187 $mg \cdot kg^{-1} \cdot day^{-1}$, considerably less than the 8-day median lethal dietary concentrations (MLDC) of 4640 $mg \cdot kg^{-1} \cdot day^{-1}$ for

bobwhite quail as reported by Sassman et al. (1984) for glyphosate. Similarly, the time-weighted average concentration for triclopyr acid (546.5 $mg \cdot kg^{-1} \cdot day^{-1}$) was much less than the minimum 8-day MLDC of 1923 $mg \cdot kg^{-1} \cdot day^{-1}$ observed for various bird species, including zebra finch (Holmes et al. 1994) and bobwhite quail, japanese quail, or mallard duck (Sassman et al. 1984). Thus, comparison of foliar residue data with the minimal data available for avian toxicity provides no evidence to conclude a substantive risk of lethality to forest bird species. However, with the exception of the zebra finch data, none of the laboratory test species are appropriate surrogates for forest songbirds inhabiting hardwood canopies. Further, results of studies on organophosphate insecticides (Driver et al. 1991) suggest that dermal and preening exposures may contribute significantly to total dose levels in birds. In this regard, reasonable estimates of total exposures for canopy-dwelling forest songbirds and pertinent data on glyphosate and triclopyr herbicide toxicity to avian species under combined exposure regimes are lacking.

Residue levels of glyphosate and triclopyr remaining in foliage at the point of 90% leaf drop averaged 3.1 and 146.9 $mg \cdot kg^{-1}$, respectively. Thus, higher residues of triclopyr would be transported to the litter and soil compartments of the environment. The comparative environmental behaviour of glyphosate and triclopyr residues in soils will be the subject of a future publication.

Summary

Results of this field study indicate that initial foliar residues were highly correlated with application rates and increased by a similar factor approximating 233 to 313 $mg \cdot kg^{-1}$ for each $kg \cdot ha^{-1}$ of active ingredient applied, irrespective of formulation type. Initial deposit levels observed in this study were generally consistent with previously published estimates for aerial applications in forestry, but varied substantially in relation to hardwood CVI. Foliar residues of glyphosate were characterized by rapid exponential decline, with DT_{50} values averaging 2 days irrespective of the formulation tested. The general equivalence in foliar impingement and persistence among glyphosate formulations suggest a similar pattern of behaviour in or on target plant species. Initial foliar deposits of triclopyr were proportionally higher than those for glyphosate, reflecting the difference in maximum label rates for forest use. The relatively rapid dissipation of triclopyr ester (average DT_{50} = 1.5 days) as compared with triclopyr acid dissipation rates (average DT_{50} = 4.0 days) was attributed to rapid cuticular penetration of the ester and metabolic conversion to the acid in planta. The slow acid dissipation rates observed for triclopyr support the postulate that uptake and translocation processes were inhibited under these experimental conditions, providing a plausible explanation for the comparatively poor efficacy observed for RELEASE® as compared with glyphosate treatments, in contradiction to previous work.

Although clearly phytotoxic to the target plants that may be of importance to wildlife habitat, the magnitude and duration of foliar herbicide residues observed in this study were substantially less than levels generating acute or chronic toxic effects in laboratory studies on birds. However, we note that the relevance of simplistic extrapolations based on laboratory data to field exposures and risks associated with free-living wildlife species is questionable at best. Substantive improvements in risk estimation for wildlife exposed to pesticides could be made by scientifically integrated research in environmental chemistry and toxicology disciplines. Such an approach necessitates a better understanding of the relative importance of various mechanisms of exposure of species most at risk and collection of field residue data from all matrices of direct toxicological relevance. Such data, together with bioavailability information, could be used as inputs for integrated fate and effects modeling (e.g., AVPEST, Driver et al. 1993) for more scientifically defensible risk estimation. An alternative, more direct approach could involve direct sampling and quantification of body burden residues in wildlife exposed under operational or semi-operational scenarios if such sacrifices were warranted in an effort to ensure the protection of high-risk wildlife populations at large.

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On-target deposit and vertical distribution of aerially released herbicides

by Dean G. Thompson[1], Douglas G. Pitt[1], Bozena Staznik[1], Nicholas J. Payne[1], David Jaipersaid[1], R.A. Lautenschlager[2] and F. Wayne Bell[2]

As a component of the Fallingsnow Ecosystem Project, glyphosate and triclopyr herbicides (Vision®, Release®) were each applied to four experimental spray plots at nominal rates of 1.5 and 1.9 kg a.e. ha^{-1} respectively. Empirical studies were undertaken on these plots with the objectives of; a) quantifying mean on-target deposit and variability b) assessing the vertical distribution of active ingredient deposits through the vegetative complex and c) comparing herbicide deposit estimates on excised natural foliage with those on proximal 2-dimensional (2D) and 3-dimensional (3D) collectors. Experimental conditions were representative of difficult aerial application scenarios since the spray plots were small (4.9 to 10.4 ha), with irregular boundaries of mature timber, and in some cases substantial topographical relief. Deposit analysis confirmed that, in some circumstances, locations well within target areas were missed completely owing to inappropriate track spacing or swath offset. Excluding these points from the data analysis, results demonstrated overall mean deposition (mean ± SE) of glyphosate and triclopyr on aspen foliage equating to 68.45 ± 6.13 and 50.28 ± 6.01% of the nominal application rates (1.5 and 1.9 kg ha^{-1}), respectively. A high degree of variation in deposit both within and between plots demonstrate that variation in operational parameters (e.g. track spacing, offset, release height and aircraft speed) as influenced by local site factors (e.g. proximity of standing timber, topographical relief) can be important determinants in uniformity and accuracy of herbicide deposit. A consistent trend ($P < 0.001$) in the deposition profile through tiered vegetative canopies was observed, with greatest impingement of the spray in the upper target canopy as noted above, and average 25% and 12% in the shrub and ground-level tiers respectively. Results suggest that for sites characterized by complex canopies, differential vertical deposition may be an important factor constraining the potential use of lower herbicide application rates, particularly where shrub or groundcover species are important competitors. In contrast, given that only a small proportion of the spray cloud penetrates and impinges in the lower vegetative tiers, animals foraging or living therein may receive substantially reduced exposures, mitigating against any potential direct effects. In general, poor correlations ($r = 0.22$ to 0.78) in deposit estimates based on either two-dimensional or three-dimensional artificial collectors as compared to excised natural foliage were observed. Significant differences ($P < 0.05$) also were detected among deposit estimates with no consistent trend in relation to herbicide treatment, sampler type or sampling height. These comparisons suggest that none of the artificial collector types tested accurately or consistently estimated true foliar deposit.

Key words: alternative conifer release treatments, Fallingsnow Ecosystem Project, glyphosate, herbicides, herbicide deposit, tending, triclopyr, vegetation management, vertical distribution

Au cours du projet écosystémique de Fallingsnow, les phytocides glyphosate et triclopyr (Vision®, Release®) ont été pulvérisés sur quatre parcelles d'essai de pulvérisation selon des taux nominaux de 1.5 et de 1.9 kg é.a. ha^{-1} respectivement. Des études empiriques furent entreprises sur ces parcelles dans le but de: a) quantifier le dépôt moyen sur la cible et sa variabilité; b) d'évaluer la distribution verticale de l'ingrédient actif des dépôts au travers du complexe végétatif; et c) comparer les estimés de dépôt de phytocide sur du feuillage naturel récolté par rapport aux collecteurs d'échantillons selon 2 et 3 dimensions situés à proximité. Les conditions expérimentales étaient comparables aux scénarios de pulvérisation sous conditions difficiles puisque les parcelles d'essai étaient petites (4.9 à 10.4 ha), localisées entre des peuplements de bois à maturité ayant des limites irrégulières, et dans certains cas situées dans un contexte topographique difficile. L'analyse des dépôts a confirmé dans certaines circonstances que des points situés clairement à l'intérieur des cibles ont été complètement manqués suite à un espacement inadéquat des rampes et de l'effet de déviation. Une fois ces points exclus de l'analyse des données, les résultats ont démontré un dépôt global moyen (moyenne ± é.t.) du glyphosate et du triclopyr sur le feuillage de peuplier équivalent à 68.45 ± 6.13 et 50.28 ± 6.01% des taux nominaux d'application (1.5 et 1.9 kg ha^{-1}) respectivement. Un niveau de variation des dépôts à la fois dans les parcelles et entre les parcelles démontre que les changements dans les paramètres opérationnels (i.e., l'espacement entre les rampes, déviation, hauteur de pulvérisation et vitesse de l'appareil) tel qu'influencés par les facteurs locaux du site (i.e. proximité des peuplements vivants, relief topographique) peuvent avoir une influence déterminante sur l'uniformité et la précision des dépôts de phytocide. Une tendance constante ($P < 0.001$) dans le profil des dépôts a été observée parmi les trois tiers de la couverture végétale, la plus forte proportion de la pulvérisation se retrouvant sur le tiers supérieur de la couverture ciblée, et une moyenne de 25% et de 12% sur le tiers arbustif et herbacé respectivement. Les résultats laissent entendre que pour les sites identifiés comme ayant une couverture végétale complexze, le différentiel vertical du dépôt peut constituer un facteur important exercant une contrainte sur l'utilisation potentielle de taux plus faibles de phytocides, particulièrement lorsque les arbustes et les végétaux au sol sont d 'importants compétiteurs. Cependant, étant donné que seulement une faible proportion des pulvérisations pénètrent et recouvrent le tiers inférieur de la végétation, les animaux broutant ou vivant dans ce tiers seraient substantiellement moins exposés, le tout réduisant les effets potentiels directs. De façon générale, on a observé que de faibles corrélations ($r = 0.22$ à 0.78) parmi les estimés des dépôts établis selon les collecteurs artificielles en deux et trois dimensions lorsque comparés au feuillage naturel recueilli sur place. Des différences significatives ($P < 0.05$) ont été également notées au sein dos estimés de dépôt sans pouvoir établir de tendance constante en relation avec le traitement phytocide, le type d'échantillon et la hauteur d'échantillonnage. Ces comparaisons laissent entendre qu'aucun des collecteurs artificiels testés avec précision ou de façon constante n'a permis d'estimer avec précision le dépôt sur les feuilles.

Mots clés: traitements alternatifs de dégagement des conifères, projet écosystémique de Fallingsnow, glyphosate, phytocides, dépôt de phytocide, soins culturaux, triclopyr, contrôle de la végétation, distribution verticale.

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Introduction

While many physical, chemical and biological variables may affect the efficacy of a herbicide treatment, all do so through

some influence on the fundamental dose-response relationship. In the case of foliar-applied herbicides, effectiveness depends to a large degree on the quantity of active ingredient impinging on target foliage, as well as the size-distribution and coverage of the depositing droplets. Given a constant nominal application rate and drop-size spectra at release, the quantity and quality of deposit are in turn controlled by a variety of operational and meteorological variables. Based on parametric sensitivity analyses for the FSCBG spray model (Teske and Barry 1993), the most important of these are release height, spraying speed, wind direction and wind speed. Picot *et al.* (1993) provide empirical data which also suggest that release height is a critical factor controlling deposit and off-target drift of aerially applied insecticides.

While quantification of on-target deposit in these terms is critical to fully understanding efficacy or assessing potential non-target effects, accurate estimation under operational conditions is difficult, expensive and time-consuming. Historically, coverage and drop-size spectra of aerially released pesticides have been assessed using indirect methods involving dyed spray mixtures and Kromekote cards or other collection surfaces, even though this approach is fraught with a number of difficulties and potential extrapolative errors (Teske *et al.* 1994; Duan *et al.* 1994). Deposit of active ingredients may involve similar indirect methods or direct quantification of active ingredient on the target foliage or a simulant thereof (Riley *et al.* 1991;Thompson *et al.* 1992; Payne and Thompson 1992; Richardson *et al.* 1989, 1990; Payne 1993). In cases where artificial collection surfaces were used, the influence of differential surface characteristics and geometry as these affect impingement of the spray cloud, has been recognized. Numerous workers have promoted the use of three-dimensional (3D) collectors (Richardson *et al.* 1989; Miller *et al.* 1992; Duan *et al.* 1994). The advantage of 3D collectors is believed to be related to enhanced sampling efficiency for small droplets which may follow non-linear, often swirling trajectories (Carleton and Bouse 1987) and reduced correlation of sampling efficiency with wind-speed (Duan et al 1994). Given all of these considerations, direct quantification of the active ingredient on the critical target foliage, which involves the least number of assumptions and extrapolations, is a preferred approach to estimating actual on-target deposit.

Several field studies quantifying on- or off-target deposit of herbicides under conditions pertinent to Canadian forest regeneration practices have been published elsewhere (Payne *et al.* 1990; Riley *et al.* 1991; Payne and Thompson 1992; Thompson *et al.* 1992; Newton *et al.* 1990; Payne 1993). Experimental conditions for some of these studies involved essentially flat terrain, large spray blocks (>50 ha) and/or relatively simple vegetative canopy structures which may be associated with herbicide applications to large clearcuts. In recent years, silvicultural practices have been conducted more frequently on smaller clearcuts bounded by relatively mature standing timber, resulting in aerial application scenarios which differ markedly from those associated with the existing experimental database. As a component of a multi-disciplinary research effort referred to as the "Fallingsnow Ecosystem Project" (Lautenschlager *et al.* 1997), this study was conducted to provide chemical accountability data pertinent to aerial applications of glyphosate (Vision®[3]) and triclopyr ester (Release®[4]) herbicides under difficult operational spray scenarios inclusive of small spray block sizes, proximity of standing timber, and substantial topographical relief. The objectives of the study were to:

a) quantify mean on-target deposit and variability under difficult operational spray scenarios

b) determine the vertical distribution of foliar deposit through the vegetative complex and

c) compare deposit measured on excised natural foliage with those on 2D and 3D artificial collectors.

These objectives relate, directly or indirectly, to a number of priorities for forestry herbicide application technology research (Campbell and Howard 1993).

Methods

Site Description and Preparation

A detailed description of the Fallingsnow Ecosystem Project and experimental site is provided in preceding papers (Lautenschlager 1997; Bell *et al.* 1997). Briefly, the overall experiment comprised four blocks of varying size (28 to 52 ha), proximally located (within 6.6 km of one-another) on sites typical of the Great Lakes-St. Lawrence forest region in northwest Ontario. All blocks were classified as aspen–spruce–mixedwoods under the forest resource inventory, had been harvested three to eight years prior to initiation of this experiment, and replanted with bareroot spruce stock. In all cases, the blocks had become dominated by a tiered complex of competing vegetation comprised predominantly of trembling aspen (*Populus tremuloides* Michx.) (12.3% cover, 2.5 m height), red raspberry (*Rubus idaeus* L. var. strigosus [Michx.] Maxim) (9.6% cover, 0.6 m height) and graminaceous/herbaceous groundcover (5/38% cover, 0.5/0.3 m height) (Bell *et al.* 1997). Each block was divided into five smaller plots which were randomly assigned to one of five alternative conifer release treatments:

1) motor-manual (brush saw)
2) mechanical (Silvana Selective/Ford Versatile)
3) aerial Release® herbicide (triclopyr @ 1.9 kg a.e. ha^{-1})
4) aerial Vision® herbicide (glyphosate @ 1.5 kg a.e. ha^{-1}) and
5) control (no treatment).

For the purposes of this paper, only the herbicide treatment plots will be considered in detail. Herbicide treatment plots ranged from 4.9 to 10.4 ha in size and varied in shape, proximity to mature trees (23 m height) and geographic orientation relative to other treatment plots and to flight direction during herbicide applications (Fig. 1, Table 1).

One week prior to treatment, the mean and 95th percentile range of leaf-area-index (LAI) for all plots was determined by monitoring the incident radiation at 3.5 m (one reading above the aspen canopy) and radiation penetrating the aspen canopy (six readings at 1.0 m height within a 1 m^2 area) on a systematic 60 m grid. LAI measurements were made with a LI-COR® LAI-2000 (LI-COR Inc. Lincoln, Nebraska) plant canopy analyzer, equipped with a single LAI-2050 sensor fitted with a 270 degree view cap, following the manufacturers' suggested protocol (LI-COR 1991). Under this protocol, the effective range of view for the readings was calculated by the following equation:

$$A = f \times \pm \times h^2$$

[3]Trademark of Monsanto.
[4]Trademark of DowElanco.

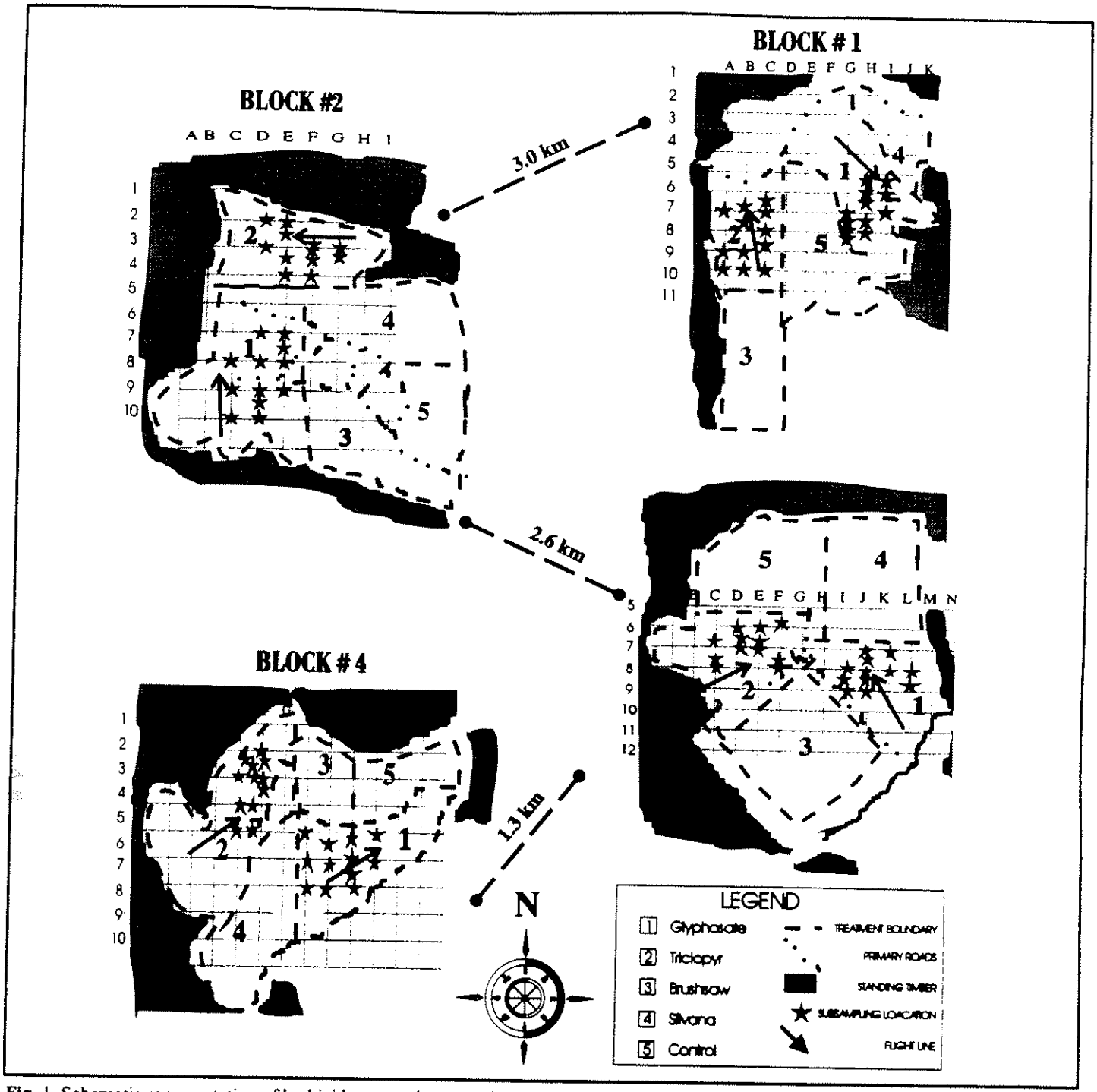

Fig. 1. Schematic representation of herbicide spray plots showing sub-sampling locations with respect to standing timber and flight lines.

where A = ground area represented by the sample, f = view fraction (0.75) correction for view cap, and h = canopy height in meters (3.0).

Based on this calculation, the LAI measurements integrate light penetration over an area of 21 m^2.

The initial LAI readings were used to establish 12 sub-sampling (SS) locations along 60 m transect lines within the inner 120 × 180 m portion of each of the treatment plots (Fig. 1). Sub-sampling positions were chosen such that six of the selected points were representative of the overall mean for all plots (0.65) and the six remaining positions spanned the 95th percentile range of LAI values for all blocks (i.e. at points where LAI = <0.1, 0.2, 0.4, 0.9, 1.2, and 1.5).

At each of the designated subsampling locations, an array of artificial and natural collectors were installed at heights of 3.5 m (above the aspen canopy), 1.0 m (above the raspberry canopy) and 0.5 m (above the ground-cover layer) (Fig. 2). Each collector array was comprised of an excised natural leaf (aspen at 3.5 m, raspberry at 1.0 m and a 10 cm segment of grass blade at 0.5 m), a 2-dimensional (2D) collector, a 3-dimensional (3D) collector and a Kromekote card to allow assessment of drop-size spectra for the impinging fraction of the spray cloud. The mean (± SD) silhouette area of natural aspen (19 ± 4 cm^2; n = 50), raspberry (20 ± 6 cm^2; n = 50) and 10 cm grass (4.4 ± 0.7 cm^2; n = 1 to 10) foliage was determined using video image analysis (Artek Model 982, Farmington, New York).

Table 1. Characteristics of herbicide treatment plots - Fallingsnow ecosystem project

Characteristic	Plot 1	Plot 2	Plot 3	Plot 4
Longitude (west)	89°49'	89°49'	89°50'	48°0'
Nominal (actual) plot size (ha)				
Glyphosate	9.9(7.6)	6.5(5.5)	10.4(7.1)	5.3(3.6)
Triclopyr	4.9(3.9)	6.5(3.7)	8.1(6.6)	9.3(7.4)
Mix volume applied (L)				
Glyphosate	310	202	286	141
Triclopyr	198	184	209	260
Calculated rate (kg a.e. ha^{-1})				
Glyphosate	1.79	1.62	1.77	1.72
Triclopyr	2.84	2.78	1.77	1.97
Treatment time				
Glyphosate	7:22-7:41	7:45-7:56	6:55-7:06	7:11-7:18
Triclopyr	9:20-9:30	21:03-21:10	21:14-21:25	9:34-9:45
Flight line (azimuth)				
Glyphosate	145	003	338	58
Triclopyr	358	280	243	214
Wind speed m s^{-1} (@ 18.5 m a.g.l.)				
Glyphosate	0.9	1.0	1.9	1.5
Triclopyr		2.7	2.8	
Wind direction (azimuth @ 18.5 m)				
Glyphosate	291	268	299	289
Triclopyr		320	313	
Air temperature (°C @ 18.5 m)				
Glyphosate	12.8	13.0	13.1	13.2
Triclopyr		15.3	15.1	
Relative humidity (% @ 18.5 m)				
Glyphosate	98	98	98	98
Triclopyr		89	89	

Notes: Mix concentrations were: 123.5 L Vision® / 1000 L total = 0.044 kg a.e L^{-1} and 105 L Release® / 900 L total = 0.056 kg a.e. L^{-1}.
Calculated rate = mix volume applied(L) × mix concentration (kg a.e. L^{-1})/actual treatment area (ha)
Data for triclopyr plots one and four unavailable due to equipment malfunction.
Boundary layer stable for all plots in both treatments.

For sampling at the 3.5 and 1.0 m levels, Whatman #1 glass fibre filter paper (surface area 38.48 cm^2) and a small Teflon ball (surface area 45.34 cm^2) were used as 2D and 3D collectors, respectively. Based on preliminary spray chamber results, the expected and realized drop-size spectra, and our subsequent field observations which showed essentially no deposition to the lower half of Teflon balls, deposit for these collectors were calculated based on the assumption of active-ingredient deposition primarily through vertical mass flux (Duan *et al.* 1994) and using only half of the total surface area (i.e. 22.67 cm^2). At the groundcover sampling level, a Teflon microtube (3D surface area 5 cm^2) and a Teflon strip (2D surface area 5 cm^2) were used to simulate grass blades and a Teflon ball was included to provide cross-comparison of deposit at the 1.0 and 3.0 m sampling height.

To minimize effects of sampler interaction, all collectors were affixed to small gauge copper wire frames using small aligator clips arranged at 90° separation angles. Wire frames were attached to hinged 2.54 × 5.08 cm aluminium or wooden stakes, such that the sampler array was ~0.3 m above the stake and at the appropriate height above ground (3.5 m, 1.0 m, and 0.5 m). Stakes were guyed with nylon rope to ensure that the sampler remained perpendicular to the ground surface. On the day prior to application, all collectors with the exception of natural foliage, were installed and covered with plastic bags to protect against dew. Immediately prior to chemical application (i.e. within 1.5 h), plastic covers were removed, excised natural foliage from surrounding vegetation was installed, and collector arrays were raised to the appropriate sampling height and tied.

Meteorological Monitoring

Meteorological stations established immediately adjacent to blocks one, two and three were used to monitor wind speed and direction, air temperature, and relative humidity during the spray periods (approx. 10 min for each plot). Given the proximal location of blocks three and four (Fig. 1) the main meteorological station located adjacent to block three served for monitoring conditions for both blocks. For the main station, monitoring equipment was mounted on a 18.5 m aluminum lattice tower and included propeller anemometer triads (Gill UVW, R.M. Young, Traverse City, MI) and air temperature sensors (thermistors, YSI, Yellow Springs, OH) at heights of 3.1, 9.2 and 18.5 m above ground level, as well as a humidity sensor (capacitance hygrometer, Heath Co. Benton Harbour, MI) at a height of 18.5 m. Electrical signals from these instru-

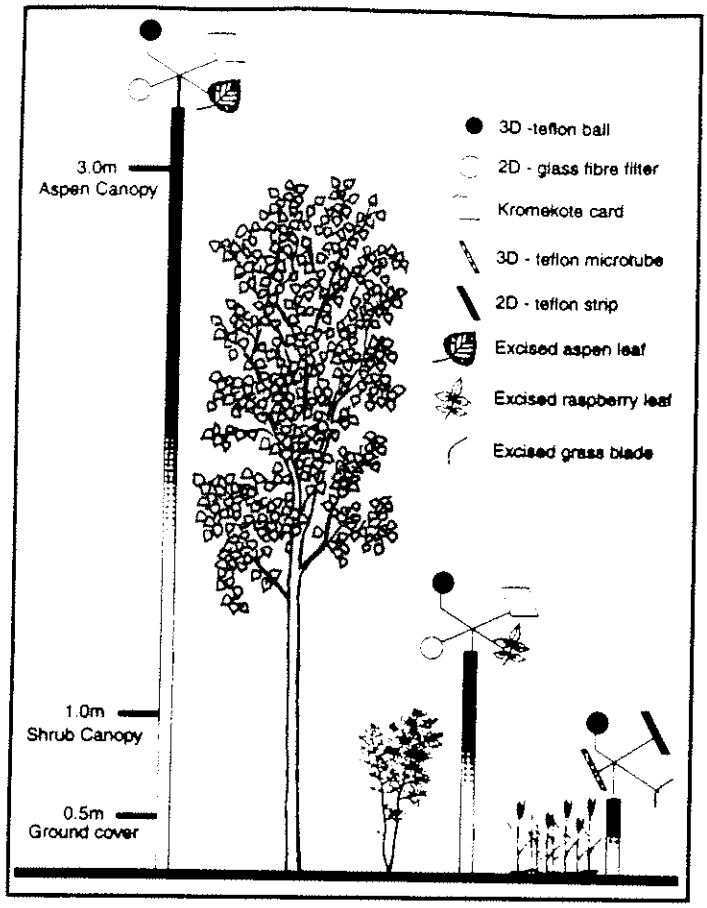

Fig. 2. Schematic representation of collector arrays established at three heights in relation to the tree, shrub and ground-cover vegetative tiers.

ments were sampled instantaneously at frequency of 0.2 Hz and averaged over successive 10 min periods using a data acquisition system (Isaac 91, Cyborg, Newton, MA). At blocks one and two, additional stations were established using 9.2 m towers instrumented with a vane-mounted anemometer (R.M. Young, Traverse City,MI) at 9.2 m, three air temperature sensors (Campbell Scientific, Edmonton, ALB.) at heights of 9.2, 4.6 and 2.3 m, as well as a relative humidity sensor (Campbell Scientific, Edmonton, AB) at a height of 4.6 m. Signals were logged at a frequency of 1 Hz using a CR10 datalogger (Campbell Scientific, Edmonton, AB).

Herbicide Application

A Bell 206B helicopter, equipped with a SIMPLEX® boom and 30 TEEJET® D8-46 nozzles, calibrated to deliver a volume application rate of 35 L ha^{-1}, was used for all applications. The track spacing was planned to be 20 m and the spray was released at 10 m above the target canopy. Glyphosate (Vision®) and triclopyr (Release®) herbicides were applied at nominal rates of 1.5 and 1.9 kg a.e. ha^{-1} respectively during morning and evening spray sessions of 16 August 1993. Erio acid red (0.1% w/v) dye was added to spray mixtures to facilitate assessment of drop-size spectra. Prior to chemical applications, a few residual trees were felled on all plots in Block one and the triclopyr plots in blocks two and three to allow for uniform spray release height. No other attempts were made to alter the non-uniform shape and size of plots or their proximity to mature timber stands.

Table 2. Aircraft and associated specifications for application of herbicide treatments.

Parameter	Specification
Contractor	Zimmer Air Services Inc.
Pilot	Mr. Paul Zimmer
Aircraft	Bell 206B; Registration #CG-FEC
Rotor length	10.2 m
Dispersal system	Simplex Hydraulic Boom
Boom length	10.9 m
Nozzles	Teejet (30 evenly spaced within 8.18 m of boom length)
Nozzle orientation	90° (straight down)
Tips & swirlplates	D8-46 (all new immediately prior to experiment)
Swath width	20 m
Track spacing	20 m
Release height	10 m above canopy
Air speed	44 km h^{-1}
Average load capacity	375 L
Avg. calibrated VMD	535.2 µm
Avg. calibrated NMD	204.82 µm
Avg. calibrated density	7.2 drops cm^{-2}
Avg. calibrated coverage	3.5 % of Kcard surface area
Calibrated volume rate	35 L min^{-1}

Details of the aircraft specifications and calibration, as conducted on site at 18:00 h 16 August 1993 are provided (Table 2). Aircraft calibration tests were conducted in an open area adjacent to block three, and involved over spraying, at a release height of 10 m, a series of 40 Kromekote cards placed along a tertiary logging road and spaced at 2.0 m intervals. Cards were set out in a straight line perpendicular to the aircraft flight line. Atmospheric conditions at the time of the calibration test were characterized as average wind speed of 1.22 m s^{-1}, from 215°, an average air temperature of 18.0°C and an average relative humidity of 75.7%. Resulting dropsize spectra were measured on the interior 20 cards having visible spray deposit using both an automated card reader (SWATHKIT®) and following general microscopic methods as described below.

Deposit Quantification

All deposit collectors were recovered from the field within four to six h post-application, placed in labeled containers, and stored frozen until processed. Care was taken to minimize dislodging of deposits during sampler collection by handling collectors with small stainless steel tweezers or by direct transfer into the containers using the alligator clips. To avoid potential sorptive losses of triclopyr butoxyethyl ester, all samples from triclopyr treated plots were placed in glass or Teflon containers; glyphosate samples were stored in plastic containers.

Deposits of glyphosate free acid and triclopyr butoxyethyl ester were quantified for each collector using either high performance liquid chromatographic (HPLC) or gas liquid chromatographic (GLC) techniques respectively (Thompson *et al.* 1989; Thompson *et al.* 1995). Triclopyr ester residues were converted to acid equivalents based upon the mass ratio of the two compounds. Specifics of the analytical methods are provided (Table 3). Analytical results were converted to amount of active ingredient per unit area of the collection surface (µg cm^{-2}) and normalized by calculating deposit of active ingredient as a percentage of the nominal application rate for each compound. Analytical methods were validated prior to experiment initiation and a concurrent quality control (QC) program

Table 3. Analytical method specifications used in quantifying glyphosate and triclopyr ester deposits

Specification	Glyphosate method	Triclopyr method
Chromatographic	HPLC	GLC
System	Varian 5560	Varian Vista 6000
Detector	UV-200 @ 570 nm	^{63}Ni Electron Capture
Autosampler	Varian 8085	Varian 8000
Injection technique	n/a	Hot on-column
Final sample volume	10 mL mobile phase	10 mL iso-octane
Injection volume	100 µL	2 µL
Column type	Aminex A-9 (10 cm × 4.6 mm i.d.)	DB-5 0.25 µm film (30 m × 0.25 µm i.d.)
Mobile phase	0.005 KH_2PO_4 (pH = 1.9) with 4% methanol 0.55 mL min^{-1}	Nitrogen 1 + 29 mL min^{-1}
Operating temperatures		
Injector	n/a	200°C
Column	50°C	85-250 @ 30°C min^{-1}
Detector	n/a	325 °C
Post-column reactor	135°C	n/a
Derivatization	ninhydrin post-column flow 0.5 mL min^{-1} coil (284 cm × 0.02 cm i.d.) Varian Model V reactor	methylation @ 95°C (14% BF_3 in methanol)
Retention time	7.1 min	11.44 min
Detector linearity	100-2500 ng injected	10 – 40 µg injected
Limit of detection natural & artificial matrices	0.10 µg g^{-1}	0.02 µg g^{-1}
Validated limits of quantification		
natural matrix	0.5 µg g^{-1}	0.1 µg g^{-1}
artificial matrix	2.0 µgcm^{-2}	0.025 µg cm^{-2}

was run throughout the duration of field sample analyses. The QC program involved duplicate blank matrices fortified at various levels of active ingredient, equilibrated for a minimum of 18 h under cold, dark conditions. Duplicate QC samples were analyzed simultaneously with each set of field samples. For each collection surface, mean recovery efficiency, standard deviation and coefficient of variation were calculated. Means from the QC data were used to correct raw field deposit data for analytical recovery efficiency. Additionally, quality assurance samples involving all collector types, fortified at levels equivalent to the nominal application rate for each herbicide, were deployed and left exposed for 4 h to environmental conditions extant on block one. The field quality assurance (QA) samples (n = 3 per collector type/herbicide treatment combination) were collected, transported and stored under conditions identical to those for actual field deposit samples. Thus QA sample analyses allowed assessment of potential degradative losses for herbicide active ingredients between the time of deposition and time of analysis.

Results for analytical QC and QA samples are presented (Table 4). Results of the laboratory QC program were highly consistent with preliminary validation studies and demonstrated excellent recovery efficiency (>76%) and precision (<6.5% CV) for all analyte/matrix combinations. Recovery efficiency for glyphosate analyses were essentially quantitative and thus correction for analytical losses was unnecessary. For the triclopyr ester analyte, recovery efficiency ranged from 76 to 82% depending upon the matrix and thus appropriate factors (Table 4) were applied to correct raw deposit data for analytical recovery efficiency. Mean recovery efficiencies observed for field QA samples were similar to those for laboratory QC samples indicating no significant degradative losses for these analytes under the conditions of sampling, transport and storage employed.

Assessment of Dropsize Spectra

Kromekote cards (5 × 5 cm) were deployed to enable drop-size spectra and density assessment. Kromekote cards were labeled on the underside and placed in individual plastic petri-dishes by block and treatment. Drop-size spectra were determined by optical analysis of stains following the general recommendations of Sundaram *et al.* (1993). Drop densities (drops cm^{-2}) were measured in five one cm^2 areas card^{-1} using a binocular microscope at 12 × magnification and sized using 50 × magnification and an eyepiece equipped with a graticule. Volume median diameter (VMD), volume average diameter (VAD), number median diameter (NMD) and the diameters at the 10th and 90th percentiles of the distribution ($DV_{0.1}$ and $DV_{0.9}$ respectively) were calculated for each sample using an empirically determined spread factor of 2.5 (Payne 1993). In a number of cases, drop-size spectra and density could not be determined owing to the deleterious effects of dew on stain shape and visibility.

Table 4. Summary of analytical quality control and quality assurance results

Collector type	Analyte	Fortification rate equiv. (μg cm^{-2})	n	Mean rec.(%)	STDS	CV	CF
Laboratory quality control sample results							
2D	Triclopyr	20-30	11	82.19	3.33	4.05	1.22
	Glyphosate	19	5	99.61	2.39	2.40	1.00
3D	Triclopyr	14.6	23	80.32	5.03	6.26	1.25
	Glyphosate	14.7	12	99.14	2.82	2.85	1.01
FO(aspen)	Triclopyr	34.5	12	76.4	3.82	5.00	1.32
	Glyphosate		6	99.74	2.25	2.25	1.00
Field quality assurance sample results							
2D	Triclopyr	19	3	82.61	5.51	6.66	
	Glyphosate	15	3	93.33	4.57	4.89	
3D	Triclopyr	19	3	78.02	12.65	16.21	
	Tlyphosate	15	3	87.09	5.49	6.30	
FO(aspen)	Triclopyr	19	3	83.86	2.68	3.20	
	Glyphosate	15	3	94.9	0.78	0.82	

Note: STDS = sample standard deviation; CV= coefficient of variation; CF = correction factor applied to correct field residue data for analytical recovery; 2D = 2-dimensional artificial collector, Whitman #1 glass fibre filter paper surface area 38.48 cm^2; 3D = 3-dimensional artificial collector, Teflon ball with surface area 45.34 cm^2; FO = excised natural aspen foliage aspen, surface area (n=40) 19 ± 4 cm^2).

Statistical Analysis

For the purposes of spray deposit assessment, each plot was considered as a unique combination of site and meteorological conditions. Accordingly, a completely randomized design (CRD) was used for subsequent analysis, with two treatments (triclopyr or glyphosate), each applied to four replicate spray plots. The statistical model employed to examine differences in mean on-target deposit was:

$$Y_{ijk} = \mu + H_i + P_{j(i)} + S_{k(ijk)}$$

where Y_{ijk} = deposit observed on the kth sample in the jth plot of the ith herbicide treatment μ = overall mean, H_i = the fixed effect associated with the ith herbicide (i = 1, 2), $P_{j(i)}$ = the random effect of the jth plot within the ith herbicide treatment, $S_{k(ijk)}$ = the random effect of the kth sampler within plot j and herbicide i.

To meet the assumptions of this model, variances of the data from each plot were checked for normality and subjected to Bartlet's test for homogeneity of variance. Residuals were also examined for normality and signs of heteroscedasticity.

Within each spray plot, 12 SS locations were established as described above. Deposit data resulting from the six SS locations corresponding to the overall mean LAI value were used to investigate deposit under constant canopy density, while data from all SS locations were regressed with corresponding LAI measurements to examine the functional relationship between deposit and the immediately surrounding target canopy density. As there was no relationship between LAI and on-target deposit for any of the eight spray plots (P = 0.746 for glyphosate, 0.233 for triclopyr) data for all over-sprayed SS locations were pooled for assessment of mean on-target deposit.

To examine the vertical distribution of herbicide deposit, the general model was employed in a repeated-measures multivariate analysis of variance (RM-MANOVA). This permitted the testing of differences between the deposits on the 3D collectors deployed at each of the three collection levels. Each observation in the above model was considered as a trivariate response representing the deposit at each of the three levels. The assumptions for this analysis are that the deposit values in the model have a multivariate normal distribution with a common covariance matrix across treatments. In this experiment, simple correlation and a parallel RM-ANOVA analysis was conducted to examine the relationship between deposits on excised natural foliage, 2D and 3D artificial collectors. Further, each observation in the model was considered as a multivariate response representing the deposit measured by each collector type at a particular level.

Results and Discussion

Meteorological Conditions During Herbicide Applications

Meteorological conditions during the periods of herbicide applications are provided (Table 1). All applications were completed during early morning or late evening spray sessions on 16 August 1993, with the time required to treat an individual plot being 10 minutes or less. The meteorological data were incomplete owing to equipment malfunction. However the data set suggested generally similar conditions at all sites and thus conditions at block three are reported. Meteorological conditions were typical for operational silvicultural herbicide applications, with moderate windspeeds (<2.8 m s^{-1} at 18.5 m), stable atmospheric boundary layers, moderate air temperatures (12.8 < T <15.3°C) and high relative humidities (89-98%) in all cases.

Droplet Density and Spectral Assessment

Droplet densities on Kromekote cards deployed at the three sampling heights for each subsampling station were determined, and mean values at each sampling height, for each treatment plot, are presented (Table 5). Droplet densities at the upper sampling height suggested greater coverage (22 drops cm^{-2}) for glyphosate treatments as compared to triclopyr treatments (15 drops cm^{-2}). In both cases, substantial canopy

Table 5. Drop density and spectra of impinging spray cloud by sampling height

Active ingredient	Sample Height	Drop density cm^{-2} Plot 1	Plot 2	Plot 3	Plot 4	Overall
Glyphosate	3.5	21 ± 8	40 ± 25	10 ± 3	15 ± 8	22
	1.0	16 ± 8	21 ± 11	8 ± 3	14 ± 5	15
	0.5	12 ± 5	14 ± 12	6 ± 5	8 ± 6	10
Triclopyr	3.5	16 ± 13	12 ± 6	20 ± 10	12 ± 10	15
	1.0	10 ± 8	6 ± 4	14 ± 10	18 ± 6	10
	0.5	8 ± 8	7 ± 4	11 ± 7	6 ± 5	8

Dropsize spectra results from plot one — Triclopyr

	n	VMD	VAD	NMD	Dv0.1	Dv0.9
3.5	209	648	322	147	301	905
1.0	158	541	327	167	270	756
0.5	114	564	361	189	321	757

penetration was observed with droplet densities at the 0.5 m and 1.0 m ranging from 45–53% and 66–68% of those at the 3.5 m level for glyphosate and triclopyr treatments respectively. A strong predominance of droplets on the upper-side of the cards was observed at all heights, suggesting that under the conditions of this study, characterized by a stable atmospheric boundary layer, impingement of small droplets associated with the horizontal component of mass flux (Duan *et al.* 1994) was relatively unimportant. Spectral assessment of many Kromekote cards was obviated by dew deposition which caused distortion of droplet stains in some cases and differential surface properties of cards in other cases, thereby negating accurate assessment of stain sizes. Such problems are commonly encountered in field studies employing Kromekote cards (Teske *et al.* 1994). Droplet spectra observed for the triclopyr application to block one, where no dew effect occurred, are presented (Table 5) as an example of spectral differences for the impinging spray cloud relative to sampling height. The dropsize data were analyzed by a χ^2 test (Volk 1980) and found to be non-homogeneous ($P < 0.05$), indicating significant differences between the dropsize spectra at different heights above ground. Larger VMD and $DV_{0.9}$ were observed near the top of the canopy as compared to lower sampling heights, indicating that the largest drops impinged within the target canopy as has been observed previously (MacNichol 1996)

Table 6. Analysis of variance for on-target deposit[1] of glyphosate and triclopyr herbicides

Source	d.f.	SS	F	P>F
Between herbicides	1	4,598.94	3.86	0.053
Plots (within herbicides)	6	22,344.63	3.12	0.009
Plots (within glyphosate)	(3)	8,915.71	2.49	0.066
Plots (within triclopyr)	(3)	13,428.92	3.75	0.014
Samples (within plots (within herbicides))	78	93,023.97		
Total	85	119,967.54		

[1]data for deposit to 2-D artificial collectors (glass fibre filter papers) at 3.5 m sampling height p(normality for plots (within herbicides): >0.196 p (homogeneity of variance): 0.630.

On-target Deposit

Due to operational factors, all glyphosate applications were made prior to triclopyr applications. During the morning spray session, wind gusts began to exceed regulatory limits (2.2 m s^{-1} at spray release height) for operational spraying between 9:30 and 10:00 am, forcing a delay in triclopyr application to plots two and three until late in the evening (21:00 h) when winds had again diminished to levels consistently below regulatory thresholds (2.2 m s^{-1} at spray release height) (Table 1). As such, the marginally significant differences ($P = 0.053$) in overall deposit means at the top of the aspen canopy (Table 6) were expected and are primarily a function of different meteorological conditions rather than inherent formulation characteristics. As a result, only within-treatment comparisons are of relevance here. Further, drop density on Kromekote cards, quantification of active ingredient on all sampler types, and post-application aerial photography, all confirmed that occasional strips were left untreated due to inappropriate swath positioning. Such errors, common to aerial application of forest pesticides (Barry 1977; Richardson *et al.* 1993) may have been exacerbated in these trials owing to the combined effects of small spray block sizes (4.9 to 10.4 ha), proximity of standing timber or other no-spray zones, and notable topographical relief. Therefore reported deposit data are for subsampling locations that were clearly over sprayed (subsamples varying from 9–12 depending upon treatment and plot, $n = 43$ for both treatments) and exclude those subsampling stations which clearly did not receive any deposit.

Overall mean deposit (mean ± SE) of glyphosate and triclopyr on aspen foliage equated to 68.45 ± 6.13 and 50.28 ± 6.01% of the nominal application rates (1.5 and 1.9 kg ha^{-1} respectively) (Fig. 3). While the overall mean foliar deposits were not significantly ($P = 0.747$) different from those derived from 2D artificial collection surfaces (71.20 ± 5.34% and 56.06 ± 5.31%) when averaged across treatments, there were statistically significant differences ($P < 0.05$) between foliar deposit and 2D estimates when data were considered within a given treatment. Thus, the average on-target deposit estimates and variability observed in this study depended on the type of collection surface employed (see following detailed comparison of collectors). Overall, on-target deposit averages estimated from natural foliage or 2D collector results were similar to those in previous studies using similar spray equipment on small spray blocks (Newton *et al.* 1990; Thompson *et al.* 1992). The graphical presentation (Fig. 3) emphasizes the degree of variation in mean deposit among plots within both treatments. Although such differences were only marginally significant

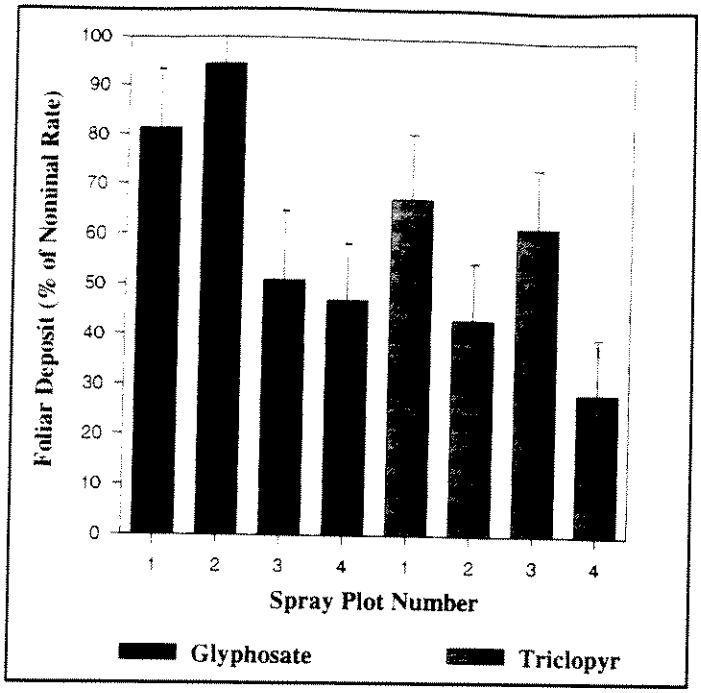

Fig. 3. Mean deposit of glyphosate and triclopyr herbicides (Vision®, Release®) on excised aspen foliage held at 3.5 m above ground level in (n=4) spray plots. (Values are least squares means ± standard errors).

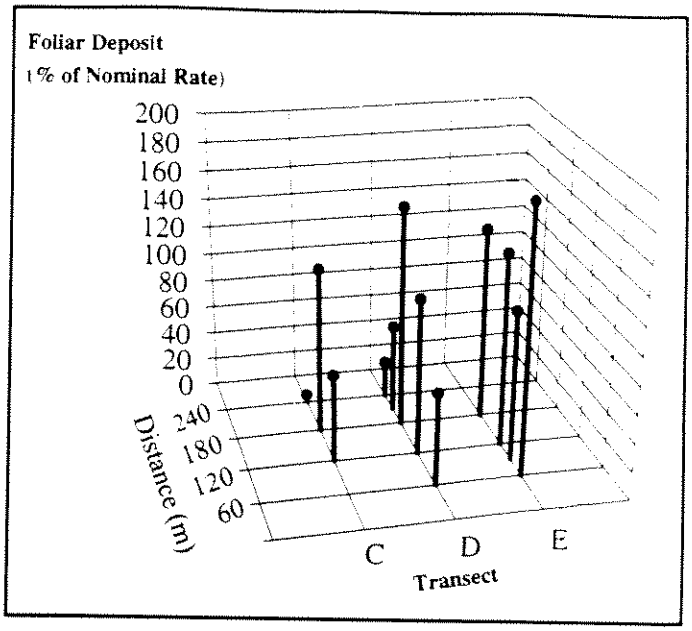

Fig. 4. Distribution of glyphosate deposit on excised aspen foliage held at 3.5 m above ground level for each of 11 sub-sampling stations on spray plot two; flight path parallel to sampling transect lines and perpendicular to wind direction (from 268° or L-R on diagram). (Values are least squares means ± standard errors).

for glyphosate treatments ($P = 0.066$), they were highly significant for triclopyr treatments ($P = 0.014$) (Table 6). The ratio of variances for plots versus samples ($F = 3.12$, Table 6) indicates that variation between plots was greater than variation within plots and suggests that meteorological, operational, and/or site conditions which differ for each plot had a strong influence on deposit. For glyphosate treatments, the lowest mean foliar deposits were observed on plots three and four (Fig. 3) for which higher windspeeds were recorded (Table 1). In the former case, lowest deposits for individual sample locations were observed in the northwest corner of the plot where boom times were very short and efforts to protect the integrity of other treatment plots resulted in poor deposit near plot boundaries. In fact, the three sub-sample locations on transect I of this plot (Fig. 1) showed only trace deposits and were omitted from statistical analysis and mean calculations. In the case of glyphosate treatment, plot four, flight lines were oriented East-West along the contours of the steep slope on the southern portion of the plot. Based on subsequent aerial photography, the low observed deposits (locations H7 & I7) resulted from inappropriate swath placement.

For triclopyr treatments to plots two and three, wind speeds and other meteorological conditions were very similar during applications (Table 1) yet foliar deposit varied markedly, with lower mean deposit (43.43 ± 11.44% of nominal rate) on plot two which was characterized by proximal standing timber on the northern, eastern and western boundaries (Fig. 1). In comparison, higher mean deposits (67.40 ± 13.21; 65.45 ± 12.54) were observed on plots 1 and 3, respectively, where flight lines were not impaired by standing timber. On triclopyr spray plot four, lowest deposits were observed at locations E2 and F2, closest to the northern edge of the plot bounded by standing timber and may be attributed to either insufficient upwind offset of the initial spray swath or higher than optimal spray release height resulting from the need to fly above proximal standing timber. The degree of within plot variation which may occur under these experimental conditions is shown for glyphosate plot two (Fig. 4). The data demonstrate the typical pattern of least deposit at sampling locations nearest the upwind side or at the extremes of sampling transects running parallel to the flight lines. In this case, a good mean deposit (94.55 ± 11.94) was achieved, largely owing to high deposition on samplers located on the downwind and mid portions of the plot.

Given that meteorological conditions for spray plots were generally similar, these results suggest that differences in operational parameters (e.g. swath offset and overlap, release height, spraying speed) induced by difficult site conditions (proximity of standing timber, steep terrain, irregular spray boundaries) may have contributed substantially to overall spray deposit variation. Newton *et al.* (1990) also noted highest variability in deposit associated with treatments where aircraft followed contours of steep side slopes or flew up and down steep gradients. While instantaneous release height and spraying speed were not monitored in this experiment, these results appear to generally support the findings of Teske and Barry (1993a) who identified release height, spraying speed, wind direction and wind speed as critical operational factors controlling deposit based on parametric sensitivity tests using the FSCBG aerial application computer model (Teske and Barry 1993b).

Vertical Distribution of Foliar Deposits

Vertical distribution of herbicide deposits through the tiered vegetative canopy was assessed using RM-MANOVA of deposits on both excised foliage and on the 3D artificial collector. Both analyses demonstrated no significant differences

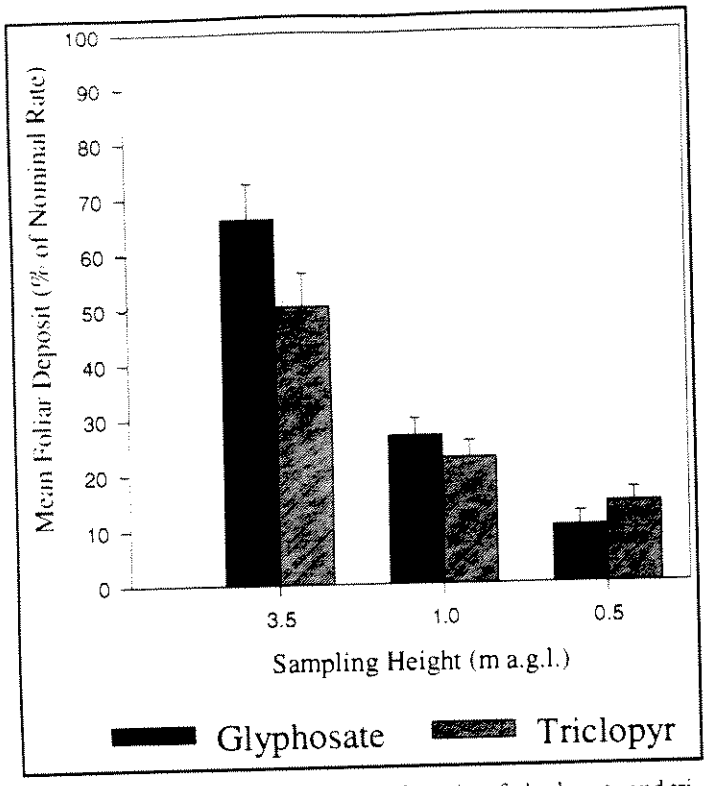

Fig. 5. Vertical distribution of foliar deposits of glyphosate and triclopyr herbicides on excised aspen, raspberry and grass foliage held at 3.5, 1.0 and 0.5 m sampling heights respectively. (Values are least squares means ± standard errors.)

in the distribution pattern among the two herbicide treatments ($P = 0.245$ and $P = 0.534$ respectively) and a significant degree of variability between plots within a given herbicide ($P < 0.012$). Most importantly, both analyses demonstrated highly significant differences in deposit estimates in relation to sampling height ($P < 0.001$). Overall mean foliar deposit for each treatment and sampling level are presented (Fig. 5), showing a consistent trend in both treatments, with substantially lower deposit at the herbaceous and ground levels as compared to those in the aspen canopy. Results for triclopyr plot three, where similar deposits at the 3.5 and 1.0 m levels were observed, represent the singular exception to this general trend.

The high proportion of deposit intercepted by the target canopy are consistent with findings of previous studies (Rafferty *et al.* 1981; Newton *et al.* 1990; Barry 1984). Our results demonstrate that on average, low-shrub and ground-cover vegetation received less than 25% and 12% respectively of the nominal application rate for the herbicide treatments, demonstrating the point made by the latter authors that upper tiers of a vegetative canopy can protect lower layers from receiving a phytotoxic dose. The results are consistent with the concept of canopy penetration as a progressive removal of airborne drops incident from above by deposition through sedimentation and inertial impaction. While substantially lower deposits to herbaceous and ground-cover vegetation may have beneficial effects in terms of reduced potential for direct or indirect effects on wildlife, it may be detrimental to efficacy in cases where herbaceous and graminaceous competition is equivalent or greater than that resulting from competitive hardwood tree species. This effect may be an important consideration given previous studies which have documented the potential for decreasing nominal rates of glyphosate without a significant reduction in efficacy (Nova Scotia Department of Lands and Forests 1989; Pitt *et al.* 1993). Although herbicide rates may be reduced without compromising efficacy in single-layer competitor scenarios, or where species in the complex are all quite susceptible, this may not be the case in tiered canopies where tree species may intercept the majority of the spray cloud, impairing efficacy on herbaceous or ground-competitors and necessitating re-treatment to achieve effective conifer release.

Comparison of Deposit on Foliage and Artificial Collectors

The inconsistencies in observed deposit estimates for collectors deployed at each sampling height are illustrated (Fig. 6). Comparison of histogram bars at the 3.5 m sampling height demonstrate reasonable agreement between deposit on 2D collectors and excised aspen foliage for both glyphosate and triclopyr treatments as evidenced by the simple correlation coefficients ($r = 0.62$ and 0.74 respectively). However, this trend was not always consistent among plots within each treatment, as evidenced by significant differences ($P = 0.004$ for glyphosate, $P = 0.05$ for triclopyr) detected for comparisons of deposit on 2D collectors and excised aspen foliage. At the 3.5 m sampling height, the 3D collectors underestimated both foliar and 2D collector deposits for glyphosate treatments, but gave essentially equivalent estimates to both natural and 2D artificial collectors for triclopyr treatments (Table 7, Fig. 6). In contrast, the 3D collectors tended to overestimate deposits on excised natural foliage for both compounds at the 1.0 m sampling height, but to a lesser degree than the artificial 2D collectors in most cases. The lack of consistency in these relationships suggest substantive differences in catch efficiencies of the various collectors in relation to sampling height. Such differences may reflect complex interactions between the physical/chemical properties of the formulations or active ingredients, surface conditions of the collector and micro meteorological conditions induced by the canopy, all of which in turn control size and trajectory of droplets, degree of adherence to the collector, as well as wind speed and direction at the specific point of sampling. For example, the low bias observed for 3D collectors and glyphosate treatments at the 3.5 m height may reflect a lower potential for retention of larger droplets on rounded surfaces, particularly for highly water soluble compounds and where the surface has been moistened by dew, as was the case for at least some of the spray blocks. Conversely, in the case of triclopyr treatments, where the ester form of the compound is less water soluble and dew was not a factor, deposit estimates from 3D collectors at the 3.5 m sampling height exceeded those on natural foliage in three of four cases.

These results conflict somewhat with the findings of other researchers who have demonstrated relatively higher collection efficiencies for 3D as compared to 2D artificial collectors on both a theoretical and empirical basis (Richardson *et al.* 1989; Miller *et al.* 1992; Duan *et al.* 1994). The difference in results may be partially explained by the relatively large droplet spectrum (VMD = 648 μm and size range $DV_{0.1}$ to $DV_{0.90}$ = 301 to 905 μm) in this study as compared to previous studies (VMD=282 [Richardson *et al.* 1989] size range < 500 μm [Miller *et al.* 1992; Duan *et al.* 1994]) and by the softer, rougher and more sorptive surface characteristics of glass fibre filter papers used as 2D collectors in this study as compared to

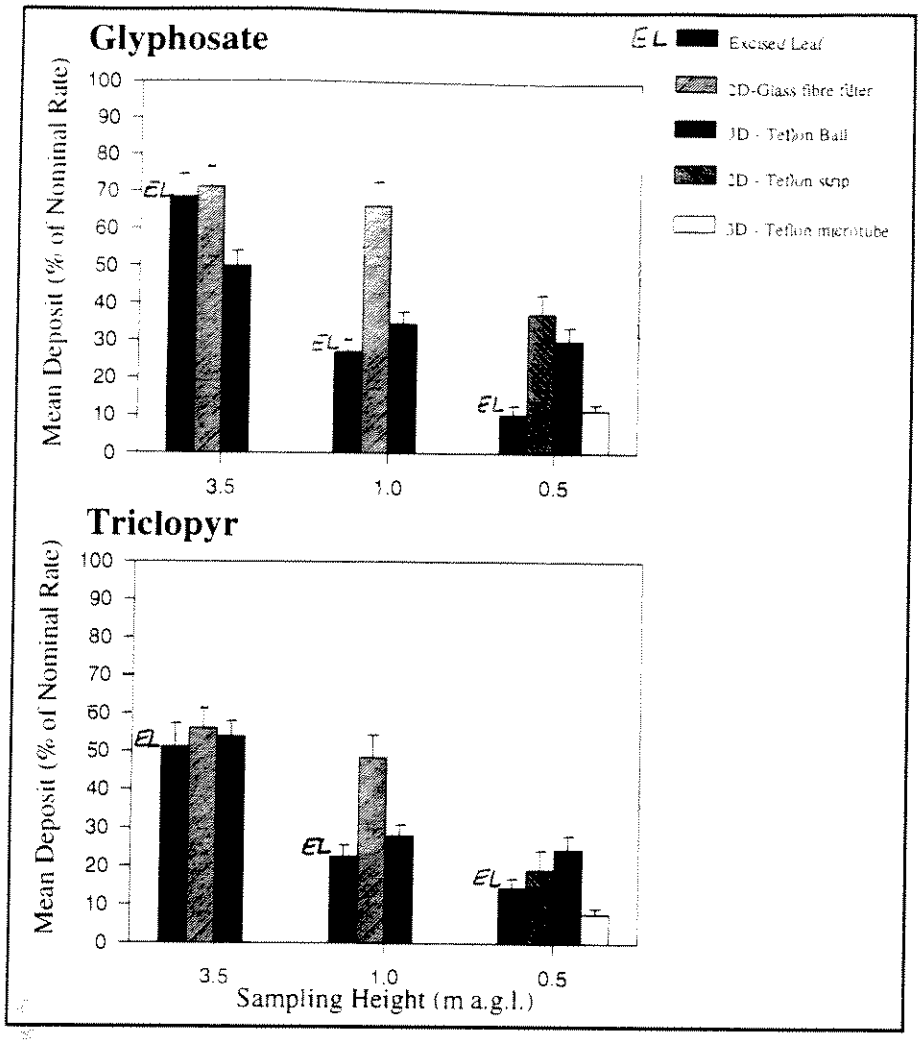

Fig. 6. Comparison of glyphosate and triclopyr deposits on various artificial surfaces and excised foliage by sampling height. (Values are least squares means ± standard errors.)

Table 7. Mean, standard error and simple correlation coefficients for foliar and artificial collector deposits by treatment and sampling height

Treatment	Sample collector		Least squares mean	SE	Corr. coeff.
	Height	Type			
Glyphosate	3.5	FO	68.45	6.13	–
		2D	71.20	5.34	0.62
		3D	49.84	4.15	0.63
	1.0	FO	27.01	3.24	–
		2D	66.02	6.38	0.53
		3D	34.46	3.34	0.51
	0.5	FO	10.22	2.48	–
		2Dts	37.38	5.13	0.49
		3D	28.98	3.71	0.78
		3Dmt	11.29	1.63	0.60
Triclopyr	3.5	FO	51.17	6.09	–
		2D	56.06	5.31	0.74
		3D	53.83	4.12	0.62
	1.0	FO	22.59	2.99	–
		2D	48.44	5.90	0.22
		3D	27.94	3.08	0.41
	0.5	FO	14.60	2.41	–
		2Dts	19.21	4.98	0.76
		3D	24.42	3.61	0.60
		3Dmt	7.69	1.58	0.58

Where: FO = excised natural foliage (aspen @ 3.5, red raspberry @ 1.0 m and grass @ 0.5 m); 2D = 2-dimensional artificial collector, Whitman #1 glass fibre filter paper 11 cm diam.; 2Dts= 2-dimensional Teflon strip; 3D = 3-dimensional artificial collector, Teflon ball; 3Dmt = 3-dimensional artificial collector, Teflon microtube.

Kromekote cards, mylar or acetate sheets and/or glass plates as used in previous studies. Based on the published theory and empirical evidence, 3D collectors were expected to have relatively higher deposit than artificial 2D collectors, particularly at the 3.5 m sampling height where the capture efficiency of 2D collectors would be theoretically minimized (Duan *et al.* 1994). However, based on the mean deposits ($n = 4$) for both glyphosate and triclopyr herbicide treatments in this study, deposits on spherical Teflon balls (3D collectors) were either equivalent to or less than those on glass fibre filter papers (2D collectors) at both 3.5 and 1.0 m sampling heights (Table 7 and Fig. 6). At the 0.5 m sampling height neither 3D collectors (Teflon ball or Teflon microtube) provided consistently higher deposits than artificial 2D collectors (Teflon strips) designed to mimic excised grass blades (Fig. 6).

In practical terms, none of the artificial collectors employed in this study provided deposit estimates which were consistently highly correlated ($r > 0.60$) (Table 7), or statistically ($P > 0.05$) equivalent, with proximally located natural excised foliage held at the same sampling height. Thus, based on the results of intensive sampling for this semi-operational experiment, we are in agreement with the general conclusion of Richardson *et al.* (1989) who stated "...for information on deposition on the target, usually foliage, there is no substitute for sampling the target itself".

Conclusions

Results of this experiment demonstrate overall mean deposition (mean ± SE) of glyphosate and triclopyr on target aspen foliage equating to 68.45 ± 6.13 and 50.28 ± 6.01% of the nominal application rates (1.5 and 1.9 kg ha^{-1}) respectively. A high degree of variation in deposit both within and between plots indicated that variation in operational parameters (e.g. release height, spraying speed) as influenced by local site factors (e.g. proximity of standing timber, topographical relief) can be important determinants in uniformity and accuracy of herbicide deposit. A consistent, significant ($P < 0.001$) trend in the deposit profile through tiered vegetative canopies was observed with highest impingement (>50% of the nominal application rate) in the aspen canopy and approximately 25% and 12% in the low-shrub and ground-cover tiers, respectively. Deposit comparisons showed that none of the artificial collectors tested were consistently effective at estimating the foliar deposit on proximally held excised natural foliage.

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Comparative fate of glyphosate and triclopyr herbicides in the forest floor and mineral soil of an Acadian forest regeneration site

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Abstract: Following applications of three different salt formulations of glyphosate (Vision®,[2] Touchdown®,[3] and Mon14420[2]) and an ester formulation of triclopyr (Release®[4]) to an Acadian forest regeneration site in New Brunswick, Canada, the fate and persistence of herbicide residues in the forest floor and underlying mineral soil were investigated. Within 14 days of treatment, maximal residue levels (average 8.3 μg·g dry mass^{-1}) were observed in the forest floor matrix following application of the glyphosate formulations, with higher values (45.7 μg·g dry mass^{-1}) observed for triclopyr. Residue maxima in the underlying mineral soil were, on average, 5.7-fold lower than those in the forest floor. In both matrices, glyphosate residues declined exponentially with time, irrespective of the formulation applied. Among the glyphosate treatments no significant differences ($p = 0.16$, $p = 0.97$, for forest floor and mineral soil, respectively) were observed in the estimated times to 50% dissipation (DT_{50}). Overall, average DT_{50} values for glyphosate were estimated as 12 ± 2 and 10 ± 3 days for the forest floor matrix and mineral soil, respectively. Triclopyr residues, particularly in the forest floor, were characterized by a series of transient increases, possibly reflecting temporally varying inputs from dew, rainwash, or litter fall from surrounding treated vegetation. Triclopyr residues also dissipated with time, with approximate DT_{50} values ranging from 39 to 69 days in the forest floor and mineral soil, respectively.

Résumé : Suite à l'application de trois formulations différentes de sels de glyphosate (Vision®,[2] Touchdown®[3] et Mon14420[2]) et d'une formulation d'un ester de triclopyr (Release®[4]) dans un site forestier acadien en régénération du Nouveau-Brunswick, au Canada, le sort et la persistance des résidus d'herbicide dans la couverture morte et le sol minéral sous-jacent ont été étudiés. Durant les 14 jours qui ont suivi le traitement, des niveaux maximums de résidus (moyenne de 8,3 μg·g de poids sec^{-1}) ont été observés dans les composantes de la couverture morte suite à l'application des formulations de glyphosate. Les valeurs les plus élevées (45,7 μg·g de poids sec^{-1}) ont été observées avec le triclopyr. Le résidu maximum dans le sol minéral sous-jacent était en moyenne 5,7 fois plus faible que dans la couverture morte. Le glyphosate diminuait de façon exponentielle avec le temps dans les deux cas, peu importe la formulation utilisée. Aucune différence significative ($p = 0,16$, $p = 0,97$ pour la couverture morte et le sol minéral, respectivement) n'a été observée dans le temps estimé pour que 50% de l'herbicide disparaisse (DT_{50}) parmi les traitements au glyphosate. La moyenne globale a été estimée respectivement à 12 ± 2 et 10 ± 3 jours pour les composantes de la couverture morte et du sol minéral. Les résidus de triclopyr, particulièrement dans la couverture morte, ont été caractérisés par une série d'augmentations passagères, reflétant vraisemblablement des apports variables dans le temps provenant de la rosée, du délavage par la pluie ou de la chute de litière provenant de la végétation environnante traitée. Les résidus de triclopyr se dissipaient aussi avec le temps selon des valeurs approximatives de DT_{50} allant respectivement de 39 à 69 jours dans la couverture morte et le sol minéral.

[Traduit par la rédaction]

Introduction

Application of synthetic herbicides continues to be the primary means of intensively managing competing vegetation in Canadian forestry. Vision®,[2] which contains glyphosate as the isopropylamine salt, is the most commonly used herbicide in Canadian forestry (Campbell 1990). Its use in controlling competition and regenerating cutover forest lands is increasing worldwide (Lund-Hoie 1984). Owing largely to patent expiration for this product, there has been a marked increase in research and development of alternative formulations containing glyphosate as the active ingredient (a.i.). Two such products are Touchdown®,[3] which contains glyphosate formulated as the trimethylsulfonium salt, and Mon14420,[2] which contains glyphosate as the monoammonium salt. Another alternative herbicide is Release®,[4] a product containing triclopyr butoxyethyl ester (TBEE). In Canadian forestry, glyphosate and triclopyr are used primarily to control competing brush species in conifer release programs. In planta, all glyphosate salts dissociate to yield the

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[2]Registered trademark of Monsanto Co., St. Louis, Mo.
[3]Registered trademark of ICI Chipman Inc., Stoney Creek, Ont.
[4]Registered trademark of DowAgrosciences Inc., Indianapolis, Ind.

glyphosate free acid (Franz 1974) and thus exert their phytotoxic effects through inhibition of the shikimic acid pathway (Jaworski 1972; Steinrucken and Amrhein 1980). This fact leads to the postulate that various salt forms of glyphosate would behave similarly following application to foliage and provide similar efficacy. Recently, field study results have supported this postulate (Thompson et al. 1994; Pitt et al. 1993). Foliar residues of glyphosate are short-lived (Newton et al. 1984; Thompson et al. 1994) and may be susceptible to wash off with rain or heavy dew occurring shortly after application (Leung and Webster 1993). Similarly, TBEE hydrolyzes in plants to yield triclopyr free acid, which in turn acts as a plant hormone mimic, disrupting normal plant growth, and ultimately leading to phytotoxic symptoms and plant death. Triclopyr ester residues on treated foliage tend to be relatively nonpersistent, dissipating through either rapid penetration (i.e., within 14 h) and translocation processes, or via photolytic degradation of surficial residues (Bentson and Norris 1991). The acid form of triclopyr may persist somewhat longer in treated foliage (Thompson et al. 1994) and may be susceptible to wash off with rain (Michael et al. 1992). The primary dissipation processes are both temperature and species dependent; however field, studies suggest that foliar residues of triclopyr may be comparatively more persistent than glyphosate (Newton et al. 1990; Thompson et al. 1994).

Laboratory and field experiments documenting the general environmental fate of glyphosate and triclopyr herbicides have been previously reviewed (Torstensson and Stark 1978; Torstensson 1985; Ghassemi et al. 1982; Stewart 1991; USDA Forest Service 1984; Neary et al. 1993). Subsequent to application in the environment, glyphosate salts dissociate to yield the free acid, which is in turn dissipated primarily through microbial mechanisms to yield aminomethylphosphonic acid (AMPA) as the principal degradation product (Rueppel et al. 1977). Several studies (Hance 1976; Sprankle et al. 1975; Piccolo et al. 1996) have demonstrated that glyphosate is strongly sorbed to organic matter and to ion-exchange sites in soils and,as a result, it has a low potential for leaching with soil water. Similarly, in matrices with sufficient free moisture, TBEE degrades via base-catalyzed hydrolysis to yield triclopyr acid (Szeto 1993), which in turn may further degrade by either photolytic or biological means to yield the principal metabolite trichloropyridinol (PYR) (USDA Forest Service 1984; Stewart 1991; Johnson and Lavy 1994). As with many ionizable herbicides, the sorptive behaviour of triclopyr is dependent upon organic matter content, pH, and the cation-exchange capacity of the soil (Johnson et al. 1995*a*; Pusino et al. 1994). Both laboratory and field study results suggest that triclopyr exhibits limited to moderate mobility in soils (Lee et al. 1986; Johnson et al. 1995*b*; Stephenson et al. 1990; Norris et al. 1987; Newton et al. 1990).

Terrestrial compartments in forested ecosystems, quite unlike those typically studied in laboratory or agricultural field experiments, are often characterized by poorly humified but highly organic layers, which are variously referred to as litter, forest floor, or duff. Typically, the upper horizons of underlying mineral soils also have very high (20–30%) organic matter content by weight. Finally, the microenvironment (i.e., incident UV irradiation, moisture, temperature, pH, etc.) varies widely depending upon many site factors, including latitude; type, density, and size of the surrounding vegetation; and underlying geomorphology. Microenvironment conditions typical of forest-use scenarios may differ substantially from soil conditions in standard laboratory tests or those in agricultural field studies, from which the bulk of data on the fate and dissipation rate for herbicides are generated.

A number of previous studies have examined the terrestrial fate and persistence of glyphosate (Newton et al. 1984; Roy et al. 1989; Feng and Thompson 1990; Newton et al. 1994) and triclopyr (Stephenson et al. 1990; Newton et al. 1990; Johnson et al. 1995*a*; Johnson and Lavy 1994; Norris et al. 1987) in litter and soils of forest environments. However, none of the available studies have been conducted under environmental conditions pertinent to the Acadian forest region, which has one of the highest use rates for forest herbicides (Campbell 1990). Further, studies comparing the fate of various formulations of the same active ingredient or of different active ingredients with similar potential use patterns are generally lacking. Therefore, a field study was designed and conducted in a regeneration site dominated by sugar maple (*Acer saccharum* L.) that typified the potential use scenarios in the Acadian forest region. The study was designed with the objective of comparing three different formulations of glyphosate and one formulation of triclopyr in terms of efficacy as well as fate and persistence in foliar and terrestrial compartments. Previous publications (Pitt et al. 1993; Thompson et al. 1994) have documented results relating to the comparative efficacy and foliar persistence of these compounds. In this paper, we report on the comparative fate and persistence of the active ingredients in the forest floor and mineral soils following application of the various formulated products.

Materials and methods

Experimental design and chemical application

The study site (46°10′N; 66°56′W) was located approximately 30 km north of Fredericton, N.B., Canada. The previous stand, consisting primarily of tolerant hardwoods, had been full-tree harvested during the winter of 1986–1987, leading to revegetation with a thick uniform cover of saplings, seedlings, and stump sprouts of various deciduous species, including principally sugar maple, striped maple (*Acer pensylvanicum* L.), yellow birch (*Betula alleghaniensis* Britton), mountain maple (*Acer spicatum* Lam.), hazel (*Corylus cornuta* Marsh.), white ash (*Fraxinus americana* L.), beech (*Fagus grandifolia* Ehrh.), and elderberry (*Sambucus pubens* Michx.). The forest floor material included a thick, distinct litter (L) layer (2–3 cm deep) composed principally of sugar maple leaves, an indistinct fermented (F) layer, and a well-developed, highly humified mull (H) layer with granular structure. The combined LFH layers, composing the forest floor, approximated 5 cm in depth. Underlying mineral soils were highly organic (20.8% organic matter by weight), derived from lithic-feldspathic sandstones, coarse to medium textured, and moderately to imperfectly drained (Colpitts et al. 1995). The upper Ah horizon (approximately 10 cm deep) was very dark, only weakly eluviated, somewhat acidic (pH 5.6), nutrient rich (N–P–K, 0.6:31.8:331.1 ppm), and characterized by a high calcium content (2788.2 ppm); it was therefore classified as a Black Chernozem based on *The Canadian System of Soil Classification* (Agriculture Canada Expert Committee on Soil Survey 1987).

Full details of the experimental design and chemical application aspects of this study are provided in previous publications (Pitt et al. 1993; Thompson et al. 1994). Briefly, the study was designed as a randomized complete block (RCB), with crown volume index of the competing brush species serving as the blocking variable. Thus, the site was stratified into three blocks having low (1115–2829 $m^3 \cdot ha^{-1}$), medium (2890–4145 $m^3 \cdot ha^{-1}$), or high (4230–7228 $m^3 \cdot ha^{-1}$) crown volume index (CVI) values. Within blocks, experimental units, consisting of 40 × 25 m plots, were randomly assigned to one of five treatments (Vision®, Touchdown®, Mon14420®, Release®, or untreated control) applied at high, medium, or low rates of active ingredient. The terrestrial fate substudy was designed to overlay the basic RCB design and involved only those plots assigned to highest rates of each treatment (4 chemical treatments × 3 blocks × 1 rate = 12 plots). Nominal application rates pertinent to this terrestrial fate study were 2.14, 1.98, 2.14, and 3.98 kg a.i.·ha^{-1} for Vision®, Touchdown®, Mon14420®, and Release®, respectively. Each chemical was applied using a specific CO_2-pressurized backpack sprayer (model 4F, R&D Sprayers Inc., Opelousas, La.). All sprayers were adapted for applying chemical to the brush canopy using an extended aluminum boom fitted with a single one-quarter KLC-9 nozzle oriented to 0° (straight up) and held at 4.5 m above ground. All sprayers were calibrated to yield equivalent volume application rates (4.32 L·min^{-1}). With a metronome-controlled walking speed of 0.76 m·s^{-1} and a 9.5-m swath width at 2 m above ground, the resultant volume application rate was 100 L·ha^{-1}.

Chemical treatments were applied to the low, medium, and high CVI blocks on the mornings of September 4, 5, and 6 of 1989, respectively. Immediately prior to application, premeasured water and herbicide volumes were thoroughly mixed in 12-L plastic containers and the entire mixture was decanted into the stainless steel sprayer tank. Applications were made to each plot using a total of four passes along premarked track spacing (9.5 m) to span the plot width. Immediately after application, the volumes of the residual spray mixture were measured and subsamples were taken. Active ingredient concentrations in each subsample were analytically determined and used in combination with the recorded application volumes to verify nominal application rates (Thompson et al. 1994).

Meteorological monitoring

Meteorological parameters were monitored to characterize conditions during application of herbicide treatments and subsequently in conjunction with the dissipation of the forest floor and soil residues. During early morning treatment sessions, wind speed and direction were measured at 4.5 m above ground using a cup anemometer (threshold speed 1.2 km·h^{-1}) and wind vane (Heathkit Digital Weather Computer model ID4001, Heath Co., Benton Harbour, Mich.). Visual estimates of leaf wetness were also recorded. Air temperature, relative humidity, and rainfall were monitored continuously using shaded thermocouples set at 1.5 and 4.5 m above ground, a wet–dry bulb psychrometer (Campbell Scientific Inc., Logan, Utah) at 4.5 m above ground, and a tipping bucket rain gauge (model RG 2501, Campbell Scientific Inc., Logan, Utah), respectively. The rain gauge was set to provide contact closure for every 1 mm of rainfall. Air temperature, relative humidity, and rainfall were monitored at 5-min intervals and recorded as 24-h averages using a CR21X datalogger (Campbell Scientific Inc., Logan, Utah).

Forest floor and mineral soil matrix sampling

Within each of these plots, four subplots (2 m^2) were staked and cleared of vegetation to ground level in an effort to maximize chemical deposition to the forest floor layer. Sampling of the forest floor and mineral soil matrices was initiated on the day of treatment and continued until the time of freeze-up. The sampling schedule, which comprised a total of eleven events (0, 3, 7, 15, 22, 29, 36, 43, 50, 63, and 77 days after treatment), was established to maximize definition of expected dissipation curves. On each sampling day, samples of the forest floor and mineral soil layers taken from each of the four subplots were pooled in large plastic bags to form a composite sample for each main plot and layer. Forest floor samples were obtained using a stainless steel box corer (10 × 10 × 2.5 cm depth) and trowel. The box corer was driven into the forest floor until flush with the surface using a 2.25-kg mallet. Forest floor material was excavated from within the defined area and depth of the box corer and transferred into a labeled plastic bag. A bucket auger was then inserted and used to remove the upper 10 cm of mineral soil, which was thus exposed and transferred into a second plastic bag. To minimize potential for cross-contamination, the sampling equipment (box corer, trowel, bucket auger) was washed in soapy water, followed by clear water, and then rinsed in acetone prior to sampling each new plot. Immediately after collection and during transportation to the local frozen storage facility, samples were kept on ice in insulated cardboard boxes to minimize potential microbial, chemical, and photolytic degradation. Within 12 h after collection, samples were placed in frozen storage (–15°C) and kept frozen during storage and transportation to the analytical facility.

Following reception and cataloguing of samples at the analytical facility, samples were thawed, macerated, and thoroughly tumble-mixed to ensure homogeneity. From each homogenized sample, two subsamples of exact mass approximating 5 g were obtained. One subsample was used for moisture content determination, while the second subsample was extracted and analyzed for quantification of glyphosate or triclopyr residues.

Analytical chemistry

Details of the analytical methods used in quantifying glyphosate and triclopyr residues have been previously described (Thompson et al. 1990, 1991). Briefly, quantification of glyphosate residues involves matrix maceration and repetitive aqueous base extraction, followed by centrifugation, filtration, and analyte isolation by ion-exchange chromatography of the supernatant. Purified extracts were subjected to high-performance liquid chromatography with visible wavelength detection. Triclopyr residues were extracted in acetone–hexane (2:1) with aliquots subsequently purified by liquid–liquid partition to diethyl ether. Ether extracts were concentrated, transferred to methanol, and derivatized with boron trifluoride reagent (BF_3 in 14% methanol, Product 27419, BDH Inc., Toronto, Ont.) at 95°C for 1 h to yield the triclopyr methyl ester. Postmethylation, samples were further purified by liquid–liquid partitioning to hexane and Florisil® fractionation of the hexane extracts. Final samples of triclopyr methyl ester were prepared in isooctane and injected on a capillary gas–liquid chromatograph equipped with an electron capture detector for quantitation.

Prior to analyses of field samples, analytical methods were validated by fortifying the replicate matrix blanks taken from control plots of the experimental site. Validation study samples were fortified with appropriate formulations of each herbicide (four replicates per concentration), thoroughly mixed, and equilibrated for approximately 18 h under cool, dark conditions prior to extraction and analyses. Validation samples were then analyzed to determine mean analytical recovery efficiency and precision (coefficient of variation) (Table 1) as well as chromatographic resolution, estimated limits of quantification, and estimated limits of detection. For glyphosate and triclopyr, respectively, the limits of detection were 0.05 and 0.005 μg·g dry $mass^{-1}$ while the limits of quantification were 0.10 and 0.01 μg·g dry $mass^{-1}$.

Statistical analysis

Following the occurrence of peak residues, similar exponential

Table 1. Validation data for the analytical methods used in quantitating glyphosate and triclopyr residues in forest floor and mineral soil matrices derived from an Acadian forest regeneration site.

Fortification (analyte)	Block	Concentration ($\mu g \cdot g^{-1}$)	Recovery efficiency (%)		
			Mean (n = 3)	SD	C.V.
Vision® (glyphosate)	High	4.272	82.44	2.55	3.10
	Med.	1.956	94.79	5.64	5.95
	Low	0.427	79.30	1.94	2.45
Touchdown® (glyphosate)	High	3.96	78.93	7.79	9.87
	Med.	1.815	79.75	2.62	3.29
	Low	0.396	76.87	4.41	5.74
Mon14420 (glyphosate)	High	3.264	92.34	5.83	6.32
	Med.	1.795	80.16	1.42	1.77
	Low	0.489	78.68	2.38	3.02
Release® (triclopyr)	High	5.0	95.75	8.67	9.05
	Med.	0.5	84.19	7.80	9.27
	Low	0.1	86.32	7.48	8.67

patterns of residue decline through time were observed for all glyphosate herbicide formulations. Considering these trends, an exponential decline model was fit to the repeated measures of glyphosate residues from each experimental unit, including peak residues and estimates subsequent thereto. The exponential model took the form of

$$y = a\ e^{-bx} + \varepsilon$$

where y is the residue ($\mu g \cdot g^{-1}$) in the forest floor or mineral soil matrix, a is an estimable parameter, e is the Euler number, b is an estimable parameter, x is time (days after treatment), and ε is the random variation.

Least square estimates of the parameters were obtained by nonlinear regression using the Marquardt algorithm (SAS Institute Inc. 1994). Time to 50% dissipation (DT_{50}) values were then derived from each nonlinear regression equation by solving for the number of days after treatment required to reach half of the observed maximal residue. The DT_{50} values thus obtained were subjected to an analysis of variance to test the hypothesis of equality between treatments, using the linear model shown below to accommodate the underlying randomized block design:

$$Y_{ij} = \mu + \tau_i + B_j + \varepsilon_{ij}$$

where Y_{ij} is the DT_{50} value (forest floor or mineral soil) observed for treatment i and block j, μ is the overall mean, τ_i is a fixed effect associated with the ith treatment (i = 1, 2, or 3), B_j is a random effect associated with the jth block (j = 1, 2, or 3), and ε_{ij} is the random variation.

Model residuals were examined to ensure that the standard assumptions of normality and homogeneity of variance were met.

Results and discussion

Meteorological conditions

During the three morning spray sessions (06:00–08:00, 4–6 Sept. 1989), wind speed monitored at spray release height did not exceed 4.6 $km \cdot h^{-1}$. Visual estimates indicated that substantial amounts of dew had formed in the early morning hours (prior to 06:30), with percent foliar surface moisture ranging from 75 to 100%; by 08:30 surface moisture had diminished, with values ranging from 30 to 50%. Average daily temperatures during the days of application ranged from 17 to 19°C. No rainfall occurred from 2 to 14 Sept., resulting in a minimum rain-free period for all treatments of approximately 1 week after application. The first rainfall event occurred on 14 Sept. 1989 with minimal total precipitation of 0.254 mm. Subsequent major rain events (>0.5 mm) occurred on 15 Sept. (1.0 mm), 17 Sept. (2.54 mm), 20 Sept. (5.59 mm), 23 Sept. (1.8 mm), 24 Sept. (2.29 mm), and 26 Sept. (3.05 mm).

Residues in the forest floor layer

Peak glyphosate residues in the forest floor matrix occurred at various times within 14 days after treatment, with overall averages of 8.25 $\mu g \cdot g$ dry $mass^{-1}$ for glyphosate formulations and 45.7 $\mu g \cdot g$ dry $mass^{-1}$ for triclopyr (Table 2). Higher peak residues for triclopyr than for glyphosate formulations reflect, in part, differences in maximal application rates (2.1 kg a.i.$\cdot ha^{-1}$ for glyphosate and 3.8 kg a.i.$\cdot ha^{-1}$ for triclopyr) and are similar to values reported for triclopyr in tanoak (*Lithocarpus densiflorus* (Hook. & Arn.) Rehd.) litter following aerial applications (Newton et al. 1990).

Initial glyphosate residues in the forest floor were variable, likely owing to differential interception by the brush and ground vegetation. Similar initial variability, delayed peak concentrations, and general magnitude of residues in the forest floor or litter layers have been reported previously (Newton et al. 1984, 1994; Feng and Thompson 1990) following aerial applications of glyphosate. Although glyphosate residues on foliage are susceptible to rainwash (Leung and Webster 1993), no rainfall occurred on the experimental site within the first 7 days after treatment. Therefore, increasing and variable residues in the forest floor compartment observed during the first 14-day period are most likely attributable to inputs via dew or leaf fall from the surrounding treated groundcover and brush foliage. Given the relatively high residues on brush (499–624 $\mu g \cdot g$ dry $mass^{-1}$), as reported previously (Thompson et al. 1994), even small transfers via these mechanisms could potentially yield significant increases in herbicide residues within the forest floor.

However, since glyphosate foliar residues dissipated rapidly (DT_{90} < 16 days; Thompson et al. 1994), no significant inputs to the forest floor were observed beyond 14 days after

Table 2. Summary of peak residue and DT_{50} estimates for residues in the forest floor compartment.

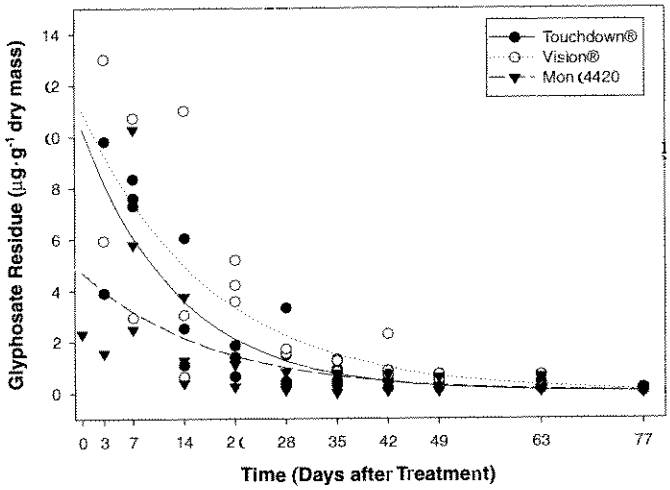
Treatment	Block	Peak residue* ($\mu g \cdot g^{-1}$)	DT_{50}† (days)
Vision®	High	11.0	18.9
	Med	5.95	14.0
	Low	13.0	11.9
	Mean (±SE)‡		14.9 (1.47)
Mon14420	High	5.78	10.5
	Med.	2.49	8.50
	Low	10.2	11.9
	Mean (±SE)‡		10.3 (1.47)
Touchdown®	High	8.35	10.8
	Med.	9.80	12.1
	Low	7.60	9.70
	Mean (±SE)‡		10.9 (1.47)
	Overall mean (±SE)§		12 (0.85)
Release®	High	22.41	43
	Med.	90.31	43
	Low	24.26	39
	Mean (±SE)‡		42 (1.33)

*Maximal residue concentration observed in the matrix.
†Time required for residues to dissipate by 50% (estimates for Release® were approximated by graphic interpolation).
‡Standard error of the least squares means.
§Standard error of mean.

treatment. As a result, glyphosate residues in the forest floor declined following monotonic curvilinear patterns (Fig. 1) that were effectively modeled by exponential decline functions. Exponential models accounted for 77, 85, and 54% of the variation in the forest floor residue data for Vision®, Touchdown®, and Mon14420, respectively. DT_{50} values derived from nonlinear regression equations for each treatment block combination are presented in Table 2. Differences in the DT_{50} estimates between treatments were not significant ($p = 0.16$) and it took an average of 12.0 ± 0.9 days for residues in the forest floor layer to degrade by 50%. This value is considered accurate to within ±2 days 19 times out of 20 and is similar to previously published estimates of 14 days (Newton et al. 1984) and 8–9 days (Feng and Thompson 1990). Brush density accounted for little variation (<12%) in DT_{50} values, suggesting that brush density is not a key factor controlling glyphosate dissipation in the forest floor.

In contrast with the exponential dissipation patterns observed for glyphosate, triclopyr residue behaviour in the forest floor layer varied substantially by block (Fig. 2). Although residues in the forest floor were initially similar among blocks, those in the low and high density blocks followed a slow linear pattern of decline, while those of the medium density block were characterized by a series of transient peaks, with maxima at days 14, 35, and 63. Coincidentally, triclopyr residues in foliage of the medium density block were also relatively high and more persistent than in other blocks (Thompson et al. 1994). In contrast with foliar residues of triclopyr ester (DT_{90} = 11 days), foliar residues of triclopyr acid, particularly those for the medium density block (DT_{90} = 48 days), were quite persistent. These results

Fig. 1. Dissipation of glyphosate residues in the forest floor compartment following application of Touchdown®, Vision®, or Mon14420 formulations to an Acadian forest regeneration site.



indicate that surrounding treated vegetation in the medium density block carried differential and significant loads of triclopyr acid residues and provide a possible explanation for the discrepant residue patterns in the forest floor of this block, if such residues were transferred to the sampling sites via leaf fall or rainwash. The timing of residues in the forest floor for the medium density block is approximately coincident with major rainfall events. Michael et al. (1992) previously reported considerable wash off of triclopyr residues from treated vegetation, further supporting this postulate. Irrespective of the underlying mechanisms, differential block effects and general variability of the forest floor residue data prevented effective modeling or mathematical estimation of DT_{50} values. However, graphical interpolation allowed DT_{50} values from 39 to 43 days to be approximated. These estimates are similar to the value of 31 days reported by Newton et al. (1990) for persistence in tanoak litter.

Residues in the mineral soil layer

Peak concentrations for herbicide residues in the mineral soil layer (Table 3) were on average 5.7-fold (3.0- to 11.9-fold range) lower than those in the forest floor layers. In mineral soil, peak glyphosate residues averaged 1.52 $\mu g \cdot g$ dry mass^{-1} and occurred typically on day 3 or 7. The magnitude of peak residues is similar to that of 1.40 $\mu g \cdot g^{-1}$ reported by Newton et al. (1994) for soil under litter. Relatively lower glyphosate residues in the underlying mineral soil indicate a general lack of vertical mobility irrespective of the formulation applied. This result is consistent with the fact that all formulated products dissociate to yield the same glyphosate free acid moiety, the characteristic binding affinity of glyphosate for organic and ion-exchange sites (Sprankle et al. 1975; Rueppel et al. 1977; Piccolo et al. 1996), the highly organic nature of the forest floor matrix, and the rapid dissipation of glyphosate residues therein. The lack of rainfall during the first week after treatment precluded potential vertical movement during the earliest phase of this study. Thereafter, despite some significant rainfall events (2.54 and 5.59 mm) occurring between 14 and 21 days after treatment, no substantial inputs into the underlying mineral

Fig. 2. Mean triclopyr acid residues in the forest floor compartment following application of Release® to an Acadian forest regeneration site.

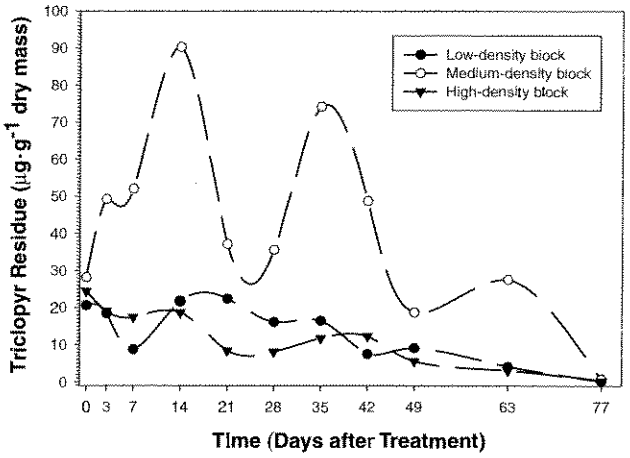


Table 3. Summary of peak residue and DT_{50} estimates for residues in the mineral soil layer.

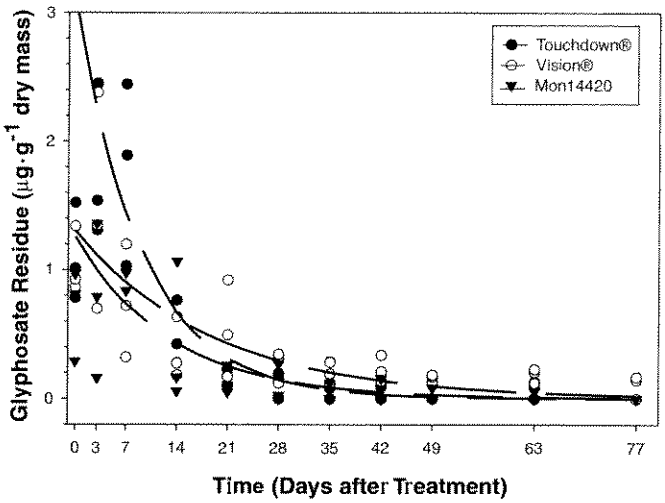
Treatment	Block	Peak residue* (µg·g^{-1})	DT_{50}† (days)
Vision®	High	0.93	15.0
	Med	1.35	5.30
	Low	2.38	10.0
	Mean (±SE)‡		10.1 (2.15)
Mon14420	High	0.97	8.60
	Med	0.84	9.0
	Low	1.36	14.7
	Mean (±SE)‡		10.8 (2.15)
Touchdown®	High	2.45	11.6
	Med	1.89	10.5
	Low	1.54	8.30
	Mean (±SE)‡		10.1 (2.15)
	Overall mean (±SE)§		10 (1.4)
Release®	High	3.91	69
	Med	11.7	42
	Low	6.94	44
	Mean (±SE)‡		52 (8.7)

*Maximal residue concentration observed in the matrix.
†Time required for residues to dissipate by 50% (estimates for Release® were approximated by graphic interpolation).
‡Standard error of the least squares means
§Standard error of mean.

soils were observed. This reflects the fact that most of glyphosate residues in the forest floor matrix had already dissipated by this time and suggests that the remaining residues were strongly bound. The lack of vertical mobility observed in this study adds to previously published literature showing that glyphosate does not leach significantly in forest soils (Roy et al. 1989; Feng and Thompson 1990; Newton et al. 1994).

Exponential models accounted for 76, 88, and 85% of the variability in the glyphosate residue data, depending upon treatment (Fig. 3). No differences ($p = 0.97$) were observed

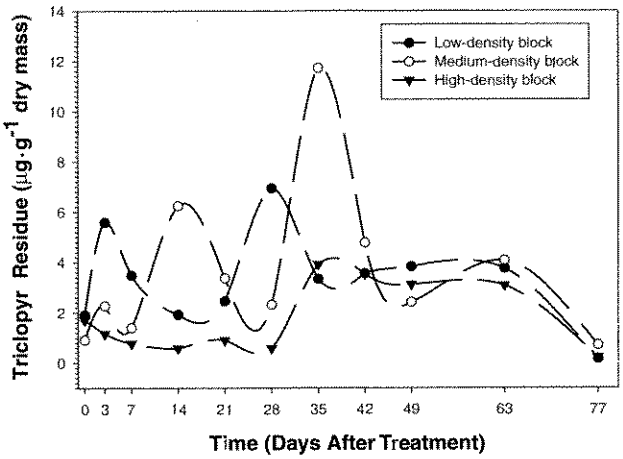
Fig. 3. Dissipation of glyphosate residues in the mineral soil following applications of Touchdown®, Vision®, or Mon14420 formulations to an Acadian forest regeneration site.



in DT_{50} estimates among the various glyphosate formulations and it took an average of 10.3 ± 1.2 days for glyphosate residues in the mineral soil layer to degrade by 50% (Table 3). As noted for the forest floor residue dissipation results, brush density had little effect (<26%) on the variation in DT_{50} values and does not appear to directly or indirectly influence glyphosate persistence in the mineral soil layer. DT_{50} estimates for glyphosate derived from this study are substantially less than estimates of 45–60 days (Feng and Thompson 1990), 24 days (Roy et al. 1989), or 29.2– 40.2 days (Newton et al. 1984) reported for studies in other forest ecosystems. Since equivalent information on the multitude of critical variables influencing overall dissipation rates is not available for all of these studies, it is impossible to determine causality for such differentials. However, given that microbial degradation is the principal mechanism of dissipation for glyphosate in soils, it is probable that variation in persistence is a function of one or more factors controlling soil microbial populations or activity on site (e.g., relative microbial abundance or species composition, organic matter content, soil moisture, pH, temperature).

In contrast, triclopyr residues in the organic layer were characterized by a series of asynchronous peaks depending upon the treatment block, with the most significant increases associated with the medium density block on day 35 (Fig. 4). The pattern reflects residue behaviour in the overlying forest floor layers and indicates that some portion of the triclopyr residues moved through and into the mineral soil layer. This finding is consistent with previous studies demonstrating moderate mobility of triclopyr in forest soils (Jotcham et al. 1989; Stephenson et al. 1990; Newton et al. 1990). While triclopyr residues were comparatively more persistent than those of glyphosate, there was evidence of triclopyr dissipation following the occurrence of peak average residues in all blocks. The maximal mineral soil residue level (11.72 µg·g^{-1}) observed in this study is similar to that of 7.32 µg·g^{-1} reported by Newton et al. (1990) for residues in mineral soils (0–15 cm) following aerial application of triclopyr ester at 3.3 kg a.i.·ha^{-1}. Again, the periodic flux in

Fig. 4. Mean triclopyr acid residues in mineral soil following application of Release® to an Acadian forest regeneration site.



triclopyr residues precluded effective modeling and mathematical estimation of DT_{50} values. However, based on graphical interpolation of the time needed to reach levels consistently below one-half of the maxima, approximate DT_{50} values of 42–69 days were derived. These estimates are within the range of values reported in the literature, which vary from 10 to 138 days (Norris et al. 1987; Stephenson et al. 1990; Johnson and Lavy 1994; Newton et al. 1990). In all the blocks, less than 6% of the observed maxima remained in the mineral soil layer at the end of the 77-day period of observation.

Biological significance of forest floor and mineral soil residues

Direct deposition to the forest floor layers during herbicide applications resulted in average initial residue levels of 7.12 and 24.4 µg·g dry mass^{-1} for glyphosate and triclopyr, respectively. These levels were markedly less than those in treated foliage (529–1630 µg·g dry mass^{-1}) previously reported by Thompson et al. (1994). Residue transfer from the foliar compartment via dew, rainwash, or litter fall resulted in transient increases in glyphosate and triclopyr residues in the forest floor. The resultant average maxima were 8.25 and 45.7 µg·g dry mass^{-1} for glyphosate and triclopyr, respectively. Residues in mineral soil were on average 5.7-fold lower than those in the forest floor, suggesting that these herbicides have a low potential to percolate downward to contaminate groundwater, and that potential impacts are most likely to be associated with biota that are active in the forest floor where residues are greatest.

Maximal glyphosate residues in the forest floor layer are well below the threshold concentrations (>5000 µg·g^{-1}) affecting litter decomposition rates (Fletcher and Freedman 1986). The observed maxima are also generally below no-effect concentrations (>100 µg·g^{-1}) reported for soil microbial growth (Rueppel et al. 1977), soil respiration (Stratton and Stewart 1992), and soil nitrogen fixation or nitrification (Grossbard 1985; Muller et al. 1981; Ghassemi et al. 1982; Stratton and Stewart 1991). However, a study by Jaworski (1972) demonstrated that certain soil microbes may be affected by glyphosate levels as low as 5 ppm, which approximates the maximal concentrations observed here. The maximal triclopyr residues observed in this study are substantially lower than 200 and 500 ppm no-effect thresholds for earthworms and soil microbes, respectively, as reported by Stewart (1991). Considering the nonpersistent nature of these compounds in terrestrial matrices, the maximal residues in forest floor compartments observed in this study, and their relation to available data on effect thresholds we suggest that significant effects on soil microbial populations or functional processes are improbable, further supporting similar conclusions drawn by Newton et al. (1994), Ghassemi et al. (1982), and Fletcher and Freedman (1986).

Conclusions

Regardless of the formulation applied, glyphosate residues in both the forest floor and mineral soil layers dissipated rapidly following similar exponential kinetics, with overall average DT_{50} values of 12 ± 2 and 10 ± 3 days, respectively. Comparatively, triclopyr residues were more variable and persistent, with approximate average DT_{50} values ranging from 39 to 69 days in the forest floor and underlying mineral soils, respectively. Results of this study are generally consistent with previous work in other forest ecosystems, supporting the weight of scientific evidence that suggests that neither glyphosate nor triclopyr is persistent or mobile in forest terrestrial substrates. Results of this study also support the postulate that different salt formulations of glyphosate show equivalent environmental fate and persistence. Finally, relations between the observed maximal residues and the threshold concentrations for toxicity to soil biota suggest that sustained deleterious effects on soil organisms or functional processes are unlikely to result from normal operational use of these herbicides in forestry. Although investigations in this general area have increased dramatically in recent years, the statement of Ghassemi et al. (1982) remains relevant: "Field studies in which environmental persistence and impacts are evaluated under 'real world' conditions can provide the database needed for more accurate assessment of environmental effects of subject herbicides in forest applications." In this regard, both the cost-effectiveness and interpretability of environmental assessments could be maximized by conducting more comprehensive, multicollaborator studies on fate and effects, concurrently, on selected sites as opposed to conducting smaller, separate studies on fate and effects aspects.

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