Research Article

Herbicides in unexpected places: non-target impacts from tree root exudation of aminopyralid and triclopyr following basal bark treatments of invasive chokecherry (*Prunus padus*) in Alaska

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Abstract

Basal bark treatment of invasive trees is an approach designed to limit damage to non-target vegetation in the vicinity, but non-target injury is still documented. No study of basal bark treatments has examined the release of herbicide residues from roots of treated plants and resulting non-target impacts. Studies were conducted in Alaska interior and coastal boreal forests on basal bark treatments with aminopyralid and triclopyr on active-growth and dormant invasive chokecherry (*Prunus padus* L.). The study assessed non-target damage and soil herbicide residue using a combination of visual evaluations, bioassays, and soil residue analyses. Non-target damage from herbicide residues were identified in 40% of treatments containing aminopyralid with triclopyr, 60% of treatments containing aminopyralid alone, and 5% of treatments containing only triclopyr. Laboratory studies of aminopyralid treatments to saplings isolated the effects of herbicide exudation from roots, which was found to be significant, and the magnitude was dependent on dose. Herbicide soil residues in field and laboratory experiments were quantified with analytical detection and plant bioassays. Aminopyralid soil residues were identified in 57% of field treatments receiving 8 to 60 ml of herbicide solution (2% ai) and 70% of laboratory treatments receiving 10 μl of herbicide solution (2% to 16% ai). Triclopyr residues were found from one field treatment following dosage with 28 ml of herbicide solution (18.5% ai). Anatomically, plants grown in soils associated with herbicide-treated trees, both in the field and lab, grew less dry mass than non–herbicide treated controls. This study provides the first evidence that root exudation of herbicide following basal bark treatments contributes to non-target damage of adjacent vegetation and to accumulation of soil herbicide residues. This is an important new factor for integrated pest management within basal bark treatment systems and has implications for other herbicide application types such as injections and frill, as well as determining whether root exudation is species or herbicide specific.

Introduction

Plants exchange compounds through their roots to interact with their surrounding environment in ways that can have profound impacts on pesticides and nutrients (Brink 2016). Among these, root exudates are well known to act as chemical defense mechanisms to protect border cells and respond to possible infections (Baetz and Martinola 2014). Herbicides, when applied to susceptible and non-susceptible plants, may alter or become a component of root exudates (Barker and Dayan 2019; Boydston and Al-Khatib 2008; Dinelli et al. 2007; Hickman et al. 1989; Kremer et al. 2005). Studies of herbicide translocation of pyridine carboxylic acids have indicated that the distribution variability of herbicides to roots and other plant parts is wide, and the compounds are not metabolized within the plants (Bukun et al. 2009; Lewis et al. 2013). When released from treated plants, root exudates may have the potential to cause non-target impacts to species that are sensitive to the applied herbicide (Boydston and Al-Khatib 2008; Dinelli et al. 2007; Hickman et al. 1989).

Stem runoff alone may not account for all the soil herbicide residues, as roots can also exude the applied chemical. Herbicide labels warn that non-target damage is possible from root uptake of soil-active chemicals such as aminopyralid and triclopyr, but do not indicate whether the

source is from drift, runoff, or root exudates (Anonymous 2015, 2018). Runoff, the physical settling of non-absorbed herbicide down the exterior of the plant stem into the soil environment, is a likely explanation for how basal bark treatments can result in soil herbicide residues. Previous field studies involving basal bark treatments of invasive figs (*Ficus carica* L.) with triclopyr identified soil residue concentrations indicative of high rates of application and suspected this was due to residues physically washing (dripping) down the trunk during subsequent precipitation events (Holmes and Berry 2009). Additional field and laboratory studies on the control of herbaceous weeds determined that herbicide leakage from roots results in impacts to surrounding vegetation (Boydston and Al-Khatib 2008; Hickman et al. 1989).

A wide, ubiquitous number of methods exist that facilitate herbicide introduction into the plant vascular system to facilitate death. Among these, basal bark treatment, in which herbicide is carried in mineral oil and applied to the tree trunk from the ground up to 50 cm, is a popular method in Alaska. This requires the herbicide to absorb through the bark, which can extend time to death after application one to two growing seasons. During these long periods between application and plant death, herbicides are translocated through the vascular system. This situation provides a potential for herbicide exudation into the rhizosphere. To date, there are no studies that have examined this process, which may be highly relevant in explaining unaccounted for non-target damage.

The pyridine carboxylic acid herbicides, aminopyralid (Milestone®) and the butoxy ethyl ester of triclopyr (Garlon 4®) are Group 4 auxin mimics labeled for basal bark treatment of unwanted trees (Anonymous 2015, 2018; Shaner 2014). Aminopyralid and triclopyr products differ, in that aminopyralid is more persistent and provides multiple seasons of control (Anonymous 2015, 2018; Tomco et al. 2016). Aminopyralid persists in a biologically available state for several years after application and accumulates in non-susceptible grasses, bioaccumulates in manures from animals fed contaminated grasses, and impacts sensitive crop species several years after application (Anonymous 2018; Seefeldt et al. 2013). In Alaska, significant changes to microbial communities after application of aminopyralid have not been detected, indicating microbial degradation of aminopyralid is not a major factor in its environmental fate (Tomco et al. 2016). Other evidence for the process of aminopyralid degradation in Alaska is lacking. Herbicides with high persistence, such as aminopyralid, require recognition of sensitive desirable vegetation and soil properties affecting persistence to control the target species and avoid negative impacts to susceptible non-target species.

Similar application techniques and activity of aminopyralid and triclopyr make them good model herbicides for assessing the factors associated with herbicide persistence and its non-target effects from accumulation of pesticide residues released from target plants. Both herbicides when absorbed by plants are transported in vascular tissue to accumulate in growing points of the plants (Shaner 2014). The compounds differ in persistence and thus potential for exposure from the soil in the long term. Triclopyr is less persistent (half-life of 10 to 46 d) than aminopyralid (half-life of 14 to 143 d), likely due to aminopyralid binding with soil increasing in strength over time (Shaner 2014). In Alaska, biologically active aminopyralid residues have persisted several years after application (Tomco et al. 2016).

Chokecherry (*Prunus padus* L.) is an excellent model organism to study the release of pesticide residues from basal bark treatments

and their persistence at high latitudes. These trees were introduced to Alaska in the 1950s as hardy, fruit-bearing ornamentals. However, by the early 2000s, *P. padus* trees had become well distributed throughout natural forests in the Anchorage Municipality, with dense infestations that require control (AKEPIC 2011). These plants are toxic to moose (*Alces alces* L.) and have the potential to decrease terrestrial food subsidies for salmon (*Oncorhynchus* sp.) in streams (Roon et al. 2016; Woodford and Harms 2011). Because these invasive plants alter ecosystems in Alaska to impact species such as moose and salmon, invasive plant managers commonly pursue control with basal bark applications.

The objectives of this study were to determine the potential for aminopyralid and triclopyr to exude from roots of invasive *P. padus* following basal bark treatments. Laboratory experiments were conducted to isolate the root exudation process and quantify potential impacts to vegetation using bioassay and residue quantification analytical chemistry approaches. Field experiments were designed to quantify the impact to surrounding vegetation from herbicide residue in soil, coupling this with bioassay and analytical detection of the herbicide. Summer active-growth and fall dormant treatments were performed to correlate the timing of herbicide application to non-target impacts and control efficacy. We hypothesized that (1) *P. padus* can exude herbicide residues at phytotoxic levels following basal bark application, and (2) aminopyralid residues are preferentially accumulated in soil over triclopyr.

Materials and Methods

Study Sites

The study involved field sites in interior (64.854°N, 147.853°W) and south-central (61.170°N, 149.871°W) Alaska locations representative of high-latitude continental (interior) and maritime (south-central) climates within boreal forests. Studies were conducted from 2017 to 2019 at four sites, two in Fairbanks (interior) and two in Anchorage (south-central).

The Fairbanks sites were both on the University of Alaska Fairbanks campus at the Agriculture and Forestry Experiment Station, one a mixed-hardwood windrow and the other in an experimental spruce forest. Both Fairbanks sites were moderately invaded (60% cover in site 1 to 15% cover in site 2), with *P. padus* ranging in size from 2-cm basal diameter saplings to 20-cm basal diameter mature trees. Other woody vegetation in the windrow included cottonwood (*Populus balsamifera* L.), green alder [*Alnus viridis* (Chaix) DC.], and the nonnative Siberian pea shrub (*Caragana arborescens* Lam.). The experimental spruce forest consisted of mature white spruce [*Picea glauca* (Moench) Voss] and *A. viridis* at varying densities. The understory vegetation at both Fairbanks sites was dominated by quackgrass [*Elymus repens* (L.) Gould] and nonnative bird vetch (*Vicia cracca* L.) located primarily on the forest edge. The site had a 0% to 2% slope and a mucky silt loam soil (Tanana series).

The Anchorage sites were both located in the Campbell Creek Greenbelt in a mixed spruce–hardwood forest. The sites were moderately (50% cover) invaded with *P. padus* and contained mixed woody vegetation including *P. balsamifera*, birch (*Betula papyrifera* Marshall), willow species (*Salix bebbiana* Scarg and *Salix scouleriana* Barratt ex Hook.), and *A. viridis*. Understory shrubs and herbaceous vegetation were diverse, including species such as dwarf dogwood (*Cornus canadensis* L.), cow parsnip (*Heracleum maximum* W. Bartram), and horsetail (*Equisetum arvense* L.). The sites had a 0% to 3% slope and silt loam soil (Moose River-

Niklason complex series, coarse-loamy, mixed, superative nonacid Typic Cryaquents and coarse-loamy over sandy or sandy-skeletal, mixed, superactive, nonacid Typic Cryofluvents).

Efficacy of Control and Non-target Impacts

Each site in Anchorage and Fairbanks was assigned an active-growth or dormant treatment. A completely randomized experimental design was applied at each site with individual *P. padus* trees as experimental units. Each herbicide treatment was replicated five times at each site and application timing. Each treated tree was spaced the greater of 4 m or twice the drip line distance from the nearest treated tree to ensure no root-to-root interaction. Drip line distance was defined as the maximum distance that the branches spread from the base of the tree and is used to approximate the spread of belowground root systems (International Society of Arboriculture 2022). Herbicide treatments were mixed in a paraffin oil carrier (Basal Oil Blue®, Alligare, Opelika, AL 36801), and included full label rates of aminopyralid (Milestone®, 6 g ai L^{-1} [2% ai], Dow AgroSciences, Indianapolis, IN 46268), butoxyethyl ester formulation of triclopyr (Garlon 4®, 143 g ai L^{-1} [18.5% ai], Dow AgroSciences), a mix of both aminopyralid and triclopyr at 6 g ai L^{-1} (2% ai) and 143 g ai L^{-1} (18.5% ai), respectively. Two control treatments were included, one consisting of application of the paraffin oil carrier alone, and the other an application of water alone.

Low-volume basal bark treatments were applied on the lower 46 cm of the tree trunk with a 1.5-L hand pump pressurized sprayer with a single, coarse cone nozzle affixed to a knapsack sprayer (Chapin 10030, Chapin International, Batavia, NY 14021). Treated trees received approximately 4 ml of herbicide solution cm^{-1} of basal diameter, as calculated from the sum of the basal diameter of each tree divided by the total volume used in the treated location. Summer active-growth treatments were applied on August 29, 2017 (13 C, wind 10 km h^{-1}, 70% relative humidity) and September 11, 2017 (11 C, wind 8 km h^{-1}, 60% relative humidity) in Fairbanks and Anchorage, respectively. Fall dormant treatments were applied on October 5, 2017 (11 C, no wind, 60% relative humidity) and October 11, 2017 (8 C, 8 km h^{-1}, 80% relative humidity) in Fairbanks and Anchorage, respectively. It is unknown whether trees were truly dormant during the October treatments, as these trees are typically in various stages of senescence at that time.

Evaluations of treated trees and non-target impact occurred in the last 2 wk of June 2018 and 2019 (9 and 21 mo after active-growth treatments; 8 and 20 mo after dormant growth treatments). Percent defoliation was visually estimated for each target tree to determine control efficacy. To evaluate damage to non-target vegetation, symptoms of herbicide damage such as leaf cupping or curling were recorded as the number of incidences of herbicide damage to an individual plant, and the affected species was documented.

Soil Sampling

Soil samples were collected underneath the drip line of treated trees and frozen (−20 C) until bioassay and instrumental analysis testing for both herbicides. Soil sample collection occurred in September to October of 2018 to allow for tree death. Samples of soil (0.5 kg each) were dug from holes approximately 15-cm deep spaced a minimum of 31 cm apart to cover the whole drip line distance dug in two different directions from the tree's trunk, resulting in two to eight total soil samples dug from underneath each tree. The samples dug from under each tree were composited from each direction, making two samples per tree for herbicide analysis and bioassay.

Aminopyralid Root Exudation Isolation of Drip-Off Effect

Laboratory manipulations to detect and identify root exudation of herbicide residues were performed with only aminopyralid, because triclopyr was shown not to have significant herbicide residues or non-target damage in field experiments. *Prunus padus* saplings, approximately 31-cm tall and 5 to 6 mm in stem diameter, were removed from forested areas around Anchorage, AK, in June 2018 and replanted in 6-cm^3 square pots with drainage holes with soil from the Anchorage area forest sieved to 2 mm to remove larger organic matter. Saplings were grown outside in a shaded spot for the duration of the summer to allow saplings to isolate root damage from harvest, recover from transplant shock, and grow more fine roots in the pots. We placed trees under fluorescent lights (38 μmol m^{-2} s^{-1}) at ambient room temperature in August 2018 to prevent senescence before treatments were applied.

Saplings were placed in a growth chamber (Thermo Scientific Classroom Plant Growth Chamber, Thermo Fisher Scientific, Marietta, OH 45750, 38 μmol m^{-2} s^{-1}, 20 C) for 3 d before treatment to allow them to acclimate. Saplings were randomly assigned herbicide treatments that included an aminopyralid-basal oil solution (10 μl) at concentrations of 0, 6, 12, 24, and 48 g ai L^{-1}, with 13 saplings in each herbicide treatment. Herbicide solution was applied to saplings lying on their sides, using a micropipette just below the first major branch. Treated saplings remained on their sides for 90 min to allow the herbicide mixture to dry and absorb on the stem without running off to the soil. White paper towels were placed below saplings lying on their sides, and no dripping of the blue herbicide mixture was seen. After 90 min, three saplings from each treatment were sealed in individual plastic bags and placed in a freezer on their sides. The remaining 10 saplings from each treatment were placed upright into the growth chamber and wet with enough water-based fertilizer solution (Miracle-Gro® All Purpose Plant Food, Scotts Miracle-Gro Products Inc., Marysville, OH 43041; 24-8-16, 0.5 g L^{-1} water) to wet the soil without seeping through the pot (approximately 5 ml) three times a week.

At 3 wk after treatment, herbicide damage to saplings was assessed on a scale of 1 to 10 adapted from Washington State University (2002) to categorize observed plant damage (Figure 1). Curling and cupping of leaves is the indication of herbicide damage, and this is sometimes most evident on newest growth. After the evaluation, plants were sealed in plastic bags and placed in a freezer (−20 C) until the soils in which they were planted were used for bioassays and extraction of aminopyralid to determine the soil herbicide concentration.

Bioassays

The biological impacts of herbicide residues from field and lab treatments were assessed using bioassays of the soil sampled from beneath treated trees. Narrow leaf hawksbeard (*Crepis tectorum* L.) was used as the bioassay species, because it is particularly sensitive to aminopyralid (Seefeldt et al. 2013). The four soil samples taken from each direction under field-treated trees were combined for each individual tree. Soils from the lab treatments were gently shaken from the root system of the treated tree. *Crepis tectorum* seeds (six) were planted in labeled petri dishes (3.5-cm diameter by 1-cm height) containing soils from a treated tree and placed in a growth chamber (Thermo Scientific Classroom Plant

Figure 1. Examples of curling and cupping of leaves from herbicide damage to treated saplings and their associated scores. A score of 0 indicates no visible damage from herbicide. A score of 1–3 (slight damage) indicates that there is some sign of herbicide cupping on a minority of the leaves, but not severe cupping. A score of 4–6 (moderate damage) indicates cupping on most but not all of the leaves. A score of 7–9 (severe) damage indicates cupping on all leaves with portions of leaves becoming brittle. A score of 10 was given if all leaves were cupped, brittle, and appeared dead.

Growth Chamber, 38 µmol m^{-2} s^{-1}, 20 C). Bioassays were watered with enough fertilizer water solution (Miracle-Gro® All Purpose Plant Food, 24-8-16, 0.5 g L^{-1}) to bring the soil to field capacity three times per week. At 20 d after seeding, symptoms of herbicide damage and dry plant biomass were recorded.

Herbicide Soil Quantification

Herbicide residues were extracted from soil as described in Tomco et al. (2016), with minor modifications. Briefly, soil (10 g wet weight) was extracted with 9:1 acetonitrile:1 N HCl (10 ml) on a rotary shaker for 1 h, then centrifuged at 1,200 rpm for 10 min. The soil–acetonitrile mixture was then frozen overnight, allowing the organic phase to stratify, and the supernatant liquid organic layer was removed and evaporated to 0.3 ml. The sample was resuspended in 2 ml 1 N HCl and cleaned using solid-phase extraction with Macherey-Nagel Chromabond® HR-P cartridges (200 mg, 3-ml tube, Peeke Scientific, Sunnyvale, CA 94085). SPE cartridges were conditioned with 3 ml of methanol, followed by 3 ml of 1 N HCl. Extracts were loaded onto cartridges, washed with 9 ml 1 N HCl, and dried under vacuum for 15 min, and then the analyte was eluted with three 1-ml ethyl acetate additions (1% formic acid). Eluates were evaporated to near dryness, resuspended in 1.0 ml of liquid chromatography-mass spectrometry (LC/MS)-grade methanol, filtered (0.45 um) into 2-ml autosampler vials, and stored at −20 C until analysis.

Aminopyralid and triclopyr in soils were quantified as described in Tomco et al. (2016). Briefly, extracted residues were analyzed with an Agilent LC/MS-MS system with a 1200 (SL) series high-performance liquid chromatograph coupled to a 6410B triple quadrupole mass spectrometer. A Restek Ultra C18 150 mm by 2.1 mm I.D. by 3 µm column (Restek, Bellafonte, PA 16823) was used. The two mobile phases used were methanol with 0.1% formic acid (A) and water with 0.1% formic acid (B). The column was equilibrated at 25% Solvent A, then the sample was injected with a gradient increasing from 25% to 40% completed at 3 min, followed by a gradient to 90% concluded at 7 min and held for an additional 3 min. Injection volumes were 5 µl with a flow rate of 0.3 ml min^{-1}. Samples were ionized in electrospray positive mode for aminopyralid and negative mode for triclopyr and detected with multiple reaction monitoring. One quantifier and one qualifier were

optimized from m/z mass transitions. Cell accelerator voltage was 7 V and capillary voltage was 2,000 V for aminopyralid and 4,000 V for triclopyr. Compound-specific mass spectrometer parameters and mass transitions were optimized for each analyte. A six-point linear internal standard calibration curve was used. The instrument detection limit and limit of quantification for both aminopyralid and triclopyr was 0.5 ppb and 9 ppb, respectively. Moisture content was calculated for each soil sample by drying at 90 C for 48 h, and subtracting the dry weight from the wet weight.

Statistical Analysis

Efficacy of control for field treatments was compared with ANOVA using percent defoliation measured in June 2018 (arcsin square-root transformed). Non-target impacts and detection of herbicide residues resulting from field treatments were compared with Pearson chi-square using the number of times that a treatment unit (treated tree) resulted in at least one incidence of non-target damage or detection of herbicide residue. Detections of herbicide from dose applied in laboratory experiments were compared using logistic regression. The biological activity of herbicide residues in soil was assessed with bioassays by comparing the number of live plants and the plant dry mass resulting from each treatment using factorial ANOVA. Field and lab treatments were separated for the bioassay analysis because they addressed different questions.

All statistical analyses were performed with SPSS Statistics 25 (IBM SPSS Statistics, Armonk, NY 10504). Significant differences were identified with P-values < 0.05. ANOVA was performed for *C. tectorum* bioassays following lab treatments of saplings, with treatment as a fixed factor and treatment group as a random factor to determine differences in the resulting live plants and plant dry mass Factorial ANOVA was performed on the resulting defoliation of treated trees and weights of *C. tectorum* bioassays from field treatments with treatment, phenophase, and location (Anchorage and Fairbanks) as fixed factors and included the interaction of each. Pairwise comparisons with LSD identified differences between individual treatments. A Pearson chi-square test was performed on the herbicide detection and observations of non-target impact data that included treatment location and
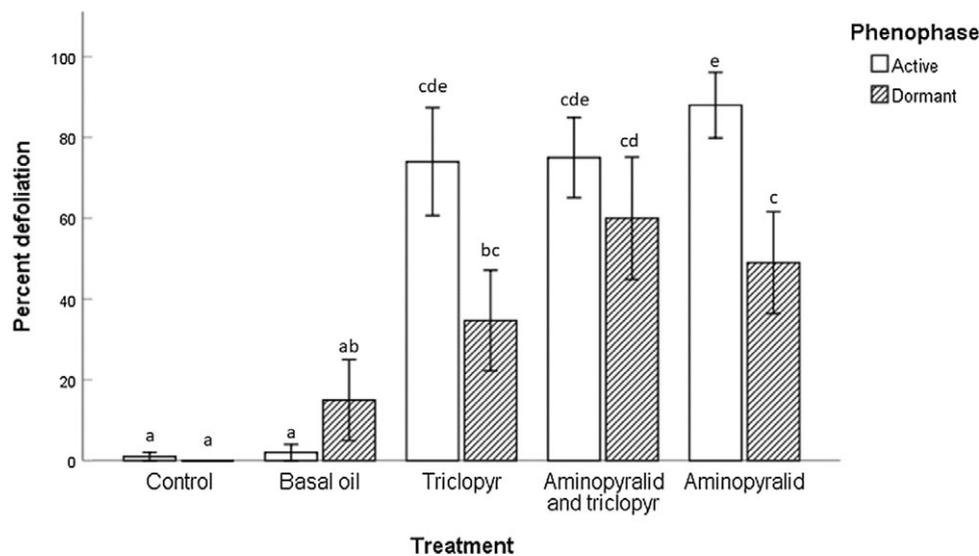
Figure 2. Effects of treatment and phenophase on mean percent defoliation. Error bars are 1 standard error of the mean ($N = 100$). Letters indicate statistical differences determined by LSD test ($P < 0.05$).

phenophase of treatment as categorical predictor variables of herbicide detection and observations of non-target damage in the models. Logistic regression was performed on herbicide detection in soils from lab treatment of saplings with treatment rate and herbicide damage score as predictor variables of herbicide detection. Significance was tested with Wald statistic (W) for the overall model, and Rao's score statistic (RSS) for importance of predictor variable based on whether it is removed from the model. Variables were stepwise removed from all logistic regression models.

Results and Discussion

Field Experiments

The field experiment results indicated that defoliation was increased by herbicide treatment ($F(4, 92) = 11.10$, $P = 0.022$). Efficacy of treatments containing aminopyralid or aminopyralid and triclopyr was similar within the same phenophase (Figure 2). Treatments containing aminopyralid applied to actively growing trees were more effective than all dormant treatments ($P < 0.05$; Figure 2).

Basal Bark Treatments

In this study, basal bark treatments of invasive *P. padus* trees in Alaska completed during the active-growth phenophase may result in quicker defoliation the following season, and some early signs of herbicide damage were observed within weeks of the August applications. However, all treatments resulted in adequate control regardless of timing. This is likely because the invasive *P. padus* in August (active growth) and October (dormant) are both transporting nutrients to the root system. The auxin herbicides follow the nutrient flow, effectively killing the plants. Basal bark treatments can be applied to actively growing or dormant trees, effectively extending the season for application. After application, the herbicide absorbs through the bark and moves with xylem or phloem to the shoot or root endpoints, respectively. Whether transport is primarily in xylem or phloem can vary by species and whether the plant is actively growing or preparing for dormancy. For these reasons, it is widely understood that application

timing can impact the efficacy of an herbicide treatment and should be studied for specific species and herbicides used. For example, Chinese privet (*Ligustrum sinense* Lour.) basal bark treatments were more effective in fall than spring (Enloe et al. 2018). Conversely, control of scotch broom [*Cytisus scoparius* (L.) Link] is not impacted by application timing (Oneto et al. 2010). *Cytisus scoparius* fixes nitrogen and does not require as much transport of nutrients to roots before dormancy, while *L. sinense* and the invasive *P. padus* do not fix nitrogen. Invasive *P. padus* retain green leaves longer than many native trees and shrubs in Alaska, and the primary transport (xylem or phloem) occurring at these times is not known.

Non-target Impacts to Vegetation

Herbicide damage to non-target vegetation was observed in association with 11 of the treated trees. Treatments containing aminopyralid had higher frequency of treated trees with associated non-target damage ($\chi^2 = 12.757$, df = 4, $P = 0.013$; Table 1). The majority of herbicide damage resulted from treatments containing aminopyralid and only one treatment that contained triclopyr alone (Table 1). Neither the location nor the phenophase of treatment influenced observations of non-target impacts ($\chi^2 = 0.595$, df = 1, $P = 0.440$). Species that suffered non-target damage near the herbicide treatment are summarized in Table 2.

Herbicide Exudation from Plant to Soil

Herbicide released into the soil surrounding treated trees was compared among treatments with analytical detection of aminopyralid in soils. Aminopyralid was detected in soils collected underneath 57.5% of the treated trees that had aminopyralid in their treatment, while triclopyr was only detected once (Table 1). Aminopyralid in treatments resulted in detection of herbicide ($\chi^2 = 46.955$, df = 4, $P < 0.001$). Aminopyralid was detected in 80% of treatments in Fairbanks but only in 35% of treatments in Anchorage, and the effect of location on detection was significant ($\chi^2 = 3.919$, df = 1, $P = 0.048$). Detections of aminopyralid did not differ between active and dormant treatments ($\chi^2 = 0.167$, df = 1, $P = 0.683$). Trace quantities of aminopyralid were detected, though still

Table 1. Number of detections of aminopyralid in soil from beneath treated trees and non-target observations.

Treatment[a]	Detections	Mean herbicide ppb (N)[b]	Non-target impact observations	Herbicide detections that co-occurred with non-target observations[c]
Aminopyralid	11	12 (3)	6	3
Aminopyralid + triclopyr	12	4 (3)	4	3
Triclopyr	1	NA	1	0

[a]$N = 19–20$ for each treatment.
[b]Mean herbicide concentration (ppb) is calculated from the detections that are above the limit of quantification. The limit of detection is 0.5 ppb, while the limit of quantification is 1.2 ppb.
[c]Herbicide detections that co-occurred with non-target damage are those treated trees with detections of herbicide and an observation of non-target damage.

Table 2. Species with herbicide damage symptoms from within the drip line of treated trees.

Location	Aminopyralid	Aminopyralid + triclopyr	Triclopyr
Anchorage	European mountain ash (*Sorbus aucuparia* L.)	Prickly wild rose (*Rosa acicularis* Lindl.) *Heracleum maximum* *Sorbus aucuparia*	*Populus balsamifera*
Fairbanks	*Caragana arborescens* *Picea glauca* *Alnus viridis*		

herbicidal, with most samples just above the method detection limit and several above the quantification limits. Treatments containing aminopyralid alone had a higher mean concentration (12 ppb) than treatments containing aminopyralid with triclopyr (4 ppb) (Table 1). However, the small sample size ($N = 3$) does not lend itself to robust comparisons, and the difference was driven by one sample with a concentration of 29 ppb. Of the 20 trees that had aminopyralid detections, only 5 trees had aminopyralid detected in both soil samples, indicating that herbicide may have been missed if sampling was not done in close enough proximity to a root. The sampling area in this study is much smaller (0.157 to 0.628 m^2) than the entire area under a tree that the roots can explore (minimum 0.707 m^2). Detections of herbicide and observations of non-target damage co-occurred in six trees, which represents 26% of the total aminopyralid detections and 60% of the damage observations (Table 1).

Compounds that are herbicidal at low doses still contain bioavailable residues that may be below the limits of analytical detection. One such compound with an application rate for basal bark treatments using the full label rate is aminopyralid at 6 g L^{-1}, which is much lower than triclopyr at 143 g L^{-1} (Anonymous 2015, 2018). Canada thistle [*Cirsium arvense* (L.) Scop.] is more susceptible to aminopyralid than a similar compound, clopyralid, as shown by less absorption of aminopyralid being measured but control efficacy being higher (Bukun et al. 2009). Detection of bioavailable pesticide residues below limits of analytical detection is completed with bioassays using the growth response of highly sensitive species as evidence of the presence of pesticide residues at concentrations under limits of detection (Ranft et al. 2010). *Crepis tectorum* is fast growing and highly sensitive to synthetic auxin herbicides such as aminopyralid and triclopyr, making it an ideal candidate for detection of herbicide residues below analytical detection limits (Anonymous 2015, 2018; Seefeldt et al. 2013).

Bioassays were used to detect the bioavailability of herbicide in soil. Multiple factors (treatment, phenophase, and location of the treatment) were assessed to evaluate the impacts on bioassay weight. Treatment was significant ($F(4, 191) = 7.230$, df = 4, $P = 0.041$); however, phenophase ($F(1, 194) = 1.162$, df = 1, $P = 0.476$) and location ($F(1, 194) = 1.431$, df = 1, $P = 0.460$) were not. Plants receiving herbicide application expressed lower growth than controls by 46% for aminopyralid, 42% for aminopyralid with triclopyr, and 28% for triclopyr (Figure 3). Treatment was the only factor with an effect on the number of plants that were alive at the end of the growing period ($F(4, 191) = 10.272$, df = 4, $P = 0.022$), but the treatment containing aminopyralid with triclopyr was the only treatment that differed significantly from the control ($P = 0.003$) with a 14% reduction in plants alive at the end of the experiment.

This study determined that phenophase timing of herbicide application did not alter non-target impacts or soil herbicide residues. Compared with aminopyralid, triclopyr had less impact, and nominal herbicide residue occurrences were detected in soil. Triclopyr, both butoxy-ethyl ester and free-acid forms, is less persistent in and has lower soil adsorption rates, resulting in less exposure to non-target plants due to leaching (Shaner 2014). Herbicide fate and persistence at the site of application is dependent on leaching potential. The organic carbon partition coefficient (K_{oc}) is a measure of the portion of the tested compound present in water and soil and describes the potential for leaching. Some compounds such as glyphosate ($K_{oc} = 24,000$ L kg^{-1}) bind to soils so strongly they are not bioavailable to plants (Shaner 2014). Other products remain bioavailable with varying leaching potential. Triclopyr butoxy ethyl ester has a lower leaching potential ($K_{oc} = 780$ L kg^{-1}) than aminopyralid ($K_{oc} = 10.8$ L kg^{-1}) (Shaner 2014). These results indicate that triclopyr treatments are preferable when non-target damage is not acceptable, and aminopyralid should be used when control efficacy must be optimized. Further work is necessary to determine whether decreasing the dose of aminopyralid results in less exudation and non-target damage.

Aminopyralid detections occurred more frequently at Fairbanks sites, which is consistent with a previous study that indicated aminopyralid is more persistent in interior Alaska compared with south-central Alaska (Tomco et al. 2016). However, the Fairbanks treatments did not have higher occurrence of non-target damage than the Anchorage treatments, likely due to vegetation differences between sites. The Fairbanks site was in a windrow and manipulated forest, while the Anchorage site was in a natural forest. The increased biodiversity of vegetation present in Anchorage natural forests where the treatments occurred may have provided an increased opportunity for non-target damage, as increased diversity increases the likelihood of an exudated herbicide residue interacting with a sensitive species. This may have masked the effect of increased persistence in the soils in Fairbanks.
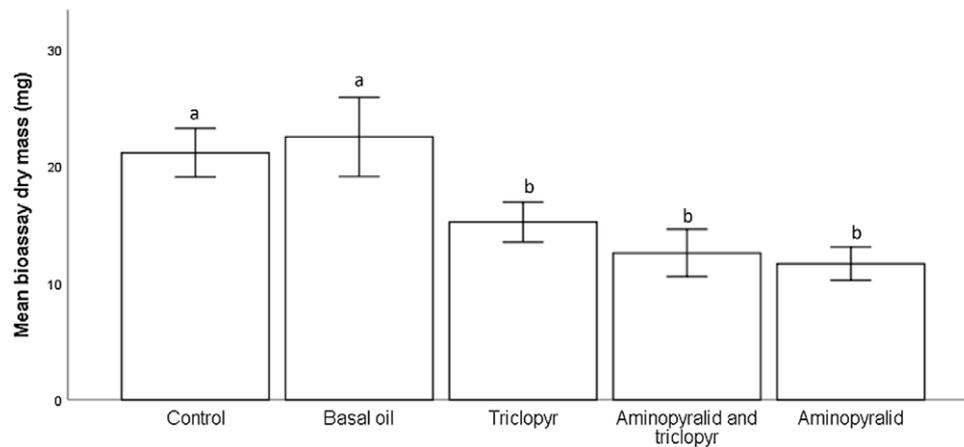
Figure 3. Mean biomass (mg) of *Crepis tectorum* bioassays by treatment. Error bars are 1 standard error of the mean ($N = 100$). Means followed by the same letter are not statistically different as determined by LSD test ($P < 0.05$).

In this study, drift was prevented by selectively applying herbicide with a coarse spray from an adjustable cone nozzle at low pressure to create a stream of liquid that is more direct than what results from using a flat-fan nozzle. One previous study measured drift from basal bark treatments 0.7 m from the base of the target tree after applications using a flat-fan nozzle at 15 to 85 psi (Voinorosky and Stewart 2021). Chances of drift should increase with application to smaller-diameter trees. In this present study, no effect of basal diameter on detection of herbicide was seen, indicating that herbicide residues detected in soil are more likely a result of root exudation than drift. These findings are consistent with other studies that observed non-target damage from root-exuded herbicides (Boydston and Al-Khatib 2008; Hickman et al. 1989; Rodrigues et al. 1982). A previous study of basal bark treatments at high densities of the target species determined that herbicide residues can accumulate in soils at much higher rates than those of regular field application (Holmes and Berry 2009). This study treated individual trees and indicated that non-target impacts are probable from basal bark treatments at low densities of application to invasive *P. padus* trees in Alaska.

Aminopyralid Root Exudation Isolation of Drip-Off Effect

Aminopyralid was detected in soil from 70% of treated saplings and increased with the highest dose (RSS = 15.863, df = 4, P = 0.003; Table 3). In a separate laboratory manipulations experiment to isolate herbicide residues released from the roots and not due to runoff (drip-off) of the applied formulation down the bark, 25% of treated saplings had detectable residues (Table 3). Analysis of the co-occurrence of herbicide damage score with detections of herbicide concluded that observable damage predicted detections (RSS = 21.689, df = 9, P = 0.010; Table 3). Treatment had an effect on biomass ($F(4, 36) = 8.597$, P < 0.001) and live plants ($F(4, 36) = 5.223$, P = 0.002). Bioassays from controls were more than quadruple the mass of all bioassays from herbicide-treated trees, while none of the treatments containing herbicide differed from one another (Figure 4). Live *C. tectorum* were present in control treatments at more than twice the rate of those present in herbicide treatments, while germination from herbicide treatments did not differ.

These findings represent a novel, previously uncharacterized pathway for aminopyralid to injure non-target plants through root exudation. Previous efforts to determine non-target impacts from basal bark treatments have asserted that soil herbicide residues are from drift or runoff from rain that washes the herbicide off the stem (Holmes and Berry 2009; Voinorosky and Stewart 2021). The lab experiments controlled for drift and runoff of herbicide down the stem after application. These results isolate roots as the source of the soil herbicide residues. Concentrations of soil herbicide residues from treated trees were so low that those concentrations only increased at the highest rate studied, which is eight times the label rate of application (Anonymous 2018). The association of damage score with detection of herbicide (Table 3) demonstrates that the herbicide translocated through the plant. These results are consistent with other studies detecting herbicide residues released from roots; however, this study used soil as the medium for detection, because soil provides a more realistic inference of the bioavailable fraction of herbicide. Previous studies have optimized the detection of herbicide in root exudates by using non-sorptive media such as perlite and inert sands (Boydston and Al-Khatib 2008; Hickman et al. 1989; Rodrigues et al. 1982). Use of media other than soil or improving soil herbicide extraction techniques with strong solvents can overestimate biological availability of the herbicide in comparison to that of natural soil settings (Ortega-Calvo et al. 2015; Ranft et al. 2010). In this study, bioassays confirmed the effect of herbicide did not differ between applications that included the herbicide. These bioassays were performed with a small volume of soil for a very short time (20 d). Growing plants for bioassays in larger volumes of soil for longer periods may result in a better association of initial herbicide concentration applied with impact to growth.

We suggest that invasive plant managers should expect herbicide release to the environment from plants after direct treatments such as basal bark. The amount of herbicide appears slight and tolerable when applied to an individual tree; however, this effect may be exacerbated in the case of dense stands of invasive tree infestations. The quantity and fate of the herbicide may vary by species, region, herbicide, and application method. Direct treatments such as basal bark use high concentrations of herbicide compared with foliar applications and may result in more herbicide residues in soil than expected (Holmes and Berry 2009; Voinorosky and Stewart 2021). Observation periods for non-target damage should span multiple seasons, because the target plant may release herbicide through root exudation or plant decomposition at any time after

Table 3. Results of the lab experiments include the number of samples above the limit of detection (3 ppb) in each treatment, the calculated concentration of aminopyralid from the samples (N) that are above the limit of quantification (9 ppb), and the mean herbicide damage score.[a]

Aminopyralid treatment g L^{-1}	Aminopyralid detection (dripoff)	Aminopyralid ppb (N)	Damage score
0	0 (0)	NA (0)	0
6	7 (1)	NA (1)	4
12	5 (1)	NA (2)	3
24	6 (1)	NA (2)	4
48	10 (0)	23 (7)	5

[a]Aminopyralid concentrations were not calculated if fewer than three samples had detections above the limit of quantification. $N = 10$ for each treatment.
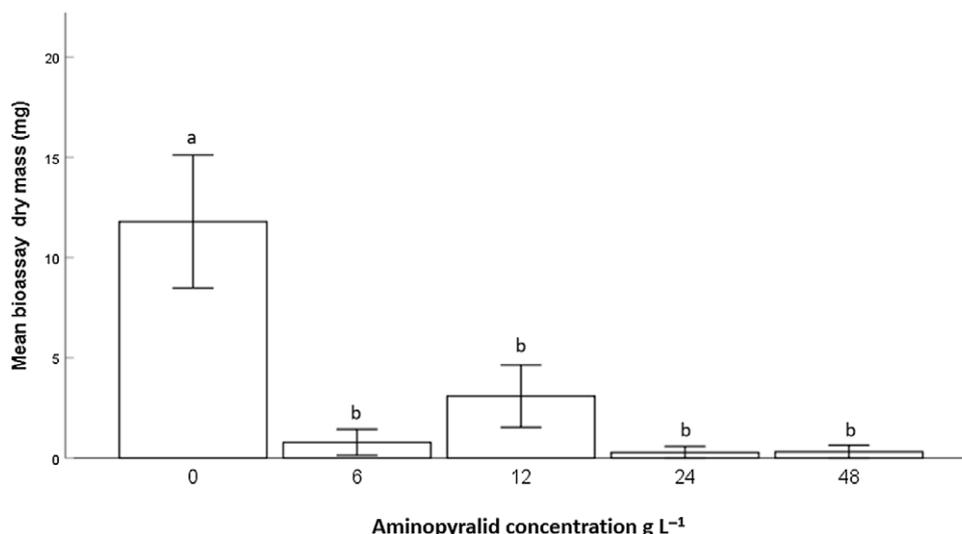
Figure 4. Mean biomass (mg) of *Crepis tectorum* bioassays for each aminopyralid treatment concentration. Error bars are 1 standard error of the mean ($N = 50$). Letters indicate statistical differences determined by LSD test ($P < 0.05$).

application. Further studies should address applications to multiple trees in plots of varying density to determine how root exudates affect herbicide residues in soil under realistic management levels.

References

[AKEPIC] Alaska Exotic Plants Information Clearinghouse (2011) European Bird Cherry (*Prunus padus* L.). Anchorage: University of Alaska Anchorage, Alaska Center For Conservation Science, Alaska Exotic Plants Information Clearinghouse. https://accs.uaa.alaska.edu/wp-content/uploads/Prunus_padus_BIO_PRPA5.pdf. Accessed: April 23, 2021

Anonymous (2015) Garlon® 4 herbicide product label. DOW AgroSciences LLC Publication No. 62719-40. Indianapolis, IN: DOW. 8 p

Anonymous (2018) Milestone® herbicide product label. DOW AgroSciences LLC Publication No. 67219-519. Indianapolis, IN: DOW. 10 p

Baetz U, Martinola E (2014) Root exudates: the hidden part of plant defense. Trends Plant Sci 19:90–98

Barker AL, Dayan FE (2019) Fate of glyphosate during production and processing of glyphosate-resistant sugar beet. J Agric Food Chem 67:2061–2065

Boydston RA, Al-Khatib K (2008) Exudation of mesotrione from potato roots injures neighboring plants. Weed Sci 56:852–855

Brink S (2016) Unlocking the secrets of the rhizosphere. Trends Plant Sci 21:169–170

Bukun B, Gaines TA, Nissen SJ, Westra P, Brunk G, Shaner DL, Sleugh BB, Peterson VF (2009) Aminopyralid and clopyralid absorption and translocation in Canada thistle (*Cirsium arvense*). Weed Sci 57:10–15

Dinelli G, Bonetti A, Marotti I, Minelli M, Busi S, Catizone P (2007) Root exudation of diclofop-methyl and triasulfuron from foliar-treated durum wheat and ryegrass. Weed Res 47:25–33

Enloe SF, O'Sullivan SE, Loewenstein NJ, Brantley E, Lauer DK (2018) The influence of treatment timing and shrub size on Chinese privet (*Ligustrum sinense*) control with cut stump herbicide treatments in the Southeastern United States. Invasive Plant Sci Manag 11:49–55

Hickman MV, Messersmith CG, Lym RG (1989) Picloram release from leafy spurge (*Euphorbia esula*) roots in the field. Weed Sci 37:167–174

Holmes KA, Berry AM (2009) Evaluation of off-target effects due to basal bark treatment for control of invasive fig trees (*Ficus carica*). Invasive Plant Sci Manag 2:345–351

International Society of Arboriculture (2022) Protecting Trees from Damage. Westminster, CO: International Society of Arboriculture, Rocky Mountain Chapter. https://isarmc.org/Protecting_Trees_From_Damage. Accessed: October 18, 2022

Kremer R, Means N, Kim S (2005) Glyphosate affects soybean root exudation and rhizosphere micro-organisms. Int J Environ Anal Chem 85:1165–1174

Lewis DF, Roten RL, Everman WJ, Gannon TW, Richardson RJ, Yelverton FH (2013) Absorption, translocation, and metabolism of aminocyclopyrachlor in tall fescue (*Lolium arundinaceum*). Weed Sci 61:348–352

Oneto SR, Kyser GB, DiTomaso JM (2010) Efficacy of mechanical and herbicide control methods for scotch broom (*Cytisus scoparius*) and cost analysis of chemical control options. Invasive Plant Sci Manag 3:421–428

Ortega-Calvo JJ, Harmsen J, Parson JR, Semple KT, Aitken MD, Ajao C, Eadsforth C, Galay-Burgos M, Naidu R, Oliver R, Peijnenburg WJBM, Rombke J, Streck G, Versonnen B (2015) From bioavailability science to regulation of organic chemicals. Environ Sci Technol 49:10255–10264

Ranft RD, Seefeldt SS, Zhang M, Barnes DL (2010) Development of a soil bioassay for triclopyr residues and comparison with a laboratory extraction. Weed Technol 24:538–543

Rodrigues JJV, Worsham AD, Corbin FT (1982) Exudation of glyphosate from wheat (*Triticum aestivum*) plants and its effects on interplanted corn (*Zea mays*) and soybeans (*Glycine max*). Weed Sci 30:316–320

Roon DA, Wipfli MS, Wurtz TL, Blanchard AL (2016) Invasive European bird cherry (*Prunus padus*) reduces terrestrial prey subsidies to urban Alaskan salmon streams. Can J Fish Aquat Sci 73:1679–1690

Seefeldt SS, Boydston RA, Kaspari PN, Zhang M, Carr E, Smeenk J, Barnes DL (2013) Aminopyralid residue impacts on potatoes and weeds. Am J Potato Res 90:239–244

Shaner DL, ed (2014) Herbicide Handbook. 10th ed. Lawrence, KS: Weed Science Society of America. Pp 43–461

Tomco PL, Duddleston KN, Schultz EJ, Hagedorn B, Stevenson TJ, Seefeldt SS (2016) Field degradation of aminopyralid and clopyralid and microbial community response to application in Alaskan soil. Environ Toxicol Chem 35:485–493

Voinorosky CL, Stewart KJ (2021) Drift, dissipation and risk to wildlife following targeted herbicide applications on boreal transmission rights-of-way. J Environ Manag 280:111860

Washington State University (2002) Bioassay Test for Herbicide Residues in Compost: Protocol for Home Gardeners and Researchers in Washington State. Washington State Department of Ecology. https://s3.wp.wsu.edu/uploads/sites/411/2014/12/PDF_Clopyralid_Bioassay.pdf. Accessed: October 18, 2022

Woodford R, Harms C (2011) Cyanide-poisoned moose ornamental chokecherry tree a devil in disguise. Alaska Fish & Wildlife News. https://www.adfg.alaska.gov/index.cfm?adfg=wildlifenews.view_article&articles_id=501. Accessed: April 23, 2021