

Research Article

Cite this article: Rolando CA, Fields F, Scott MB, Sapsford S, and Paul TS (2024) Impacts of herbicides used for control of invasive *Pinus contorta* on the potential for reinvasion and germination of restoration species. *Invasive Plant Sci. Manag* **17**: 287–296. doi: [10.1017/inp.2024.30](https://doi.org/10.1017/inp.2024.30)

Received: 26 February 2024
Revised: 13 June 2024
Accepted: 30 August 2024
First published online: 29 October 2024

Associate Editor:

Michala Phillips, US Geological Survey

Keywords:

Herbicide persistence; lodgepole pine; mycorrhizal infection; phytotoxicity; seedbank; wilding pine

Corresponding author:

Sarah Sapsford;
Email: Sarah.Sapsford@murdoch.edu.au

Impacts of herbicides used for control of invasive *Pinus contorta* on the potential for reinvasion and germination of restoration species

Carol A. Rolando¹ , Fiona Fields² , Matthew B. Scott¹ , Sarah Sapsford³  and Thomas S. Paul¹ 

¹Senior Scientist, Forest Ecology and Management, New Zealand Forest Research, Scion, New Zealand; ²Senior Technician, Forest Ecology and Management, New Zealand Forest Research, Scion, Rotorua, New Zealand and ³Postdoctoral Researcher, School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; current: DECRA Research Fellow, Harry Butler Institute, Murdoch University, Murdoch, Western Australia

Abstract

Herbicides that persist in the forest litter and soil following their use for managing invasive plant species may negatively affect restoration efforts as well as minimize reinvasion via their residual phytotoxic activity. This study determined the impact of an herbicide mixture comprising triclopyr, dicamba, picloram, and aminopyralid (TDPA) for the control of dense infestations of a woody invader, *Pinus contorta* Douglas ex Loudon, on the germination of reinvading *P. contorta* and three New Zealand native species (*Chionochloa rubra* Zotov, *Nothofagus cliffortioides* (Hook. f.) Oerst., and *Leptospermum scoparium* J.R. Forst. & G. Forst.) used in restoration. Given the essential role of ectomycorrhizal fungi in facilitating conifer reinvasion, the impact of residual herbicides present in mineral soil on the ectomycorrhizal infection of *P. contorta* seedling roots was also examined. Germination trials were conducted using intact forest litter–soil cores collected at 27, 112 and 480 d (after herbicide spraying) from sprayed and adjacent unsprayed dense *P. contorta* infestations. At the same time, mineral soil was also collected for the ectomycorrhizal infection study. Post-spray herbicide residue bound in the litter significantly decreased survival, germination rate, root and shoot growth, and also caused malformation of *P. contorta* seedlings. Similar results were recorded for native species' germination; however, overall viability of native seed was poor, resulting in low germination rates. There was no difference in levels of ectomycorrhizal infection rates of *P. contorta* between treatments. Results indicate residual levels of TDPA herbicide in forest floor litter negatively affect *P. contorta* reinvasion, native recruitment, and active restoration management. Ectomycorrhizal fungi, however, are unaffected by this herbicide mixture and therefore remain a risk to facilitating reinvasion as residual herbicide declines.

Introduction

Herbicides are a cost-effective and efficient method for controlling invasive woody species, particularly in cases of extensive and dense infestations (Douglass et al. 2016). However, the application of herbicides without effective follow-up revegetation management risks areas being reinvaded or subjected to secondary invasions, in which non-target invaders expand following the eradication of the target invader (Pile Knapp et al. 2023; Shen et al. 2023). To reduce the likelihood of reinvasion or secondary invasion and to meet management objectives, land managers often pair herbicide treatments with reseedling or replanting efforts (Rinella et al. 2009; Wagner et al. 2017) using native species or non-native/crop species that will rapidly establish to occupy the site (Douglass et al. 2016; McManamen et al. 2018). The risk to revegetation after herbicide control is that the herbicides used for control may negatively impact reseedling or post-control management efforts through either direct impacts to native plant populations at the time of application or persistence in the soil preventing germination (and survival) of existing or supplemented seeds in the soil bank (Douglass et al. 2016; Rinella et al. 2009). Getting the timing correct is critical to the success of post-herbicide management interventions.

Initially established for erosion control or to provide shelter to livestock, invasive trees in the family Pinaceae, also known as wilding conifers, particularly *Pinus contorta* Douglas ex Loudon (Lodgepole pine), *Pinus nigra* Arnold (Black pine), and *Pinus mugo* Turra (Dwarf Mountain pine), are among the most problematic invasive plant species naturalized in New Zealand (Brandt et al. 2021) and many other countries (Engelmark et al. 2001; Langdon et al. 2010; Nuñez et al. 2017). In 2014, it was estimated that if ongoing spread of these species was not restricted in New Zealand, wildings would invade significant areas within 15 to 30 yr, resulting

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Management Implications

Herbicides are a cost-effective and efficient method for controlling invasive woody species, particularly in cases of dense infestations where manual control is not possible. However, herbicides may also negatively impact post-control management efforts either directly through effects to native plant populations seeded at the time of application or persistence in the soil preventing germination of restorative species already present in the seedbank. This conundrum applies to the use of aerial herbicide application for the management of invasive pines, notably *Pinus contorta*. While the treatment used (in New Zealand) is effective at removing the dense woody canopy, the longer-term impact of the herbicides used (triclopyr, dicamba, picloram, and aminopyralid [TDPA]) on reinvasion with pines and establishment of restorative species is unknown.

To better inform management efforts after control, we conducted a sequential series of germination trials (at ~1 mo, ~4 mo, and ~18 mo after spraying) with an invasive pine (*Pinus contorta*, Lodgepole pine) and three New Zealand native species (mountain beech [*Nothofagus cliffortioides* (Hook. f.) Oerst., Nothofagaceae], mānuka [*Leptospermum scoparium* J.R. Forst. & G. Forst., Myrtaceae], and Red tussock [*Chionochloa rubra* Zotov, Poaceae]) in forest litter-soil cores extracted from unsprayed areas and areas that had been aerially sprayed. Given the important role of ectomycorrhizal infections on reestablishment of pines, we also examined the effect of the residual herbicides on ectomycorrhizal infection of emerging *P. contorta* seedling roots.

Our study indicated that residual levels of herbicide (triclopyr, dicamba and picloram) following spraying had the potential to reduce reinvasion of *P. contorta* for up to 18 mo after spraying. While this is a promising result for management, our results also indicated that residual herbicide levels affected germination of native species, suggesting that site restoration efforts using seed material should also not commence before 18 mo after aerial application of herbicide. Our study showed that residual herbicides did not affect mycorrhizal infection of *P. contorta* seedling roots, indicating the risk of reinvasion increases after 18 mo. In practical terms this would imply that managers should increase their efforts to both revegetate the site and also remove incipient pine invasions from 18 mo to 2 yr after initial control efforts.

in massive costs to the New Zealand economy (MPI 2014). In response, the New Zealand government established the National Wilding Conifer Control Programme (NWCCP) in 2016 to contain and eradicate established areas of wilding conifers by 2030 and prevent further spread. Since then, the NWCCP has focused on removing conifers from the landscape through control (mainly manual removal or through use of herbicides applied from the ground or via aerial application) but largely has not implemented active rehabilitation or restoration activities. Of particular concern are areas of dense infestation, defined as sites with >85% cover of mature wilding pines (often with a stem density of >5,000 stems ha⁻¹) that are managed with aerial herbicide applications (NWCCP 2019). An aerial broadcast application of a mixture of triclopyr, picloram, dicamba, and aminopyralid (TDPA) is often used to manage these infestations (NWCCP 2019; Rolando et al. 2023). While the treatment has been effective at removing the mature canopy of invasive conifers, there is little information on the impact of the residual levels of the

herbicides on germination and early growth of reinvasive conifers and/or native species following control.

Plant communities may not thrive after herbicide application, as they can be significantly damaged or even eradicated by the herbicide treatments, especially if these treatments are applied aerially (Rinella et al. 2009). Germinating plants are often also particularly sensitive to growth-regulating herbicides (synthetic auxins), such as triclopyr, aminopyralid, and picloram, because they contain relatively higher natural auxin concentrations than mature plants. Synthetic auxin herbicides have been developed specifically to cause growth deformation, growth inhibition, senescence, and death in target broadleaf plants, and therefore a response to even low levels of these herbicides by newly germinating or sensitive species after a control event would not be surprising (McBean 2012). Rolando et al. (2023) showed that triclopyr, dicamba, and picloram were present in the forest litter layer for up to 2 yr following aerial application to control dense infestations of invasive pines (*P. contorta*) in New Zealand; however, they did not consider the effects of germination of both native and invasive species after application.

A common outcome following wilding conifer control, particularly by aerial application of herbicides, is the secondary invasion of other non-native species, particularly grasses, a factor probably driven by both the impacts of the herbicides used (selective for broadleaves) as well as soil legacy effects from the pine invasion (Dickie et al. 2014; Paul and Ledgard 2009). Biotic legacies following an invasion of wilding conifers include the persistence of their invasive ectomycorrhizal fungi, which may facilitate reinvasion by wilding conifers (Sapsford and Dickie 2023). In the Southern Hemisphere, pines co-invade with a few species of ectomycorrhizal fungi from their native range (e.g., *Suillus luteus* at early stages of invasion and *Amanita muscaria* at later stages of invasion; Sapsford et al. 2021). These fungi form a mantle around root tips and increase nutrient uptake to the plant through the exchange of carbon; without these mutualist fungi, pines fail to establish (Dickie et al. 2010; Sapsford et al. 2021). Even after pine removal, these ectomycorrhizal fungi remain in the soil for years with persistence after host removal independent of pine density (Sapsford and Dickie 2023). However, their relative abundance does decrease with time following removal of pines (Sapsford and Dickie 2023). The effect of soil-active herbicides such as triclopyr, picloram, and aminopyralid on ectomycorrhizal fungi, and therefore reinvasion with pines, is not well understood, particularly when applied at the rates used for invasive conifer management (Busse et al. 2004; Chakravarty and Chatarpaul 1990; Chakravarty and Sidhu 1987; Sidhu and Chakravarty 1990). Most studies indicate no to low adverse effects of selected herbicides on ectomycorrhizal development. However, observations in New Zealand indicating low numbers of pine seedlings regenerating following aerial control of dense infestations have led to questions on the impact of herbicide-based control on soil ectomycorrhizal populations and resulting impacts for reinvasions.

Understanding the relative sensitivity of plant species to herbicide residues following their application is critical to understand the legacy of control as well for optimizing a restoration approach. The purpose of the study described here was to determine the impact of aerial herbicide application for control of dense infestations of pines in New Zealand on the germination of emerging wilding pine and restoration of native plant species after such control. In parallel, the effect of the residual herbicides on ectomycorrhizal infection of emerging *Pinus contorta* seedling roots was also determined.

Table 1. Location, physiography, and soil (chemical and physical) characteristics for study sites with sprayed and unsprayed treatment blocks.^a

Site	Latitude, longitude	MAP		MAT	Elevation	Soil type ^b
	DD WSG84	mm	C	m		
Glen Eyrie Downs	44.324938, 169.87318	717		8.8	620	Allophanic brown
Pukaki Downs	44.15917, 170.08972	704		8.9	611	Allophanic brown
Kaweka Forest	39.465933, 176.283132	1,017		−10.4	780	Typic Orthic Allophanic

^aMAP, mean annual precipitation; MAT, mean annual temperature. Site temperature and rainfall data were retrieved from the National Institute of Water and Atmospheric Research (NIWA/ Taihoro Nukurangi), Virtual Climate Station Network (VCSN). The nearest VCSN station was used for each study site. The geographic location (WSG84) for each is: GE and PD: −44.325, 169.875; KF: −39.475, 176.275.

^bSoil types from S-map online (accessed: December 2022).

Materials and Methods

Germination Trial

Site Description

The germination study used soil and forest floor litter collected from sprayed and unsprayed dense stands of *P. contorta* in the Mackenzie Basin, an expansive dryland intermontane basin in the South Island, New Zealand, located at an altitude of 610 to 620 m above sea level. The sprayed stand, located at Glen Eyrie Downs (44.324938°S, 169.87318°E), was part of a larger study, in which operational spraying had been undertaken (Rolando et al. 2023; Table 1). The unsprayed stand was located nearby at Pukaki Downs (44.15917°S, 170.08972°E). These paired sites were specifically chosen due to their similarities in soil, climate, and tree invasion status. The soils at both sites are well-drained, allophanic brown soils comprising a mantle of silty loess over well-draining glacial till (Allophanic Brown). The surface is covered in a thick layer of coarse pine litter over comparatively thin fragmented and humified layers (Rolando et al. 2023; Table 1). Climate and weather at both sites are similar (Table 1), as was vegetation cover, comprising dense, mature stands of *P. contorta* (>5,000 stems ha^{−1}).

At more than 25 yr old with an average canopy height of 11 ± 0.9 m and canopy cover ranging from 80% to 90%, the trees at these sites represent forest structure that current management operations would treat using broadcast aerial boom spraying (NWCCP 2019). The formulation used, known to be effective in killing mature and dense stands of invasive *P. contorta*, includes 18 kg ai triclopyr (3,5,6-trichloro-2-pyridyloxyacetic acid), 5 kg ai dicamba (3,6-dichloro-2-methoxybenzoic acid), 2 kg ai picloram (4-amino-3,5,6-trichloropicolinic acid), 0.28 kg ai aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid), 20 L of a methylated seed oil, 0.5 L of a lecithin blend, and 2.3 kg of ammonium sulphate, all applied aerially in 400 to 600 L total volume (water) ha^{−1} (NWCCP 2019).

Broadcast spraying using the formulation described was carried out at the Glen Eyrie Downs site as per operational guidelines using a MD 520N (MD Helicopters) helicopter equipped with 39 CP-09-3P nozzles (30° deflection) on a 7.45-m boom on January 26, 2018 (NWCCP 2019; Rolando et al. 2023). This setup produced a coarse droplet spectrum of spray that was applied 3 to 4 m above the tree canopy using the half-overlap, opposite-pass technique (Richardson et al. 2020). Weather on the day of spraying was 26 C, 78% relative humidity, and a southeasterly wind of 0.5 to 1 m s^{−1}. The Pukaki Downs site, representing the study control, had no history of chemical use, and the *P. contorta* stands were uncontrolled.

Field Sampling

Cores containing soil, decomposing organic matter, and forest floor litter (LFH) were collected at Glen Eyrie Downs and Pukaki



Figure 1. Sampling the forest floor for the germination trials, showing inclusion of both litter and mineral soil in the extracted core.

Downs in February 2018, May 2018, and May 2019 (Figure 1). On each occasion, 12 intact core samples were taken from each of five replicate plots using a PVC pipe measuring 8-cm deep by 6.5-cm diameter (60 cores from each site). The pipe was driven into an area of undisturbed ground using a mallet and wooden covering block. Cores were extracted from the ground using a metal spatula to prevent disturbing the profile. Cores were immediately wrapped in plastic film to keep them intact until they were used for the germination trials. Intact cores were transported to the Scion, Rotorua campus, where they were stored in a chiller at 4 C.

Soil and forest litter were concurrently sampled at each of five locations within the site to track the environmental persistence of the herbicide active ingredients (for details see Rolando et al. 2023). The results of the herbicide persistence study are reported in Rolando et al. (2023).

Table 2. Details of the key dates and duration of three glasshouse germination experiments conducted with *Pinus contorta* and selected New Zealand native species between 2018 and 2019.^a

Trial	Date		Time		Date	Time	Temp (%RH)			Species (no. seed sown/core)	Viability
	Sample	Sow	Spray to sample	Spray to sow	Harvest	Sow to measure	max.	min.	mean		%
1	February 22, 2018	March 22, 2018	27 days	55	May 24, 2018	63 days	41 C (108%)	3 C (22%)	16 C (89%)	<i>P. contorta</i> (10) <i>N. cliffortioides</i> (20)	52 1
2	May 18, 2018	August 8, 2018	112	194	November 30, 2018	114	32 C (103%)	1 C (17%)	14 C (80%)	<i>P. contorta</i> (13) <i>N. cliffortioides</i> (20) <i>L. scoparium</i> (50) <i>C. rubra</i> (20)	67 2 13 28
3	May 21, 2019	June 6, 2019	480	496	October 24, 2019	140	38 C (106%)	-1 C (22%)	12 C (89%)	<i>P. contorta</i> (13) <i>N. cliffortioides</i> (20) <i>L. scoparium</i> (50) <i>C. rubra</i> (20)	26 10 8 3

^aInformation is included on dates for core sampling and seed sowing, temperatures (Temp), relative humidity (%RH), numbers of seeds sown and their viability. Duration is the time from sowing to the shoot and root measurement.

Trial Design

Three sequential germination trials with *P. contorta* and selected native species were conducted between March 2018 and June 2019 using the undisturbed soil/litter core samples collected from the sprayed (27, 112, and 480 d after spraying) and unsprayed study sites, hereafter, Trial 1, Trial 2, and Trial 3, respectively (Table 2). One native tree species was selected for the three trials, mountain beech [*Nothofagus cliffortioides* (Hook. f.) Oerst., Nothofagaceae]. For the second and third trials, a woody shrub (mānuka [*Leptospermum scoparium* J.R. Forst. & G. Forst., Myrtaceae]), and a native red tussock (*Chionochloa rubra* Zotov, Poaceae), were also sown. Seeds for these native species were sourced from NZSeeds (Canterbury, New Zealand). *Pinus contorta* seed was sourced from the Pukaki Downs unsprayed site. Stratification measures were undertaken before sowing seeds (e.g., for *N. cliffortioides*; Ledgard and Cath 1983). For all cores, the uppermost 0.5 to 1 cm of litter was removed before sowing the seed and then replaced after sowing. The surface onto which the seeds were sown was roughed up slightly to allow the seed to be covered.

Three replicate cores from each of the 10 sampling locations ($n = 5$ from sprayed and unsprayed areas, respectively) were assigned to each species ($n = 30$ cores per species) and placed on a greenhouse bench in a randomized complete block design at the Scion nursery in Rotorua. The number of seeds sown varied between species but remained consistent within each species across the three trials (Table 2). Cores were watered immediately after sowing and consistently throughout the trials, and ambient temperature and relative humidity were recorded. Netting was installed over the cores to prevent disturbance by birds able to access the environment through the controlled ventilation system. For each of the three trials, 100 seeds of each species were also sown into viability trays of potting mix at the same time as trial initiation. The results of the viability tests are shown in Table 2.

Germination and Growth Assessments

The number of germinated seeds were recorded on a regular basis throughout each trial. On each occasion, any obvious seedling discoloration or abnormalities were also noted. Germinated seedlings that subsequently died were also recorded.

At the end of each trial (Table 2), seedlings were carefully harvested from cores so as not to damage roots, washed of adhering soil, and photographed. Images were also captured with a stereomicroscope for selected seedlings that displayed visible root growth abnormalities. Shoot and root lengths of Trial 2 and 3 seedlings, were measured and then dried at 65 C to constant weight before obtaining individual dry biomass weights. Individual shoot and root lengths were not obtained for Trial 1 seedlings, which were dried at 30 C before obtaining total dry biomass weights for all seedlings per core.

Ectomycorrhizal Infection Study

The ectomycorrhizal infection study was based on soil collected from the sites described earlier (i.e., Glen Eyrie [sprayed] and Pukaki Downs [unsprayed]), as well as a pair of sprayed and unsprayed sites in the Kaweka Forest (39.46611°S, 176.28306°E), Hawke's Bay, New Zealand (Table 1). Sprayed sites were subject to broadcast spraying of TDPA to control dense infestations of *Pinus contorta* (Rolando et al. 2023).

One liter of mineral soil was collected from each of five plots at each site at 1, 6, 9, and 12 mo after the date of spraying, with corresponding unsprayed samples collected from five plots at on the same date. Soil collected from sprayed and unsprayed plots at the study sites was placed into four 500-ml volume plastic pots (20 pots per site) within 1 wk of soil collection. Soil was watered and allowed to settle for 2 d, after which a pre-germinated *Pinus contorta* seedling grown in a vermiculite, sterile medium was planted into each pot. Watering was completed three times a week, and seedlings were grown for 3 mo to allow ectomycorrhizal development. After 3 mo, seedlings were harvested, roots were washed, and ectomycorrhizal infection was assessed as proportion infection using the intercept method (Brundrett et al. 1996).

Analyses

All analyses were performed using R v. 4.3.1 (R Core Team 2020). ANOVAs using the *aov* function in the LSMEANS (Lenth 2016) and EMMEANS (Lenth 2024) packages were conducted to compare germination, growth, and mortality rates for sprayed and unsprayed cores in the three germination trials for each

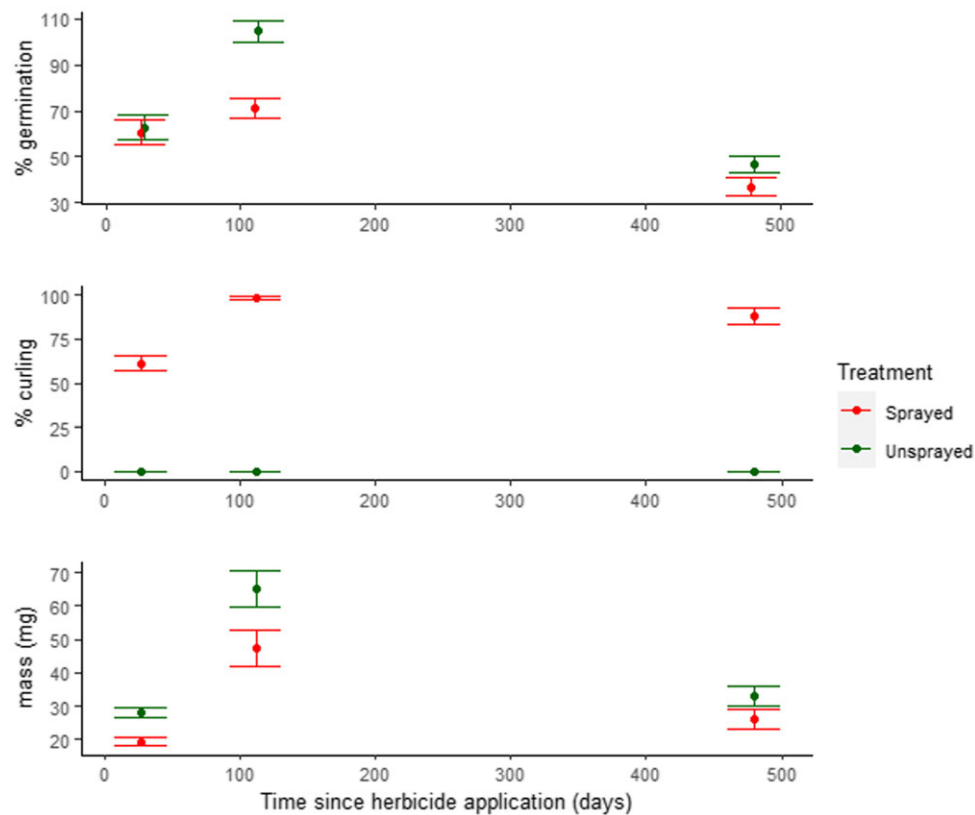


Figure 2. Percentage germination (mean and SE), curling, and mass of *Pinus contorta* seedlings in litter and soil cores sampled at 27 (Trial 1), 112 (Trial 2), and 480 (Trial 3) d from sprayed and unsprayed dense, mature stands of *P. contorta*. Treated stands had been sprayed with the operationally used mix containing the herbicides triclopyr, dicamba, picloram, and aminopyralid (TDPA).

species, with separate analyses conducted for each trial. Where necessary, data were arcsine transformed to meet the assumptions of normality.

A binomial generalized linear mixed model using function *glmer* in package *LME4* (Bates et al. 2015) was applied to determine whether ectomycorrhizal infection changed as a result of herbicide application and over time. Ectomycorrhizal infection (as successes and failures of infection) was the response variable. Fixed covariates included whether the site had herbicide applied or not (factor with 2 levels), time since application (factor with 5 levels: pre-application, and 1-, 6-, 9-, and 12-mo post-application), and their interaction. To incorporate the dependency among plots and glasshouse replicates within sites, replicate within plot within site was used as a random intercept. We used the *EMMEANS* package (Lenth 2024) to calculate estimated marginal means and chi-square tests and P-values were calculated using the *Anova* function in the package *CAR* (Fox and Weisberg 2019).

Results and Discussion

Germination Trials

The purpose of the germination study was to determine the impact of the residual herbicide following aerial application on the germination of successive wilding pines (reinvansion) and establishment of restorative native plant species. A detailed study on the persistence of herbicides in the environment following aerial spraying to control dense pine invasion was made by Rolando et al. (2023).

Pinus contorta

There was no significant effect of residual herbicide on the germination rate of *P. contorta* in Trial 1 (cores taken 27 d after spray; $F(1, 24) = 0.179$, $P = 0.676$) and Trial 3 (cores taken 480 d after spray; $F(1, 24) = 3.2$, $P = 0.08$) (Figure 2, top panel). However, significantly fewer *P. contorta* germinated in Trial 2 (soil taken 112 d after spraying; $F(1, 24) = 2.5$, $P \leq <0.01$) (Figure 2, top panel). Most *P. contorta* seedlings in sprayed cores showed significant needle curling throughout the trial (Figure 2, middle panel, and Figure 3), but significant mortality of germinated seedlings occurred only in Trial 2, with 24% of germinated seedlings dying in the sprayed cores and <1% mortality in unsprayed cores. Seedling biomass in sprayed cores in Trial 2 was significantly lower than that of corresponding unsprayed cores ($F(1, 23) = 4.8$, $P = 0.04$) (Figure 2, bottom panel). In Trials 2 and 3, mean root and shoot lengths of seedlings in sprayed cores were significantly lower than those in unsprayed cores (Table 3). Abnormal root development in sprayed cores was observed throughout the experiments (Figure 3).

Our results suggest that peak phytotoxicity in the *P. contorta* forest litter was between 90 to 180 d after spray application. The levels of herbicide in the cores extracted from sprayed areas significantly affected germination, form, root and shoot growth, and/or mortality of sown *P. contorta* seeds across all three germination trials, but symptoms of phytotoxicity were most evident in cores extracted 112 d after spraying. In the first trial, while all *P. contorta* seedlings in sprayed cores showed signs of phytotoxicity through abnormal curling of needles and lower

Table 3. Shoot and root length ($\pm$ SE) measurement data for harvested seedlings

Species	Shoot length		Root length	
	Control	Treated	Control	Treated
Trial 2^a				
<i>Pinus contorta</i>	26.6 $\pm$ 0.7	25.5 $\pm$ 0.7	97.8 $\pm$ 2.0*	92.0 $\pm$ 2.0*
<i>Leptospermum scoparium</i>	57.7 $\pm$ 4.7*	69.5 $\pm$ 7.6*	103.0 $\pm$ 3.3	107.0 $\pm$ 5.4
<i>Chionochloa rubra</i>	62.0 $\pm$ 6.1	66.0 $\pm$ 6.8	81.3 $\pm$ 5.9	76.1 $\pm$ 6.6
Trial 3^b				
<i>Pinus contorta</i>	51.4 $\pm$ 0.9*	46.2 $\pm$ 1.1*	79.7 $\pm$ 2.5	74.2 $\pm$ 2.9
<i>Nothofagus cliffortioides</i>	27.8 $\pm$ 1.7	27.5 $\pm$ 1.7	39.6 $\pm$ 3.4*	33.1 $\pm$ 3.4*
<i>Leptospermum scoparium</i>	39.1 $\pm$ 3.0	35.0 $\pm$ 4.4	80.6 $\pm$ 5.4*	59.0 $\pm$ 7.7*

^aTrial 2 (112 d) seedlings were harvested and measured 114 d after sowing. Data for *N. cliffortioides* not reported for Trial 2, as too few individuals survived.
^bTrial 3 (480 d) seedlings were harvested and measured 140 d after sowing. Data for *C. rubra* not reported for Trial 3, due to low germination in sprayed and unsprayed cores.
*P < 0.05.



Figure 3. Examples of abnormal shoot and root development and stunting of lateral roots in *Pinus contorta* seedlings grown in soil cores collected from a stand of invasive *P. contorta* where an operational herbicide mix containing the herbicides triclopyr, dicamba, picloram, and aminopyralid (TDPA) was applied 112 d before sampling.

individual seedling mass, germination rate was not significantly different between treatments. Based on this, we surmise the herbicide levels in the forest floor LFH layer continues to increase over time as spray-affected trees cast herbicide-laden needles. Cores collected at 112 d after spraying would have had significantly higher levels of all herbicides in the LFH, and this was reflected in significant differences in germination, curling, mass, and mortality of sown *P. contorta* seeds compared with

the unsprayed cores. At 480 d post-spray, the levels of herbicide in the treated cores had declined, and consequently, germination and mortality rates of *P. contorta* in the herbicide treatment were not different from those in the control. However, symptoms of phytotoxicity were still evident in the form of needle curling, root deformities, and lower seedling biomass, indicating that levels of herbicide were still sufficiently high to induce a phytotoxic response.

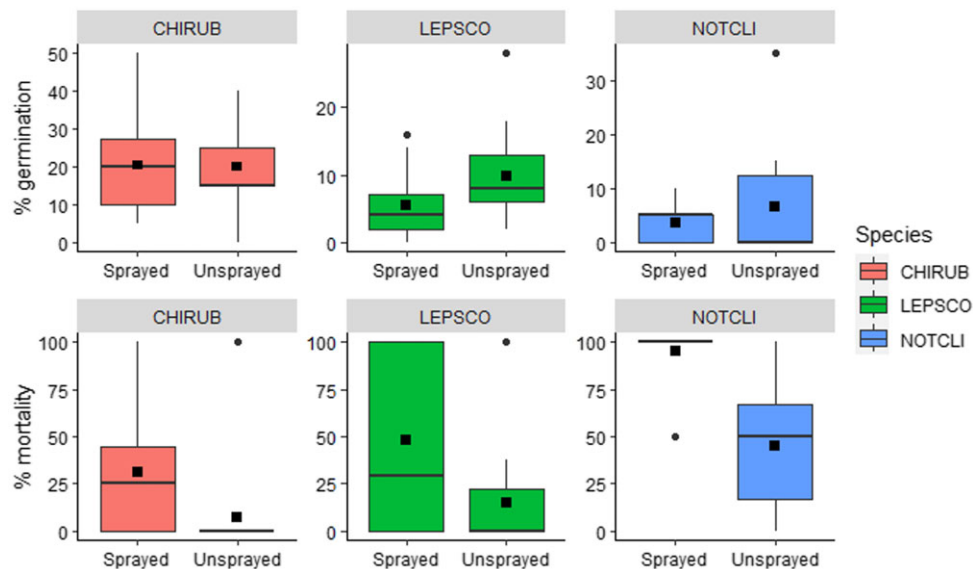


Figure 4. Percentage germination and mortality of native seedlings in cores extracted at 112 (Trial 2) d after spraying in sprayed and unsprayed dense infestations of *Pinus contorta*. CHIRUB, *Chionochloa rubra*; NOTCLI, *Nothofagus cliffortioides*; and LEPSCO, *Leptospermum scoparium*. The box plots display the interquartile range, median, and mean (displayed as a solid black square).

Native Species

Across all trials, viability, and thus germination, of native seeds was poor (Table 2). *Nothofagus cliffortioides* was the only native woody species that was sown across the three germination trials. Too few *N. cliffortioides* germinated in Trial 1 to allow meaningful statistical analyses. Overall percentage germination of *N. cliffortioides* was still low in Trial 2 (<15%) with no significant difference in the percentage of seeds that germinated in the sprayed versus unsprayed cores ($F(1, 24) = 0.0767$, $P > 0.05$) (Figure 4, top right panel). However, in Trial 2, there was significantly higher mortality of germinated *N. cliffortioides* in the cores extracted from sprayed versus unsprayed stands ($F(1, 24) = 11.7$, $P < 0.01$) (Figure 4, bottom right panel). In Trial 3, there was no significant difference in percentage germination (17.5 ± 0.03) or mortality (<1%) of *N. cliffortioides* in sprayed and unsprayed cores; however, the length of roots of *N. cliffortioides* seedlings in sprayed cores was significantly shorter than those in unsprayed cores (Table 3).

Leptospermum scoparium was only sown in Trials 2 and 3. In Trial 2 significantly fewer *L. scoparium* seeds germinated in cores collected from sprayed versus unsprayed cores (Figure 4, top centre panel; $F(1, 24) = 5.74$, $P = 0.02$), with mortality of germinated seedlings also significantly higher in the treated cores (Figure 4, bottom centre panel; $F(1, 24) = 15.6$, $P < 0.001$). The lengths of roots and shoots of seedlings germinated in sprayed cores in Trial 2 were longer than those in unsprayed cores; however, this was only significant for shoots (Table 3). This trend was not observed for *L. scoparium* seedlings germinated in Trial 3 (Table 3) when percentage germination of *L. scoparium* seeds in both sprayed and unsprayed cores was again very low (<5%) and not significantly different.

For *C. rubra*, there was no significant difference in germination between the sprayed and unsprayed cores in Trial 2 ($F(1, 24) = 0.025$, $P = 0.88$), indicating that 3 to 4 mo after spraying the germination rate of this native tussock grass was unaffected by herbicide residues (Figure 4, top left panel). However, significantly more seedlings died in sprayed than unsprayed cores ($F(1, 23) = 7.2$, $P = 0.01$) (Figure 4, left panel). Early seedling shoot and root growth was not significantly affected by the presence of

herbicides in Trial 2 (Table 3). Germination rates of *C. rubra* in Trial 3 were very low and not suitable for analysis.

The morphological characteristics of *N. cliffortioides*, *C. rubra*, and *L. scoparium* growing in sprayed cores were not dissimilar to those growing in control cores, and no obvious root abnormalities were noted.

The overall poor viability and germination of native species highlights the challenges to restoration efforts, particularly when a direct seeding approach is considered (Gibson-Roy et al. 2021). Obtaining native seeds is a labor-intensive and costly process. Furthermore, for some species, viability tends to be low. This issue is compounded by the residual impacts of herbicides on seedling establishment, leading to an even lower success rate in restoration efforts (Pedrini et al. 2022). Interestingly, the findings of our study suggest that the native red tussock, *C. rubra*, may exhibit some tolerance to the residual effects of the herbicides, which aligns with expectations, given that the herbicides used in this study are selective for broadleaved species (McBean 2012).

Rolando et al. (2023) reported residual herbicide levels in cast needles, forest floor litter (LFH), and mineral soil at Glen Eyrie Downs. The results indicated that most of the residual herbicide remained and persisted in the forest floor litter layer (LFH) (Rolando et al. 2023), detectable for up to 2 yr after spraying. Levels of triclopyr in freshly fallen needles peaked in the month after spraying at 42 ppm, declining thereafter, with dicamba and picloram residues peaking at 20 ppm and 3 ppm at around 6 mo after spraying. In the LFH, triclopyr residues reached a peak of 8 ppm at around 4 to 6 mo after spraying, with lower peak levels (<3 ppm) of dicamba and picloram detected in the first year after spraying, declining thereafter (Rolando et al. 2023). The extended period over which herbicide residues were detectable in the LFH (up to 2 yr after spraying) reflected their constant input through freshly discarded needles as the sprayed trees shed needles. The significant negative effects of residual herbicide on germination and seedling survival observed in this study is likely a response to herbicide residues locked up in the lignin-rich LFH following aerial application. Moreover, the outcome of these germination trials

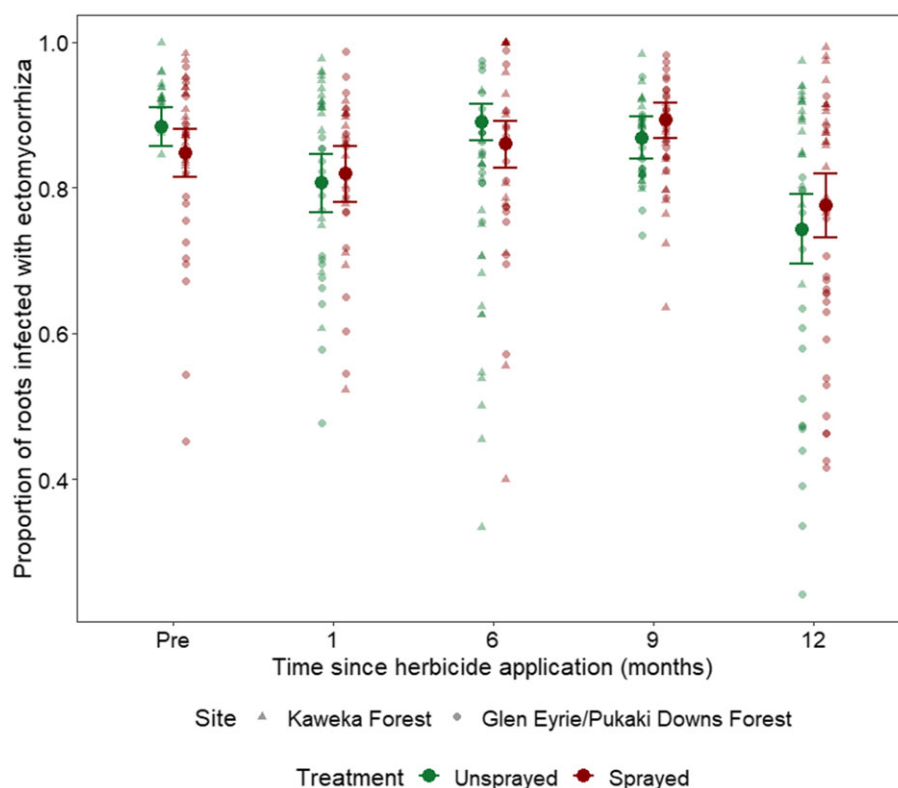


Figure 5. Estimated marginal means ($\pm 95\%$ Confidence Interval [CI]) of the best-fit model for proportion of *Pinus contorta* roots infected with ectomycorrhiza from paired sprayed and unsprayed sites at Kaweka Forest and Glen Eyrie Downs/Pukaki Downs. Time indicates infection by ectomycorrhiza before herbicide application (0 mo) followed by sampling at 1, 6, 9, and 12 mo after herbicide application. Raw individual root measurements (proportion of roots infected with ectomycorrhiza) displayed in background.

highlight that pine recruitment and growth is potentially affected by herbicide residues in the forest floor litter.

There is not a lot of published information that details the specific concentrations of herbicide residues that negatively impact germination of pines (native or exotic) or native species used in restoration programs. Further, where information is available it is very often particular to herbicide persistence in a specific mineral soil as opposed to persistence and availability when adsorbed in a thick forest floor litter layer as occurred at this study site (see Figure 1). This lack of comparable information makes it difficult to contextualize the results of this study with the findings of others. In a study to determine the effects of herbicides on germination of seeds in the forest floor, Morash and Freedman (1988) found triclopyr to significantly affect seedling germination at levels of 50 ppm and above, a level within the range observed at this study site and other sites included in the broader project (Rolando et al. 2023). Ranft et al. (2010) found that seedlings differed in their sensitivities to triclopyr residues, but that significant effects were observed when residues were above 0.1 kg ha^{-1} , which was also within the range measured for this study site (7 to 50 ppm). Douglass et al. (2016) showed that biomass of germinated restoration species was differentially affected by residual triclopyr rates, with rates of 0.015 to 1.54 kg ha^{-1} resulting in biomass reductions of up to 20% relative to where no herbicide was present. McManamen et al. (2018) found that picloram and aminopyralid applied at operational rates (4.78 and 0.52 L ha^{-1} , respectively) negatively impacted germination, causing mortality of 10 native species for up to 11 mo after the initial treatment application. While no measures of herbicide residues were made, the extended

impacts to germination rate are within the time frames observed for our study.

Ectomycorrhizal Infection Study

The best-fit model resulted in a significant interaction between time and whether a site was sprayed or not ($\chi^2 = 78.53$, $df = 4$, $P < 0.0001$) with the inclusion of the nested random intercept (Figure 5). The interaction was driven mainly by a decrease in infection in both sprayed and unsprayed sites 12 mo after application and increase in infection in the sprayed (in comparison to unsprayed sites) at 1- and 9-mo post-spraying (Figure 5). Infection of ectomycorrhizae overlapped between the treatments, with location (of the sites) potentially influencing the interaction (Table 1; Figure 5). For example, *P. contorta* grown in soils from the unsprayed site at Pukaki Downs (Mackenzie Basin) had lower ectomycorrhizal infection in the unsprayed site in comparison to the unsprayed site at the Kaweka Forest (Figure 5).

Ectomycorrhizal fungi were not affected by herbicide residues, as indicated by high infection rates ($>80\%$) throughout the study period; it therefore seems unlikely reinvasion is limited by their absence following spraying. We did observe a drop in infection 12 mo after herbicide application, but this drop was also observed in the unsprayed areas, indicating a potential environmental driver in this decline.

Ectomycorrhizal fungi are necessary for pines to establish, and thus their persistence through herbicide application serves as inoculum for future reinvasions. Various species of ectomycorrhizal fungi associated with pines remain in the soil for years after

pine has been removed (Sapsford and Dickie 2023). Early successional ectomycorrhizal species, such as *Suillus* (Suillaceae) remain present at low levels up to 12 yr after pine removal. *Suillus* is considered the only species necessary to enable pine establishment and invasion (Hayward et al. 2015; Policelli et al. 2019). The combination of the persistence of ectomycorrhizal fungi and poor native plant species germination and survival following herbicide application demonstrate the difficulties we face with native restoration following pine invasions. However, there may be potential with planted seedlings (rather than seed rain) as seedlings may be more protected or resilient to low levels of herbicide.

These results indicate that residual levels of herbicide (TDPA) in forest floor litter reduce germination of wilding *P. contorta* reinvastation for a period of approximately 18 mo after spraying. On the other hand, these results do not provide evidence that the TDPA herbicide mix has any impact to ectomycorrhizal infection of emerging *P. contorta*, meaning sprayed sites continue to be at risk of invasion after the active ingredients no longer persist in the environment.

The results suggest residual levels of herbicide in forest floor litter may negatively impact on the germination and growth of native seed sown for site restoration. Thus, it is recommended that site restoration efforts should not commence within 18 mo of spraying (TDPA), particularly if using seed material or broad-leaved species. We suggest physical manipulation (e.g., mechanical removal) of the organic litter layers (LFH) is a potential consideration if seed or young seedlings are planned for site restoration shortly after spraying. In general, native grasses (Poaceae) should be considered a good early site colonizer, and all future restoration management should consider secondary (re-) invasions.

Acknowledgments. Support from landowners and control operators, the Department of Conservation, and Scion nursery staff is greatly appreciated, including from John Meredith and Liam Wright, who supported the management of germination trials during their employment at Scion. The ectomycorrhizal trial was designed and initiated by Ian Dickie, University of Canterbury, who also obtained funding for and supervised SS. David Conder supported the herbicide trials at the University of Canterbury

Funding statement. We are grateful for support from the Wilding Pine Network and the Ministry for Primary Industries for funding this work through the Sustainable Farming Fund: Beyond Conifer Control (SFF405328). The writing and publication of this article was also funded by the New Zealand Ministry of Business, Innovation and Employment through the Endeavour Research Programme Vive la Résistance: Preventing Wilding Conifer Reinvasion (C04X2102). The New Zealand Ministry of Business, Innovation and Employment is also acknowledged for their support of SS through the Winning Against Wildings Endeavour Research Programme (C09X1611) during the experimentation period (2017 to 2020), and the Australian Research Council Discovery Early Career Research Award (DE220100833) for contribution to the article.

Competing interests. The authors declare no competing interests.

References

- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Brandt AJ, Bellingham P, Duncan RP, Etherington TR, Fridley JD, Howell CJ, Hulme PE, Jo I, McGlone MS, Richardson SJ, Sullivan JJ, Williams PA, Peltzer DA (2021) Naturalised plants transform the composition and function of the New Zealand flora. *Biol Invasions* 23:351–366
- Brundrett M, Bougher N, Dell B, Grove T (1996) Working with Mycorrhizas in Forestry and Agriculture. Canberra: ACIAR. 380 p
- Busse MD, Fiddler GO, Ratcliff AW (2004) Ectomycorrhizal formation in herbicide-treated soils of differing clay and organic matter content. *Water Air Soil Pollut* 152:23–34
- Chakravarty P, Chatarpaul L (1990) Non-target effect of herbicides: II The influence of glyphosate on ectomycorrhizal symbiosis of red pine (*Pinus resinosa*) under greenhouse and field conditions. *Pestic Sci* 28:243–247
- Chakravarty P, Sidhu SS (1987) Effect of glyphosate, hexazinone and triclopyr on in vitro growth of five species of ectomycorrhizal fungi. *Eur J For Pathol* 17:204–210
- Dickie IA, Bennett BM, Burrows LE, Nuñez MA, Peltzer DA, Porté A, Richardson DM, Rejmánek M, Rundel PW, van Wilgen BW (2014) Conflicting values: ecosystem services and invasive tree management. *Biol Invasions* 16:705–719
- Dickie IA, Bolstridge N, Cooper JA, Peltzer DA (2010) Co-invasion by *Pinus* and its ectomycorrhizal fungi. *New Phytol* 187:475–484
- Douglass CH, Nissen SJ, Meiman PJ, Kniss AR (2016) Impacts of imazapyr and triclopyr soil residues on the growth of several restoration species. *Rangeland Ecol Manag* 69:199–205
- Engelmark O, Sjöberg K, Andersson B, Rosvall O, Ågren GI, Baker WL, Barklund P, Björkman C, Despain DG, Elfving B, Ennos RA, Karlman M, Knecht MF, Knight DH, Ledgard NJ, et al. (2001) Ecological effects and management aspects of an exotic tree species: the case of lodgepole pine in Sweden. *For Ecol Manag* 141:3–13
- Fox J, Weisberg S (2019) An R Companion to Applied Regression. 3rd ed. Thousand Oaks, CA: Sage. 608 p
- Gibson-Roy P, Hancock N, Broadhurst L, Driver M (2021) Australian native seed sector characteristics and perceptions indicate low capacity for upscaled ecological restoration: insights from the Australian Native Seed Report. *Restor Ecol* 29:e13428
- Hayward J, Horton TR, Pauchard A, Nuñez MA (2015) A single ectomycorrhizal fungal species can enable a *Pinus* invasion. *Ecology* 96: 1438–1444
- Langdon B, Pauchard A, Aguayo M (2010) *Pinus contorta* invasion in the Chilean Patagonia: local patterns in a global context. *Biol Invasions* 12:3961–3971
- Ledgard NJ, Cath PW (1983) Seed of New Zealand Nothofagus species—studies of seed weight, viability, shape and the effect of varying stratification periods. *NZ J For* 28:150–162
- Lenth R (2024) emmeans: Estimated Marginal Means, aka Least-Squares Means. R Package Version 1.10.2.090002. <https://rvinlenth.github.io/emmeans>
- Lenth RV (2016) Least-squares means: the R package lsmeans. *J Stat Softw* 69:1–33
- McBean C (2012) The Pesticide Manual. 16th ed. Hampshire, UK: British Crop Protection Council. 1439 p
- McManamen C, Nelson C, Wagner V (2018) Timing of seeding after herbicide application influences rates of germination and seedling biomass of native plants used for grassland restoration: timing of herbicide impacts seed germination. *Restor Ecol* 26:1137–1148
- [MPI] Ministry for Primary Industries (2014) The Right Tree in the Right Place. New Zealand Wilding Conifer Management Strategy 2015–2030. www.wildingconifers.org.nz. Accessed: November 15, 2024
- Morash, R, Freedman, B (1988) The effects of several herbicides on the germination of seeds in the forest floor. *Can J For Res* 19:347–350
- [NWCCP] National Wilding Conifer Control Programme (2019) Good Practice Guide for Wilding Conifer Control: Aerial Foliar Spray Application (AFSA). Wellington, New Zealand: Ministry for Primary Industries, 30 p
- Nuñez MA, Chiuffo MC, Torres A, Paul T, Dimarco RD, Raal P, Policelli N, Moyano J, García RA, van Wilgen BW, Pauchard A, Richardson DM (2017) Ecology and management of invasive Pinaceae around the world: progress and challenges. *Biol Invasions* 19:3099–3120
- Paul TSH, Ledgard NJ (2009) Vegetation succession associated with wilding conifer removal. *NZ Plant Prot* 62:374–379
- Pedrinis S, D'Agui HM, Arya T, Turner S, Dixon KW (2022) Seed quality and the true price of native seed for mine site restoration. *Restor Ecol* 30: e13638
- Pile Knapp LS, Coyle DR, Dey DC, Fraser JS, Hutchinson T, Jenkins MA, Kern CC, Knapp BO, Maddox D, Pinchot C, Wang GG (2023) Invasive plant

- management in eastern North American forests: a systematic review. *For Ecol Manag* 550:121517
- Policelli N, Bruns TD, Vilgalys R, Nuñez MA (2019) Suilloid fungi as global drivers of pine invasions. *New Phytol* 222:714–725
- 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
- R Core Team (2020) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing
- Richardson B, Rolando CA, Hewitt AJ, Kimberley MO (2020) Meeting droplet size specifications for aerial herbicide control to wilding conifers. *NZ Plant Prot* 73:13–23
- Rinella MJ, Maxwell BD, Fay PK, Weaver T, Sheley RL (2009) Control effort exacerbates invasive-species problem. *Ecol Appl* 19:155–162
- Rolando CA, Scott MB, Baillie BR, Dean F, Todoroki CL, Paul TSH (2023) Persistence of triclopyr, dicamba, and picloram in the environment following aerial spraying for control of dense pine invasion. *Invasive Plant Sci Manag* 16:177–190
- Sapsford SJ, Dickie IA (2023) Slow soil enzyme recovery following invasive tree removal through gradual changes in bacterial and fungal communities. *J Ecol* 111:2614–2626
- Sapsford SJ, Wakelin A, Peltzer DA, Dickie IA (2021) Pine invasion drives loss of soil fungal diversity. *Biol Invasions* 24:401–414
- Shen C, Chen P, Zhang K, He M, Wan J, Wang Y, Tao Z, Huang W, Siemann E (2023) Dynamics and mechanisms of secondary invasion following biological control of an invasive plant. *New Phytol* 238:2594–2606
- Sidhu SS, Chakravarty P (1990) Effect of selected forestry herbicides on ectomycorrhizal development and seedling growth of lodgepole pine and white spruce under controlled and field environment *Eur J For Pathol* 20:77–94
- Wagner V, Antunes PM, Irvine M, Nelson CR (2017) Herbicide usage for invasive non-native plant management in wildland areas of North America. *J Appl Ecol* 54:198–204