

## Enhanced colonization by the blue stain fungus *Ophiostoma clavigerum* in glyphosate-treated sapwood of lodgepole pine

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The herbicide glyphosate was administered into the sapwood around the root collar of lodgepole pine trees, *Pinus contorta* var. *latifolia* Engelm., to determine its effect on invasion by the blue stain fungus *Ophiostoma clavigerum* (Robinson-Jeffrey & R.W. Davidson) T.C. Harrington. In two experiments, lesions in the sapwood were longer and wider in trees treated with glyphosate before inoculation with *O. clavigerum* than in untreated, control trees. *Ophiostoma clavigerum* was recovered in a third experiment at seven times the distance from the point of inoculation in trees treated with glyphosate 3 weeks before inoculation as in untreated, control trees. We conclude that previously observed enhancement of brood development of the mountain pine beetle, *Dendroctonus ponderosae* Hopk., was caused by glyphosate-induced inhibition of the trees' secondary defense response to invasion by the beetle's symbiotic fungi.

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Du glyphosate a été administré dans l'aubier du pin vrille, *Pinus contorta* var. *latifolia* Engelm., à la base du tronc, pour déterminer les effets sur l'invasion par le *Ophiostoma clavigerum* (Robinson-Jeffrey & R.W. Davidson) T.C. Harrington. Dans les deux expériences, les lésions dans l'aubier étaient plus longues et plus larges sur les arbres traités avec du glyphosate avant l'inoculation avec *O. clavigerum* que sur les arbres contrôles non traités. L'hypothèse que des lésions plus grandes sont le résultat d'une dispersion plus rapide du fungus a été étudiée dans une troisième expérience. *Ophiostoma clavigerum* a été retrouvé à une distance 7 fois plus grande du point d'inoculation pour les arbres traités avec du glyphosate 3 semaines avant l'inoculation que pour les arbres contrôles non traités. Nous concluons que l'observation précédente de l'augmentation de l'attaque par le dendroctone du pin ponderosa, *Dendroctonus ponderosae* Hopk., sur les arbres pré-traités avec du glyphosate était causée par une inhibition des défenses secondaires des arbres, en réponse à l'invasion du fungi symbiotique des coccinelles.

### Introduction

Reproductive success of the mountain pine beetle, *Dendroctonus ponderosae* Hopk., is contingent on host mortality (Berryman 1972; Raffa and Berryman 1982, 1983). Lodgepole pine, *Pinus contorta* var. *latifolia* Engelm., resists attack and colonization by *D. ponderosae* and its symbiotic phytopathogens, including the blue stain fungus *Ophiostoma clavigerum* (Robinson-Jeffrey & R.W. Davidson) T.C. Harrington using a combination of passive and active defenses (Reid *et al.* 1967; Shrimpton and Whitney 1968; Berryman 1972; Amman 1975). The beetles and their fungi must first overcome the copious amounts of resin released passively from severed resin ducts (Raffa and Berryman 1983). Active defenses include a series of metabolic processes that culminate in local autolysis of parenchyma cells, the formation of traumatic resin ducts, and the production of secondary resin with high concentrations of monoterpenes and phenolics (Reid *et al.* 1967; Russell and Berryman 1976). Associated with localized necrosis is the loss of starch and the accumulation of phenolics, resin acids, lignins, and other compounds (Berryman 1972; Russell and Berryman 1976), making the phloem and sapwood unsuitable for fungal spread and larval development (Shrimpton and Whitney 1968).

The broad spectrum herbicide glyphosate, *N*-phosphonmethylglycine (Lund-Hoie 1985), is a metabolic inhibitor of the shikimic acid pathway that mediates synthesis of phenol precursors used for plant defense (Duke *et al.* 1980). Colonization by *D. ponderosae* was more advanced in lodgepole pines in which glyphosate was injected into the sapwood around the root collar than in untreated, control trees (Bergvinson 1989; Bergvinson and Borden 1992). Our objective was to investigate the hypothesis that the enhanced *D. ponderosae* development in glyphosate-treated trees was due to decreased resistance to colonization by the beetle's symbiotic phytopathogens.

### Materials and methods

In 1986, a healthy 80-year-old lodgepole pine stand 40 km north east of Princeton, British Columbia (50°14'N, 120°47'W), which had no history of *D. ponderosae* attack or signs of physical injury or stress was selected for study. A random number table was used to assign trees to one of two treatments: (i) drilled and treated with glyphosate or (ii) drilled and treated with distilled water.

All experiments used the commercial formulation of glyphosate Roundup (360 mg active ingredient/mL) (Monsanto Canada, Streetsville, Mississauga, Ont.). An electric drill was used to make 0.9 × 5 cm holes that penetrated the sapwood at 45° at 5-cm intervals around the root collar. Using a calibrated Pasteur pipette, 1 mL neat (undiluted) Roundup was administered to each hole, with control trees receiving distilled water.

*Ophiostoma clavigerum* was obtained from Dr. H.S. Whitney (Pacific Forestry Centre, Forestry Canada, Victoria, B.C.) and cultured on 2% potato dextrose agar in plastic Petri dishes. One-week

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TABLE 1. Influence of glyphosate treatment on *Ophiostoma clavigerum* induced lesion development in lodgepole pine sapwood

Experiment	Treatment and sampling dates	Treatment	Mean lesion dimensions (cm)*	
			Length	Width
1	Trees drilled and treated with water or glyphosate on 15 July and inoculated 3 August; lesions measured 31 August	Water	15.8a	1.3a
		Glyphosate	27.9b	1.7b
2	Trees drilled and treated with water or glyphosate on 19 August and inoculated 25 August; lesions measured 22 September	Water	10.2a	0.7a
		Glyphosate	21.4b	0.9b

\*Mean lengths or widths within each experiment followed by the same letter are not significantly different; *t*-test,  $P < 0.05$ .

... were used to inoculate experimental trees using Wright's method. A cork borer 0.4 cm outside diameter was employed through the bark to the cambium layer at a height of 1.3 m. A 0.4 cm outside diameter cork borer was used to remove agar along the advancing hyphal margin, which was applied to the dead xylem. The excised bark pellet was reinserted and sealed with masking tape.

... experiments were conducted to assess the effect of glyphosate treatment on the size of the lesion produced in response to inoculation with *O. clavigerum*. On 15 July 1986, 15 trees were treated with glyphosate and 15 were treated with water. Inoculations were made on all trees 3 weeks later on six faces of the tree (N, NE, SE, SW). Four weeks after inoculation a 60 × 20 cm section of bark centred over one inoculation site was removed and the dimensions of the wound response lesion in the sapwood were measured. In experiment 2, on a site 50 m north of the first experiment, 40 trees were drilled, and 20 were randomly selected and treated as above. One week later each tree was inoculated on six faces. Four weeks after inoculation the dimensions of one randomly selected sapwood response lesion per tree were again measured.

... third experiment was conducted in 1988 to determine whether lesion size was associated with fungal colonization. A stand of mature lodgepole pine located 50 km northeast of Princeton, British Columbia, was chosen. On 8 July, 30 trees were selected to receive three treatments: (i) drilled and glyphosate applied on 8 July, 3 weeks prior to fungal inoculation, (ii) drilled on 8 July, 3 weeks prior to inoculation, with glyphosate applied during inoculation, and (iii) drilled with water applied (8 and 30 July). All trees were inoculated in the afternoon on the N, E, S, and W aspects. Six of the trees treated with glyphosate 3 weeks prior to inoculation were attacked by *Moniliopsis ponderosae* before artificial inoculation and were excluded from the study, resulting in four complete replicates. Four weeks after inoculation a 15 × 60 cm rectangle of bark positioned at the start of the inoculation site and orientated longitudinally up the bole was removed by chisel. A fine knife was then used to make two cuts at 45° inward into the sapwood and running into the inoculation site. The 60 cm length of sapwood (1–3 mm wide) was removed in three pieces; 5 cm long sections were cut and placed into 9-cm Petri dishes containing 2% water agar. All incisions were started from the distal to the inoculation, and after each step the tools were sterilized in 95% ethanol. The Petri dishes were incubated in the dark at 20°C for 5 days. Hyphae from a pure culture of *O. clavigerum* were plated on 2% water agar to compare morphology and growth rate of hyphae to confirm identification and as well, a dextrose agar plated wood sample was analyzed by Dr. H.S. Gentry (Pacific Forestry Centre, Forestry Canada, Victoria, B.C.) to confirm the presence of *O. clavigerum*.

All data were transformed by  $\ln(x + 1)$  to correct for heteroscedasticity, before means were compared by *t*-tests (experiments 1 and 2) or before data in experiment 3 were subjected to ANOVA and Scheffé's test ( $P < 0.05$ ), to determine the significance of mean differences (Zar 1984).

### Results and discussion

Trees treated with glyphosate prior to inoculation with *O. clavigerum* produced significantly larger lesions in the sapwood than did control trees (Table 1). The corresponding lesions in the phloem tissue of treated trees in experiment 1 had no free resin and were highly oxidized, imparting a rust colour to the infected tissue, indicating successful fungal colonization (Reid *et al.* 1967). Lesions in control trees had a light yellow traumatic resin cavity soaked in free-flowing resin around the site of inoculation, producing a restricted lesion typical of resistant trees (Berryman 1972; Raffa and Berryman 1982). Apparently, because of the shorter period between glyphosate treatment and inoculation in experiment 2, treated trees had only a narrow band of oxidation around the lesion perimeter in both the sapwood and phloem tissue, while still possessing a resin-soaked traumatic cavity comparable to control trees. Nonetheless, the lesions in glyphosate-treated trees in experiment 2 were larger than those in the control trees. Thus the fungus must have advanced further in treated trees than in control trees.

Hyphae emanating from the sapwood sections on agar in experiment 3 were visible by transmission light microscopy. Only *O. clavigerum* was present, characterized by its filamentous hyphae (Whitney 1971). *Ophiostoma clavigerum* in trees treated with glyphosate 3 weeks before inoculation had advanced vertically at seven times the rate of those in trees treated during inoculation (Fig. 1).

Glyphosate applied to needles of Scots pine, *Pinus sylvestris* L., did not prevent the incorporation of  $^{14}\text{CO}_2$  into phenolics, as synthesis occurred via quinic acid (Osipov and Aleksandrova 1986). Quinic acid is a precursor to hydroxybenzoic acids and more complicated phenolics (Goodwin and Mercer 1983), but is not known to be a precursor to stilbenes, aromatic hydrocarbons that include the fungistatic pinobanksin, pinocembrin, and pinosylvin compounds found in *Pinus* spp. (Loman 1970). If stilbenes or quinic acid derivatives were present in the inoculated trees,

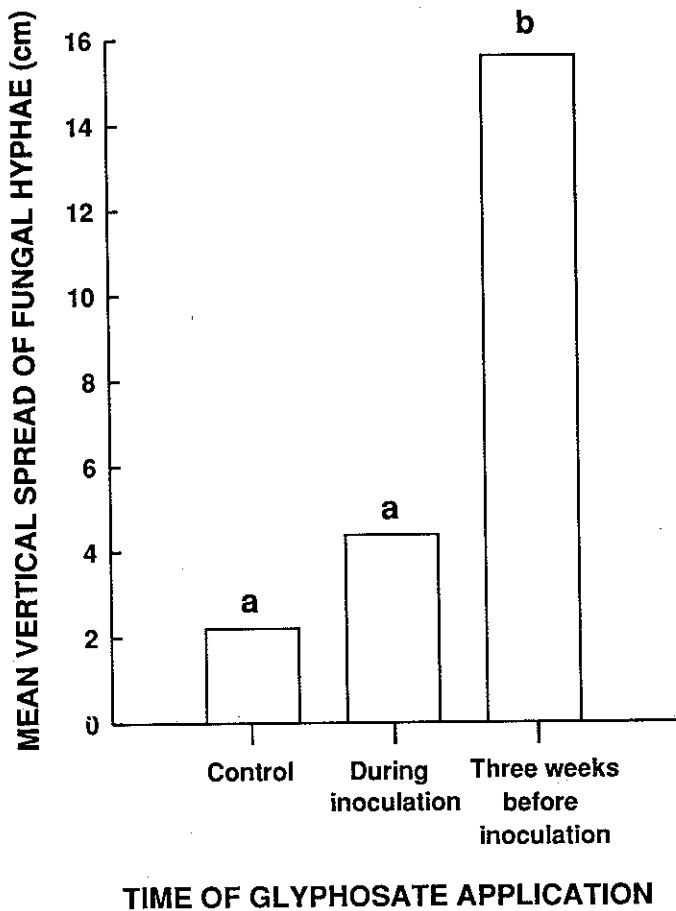


FIG. 1. Vertical spread of *Ophiostoma clavigerum* verified by recovery of the fungus from lodgepole pine trees treated with glyphosate 3 weeks before or during inoculation. Each inoculation on four faces of the bole was treated as an individual observation, as position nested within replicate was not significant (ANOVA,  $F_{[11,24]} = 0.73$ ,  $P = 0.69$ ) (Zar 1984). Bars with the same letter are not significantly different; Scheffé's test,  $P < 0.05$ .

they must have been synthesized at levels insufficient for fungal containment. Therefore, glyphosate must have debilitated the secondary or active defenses of the tree. This hypothesis is consistent with Raffa and Berryman's (1982) conclusion that the secondary wound response was the determinant of resistance for lodgepole pines. Enhanced phytopathogen colonization associated with glyphosate treatment has been previously demonstrated in agricultural systems (Johal and Rahe 1984; Brammall and Higgins 1988).

Pine phloem and xylem infected by blue stain fungi undergo desiccation, with sapwood moisture content dropping twofold 3 months after *D. ponderosae* attack, and fivefold after 1 year (Reid 1961). When penetrated by a drill, sapwood from control trees produced measurable amounts of free moisture, whereas trees treated with glyphosate 3 weeks prior to inoculation did not produce any free moisture 1 week following attack by *D. ponderosae*. Enhanced colonization of sapwood by *O. clavigerum* (Fig. 1) and the apparent restriction of water transport by parenchyma ray cells can account for the rapid desiccation of sapwood associated with glyphosate treatments, as well as for enhanced success of

brood development by *D. ponderosae* (Bergvinson and Borden 1992). Despite sapwood drying, the phloem and phloem-xylem interface in glyphosate-treated trees remained moist enough for at least a year to permit development of *D. ponderosae*. Moist phloem from treated trees decayed rapidly and was easily removed 3 months following *D. ponderosae* attack. Much of this moisture may result from impaired transpiration.

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