Seasonal Variations in Jack Pine (*Pinus banksiana*) and White Spruce (*Picea glauca*) Tolerance to Glyphosate and Triclopyr[1]

R. G. WILLIS, G. R. STEPHENSON, R. A. FLETCHER, and R. PRASAD[2]

Abstract. Seasonal variations in tolerance of jack pine and white spruce to glyphosate and triclopyr were investigated in field and growthroom studies. Foliar injury was higher in both species following field applications of herbicides in July during periods of active growth than in September and October when the plants were not growing actively. Herbicides applied to trees in the growthroom 3 weeks after bud break caused greater injury than applications either before or 14 weeks after bud break. White spruce tolerated both glyphosate and triclopyr better than jack pine. Triclopyr injured both species more than glyphosate. Chemical analysis and electron micrographs showed that the cuticular wax deposition on both species was greater in field-grown trees than in growthroom-grown trees and was greater at 14 weeks after bud break than at 3 weeks. Increased wax deposition with time after bud break may reduce herbicide uptake and may account partially for the increased tolerance of both species to glyphosate and triclopyr. Nomenclature: Glyphosate, *N*-(phosphonomethyl)glycine; triclopyr, [(3,5,6-trichloro-2-pyridinyl)oxy] acetic acid; jack pine, *Pinus banksiana* L.; white spruce, *Picea glauca* (Moench) Voss.

Additional index words: Electron microscopy, cuticular wax.

INTRODUCTION

A common silvicultural practice in forest management is to regenerate a conifer stand by replanting as soon as possible after the previous harvest. However, competing vegetation can inhibit the establishment and growth of conifers (9). Thus, intensive management to reduce competition from unwanted vegetation is important during conifer stand establishment.

Herbicides often are used in newly planted coniferous stands to release the trees from shrub competition (9, 10). The phenoxy herbicides 2,4-D [(2,4-dichlorophenoxy)acetic acid], and particularly 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid] were important for silviculture until 2,4,5-T was banned in several countries in the early 1980s (9). This created a need for other herbicides with similar or better activity than those of 2,4,5-T or other phenoxy acids.

The effectiveness of herbicides often is limited by their ability to control the unwanted vegetation selectively without injuring the coniferous crop. Most conifers exhibit less injury when herbicides are applied in the late summer or early fall than when they are applied in the spring and summer (6, 8, 10, 12). However, the factors responsible for seasonal variations of conifer tolerance to certain herbicides are obscure and are documented poorly (12). Therefore, the objectives of the present study were to document the seasonal variations of jack pine and white spruce tolerance to glyphosate and triclopyr. Wax deposition on the needle surfaces was examined visually and quantitatively to evaluate its possible role in seasonal differences of herbicide tolerance.

MATERIALS AND METHODS

Field study. A study was conducted from 1986 to 1987 on a 12- by 32-m area located at the Arkell Research Station near Guelph, Ontario. The experimental plot was characterized by a flat terrain and clay loam soil (pH 6.8; organic matter 4.0%; sand 35%; silt 43%; clay 21%). The site was prepared with a shear blade and was rototilled in April, 1986.

A randomized complete block design was used with five replications in a factorial arrangement. Nursery-grown jack pine (2 years in the seed bed, 0 years in the field) and white spruce trees (2 years in both the seed bed and field) were planted during the last week of May, 1986. There were four trees spaced 1.0 m apart for each treatment. Triclopyr at 1.5 kg ae/ha and glyphosate at 2 kg ae/ha were sprayed at three different times throughout the 1986 growing season, July 26, Sept. 9, and Oct. 1, to encompass the various stages of conifer shoot growth. The herbicides were applied with a hand pumped, 10-L sprayer, 35 cm above target, with walking speed of 7 km/h, a spray pressure of 200 kPa with a low volume 800067 flat fan nozzle (50 to 60 L/ha).

[1] Received for publication Feb. 1, 1988, and in revised form May 9, 1988.

[2] Grad. Res. Asst. and Profs., Dep. Environ. Biol., Univ. Guelph, Guelph, Ontario, Canada N1G 2W1, and Sci., For. Pest Manage. Inst., Canadian Forestry Serv., Sault Ste. Marie, Ontario, Canada P6A 5M7.

Data were collected Oct. 18, 1986, and Oct. 19, 1987. Herbicide injury to foliage was assessed using a 0 to 10 visual rating system with 0 = no injury and 10 = complete kill. Data was subjected to analysis of variance, and the LSD test was used to compare means.

Growthroom study. Nursery grown 2-yr-old jack pine (2 + 0) and 4-yr-old white spruce (2 + 2) were placed in cold storage (−4 C) for 10 weeks. The trees were thawed gradually, were planted in 25-cm diam fiber pots, and were placed in a growthroom with day/night temperatures of 25 and 20 C, respectively, and a 16-h photoperiod with a photon flux density of 280 $\mu E\ m^{-2}\ s^{-1}$. Before spraying, the soil surface in each pot was covered with plastic. The seedlings were placed in a spray chamber calibrated to deliver 195 L/ha of spray solution at 200 kPa using an 8002E nozzle. The seedlings were sprayed with either triclopyr at 1.5 kg/ha or glyphosate at 2 kg/ha 4 days before or 3 to 14 weeks after the bud break of new growth. The experiment was arranged using a completely random design with five replications and four trees per replication. Foliar injury was evaluated 40 days after application, data were subjected to analysis of variance, and the LSD test was used to compare means.

Estimation of epicuticular wax. At 3 and 14 weeks after bud break, new shoots were selected randomly for wax extraction from both field- and growthroom-grown pine and spruce seedlings. Three shoots per tree were removed and were immersed in chloroform for 10 to 15 seconds. Kolattukudy (4) reported that this technique extracts about 99% of the wax on leaf surfaces. Also, limiting the time of exposure to solvents decreases the chance of extracting internal lipids. The dewaxed shoots were freeze dried to determine the dry weight of needles. The extract was passed through a 2-cm diam by 12-cm column of anhydrous sodium sulphate to remove any water. The solvents were evaporated at room temperature using nitrogen gas until the wax was a constant weight. Wax deposition was expressed on a percentage of dry weight basis (weight of needles only).

There were five replications with four trees per replication for each species both in the field and growthroom at each growth stage. Experiments were arranged in a completely random design, and data from the field and growthroom and for each plant species were subjected to analysis of variance, and the LSD test was used to compare means.

Comparision of epicuticular wax ultrastructure. At 3 and 14 weeks after bud break, needles were collected randomly from both field- and growthroom-grown pine and spruce seedlings. The needles were freeze dried for 48 h (2) and were cut into 0.5-cm lengths with a razor blade. The specimens were placed on carriers with 2-sided tape, were coated with gold palladium (Polaron SEM coating unit), and were placed in a Phillips scanning electron microscope. The magnification of the photomicrographs ranged from 160- to 2000-fold. The freeze-drying technique (2) resulted in an undisturbed epicuticular wax layer with a high degree of resolution in the micrographs.

RESULTS AND DISCUSSION

Field study. Foliar injury to white spruce by either glyphosate or triclopyr was greater at the end of the second growing season than at the end of the first growing season (Figure 1). These results agree with those of Sutton (13) who observed that white spruce survival continued to decrease during the second season after glyphosate application. Sutton implied that this delayed mortality was not because of a 2-yr translocation period for absorbed glyphosate but rather the result of severe stress over time. The amount of apical growth in most conifers is determined largely by growth during the previous summer (9). Thus any stress, such as herbicide injury during that previous summer, could affect the next year's growth (13). This, combined with the stress of winter conditions, could explain the continued increase in foliar injury for white spruce the year after application.

Foliar injury to jack pine also was higher at the end of the second growing season but only from triclopyr (Figure 1). The increase in injury, however, was not as great as that observed for white spruce. Jack pine injury from glyphosate was lower in 1987 than it had been at the end of 1986.

Both species showed greatest foliar injury from the July application for both herbicides in both years of assessment (Figure 1). The July application date was approximately 6 weeks after bud break when shoots were actively elongating. The September application was approximately 12 weeks after bud break; and even at this growth stage, injury ratings ranged from 1.3 to 4.0 (Figure 1).

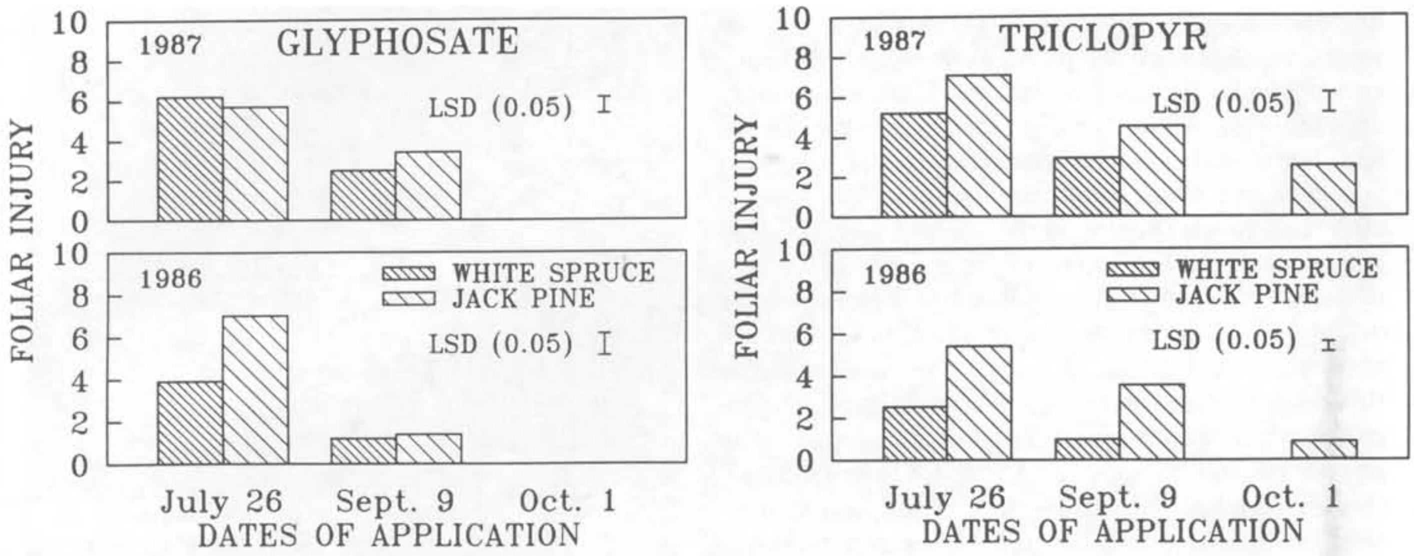


Figure 1. Foliar injury of 2-yr-old jack pine and 4-yr-old white spruce from glyphosate (left) and triclopyr (right) applied during 1986. Injury rating: 0 = no injury; 10 = complete kill. Injury assessed Oct. 18, 1986, and Oct. 19, 1987.

Visual examination suggested that the seedlings had stopped their active growth by September, but both species were injured by both herbicides applied in September (Figure 1). The October application was approximately 16 weeks after bud break; no injury was observed with glyphosate, but triclopyr injured jack pine. Most investigators (1, 3, 7, 12) concluded that conifers tolerate herbicides (glyphosate and phenoxy) applied in late summer and early fall. These field studies confirm this. However, the trees were planted late, and herbicides applied during September still caused injury.

The toxicity of both herbicides to white spruce differed little (Figure 1). However, at the final assessment in October, 1987, triclopyr was more toxic than glyphosate to jack pine regardless of 1986 application date. Jack pine was generally more susceptible than white spruce to either herbicide for each application date. These results are similar to those of other investigators who have reported that jack pine is more susceptible to herbicides than white spruce (5, 10, 12). Also, triclopyr could damage ponderosa pine (*Pinus ponderosa*) regardless of application time during the season (11).

Growthroom studies. In growthroom studies, injury was greatest to white spruce or jack pine when either glyphosate or triclopyr was applied 3 weeks after bud break in spring (Figure 2). Significantly less injury was observed at the other application times. The least injury occurred 14 weeks after bud break, which is equivalent to a late summer-early fall application. Triclopyr was significantly more toxic than glyphosate to both jack pine and white spruce. These results confirm the results of the field study.

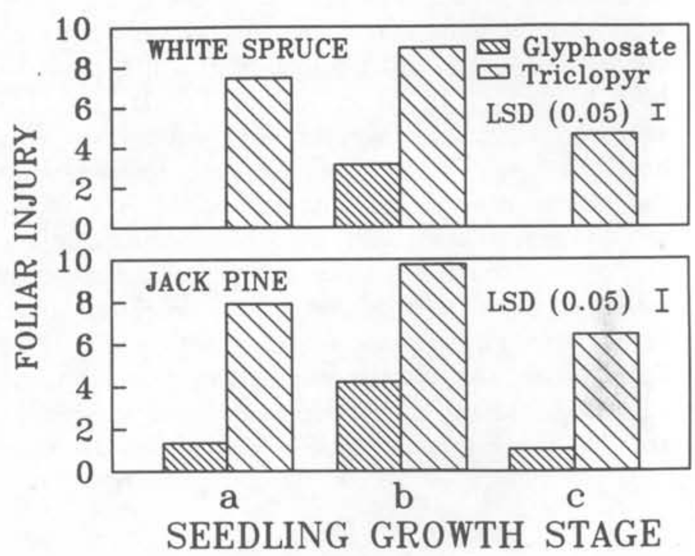


Figure 2. Foliar injury to growthroom-grown 2-yr-old jack pine and 4-ry-old white spruce 40 days after applying glyphosate and triclopyr at (a) 4 days before bud break, (b) 3 weeks after bud break, and (c) 14 weeks after bud break. Injury rating: 0 = no injury; 10 = complete kill.

Epicuticular wax quantity. Wax extraction studies were conducted at the same time as growthroom-grown trees were assessed for herbicide injury. Wax deposition on needles was greater at 14 weeks after bud break with both growthroom- and field-grown jack pine and white spruce seedlings than at 3 weeks after bud break (Figure 3). Wax deposition on field-grown white spruce increased from 1.9% at 3 weeks to 4.2% at 14 weeks after bud break. Wax deposition on field-grown jack pine needles increased from 0.7% at 3 weeks to 1.3% at 14 weeks after bud break. At the 3-week period, wax deposition on growthroom-grown white spruce was 1.8% with injury ratings by glyphosate and triclopyr at 3.0 and 8.0, respectively (Figure 2 and 3). At the 14-week period, wax deposition had increased to 3.5%, and the injury ratings had decreased to 0 and 4.0 for glyphosate and triclopyr, respectively.

Wax deposition on growthroom-grown jack pine increased from 0.6% at 3 weeks to 1.0% at 14 weeks after bud break, while the injury ratings decreased from 10 to 6.5 and from 4.5 to 1.0 for triclopyr and glyphosate, respectively (Figures 2 and 3). The increase in wax deposition may be a factor in increased tolerance to herbicides, perhaps by creating a greater barrier to herbicide penetration by decreasing the wettability and retention of the herbicide on the needles.

Field- and growthroom-grown white spruce had significantly greater wax deposition at the 3- and 14-week growth stage than jack pine (Figure 3), which could explain the greater tolerance of white spruce to both herbicides. In earlier studies, Lehela et al. (5) similarly suggested that the thin cuticle and less heavily plugged stomata of jack pine could explain the lower tolerance to aminotriazole (*1H*-1,2,4-triazole-3-amine) compared to white spruce. Injury ratings from both the field- and growthroom-grown seedlings show that white spruce tolerated both herbicides more than jack pine (Figures 1 and 2).

Epicuticular wax ultrastructure. The stoma on the adaxial surface of a white spruce needle at 3 weeks after bud break was partially occluded with wax,

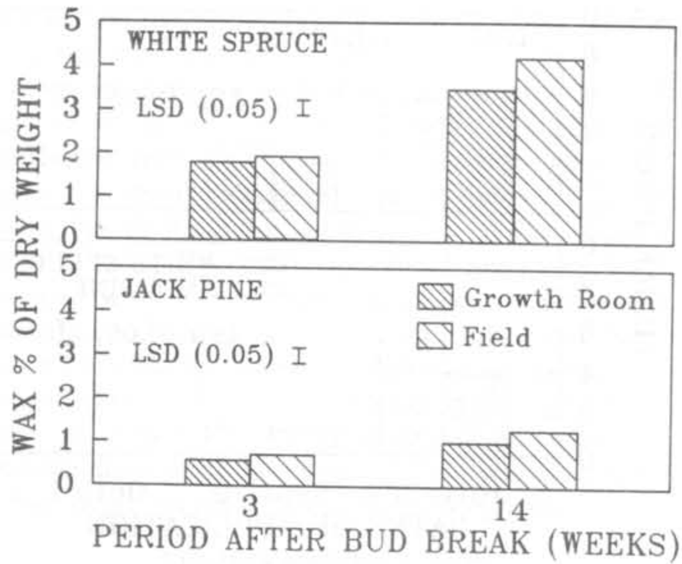


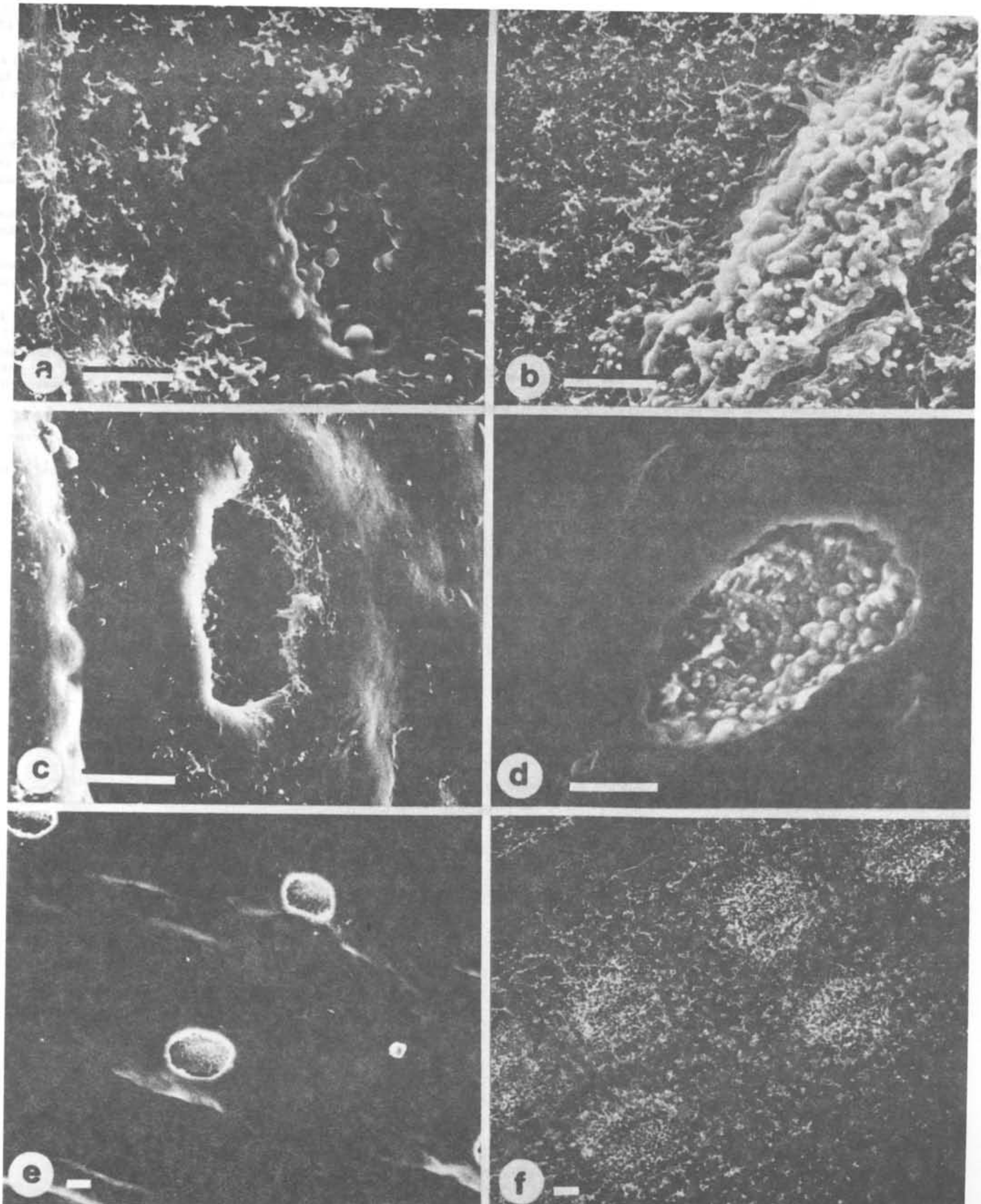
Figure 3. Wax deposition on needles of field- and growthroom-grown white spruce and jack pine at 3 and 14 weeks after bud break.

and the surrounding needle surface was sparsely coated with tubular wax projections (Figure 4a). The stoma appears out of focus because of the difference in depth of the pore. The stoma of white spruce at 14 weeks after bud break is completely occluded with wax (Figure 4b), and the surrounding needle surface is densely packed with wax projections.

The stoma and surrounding needle surface of jack pine at 3 weeks after bud break are sparsely coated with epicuticular wax (Figure 4c). The stoma of jack pine at 14 weeks after bud break was occluded with wax (Figure 4d), and the surrounding needle surface was sparsely coated with epicuticular wax.

The stoma of jack pine at 14 weeks after bud break are occluded with wax (Figure 4e), and the surrounding needle surfaces are sparsely coated with epicuticular wax. The stoma of white spruce at 14 weeks after bud break are completely occluded (Figure 4f), and the needle surface is densely packed with epicuticular wax. This greater wax deposition on white spruce may explain the greater tolerance of the species over jack pine.

Figure 4. Scanning electron micrograph of stomata on the adaxial surface of white spruce (a) 3 and (b) 14 weeks after bud break; jack pine (c) 3 and (d) 14 weeks after bud break; and a comparison of the stomata of (e) jack pine and (f) white spruce 14 weeks after bud break. Bars = 10 μm.


a
b
c
d
e
f

Increased wax deposition with time after bud break on both jack pine and white spruce may provide a greater barrier to glyphosate and triclopyr penetration and could explain the increased herbicide tolerance later in the season.

ACKNOWLEDGMENTS

This study was supported by the Ontario Ministry of Agriculture and Food and by grants from the Natural Sciences and Engineering Research Council of Canada and the Canadian Forestry Service – Program for Research by Universities in Forestry (PRUF).

LITERATURE CITED

1. Barring, U. 1979. Results of country wide trials in forestry trials into aerial application of glyphosate and triclopyr amine. Proc. 20th Swed. Weed Control Conf.:82–93.
2. Campbell, R. 1972. Electron microscopy of the epidermis and cuticle of the needles of *Pinus nigra* in relation to infection by *Lophodermella sulcigena*. Ann. Bot. 36:307–319.
3. Gratkowski, H. J. 1977. Seasonal effects of phenoxy herbicides on ponderosa pine and associated brush species. For. Sci. 23:3–12.
4. Kolattukudy, P. E. 1965. Biosynthesis of wax in *Brassica oleracea*. Biochemistry 4:1844–1845.
5. Lehela, A., R. J. Day, and Z. Koran. 1972. A close up of the stomatal regions of white spruce and jack pine. For. Chron. 48: 32–34.
6. Lund-Hoie, K. 1975. N-phosphonomethylglycine (glyphosate) an alternative to commercial pre- and post-emergence herbicides for the control of unwanted plant species in forest plantations in Norway. Sci. Rep. Agric. Univ. Norway 54:1–14.
7. Lund-Hoie, K. 1976. The correlation between the tolerance of Norway spruce (*Picea abies*) to glyphosate (N-phosphonomethylglycine), and the uptake, distribution and metabolism of the herbicide in the spruce plants. Sci. Rep. Agric. Univ. Norway 55:1–26.
8. Lund-Hoie, K. 1984. Growth responses of Norway spruce (*Picea abies*) to different vegetation managements programmes – preliminary results. Aspects Appl. Biol. 5:127–133.
9. Lund-Hoie, K. 1985. Efficacy of glyphosate in forest plantations. p. 328–338 *in* E. Grossbard and D. Atkinson, eds. The Herbicide Glyphosate. Butterworth & Co., Ltd., London.
10. McCavish, W. J. 1980. Herbicides for woody weedy control by foliar application. Proc. Br. Crop Prot. Conf. Weeds, p. 729–737.
11. Paley, S. M., and S. R. Radosevich. 1984. Effect of physiological status and growth of Ponderosa pine (*Pinus ponderosa*) and greenleaf manzanita (*Arctostaphylos patula*) on herbicide selectivity. Weed Sci. 32:395–402.
12. Radosevich, S. R., E. J. Roncoroni, S. G. Conrad, and W. B. McHenry. 1980. Seasonal tolerance of six coniferous species to eight foliage-active herbicides. For. Sci. 26:3–9.
13. Sutton, R. F. 1978. Glyphosate herbicide: An assessment of forestry potential. For. Chron. 54:24–28.