Screening Jack Pine Seedlings for Resistance to *Endocronartium harknessii*

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ABSTRACT

Burnes, T. A., Blanchette, R. A., Wang, C.-G., and French, D. W. 1988. Screening jack pine seedlings for resistance to *Endocronartium harknessii*. Plant Disease 72:614-616.

Concentrated suspensions of *Endocronartium harknessii* aeciospores in water or oil were sprayed onto 8-wk-old *Pinus banksiana* seedlings in the greenhouse. Spores, concentrated in oil, dispersed more readily and resulted in a higher incidence of infection than aeciospores in water. Red stem and needle lesions were visible 2–3 wk after inoculation, and galls developed 8 wk later. Seedlings from eight *P. banksiana* seed lots were inoculated and evaluated for resistance 12, 24, and 48 wk after inoculation. Several seed lots derived from *P. banksiana*, previously selected in the field for possible resistance to gall rusts, had significantly fewer galls 24 wk after inoculation than other seed sources tested.

Gall rust caused by *Endocronartium harknessii* (J. Moore) Y. Hirats. is a major autoecious stem rust of hard pines in North America (4,17) and causes serious losses in nurseries and young stands of jack pine, *Pinus banksiana* Lamb. (6). In Minnesota, *E. harknessii* occurs in the northernmost regions of the jack pine range (5). Resistance of jack pine to another gall rust, *Cronartium quercuum* (Berk.) Miyabe ex. Shirai f. sp. *banksianae* Burdsall and Snow, has been identified in field (7) and greenhouse studies (15) but no information is available concerning resistance of jack pine to *E. harknessii*.

Pine seedlings have been used in several previous investigations to identify rust resistance with artificial inoculation (1,3,8,11,12,16). Basidiospores suspended in water were used to infect jack pine with *C. quercuum* f. sp. *banksianae* (16) and various species of southern pines with *C. quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* Burdsall and Snow (8,11,12). Allen and Hiratsuka (1) and Blenis and Hiratsuka (3) have developed techniques to inoculate and infect lodgepole pine, *P. contorta* var. *latifolia*, with *E. harknessii*. These studies indicated that seedling age had little influence on infection, but higher spore concentrations were responsible for increased infection. A possible method to obtain uniform spore distribution and infection may be to incorporate aeciospores in a refined nonphytotoxic petroleum oil as previously used for inoculating wheat and other cereal with various spore stages of rusts (14).

The objectives were to examine the effectiveness of two inoculation techniques using water and petroleum oil to infect jack pine seedlings with *E. harknessii* and to evaluate seedlings grown from various seed sources of jack pine for resistance.

MATERIALS AND METHODS

Aeciospores were collected from galls on jack pine in May and June from three different sites in northern Minnesota. Aeciospores of *E. harknessii* were identified by their germ tube morphology and stored in gelatin capsules at −15 C (2). Different jack pine seed lots were collected from trees in a Cloquet, MN seed orchard that was established with trees previously selected for possible resistance to gall rust (French, *unpublished data*). Seed was used from eight seed sources that produced sufficient numbers of seeds for experimental purposes. In addition, a general bulk seed (#4E) from the General Andrews State Nursery, Willow River, MN was used. Seeds were sown in a mixture of vermiculite and peat (1:1 by volume) contained in 30 cavity styroblocks (4.1 cubic in. per cavity). Seedlings were grown in a greenhouse at 21–27 C with supplemental light for a 14-hr photoperiod and were fertilized every 2 wk. Five experiments were

Paper No. 15,660, Scientific Journal Series, Minnesota Agriculture Experiment Station, St. Paul 55108.

Accepted for publication 17 February 1988 (submitted for electronic processing).

completed: 1) inoculation of jack pine seedlings using aeciospores suspended in water; 2) inoculation of jack pine seedlings using aeciospores suspended in oil; 3, 4, and 5) Inoculation of jack pine seedlings grown from eight seed lots with aeciospores suspended in oil. Experiment 5 was a repeat inoculation of seedlings from several seed sources in experiments 3 and 4.

A Power Pak aerosol sprayer (Crown Ind. Prod. Co.) was used in experiment 1 to distribute 15.0 ml of a water suspension of 2×10^5 aeciospores per ml to each of seven styroblocks of 30 seedlings (15). Fifteen ml of water was sprayed on two styroblocks of seedlings to serve as controls. In experiment 2, oil (Soltrol 170, Phillips Petroleum Co.) and aeciospores were added to a gelatin capsule and agitated to suspend the spores in oil. Seedlings were sprayed with aeciospores at a concentration of 15×10^5 aeciospores per 0.75 ml of oil using a venturi atomizer connected to an air pump that created a steady stream of air (14). Seven styroblocks of 30 seedlings each were sprayed with two gelatin capsules of aeciospores concentrated in oil. Two styroblocks of seedlings were each sprayed with two capsules of 0.75 ml of oil as controls. Seedlings in experiments 1 and 2 were inoculated 8 wk after planting.

After inoculation, each styroblock of seedlings was misted with water, placed in a plastic bag, and incubated in darkness at 18 C for 48 hr. Styroblocks of seedlings were removed after 48 hr from the bags and placed in a greenhouse. Experiments 1 and 2 were repeated twice and infection of seedlings was recorded 12, 24, and 32 wk after inoculation. The percent of germination of aeciospores was determined by spraying aeciospores on 2% water agar, misting with water, and placing in a plastic bag at 18 C in darkness for 12–16 hr.

Seedlings from jack pine seed lots were used in experiments 3, 4, and 5. For each seed lot, three styroblocks of 30 seedlings were inoculated in the same manner as previously described using the venturi atomizer with one capsule of 15×10^5 aeciospores per 0.5 ml of oil. One styroblock of 30 seedlings for each seed lot was sprayed with 0.5 ml of oil as a control. Each experiment was repeated three times and infection of seedlings was recorded 12, 24, and 48 wk after inoculation.

RESULTS

Germination of aeciospores on water agar was 60–70% in all experiments. Red stem and needle lesions (Fig. 1A) were visible in seedlings 2 wk after inoculation and galls were visible approximately 8 wk later. There were no visible signs of phytotoxicity from the Soltrol 170 petroleum oil. No infection occurred on control seedlings.

Seedlings inoculated with aeciospores suspended in oil resulted in a greater percent of seedlings with galls (88.8%) than inoculating with spores in water (14.1%) (Table 1). Since infection was extremely high using 15×10^5 spores in experiment 2, the number of aeciospores used in experiments 3, 4, and 5 was reduced by 50%. The reduced number of spores in the inoculum resulted in a lower percentage of seedlings with galls (Table 2).

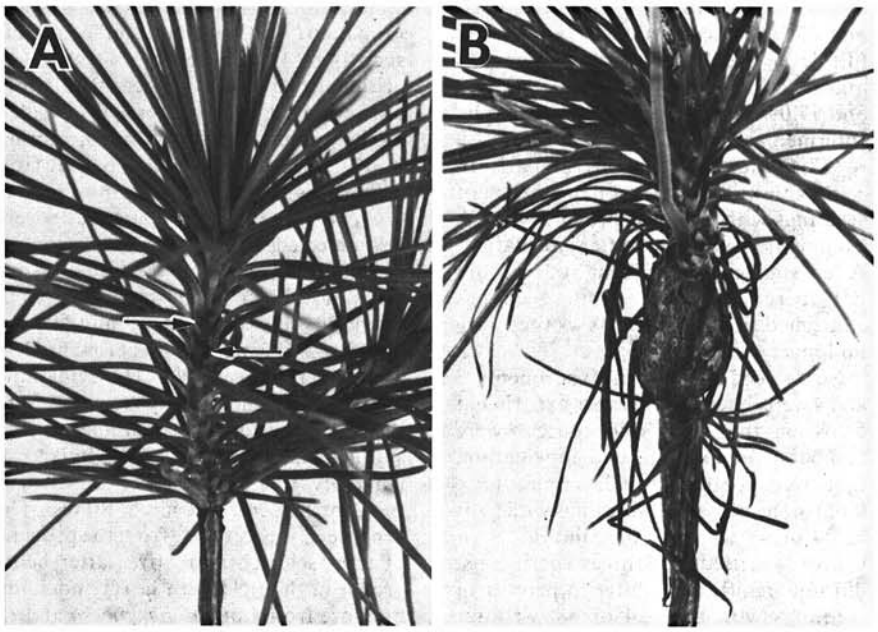

Fig. 1. (A) Red stem lesions (arrows) on *Pinus banksiana* seedlings 3 wk after inoculation with *Endocronartium harknessii*. **(B)** Gall on *P. banksiana* 48 wk after inoculation.

Table 1. Average means[a] of percent of jack pine seedlings with galls 12, 24, and 32 wk after inoculating with spores in water or oil

Experiment	Spore suspension	Seed lot	Percent of seedlings with galls		
			12 wk	24 wk	32 wk
1	Water	4E	13.7 ± 13.9[b]	13.8 ± 14.1	14.1 ± 14.6
2	Oil	4E	83.8 ± 8.6	86.6 ± 3.7	88.8 ± 1.9

[a] Means are from three replications of 210 seedlings each.
[b] Indicates standard deviation.

Table 2. Average means[a] of percent of jack pine seedlings with galls 12, 24, and 48 wk after inoculating with spores in oil

Experiment	Seed lot	Percent of seedlings with galls[b]		
		12 wk	24 wk	48 wk
3[c]	4E	14.1 ± 9.0 a[d]	43.7 ± 9.3 a	42.9 ± 21.6 a
	69	8.5 ± 7.0 ab	36.3 ± 18.8 ab	37.7 ± 23.4 ab
	53	7.4 ± 5.5 ab	29.3 ± 9.9 ab	25.5 ± 12.1 abc
	61	7.0 ± 9.3 ab	21.9 ± 9.7 bc	20.0 ± 10.0 bc
	244	1.8 ± 3.3 b	7.7 ± 6.2 c	10.0 ± 7.0 c
4	67	41.4 ± 13.0 a	72.5 ± 12.4 a	64.4 ± 13.9 a
	48	23.7 ± 14.7 abc	63.7 ± 9.2 a	61.4 ± 12.8 a
	4E	27.4 ± 8.4 bc	68.1 ± 13.1 a	56.2 ± 10.8 ab
	63	23.3 ± 9.1 bc	57.4 ± 9.3 b	37.0 ± 6.3 c
	68	16.6 ± 6.4 c	42.2 ± 13.7 c	28.1 ± 10.8 c
5	4E	51.4 ± 15.9 a	51.4 ± 18.9 a	59.6 ± 18.2 a
	68	44.1 ± 8.9 ab	45.9 ± 9.6 a	44.8 ± 10.6 ab
	53	37.4 ± 22.5 ab	32.9 ± 19.4 ab	35.5 ± 20.4 ab
	63	33.3 ± 17.5 ab	31.8 ± 16.8 ab	34.0 ± 20.1 ab
	244	22.2 ± 10.8 b	17.0 ± 9.6 b	18.8 ± 11.1 b

[a] Means are from three replicates of 90 seedlings each.
[b] For each experiment, the means within the same column and followed by the same letter were not significantly different at the 5% level using Duncan's multiple range test (13).
[c] Experiments 3, 4, and 5 were done at different times.
[d] Indicates standard deviation.

Seed lots within experiments 3 and 4 were significantly different ($P < 0.05$) in percent of seedlings with galls, and the galls were evident as early as 12 wk after inoculation (Table 2). Percent of infected trees usually increased from 12 to 48 wk. At 24 wk after inoculation, the highest infection occurred among seed lots 67, 4E, and 48 in experiment 4 with 72.5, 68.1, and 63.7% galled seedlings, respectively (Table 2). Seed lot 244 in experiments 3 and 5 had the lowest infection with 7.7 and 17.0% galled seedlings, respectively. Intermediate infection ranged from 21.9 to 57.4% for seed lots 61 and 63. In experiment 4, a lower number of seedlings with galls was present at 48 wk as compared with 24 wk after inoculation. A few smaller galls evident at 24 wk did not increase in size, while seedlings continued to grow. After 48 wk they were no longer evident.

Some seed sources in experiments 3 and 4 were inoculated again in experiment 5. When the same seed sources were compared in this repeated inoculation, there were no significant differences ($P > 0.05$) in the percent of seedlings with galls at 24 or 48 wk after inoculation. Seed source #4E, used in all three experiments, did not significantly differ in percent of seedlings with galls 24 or 48 wk after inoculation.

DISCUSSION

Aeciospores of *E. harknessii* suspended in water were dispersed poorly on seedlings using the Power Pak aerosol sprayer. In contrast, aeciospores in oil were readily suspended and evenly dispersed using the venturi atomizer. This may account for the higher infection in experiment 2.

The number of spores used in experiment 2 resulted in an extremely high infection, with 88.8% galled seedlings. To determine if seedlings from various seed sources were susceptible or resistant, a lower inoculum density of 15×10^5 aeciospores per 0.5 ml of oil was used in experiments 3, 4, and 5 that resulted in less infection as compared with seedlings inoculated in experiment 2. Laird and Phelps (8) suggested that a reduction in inoculum density of *C. quercuum* f. sp. *fusiforme* spores enhanced the sensitivity of artificial inoculation in identifying resistance among southern pine seedlings. Since needle and stem lesions could be an expression of resistance or a susceptible reaction, seed sources in this study were selected for resistance on the basis of gall formation (9,10). Resistant and susceptible seedlings from seed lots could be distinguished at 12 wk after inoculation. However, the average percent of galled seedlings increased with time. In another study using artificial inoculation techniques (15) and basidiospores of *C. quercuum* f. sp. *banksianae*, galls were best detected 24 wk after inoculation.

Among all seed sources tested in this study, lot 244 had the highest level of resistance in experiments 3 and 5. Seed lots 48, 67, 68, and 4E in experiments 3, 4, and 5 were susceptible. The remaining seed lots were moderately resistant. Seed lot 4E consists of a large number of seed sources and is typical of the bulk seed currently used in forest tree nurseries in the North Central United States. The other seed sources were from the progeny of trees selected as rustfree after being grown in the field with heavy inoculum pressure from both *E. harknessii* and *C. quercuum* f. sp. *banksianae*. The results presented here indicate that resistance to *E. harknessii* is present in various seed sources of jack pine. Although some seed sources such as 48, 67, and 68 were susceptible to *E. harknessii*, they may be resistant to *C. quercuum* f. sp. *banksianae*. Screening these seed sources with artificial inoculation techniques developed for *C. quercuum* f. sp. *banksianae* (15) will be necessary.

Jack pine seedlings inoculated with aeciospores of *E. harknessii* suspended in oil and applied using a venturi atomizer appears to be an excellent system to rapidly screen seed lots for resistance. Jack pine identified as being resistant to *E. harknessii* could be planted in areas of high infection to reduce losses (5).

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