# INOCULATION EXPERIMENTS WITH *PHAEOCRYPTOPUS GAEUMANNII* ON DOUGLAS FIR SEEDLINGS

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## ABSTRACT

Water suspensions of fragmented mycelia cultured from single-ascospore isolates of **Phaeocryptopus gaeumannii** (Rohde) Petrak were used to inoculate foliage of seedlings of Douglas fir ( **Pseudotsuga menziesii** (Mirb.) Franco). Infected 0- to 1-year-old foliage on inoculated seedlings was retained to the same extent as uninfected foliage on control plants. On the other hand, the inoculated seedlings retained a significantly lower proportion of 1- to 2-year-old needles than the controls (with one exception) 19-23 months after becoming infected. Infected 0- to 1-year-old foliage photosynthesised at a lower mean rate than uninfected needles of the same age. Despite these effects, no significant differences in total seedling dry weight were found between control seedlings and seedlings infected for 19-23 months.

## INTRODUCTION

The parasitic fungus *Phaeocryptopus gaeumannii (Rohde)* Petrak was discovered in Switzerland 50 years ago (Gäumann, 1930) associated with premature defoliation of its only known host, Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Since then there have been reports of needle casting from many other countries where plantations of this species have become infected (e.g., Blada, 1971; James, 1975; Liese, 1939; McCormick, 1939; Marks, 1975; Morton and Patton, 1970; Thulin, 1949). Despite these observations it has apparently never been conclusively proved that *P. gaeumannii* is responsible for defoliation or growth decline of its host. Lyr (1955) carried out inoculation experiments using mycelial suspensions applied to foliage of potted seedlings. He found that pseudothecia developed on foliage about one year after it was inoculated, and he was able to reisolate the fungus from infected needles. However he did not report any effect of *P. gaeumannii* on seedling health. Peace (1962, p. 348) briefly referred to unpublished inoculation experiments of Hahn without giving any details. It was therefore decided to carry out further inoculation experiments in order to investigate the effect of infection on needle retention, $CO_2$ gaseous exchange, and total seedling weight.

**Note added in proof.** Strittmatter (1974) inoculated seedlings by suspending infected foliage above them under very moist conditions. Foliage so infected was shed 18 months later, whereas no needle casting occurred on nearly uninfected control plants.

## METHODS

### *Preparation of Inoculum*

Inoculations were carried out using water suspensions of mycelium fragments, since *P. gaeumannii* has not been known to sporulate in culture (Rohde, 1937). Two single-ascospore isolates of *P. gaeumannii* obtained from infected Douglas fir foliage were cultured in flasks of 3% malt solution at 14-18°C for periods of 10-40 weeks. Mycelial mats were filtered, suspended in distilled water, disintegrated using a Waring Blendor, and diluted to concentrations of 1-6 g (wet weight) per 100 ml. Suspensions were usually used within 1 day of preparation.

### *Inoculations*

Potted Douglas fir seedlings of one seedlot (1) were obtained from an area still free from *P. gaeumannii* infection, while those of two others (2 and 3) were grown from seeds sown under glasshouse cover. About half of the seedlings in each seedlot were randomly selected for inoculations and the rest were used as controls. Controls and treatment plants were kept randomly mixed except when being inoculated.

Inoculations were started when plants were 1-1½ years old. Each seedling was inoculated two or three times between October and March over two successive growing seasons. During each inoculation seedlings were sprayed nearly to runoff point with inoculum and then placed for 3-6 days in a high-humidity environment normally at 15-19°C. Control seedlings were sprayed with autoclaved inoculum, and all seedlings within each seedlot were inoculated the same number of times each season over the same periods.

To protect them from natural infection, seedlings were stored in a dry glasshouse each year between August and March (Hood and Kershaw, 1975) and during the rest of the year they were kept outside. Glasshouse temperatures for seedlings of seedlots 2 and 3 and most of seedlot 1 (group 1) were controlled at 15-20°C. The other seedlings of seedlot 1 (group 2) were stored at a different temperature range (10-30°C) for the first season only.

Scions from three 22-year-old Douglas fir trees (clones 4, 5, and 6) were also inoculated after grafting onto potted seedlings. Both scions and rootstock material (of one seedlot) came from areas free from *P. gaeumannii* infection. Grafted plants were treated in the same way as seedlings, except that temperatures during inoculations and glasshouse storage were not controlled or recorded. Inoculations were begun on the first new scion foliage that emerged after grafting.

### *Evaluation of Results*

Infection was evaluated on current (i.e., 0- to 1-year-old) foliage 7-12 months after the start of each season's inoculations. A stereo-microscope was used to examine 50 needles on each seedling in order to determine the percentage bearing pseudothecia. Seedlot 1 seedlings were not evaluated for percentage infection after the first inoculation series.

Seedlings were destructively harvested 20-23 months after the first inoculation, plants of the same seedlot being dealt with at approximately the same time. Plants were removed from their pots and soil was washed from the roots so that, after drying, the various parts could be weighed in order to determine the percentages present of 1-year-old, 2-year-old, and any older foliage. Grafted plants were treated similarly 20 months

after the first inoculation except that foliage retention was expressed as dry weight of foliage per unit length of fresh stem tissue of the same year.

Rates of $CO_2$ gaseous exchange of current foliage were also measured on seedlot 1 seedlings. Readings were made in the dark at 18-23°C and in the light (100 watts/$m^2$) at 22-26°C, immediately before seedlings were removed from pots for drying and weighing. The shoots of each seedling bearing current foliage were enclosed in a plastic bag, after removal of older needles, and a null balance infrared analyser was used to measure the $CO_2$ gas differential between air flowing to and from the bag, in an open system.

## RESULTS

The first series of inoculations resulted in mean infection levels on current foliage equal to or greater than 84% on seedlings (seedlots 2 and 3) and 48% on grafts, while foliage on control plants remained nearly uninfected (Tables 1 and 2). Inspection of

TABLE 1—Mean values of infection, needle retention, total weight, and $CO_2$ exchange for seedlings

| Seedlot and group | Treatment | Number of plants | Infection (%) 0- to 1-year-old foliage, in successive years | | Needle retention (% total plant dry weight) foliage age-classes in years† | | Whole seedling dry weight (g) | $CO_2$ exchange 0- to 1-year-old foliage mg/g (dry wt)/hour | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1st | 2nd | 0-1 | 1-2 | | Light | Dark |
| 1 group 1 | Inoculated | 20 | — | 95 | 23.5 | 1.9 | 15.9 | 6.0 | 0.7 |
| | Control | 17 | — | 1 | 23.3 | 3.5 | 19.1 | 7.5 | 0.6 |
| | Significance | — | — | *** | NS | *** | NS | * | NS |
| 1 group 2 | Inoculated | 6 | — | 97 | 24.3 | 0.8 | 16.0 | 5.4 | 0.7 |
| | Control | 7 | — | 0‡ | 24.0 | 2.1 | 13.4 | 7.7 | 0.4 |
| | Signifiance | — | — | *** | NS | NS | NS | * | NS |
| 2 | Inoculated | 10 | 94 | 26 | 14.6 | 1.8 | 23.6 | — | — |
| | Control | 12 | 0‡ | 3 | 13.2 | 6.4 | 26.0 | — | — |
| | Significance | — | *** | *** | NS | *** | NS | — | — |
| 3 | Inoculated | 8 | 84 | 12 | 14.8 | 2.9 | 28.3 | — | — |
| | Control | 14 | 0‡ | 1 | 13.7 | 5.2 | 34.0 | — | — |
| | Significance | — | *** | ** | NS | ** | NS | — | — |

All results (except 1st-year infection) evaluated at the end of the experiment, 20-23 months after the first inoculation.

Significant levels from t test, inoculated v. control seedlings; probability of means not being different: *** $\lessapprox 0.001$

** $\lessapprox 0.01$

* $\lessapprox 0.05$

NS Not significant (>0.05)

† For seedlot 1 the small amount of older foliage is included with 1- to 2-year-old foliage

‡ Trace of infection present (mean <0.5%).

TABLE 2—Infection and needle retention for grafts; mean values and standard deviations

| Clone | Treatment | No. plants | Infection (%) (0- to 1-yr-old foliage, 1st yr)* | Needle retention at end of experiment (dry weight of needles on stem†, g/m) for foliage age-classes: | |
|---|---|---|---|---|---|
| | | | | 0- to 1-yr | 1- to 2-yr |
| 4 | Inoculated | 5 | 48 ± 19 | 4.4 ± 0.8 | 1.2 ± 0.6 |
| | Control | 4 | 1 ± 1 | 4.2 ± 0.6 | 5.4 ± 1.2‡ |
| 5 | Inoculated | 3 | 81 ± 11 | 4.4 ± 0.3 | 0.7 ± 0.6 |
| | Control | 4 | 0 ± 0 | 4.2 ± 0.4 | 3.2 ± 0.8 |
| 6 | Inoculated | 3 | 94 ± 5 | 5.4 ± 1.1 | 0.2 ± 0.1 |
| | Control | 1 | 2 | 4.2 | 5.7 |

* 1- to 2-yr-old at end of experiment, 20 months after the first inoculation.

† Total stem length per plant: 2.7 ± 1.0 m (0- to 1-yr-old) and 0.5 ± 0.2 m (1- to 2-yr-old); means and standard deviations for all plants.

‡ The very low result of 0.1 for one plant has been omitted. (If included, this value would be 4.1 ± 2.8).

seedlot 1 seedlings indicated a similar result. Full-sized ascospores were found within pseudothecia sampled from seedlings inoculated using only one single-ascospore isolate. The second inoculation series caused new seedlot 1 foliage to become heavily infected but less infection appeared on seedlot 2 and 3 foliage (Table 1), and new foliage of inoculated grafts remained almost uninfected (not more than 4% on any plant).

Current 0- to 1-year-old foliage did not noticeably change colour as a result of infection, and the same amount of current foliage was found on both inoculated and control seedlings when they were harvested at the end of the experiment (Table 1). On the other hand, with one exception (seedlot 1, group 2), a greater proportion of 1- to 2-year-old foliage was retained on control seedlings than on inoculated seedlings. Results for the smaller number of grafted plants (some died from root rot) were similar (Table 2). Much 1- to 2-year-old foliage on inoculated grafted plants turned brown in February, 13 months after the first inoculation, and was subsequently cast, while the same foliage on controls remained green until the end of the experiment. Such a distinctive colour change over a short period was not observed with seedlings but towards the end of the experiment it could be seen that a number of inoculated seedlings retained fewer 1- to 2-year-old needles than most controls (Fig. 1). Very little 2- to 3-year-old foliage was present on any seedling (mean and standard deviation for all seedlot 2 and 3 seedlings: 0.1 ± 0.1%, by weight).

No significant difference (Table 1) was found between total dry weights of control seedlings and those of seedlings continuously infected for 19-23 months (i.e., for all seedlots, from sometime between the first and last inoculations during the first season).

Current foliage of infected seedlings (seedlot 1) photosynthesised at a slightly lower mean rate than that of control seedlings. Infection did not significantly affect the respiration rates of current foliage.

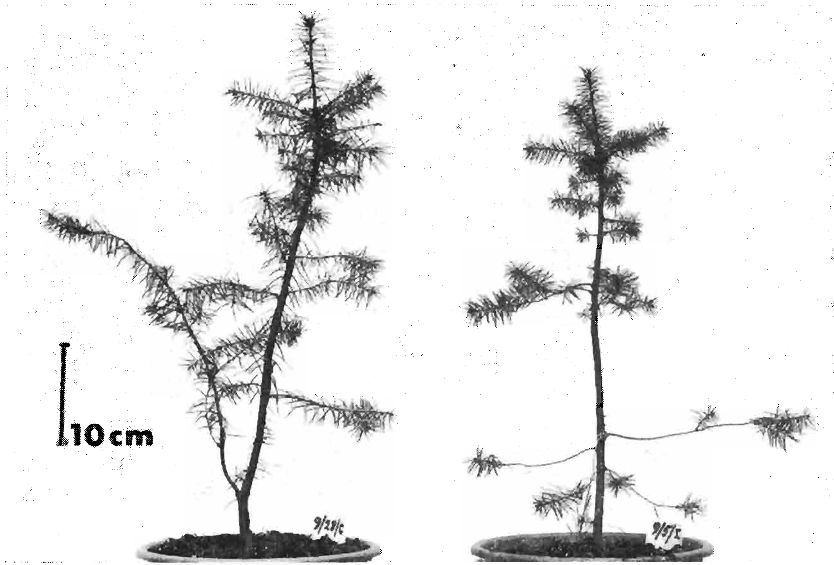


FIG. 1—Selected seedlings of seedlot 2 photographed at the end of the experiment. The 1- to 2-year-old foliage makes up 0.3% (by weight) of the right-hand plant, artificially infected 19-20 months previously, and 6.4% of the left-hand plant, treated only with autoclaved inocula.

## DISCUSSION

One of several factors may have caused the failure of the second series of grafted plant inoculations. These were not started until 25 February, which may have been too late, since infection takes place naturally in New Zealand between November and January (Hood and Kershaw, 1975). At other times first inoculations of grafts and seedlings were commenced before the end of January each season. In addition, during the second inoculation series with grafted plants, inoculum dried more quickly onto needles during the short inoculation periods, due to the greater bulk of foliage and the equipment used, and this may have hindered needle infection.

Group 2 seedlings of seedlot 1 were an exception to the result that significantly less 1- to 2-year-old foliage was retained by inoculated seedlings than by controls (Table 1). Even with this group, however, the mean for the inoculated plants was less than that of the controls and it is possible that with a greater number of seedlings the difference may have become significant. The only treatment difference between group 1 and 2 seedlings was their glasshouse incubation temperature in the first season.

Infection of new foliage on Douglas fir seedlings by *P. gaeumannii* caused a reduction in mean photosynthesis, when measured within the first year of needle life, and apparently also induced a greater shedding of needles during their second year, both on seedlings and on grafted scions from 22-year-old trees. Continuous infection for 19-23 months did not affect the total weight of seedlings but this may have been insufficient time for defoliation and photosynthesis reduction to have had full effect. The effect of

defoliation on plant growth in the field would depend on how great a contribution 1- to 2-year-old and older needles make to the total assimilation of healthy plants. The results of these experiments (Table 1; Fig. 1) suggest that, with seedlings, this older foliage may not make up a very large component of the total foliage present.

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