# *FUSARIUM CIRCINATUM* -
# - AN AGENT OF DAMPING-OFF DISEASE

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## EXECUTIVE SUMMARY

### Background

- The susceptibility of six plantation and two pasture species of plants, to damping-off caused by *Fusarium circinatum* and to *Fusarium oxysporum,* was compared.

- *Fusarium circinatum* is the agent of pine pitch canker disease and can cause damping-off of seedlings in addition to causing canker and dieback of older plants. *Fusarium oxysporum* is a common inhabitant of nursery soils in New Zealand, causing damping-off and root rot to a number of species when soil moisture is high and conditions are conducive to disease.

### Methods

- The plantation species tested were *Pinus radiata, P. pinaster, Eucalyptus fastigata, E. nitens, Pseudotsuga menziesii* and *Cupressus macrocarpa*. *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) are representative of pasture species used when nursery beds are put into fallow.

- *Pinus radiata* and *P. pinaster* were extremely susceptible to damping-off caused by *F. circinatum*. None of the *P. radiata* survived and only seven percent (of seed sown) of the *P. pinaster* were alive at the close of the experiment. Lower levels of mortality were caused by *F. oxysporum.*

- Pre-emergence and post-emergence deaths of *C. macrocarpa* occurred in both the *Fusarium* treatments but were higher in the *F. oxysporum* treatments than the *F. circinatum* treatments. As damping-off associated with *F. oxysporum* is relatively uncommon in nurseries when conditions can be considered as 'normal' it is likely that *F. circinatum* would only be a problem to *C. macrocarpa* under extreme conditions. These are only rarely encountered in bare-root nurseries.

- *Eucalyptus regnans* was also far more susceptible to *F. oxysporum* than to *F. circinatum.* Total mortality in the *F. circinatum* treatments (61%) was only slightly higher than in the control and *E. nigrum* treatments (both 50%).

- *Trifolium repens* (white clover) was very susceptible to the isolate of *F. oxysporum* used in the trial but was unaffected by *F. circinatum.*

- *Eucalyptus globulus* and *L. perenne* were not susceptible to damping-off by either *F. circinatum* or *F. oxysporum.*

- No conclusions could be drawn about the susceptibility of *Ps. menziesii* due to very poor emergence in all treatments.

- It is possible that if a wider range of species were to be tested some conifers other than pines may demonstrate a level of susceptibility to *F. circinatum.*

**ABSTRACT**

The susceptibility of six plantation and two pasture species of plants, to damping-off caused by *Fusarium circinatum,* the agent of pine pitch canker disease, and to *Fusarium oxysporum,* a common inhabitant of nursery soils in New Zealand, was compared. The plantation species tested were *Pinus radiata, P. pinaster, Eucalyptus fastigata, E. nitens, Pseudotsuga menziesii* and *Cupressus macrocarpa*. *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) are representative of pasture species used when nursery beds are put into fallow. *Pinus radiata* and *P. pinaster* were extremely susceptible to damping-off caused by *F. circinatum*. Lower levels of mortality were caused by *F. oxysporum.* Pre-emergence and post-emergence deaths of *C. macrocarpa* and *E. regnans* occurred in both the *Fusarium* treatments but were higher in the *F. oxysporum* treatments than the *F. circinatum* treatments. *Trifolium repens* (white clover) was very susceptible to the isolate of *F. oxysporum* used in the trial but was unaffected by *F. circinatum. Eucalyptus globulus* and *L. perenne* were not susceptible to damping-off by either *F. circinatum* or *F. oxysporum.* No conclusions could be drawn about the susceptibility of *P~~s~~. menziesii* due to very poor emergence in all treatments. It is possible that if a wider range of species were to be tested some other conifers other than pines may demonstrate a level of susceptibility to *F. circinatum.* As damping-off associated with *F. oxysporum* is relatively uncommon in nurseries when conditions can be considered as 'normal' it is likely that *F. circinatum* would only be a problem to such non-pine hosts under extreme conditions. These are only rarely encountered in bare-root nurseries.

# *FUSARIUM CIRCINATUM* - AN AGENT OF DAMPING-OFF DISEASE

**MA Dick and J Simpson**

## INTRODUCTION

*Fusarium circinatum,* the cause of pine pitch canker disease, has a host range of over 40 species in the genus *Pinus*. Some species of *Pinus* are far more severely affected than others and *Pinus radiata* has proven to be one of the most susceptible (Dick and Bain 1996; Dick 1998). *Fusarium circinatum* is capable of infecting *P. radiata* at all stages of growth, from very young seedlings through to mature trees. It has proved to be a particularly devastating disease in some pine-growing nurseries. In South Africa, pitch canker has been a major agent of disease in many nurseries. The scale of the epidemic has been very large on some occasions, affecting over six million seedlings (*Pinus patula*) in a single infestation (MJ Wingfield, pers. com.).

*Fusarium circinatum* is both externally and internally seed borne. It has been found on the surface of seed collected from healthy cones and both internally and externally on living seeds from infected cones of *P. radiata* (Storer *et al.* 1998; Dwinell 1999a, 1999b). When borne internally it may remain dormant until seed germination (Storer *et al.* 1998). The sources of the outbreaks of pitch canker in both South Africa and in Chile are believed to have been contaminated seed (Coutinho *et al*. 1997; MJ Wingfield pers. com.). A risk analysis carried out in New Zealand has consistently evaluated contaminated seed as the likely source of an incursion of this pathogen (Dick and Bain 1996; Gadgil *et al*. 2002).

*Fusarium circinatum* causes a variety of conditions in the nursery. It can infect the stem causing lesions and/or dieback. The fungus can survive in soil and may infect seedlings at or just above the soil-line (Viljoen *et al*. 1994). It may also act as a typical root-infecting pathogen (Dwinell *et al*. 1985). *Fusarium circinatum* can also cause both pre- and post-emergence damping-off (Muramoto *et al*. 1993). The term "damping-off" refers to the generalised collapse of very young seedlings. Young seedlings do not have fully developed natural defence mechanisms and all tissues are susceptible to infection by a number of common, non-specific soil-inhabiting fungal parasites. Such fungi normally live saprophytically on dead plant material in the soil but can cause disease in wet weather when soils are moist; hence the name 'damping-off". There are two main types of damping-off:

- Pre- emergence damping-off: germinating seeds that are attacked and rot before they break through the surface
- Post-emergence damping-off: the collapse of seedlings soon after they have emerged due to attack on either the stem, the roots or both

Because of the generalised collapse of such small plants the causal agents cannot be separated on the basis of disease symptoms alone. The symptoms of pitch canker infection in such young seedlings would therefore be indistinguishable from those of many other diseases and disorders. This occurred in South Africa in 1990 where losses in a *P. patula* nursery (Viljoen *et al.*1994) were originally attributed to *F. oxysporum* rather than to pine pitch canker disease (MJ Wingfield pers. com.).

Agents of damping-off diseases in New Zealand conifer nurseries include species of *Fusarium, Cylindrocladium, Rhizoctonia, Phytophthora* and *Pythium.* As in South Africa the most common species of *Fusarium* found in plantation forest nurseries in New Zealand is *F. oxysporum* (Dick and Dobbie 2002).

Many of the damping-off fungi are capable of infecting many different hosts, from a diversity of plant groups, when the seedlings are small and vulnerable. Although the ability of *F. circinatum* to cause nursery disease of pine species has been reported (Viljoen *et al.* 1994; Dwinell *et al.* 1985) there is little information about any susceptibility, in the period immediately after germination, of non-pine hosts. If *F. circinatum* is not capable of causing damping-off of plants in genera other than *Pinus* there is a possibility that it could become established in a nursery (hitchhiking on seed of a non-host plant) and for the establishment to remain unrecognised for a period of time if there is no contact with susceptible hosts.

The objective of this trial was to determine whether *F. circinatum* is capable of behaving as a non-specialist agent of damping-off disease of genera other than *Pinus.* Plant species representative of genera likely to be found in many forest nurseries in both New Zealand and Australia were selected for testing. As many forest bare-root nurseries use a rotation regime, in which each year some of the beds will be in fallow and planted with pasture plants, two typical species were tested.

## MATERIALS AND METHODS

### *Plant material*

Seeds of *Pinus radiata, P. pinaster, Eucalyptus fastigata, E. nitens, Pseudotsuga menziesii* (Douglas fir) , *Cupressus macrocarpa*, *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) were given the appropriate soaking and stratifying regime recommended for each seed type (Table 1). Plant species tested were selected because they are representatives of genera likely to be found in forest nurseries in both New Zealand and Australia. Species of *Lolium* and *Trifolium* are often used in beds that are retired for a fallow period.

**Table 1:** Seed numbers and pre-sowing treatment

| Plant host | Stratification period (weeks) | No. seeds per replicate | Total seeds per treatment |
|---|---|---|---|
| *Pinus radiata* | 2 | 40[1] 24[2] | 120[1] 72[2] |
| *P. pinaster* | 2 | 24 | 72 |
| *Cupressus macrocarpa* | 4 | 120 | 360 |
| *Pseudotsuga menziesii* | 6 | 24 | 72 |
| *Eucalyptus globulus* | 6 | 24 | 72 |
| *E. regnans* | 6 | 24 | 72 |
| *Trifolium repens* | nil | 2 gm /rep | not counted |
| *Lolium perenne* | nil | 2 gm /rep | not counted |

[1] First *P. radiata* test
[2] Second *P. radiata* test

***Inoculum***
In addition to *F. circinatum* one of the common damping-off fungi found throughout New Zealand, *Fusarium oxysporum* was included. Another common fungus, *Epicoccum nigrum* which is sometimes considered to have positive benefits to plants (eg Pieckenstain *et al. 2001*), was included as a positive control. An isolate each of *F. circinatum, F. oxysporum*, and *Epicoccum nigrum* (Table 2) were separately grown on 3% malt extract agar (MEA) plates at 25ºC in the dark for 5 days. From these cultures a spore suspension of approximately 1000 spores/ml of each fungus in sterilised, deionised water was prepared and used to inoculate sterilised oat seeds. The inoculated oats were incubated for 10 days at 25ºC in the dark and then mixed evenly into a potting mix (Daltons Ltd Superseed Mix) at a rate of 50 g oats per kg of moist potting mix.

The inoculated potting mix was held at approximately 20ºC for 10 days prior to sowing seed to allow the fungus to grow through the medium. The potting mix was then divided, after further stirring, in 300 gm quantities into 150 x 95 mm seed trays. Sterilised, but un-inoculated, oats were used as controls.

**Table 2:** Fungi used in treatments

| Forest Research Culture Collection No. | Fungus | Host | Source |
|---|---|---|---|
| NZFS 308B | *Fusarium circinatum* | *Pinus radiata* | Santa Cruz, California |
| NZFS 817 | *Fusarium oxysporum* | *Pinus radiata* | FR Nursery, Rotorua |
| NZFS 879 | *Epicoccum nigrum* | *Podocarpus totara* | Auckland |

***Treatments***
The stratified seed was sown in the trays containing artificially infested standard potting mix, at rates dependent on expected germination percentages (Table 1). Four treatments were applied to each seed type. In order to ensure that there was no risk attached to testing *F. circinatum* all containment criteria set by ERMA New Zealand, as outlined in their decision to application no. NOC00003 (21 December 2000), and in the Ministry of Agriculture and Forestry approved quality manual for the operation of the Forest Research containment facility were adhered to. All trays were enclosed separately in sealed plastic bags (Fig 3). Each of the 32 trays (8 host species inoculated with 3 fungi plus an inoculated control) was replicated 3 times. The *P. radiata* and the *P. menziesii* treatments were repeated.

***Assessment:*** Emerging seedlings were counted 2 weeks after sowing and thereafter weekly for 5 weeks. Disease levels and mortality were recorded, and dead plants removed at each assessment.

***Re-isolation of inoculated fungi***
Root and stem segments of representative dead plants from each treatment were plated onto MEA to check for presence of the inoculated fungus.

## RESULTS

Enclosure of each tray of seedlings in a plastic bag (Fig 3) meant that the environment was extremely humid and very conducive to disease development. A test run of the system was carried out using *P. radiata* seed. Seed began to emerge after 7 days and five weeks after sowing some seedlings in all treatments, including the control, were beginning to show signs of disease and/or breakdown. In subsequent tests two additional trays were prepared for each treatment, with the exception of the *F. circinatum* treatments, and these were not enclosed in bags. These unenclosed replicates served as extra controls for comparison with disease developing within the enclosed treatments and enabled a judgement to be made for the most appropriate time for final assessments to be carried out.

There was no disease development in the uninoculated controls (eg Fig 6), or in the *Epicoccum* treatments in the unenclosed replicates of any of the treatments. Disease levels, where they occurred, in the unenclosed *F. oxysporum* treatments were lower than those in the trays contained within the bags and took several days longer to develop. Final counts for the enclosed treatments were made between 4 and 5 weeks after seed sowing.

Results for the 6 tree species are given in Figs 1a-1g. The two repeat trials of *P. radiata* are given separately in Figs 1a and 1b. For each host species the percent emergence of the seed sown is compared with percent mortality (post-emergence damping-off) of the emerged seeds.

***Pinus radiata*****:** Results (Figs 1a and 1b, Fig 4) for the different fungi ranked in the same order in each of the 2 test runs. Emergence in all *F. circinatum* replicates was low (less than 50%) and there was 100% mortality of all emerged seedlings by 35 days. Emergence in the control and *E. nigrum* treatments varied from 80 – 92%. Mortality in the *F. oxysporum* treatments differed markedly between the two runs, averaging 78% in the first and 11% in the second.

***Pinus pinaster*****:** Results were similar (Fig 1c) to those in the *P. radiata* treatments, ranking in the same order, though mortality in the pitch canker treatments occurred a little more slowly and a few of the *P. pinaster* seedlings survived. Mortality in the *F. oxysporum* treatments was significantly higher than in the control and *E. nigrum* treatments, but significantly lower than the *F. circinatum* treatment.

***Cupressus macrocarpa***: Germination percentage was, as expected, very low (hence the high numbers of seed sown). Seedling emergence was lower in both the *Fusarium* treatments (23% for *F. oxysporum* and 32% for *F. circinatum*) than in the control and *E. nigrum* treatments (both with 40%). Post-emergence mortality in the *F. oxysporum* treatment (70%) was markedly higher than in the *F. circinatum* treatment (25%), with both being greater than the control and the *E. nigrum* at 3.5% each (Fig 1d).

***Eucalyptus globulus*****:** This species thrived. Losses were minimal in all treatments (Fig 1e) and there were no differences between treatments.

***Eucalyptus regnans*****:** In contrast to the *E. globulus* deaths occurred in all of the *E. regnans* treatments (Fig 1f). Post-emergence mortality was however lower in the *F. circinatum* treatment (33%) than in the control (45%) though emergence in the *F. circinatum* treatment was only 58% in comparison to 90% for the control. Total mortality was slightly higher in the *F. circinatum* treatment.

***Pseudotsuga menziesii***: Emergence was very low (< 20%) in all replicates of all treatments including the controls. The experiment was therefore repeated but with very similar results. Results from the two test runs were combined (Fig 1g). Either the seedlot had a low viability, or the conditions were unsuitable for *P. menziesii*. The highest post-emergence mortality occurred in the *F. oxysporum* treatment (80%), and the mortality in the *F. circinatum* treatment (33%) was lower than that in the uninoculated control (42%). However the figures were so low overall that no meaningful comparisons could be made.

Total mortality of the six tree species, as a percentage of the seed sown, is shown in Fig 2. The susceptibility of the two pine species to *F. circinatum*, in comparison to the controls for each pine, is markedly different to the same comparison made for the other tree species.

***Lolium perenne*:** Individual plants were not counted. After 6 weeks no deaths had occurred in any of the treatments (Fig 8).

***Trifolium repens*:** Individual plants were not counted but there were approximately 150 per replicate. Considerable mortality occurred in the *F. oxysporum* treatment both in the enclosed and unenclosed replicates with the unenclosed deaths lagging behind. Although a few deaths occurred in all treatments within the bags there was no discernable difference between the *F. circinatum* treatment and either the control or the *E. nigrum* treatments (Fig 7).

***Reisolation of inoculated fungi:*** *Fusarium circinatum* and *F. oxysporum* were recovered from diseased plants from the respective treatments when isolations were made onto agar media. *Trichoderma* spp., common saprophytic fungi were also obtained from a few of these plants and these were the most frequent isolates obtained from seedlings from the control and *E. nigrum* treatments. Bacteria were also obtained from all diseased plants but these were considered to be incidental and were not identified to genus.

## DISCUSSION

The test conditions, with the very high sustained humidity and moderate temperatures were very conducive to disease development and were such that even plants in the un-inoculated control treatments could be expected to eventually breakdown. This had begun to occur for some of the species after 5 weeks. Comparisons between fungal treatments for each host were made before collapse of control plants happened.

The pine species proved to be highly vulnerable to *F. circinatum* which caused both pre- and post-emergence damping-off. None of the *P. radiata* seedlings survived the pitch canker treatments, and the *P. pinaster* seedlings were only slightly less susceptible. Although losses to *F. oxysporum* were recorded in the enclosed trays there was no mortality in the unenclosed replicates. The differences in levels of mortality between the two test runs with *P. radiata* are an indication of how crucial differences in environment are for the mildly pathogenic damping-off fungi. With the exception of the soil moisture levels (higher in the first round) conditions were the same for the two tests. Losses that would be sustained by *P. radiata* and *P. pinaster* to *F. circinatum* in more ‘normal’ circumstances, i.e. those encountered under regular nursery conditions, could therefore be assumed to be less drastic, or to occur over a longer time period than those recorded here. However there are a number of reports of very rapid disease development in some pine seedlings. In these reports, when stem inoculations with *F. circinatum* were made lesion development and shoot mortality of seedlings of susceptible pine species could be seen on many plants within two weeks (Dwinell 1978; Viljoen *et al.* 1995; Reglinski and Dick 2001).

Unfortunately germination of *P. menziesii* seed was so poor that conclusions cannot be drawn about the susceptibility of this species. This result was particularly disappointing as *P. menziesii* is the only non-*Pinus* species recorded as a host of pitch canker. The record of pitch canker in *P. menziesii* was from a group of young trees adjacent to heavily infected *P. radiata*. The disease has not been recorded elsewhere in *P. menziesii*, although there are areas where this species is growing in close proximity to badly diseased *P. radiata*. It has been suggested that the infected trees are a provenance from the Rocky Mountains and not the coastal Douglas fir (W. Libby, pers. comm.). Given the apparent lack of infection elsewhere in *P~~s~~. menziesii*, it could be postulated that coastal provenances are not susceptible to pitch canker. A better result from this experiment would have been useful in understanding the relationship between *F. circinatum* and *P. menziesii.*

With the exception of the ryegrass (*L. perenne*), which remained healthy in all treatments, the other hosts tested (*C. macrocarpa, E. globulus, E. regnans* and *T. repens*) were more susceptible to *F. oxysporum* than to *F. circinatum.* Both *E. regnans* and *T. repens* sustained high levels of mortality in the unenclosed *F. oxysporum* treatments in addition to those in the enclosed replicates, whereas *C. macrocarpa* was only lightly affected and *E. globulus* was unaffected in the unenclosed replicates.

Several species of *Trifolium* are subject to root rot and damping-off caused by a range of *Fusarium* species including *F. oxysporum.* Data from this trial support other reports (Choi *et al.* 1999; Pecetti *et al.* 2002; Sarathchandra *et al.* 2000) of *F. oxysporum* as a pathogen of *T. repens.* Although there are also records of *F. oxysporum* associated with roots of *L. perenne* (Cagas *et al.* 1998; Skipp and Christensen 1989; Waipara *et al.* 1997) a clear causal relationship with disease has not been demonstrated.

In bare root forest nurseries in New Zealand *F. oxysporum* is a very common soil inhabitant. It is a typical damping-off fungus, and is also frequently isolated from older plants with root rot. Much of the time it is present in the soil without being active as a pathogen. Infection will only occur when environmental conditions, particularly elevated soil moisture, are suitable for the damping-off fungi and when plant tissues are susceptible. This indicates that *F. circinatum* will be of no concern as a damping-off fungus to those host species that demonstrated a lower susceptibility than to *F. oxysporum*.

In the *F. circinatum* treatments with all of the conifer hosts the fungus grew rapidly over the surface of the soil (Fig 6). This has been reported to occur in containerised stock in nurseries in South Africa where outbreaks of pine pitch canker have occurred. The rampant growth of the fungus on these occasions has made these outbreaks extremely difficult to control (MJ Wingfield pers. com.). Surface mycelial growth also occurred in the *F. oxysporum* replicates in trays of susceptible species (Fig 4) but to a lesser extent than the *F. circinatum* replicates.

## CONCLUSIONS

The pine species were extremely susceptible to damping-off caused by *F. circinatum* and both pre- and post-emergence damping-off was recorded. Pre-emergence and post-emergence deaths of *C. macrocarpa* occurred in both the *Fusarium* treatments but were higher in the *F. oxysporum* treatments than the *F. circinatum* treatments. As damping-off associated with *F. oxysporum* is relatively uncommon in nurseries when conditions can be considered as 'normal' it is likely that *F. circinatum* would only be a problem to *C. macrocarpa* under extreme conditions that are unlikely to be encountered, especially in bare-root nurseries. The other plants tested were not susceptible to *F. circinatum* even under these highly challenging circumstances.

It is possible that if a wider range of species were to be tested some conifers other than pines may demonstrate a level of susceptibility to *F. circinatum.* The host-pathogen specificity that is apparent in older plants may, to some degree, break down. Based on these results, it seems likely that non-conifers will not be affected.

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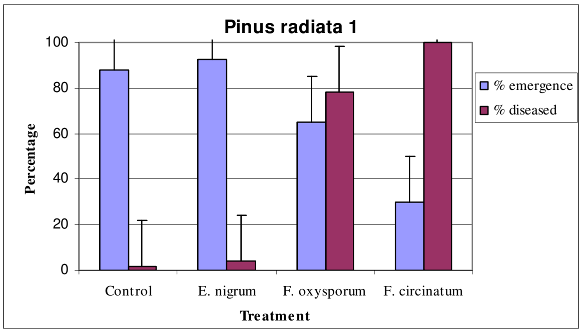


Fig 1a

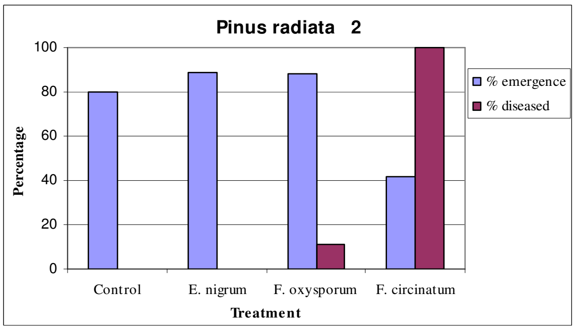


Fig 1b

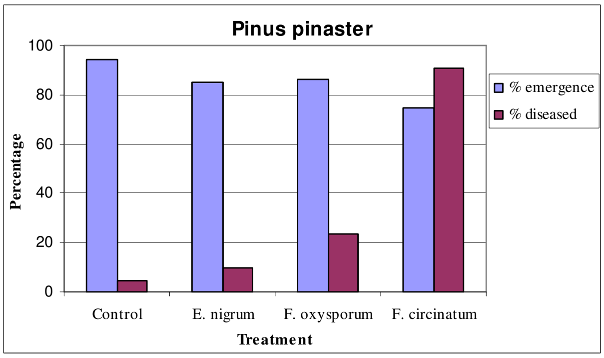


Fig 1c

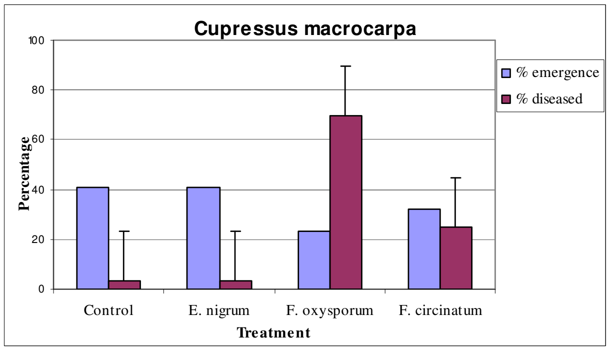


Fig 1d

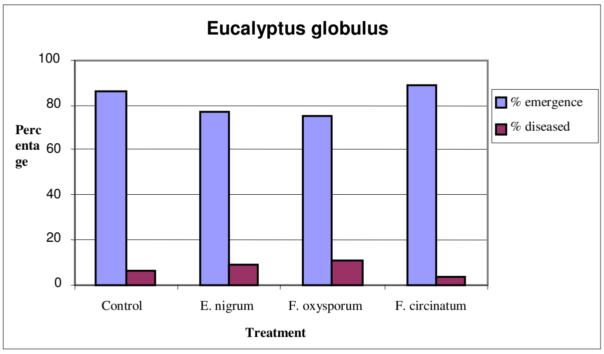


Fig 1e

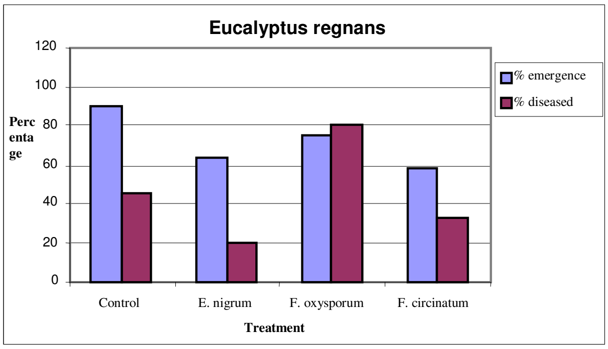


Fig 1f

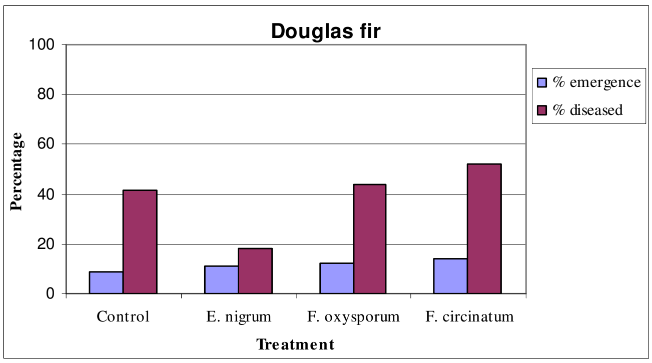


Fig 1g

**Fig 1**: Average percent emergence of seed sown and percent mortality of emerged seedlings in each of the four treatments for each of the six plantation species tested: 1a and 1b *P. radiata*; 1c *P. pinaster*; 1d *C. macrocarpa*; 1e *E. globulus*; 1f *E. regnans*; 1g *P. menziesii* (Douglas fir)

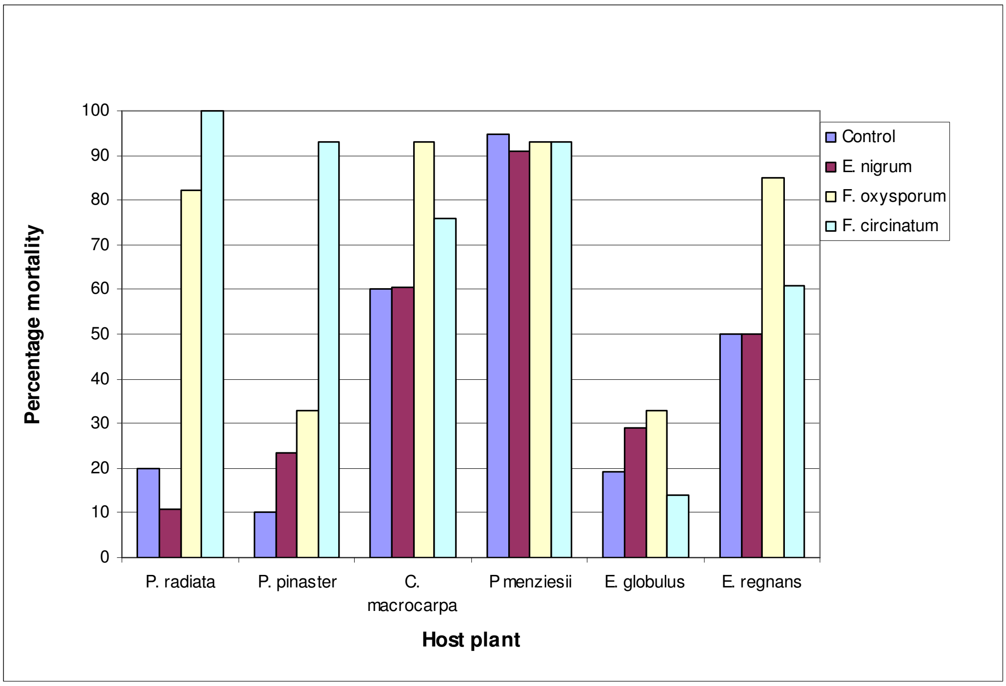


**Fig 2:** Total mortality, non-emergence plus death of emerged seedlings, at 30 days (as a percentage of seed sown)

**Fig 3:** Each replicate tray of seedlings was contained within a sealed plastic bag.


**Fig 4:** *Pinus radiata* treatments: From left; *E. nigrum, F. oxysporum*, uninoculated control, *F. circinatum*


**Fig 5:** Control treatments: *Pinus radiata* on left, the *C. macrocarpa* on right

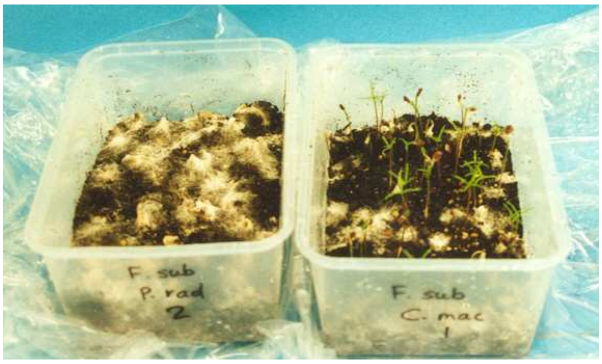


**Fig 6:** *Fusarium circinatum* mycelium grew readily on surface of the soil. *P.radiata* on left, *C. macrocarpa* on right

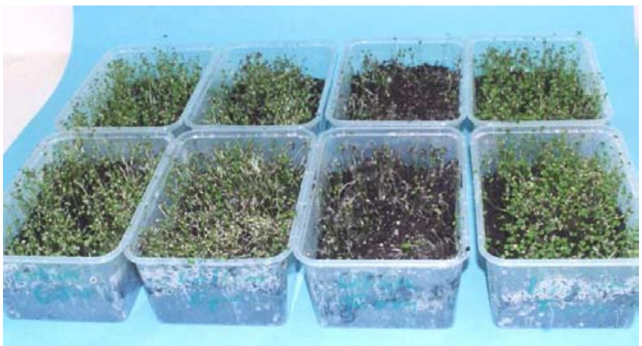

**Fig 7:** *Trifolium repens* treatments: From left uninoculated control, *E. nigrum, F. oxysporum*, *F. circinatum*

**Fig 8:** *Lolium perenne*: uninoculated control on left, *F. circinatum* on right