SUPPRESSION OF *DIPLODIA PINEA* SPORE GERMINATION AT THE SHOOT SURFACE OF *PINUS RADIATA*

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During studies on the infection of shoots of *Pinus radiata* D. Don by *Diplodia pinea* (Desm) Kickx, spore germination on the host surface was often much less than on glass slides. This reduced germination was at first thought to be caused by the possible presence of inhibitory substances in the tap water used in the misting system to which inoculated plants had been exposed. However, the tap water was found to be no different from distilled water as a medium for *D. pinea* spore germination.

A more critical examination was made to compare spore germination on glass slides and on the host surface. Young shoots of 6-month-old and 1½-year-old potted *P. radiata* seedlings were inoculated with *D. pinea* in 2 ways: (a) needles were brushed or sprayed with a spore suspension (conc. = 50 000/ml); (b) droplets of spore suspension (12 000–15 000/ml) were placed on the stem surface of potted plants laid horizontally so that the droplets stayed in place. All inoculated plants were kept in a misting chamber at 25°C. Droplets of spore suspension were also placed on glass slides in petri dishes lined with moist filter paper. These dishes were put in the incubator at 25°C as well as in the misting chamber. The incubation period was 6 h on slides and 6–24 h on the host surface; spore germination was halted by adding a drop of formalin. Epidermal strippings were prepared from the inoculated stems, stained in acid fuchsin, and examined under a microscope. Needles were examined directly under a reflecting microscope at ×300. Table 1 summarises the results. On the needle surface,

TABLE 1—**D. pinea** spore germination on **P. radiata** green stem and needle and on slides at 25°C (incubation period 6 h on slides, 6–24 h on host surface)

Treatment	% germination*	Number of spores counted
Needle surface**	38.4	1763
Stem surface***	23	2035
Slides	70.3	2000

* Average of 3 experiments
** From 16 seedlings
*** From 11 seedlings

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spore germination was reduced by almost 50% and on stem surfaces by 70% compared with that on slides. Germ tubes were 492 μm long (average of 110 germinating spores) on slides and 213 μm long on stem surfaces (average of 41 germinating spores). Spore germination varied between 67 and 88% in droplets on slides, between 4 and 68% on needles between individual plants, and between 0 and 57% for individual epidermal strippings.

In one of the experiments, spore germination on a few detached needles was incidentally included. Germination was 74%, apparently showing no sign of the suppressive effect by the host. Further experiments were carried out to determine whether spore germination can be adversely affected on the detached stems. Stem segments 3–4 cm long were cut from the shoot tip of 6-month-old to 1-year-old seedlings and placed in petri dishes lined with moist filter paper. Droplets of spore suspension (50 000–100 000/ml) in 0.5% gelatin were placed on the surface of these stems as well as on potted whole plants laid horizontally in a misting chamber at 25°C. Germination time was 6 h on slides and 6–24 h on host surfaces. Examination methods were as previously described; in addition, SEM was used in some cases. The results of 4 repeat experiments are summarised in Table 2. The marked difference between spore germination on potted whole plants and on slides or detached stems was again demonstrated. In experiments 1–3, germination was only 9–21% on whole plants in contrast to 61–86% and 72–84% on detached stems and slides respectively. In experiment 4 germination on whole plants was reasonably high (62%); however, it is still lower than that on slides (92%) and on detached stems (80%), the difference being highly significant by chi-square analysis ($\chi^2 = 63.5$).

Finally, an experiment by the drop-diffusate technique (Cruickshank 1966) was designed with a view to determining whether phytoalexin (Cruickshank 1963) production played any role in this suppression phenomenon. Droplets of distilled water with or without spores (spore conc. = 100 000/ml) were placed on the green stem surfaces of potted 1-year-old *P. radiata* seedlings laid horizontally in a misting chamber at 25°C. After 16 h the droplets (drop-diffusate) were collected with a Pasteur pipette, centrifuged, and the supernatants, some sterilised by boiling, some milipore filtered, and some untreated, together with distilled water, were used for slide germination tests as follows: 25 μl droplets of these liquids were pipetted on to slides and to each of them was added a 25 μl drop of spore suspension (30 000/ml), giving a final concentration of 15 000 spores per ml. The slides were kept in petri dishes lined with moist filter paper and placed in an incubator at 25°C. At the same time spore germination tests were carried out on segments of detached stem, on stem epidermal strippings glued to a slide in a petri dish, and on potted whole plants as previously described. The results obtained are summarised in Table 3. The drop-diffusates, whether pathogen-challenged or not, showed virtually no inhibitory activity against *D. pinea* spore germination. There were small differences between germination in distilled water and in some of the drop-diffusates, but the differences are not significant at $P = 0.05$ by chi-square analysis. Once again it was demonstrated in this experiment that there was marked reduction of spore germination on potted whole plants but not on detached plant parts.

The cause of germination suppression on the host surface remains to be elucidated. It seems unlikely that suppression is caused by peculiarities in the physical nature of the

TABLE 2—**D. pinea** spore germination on detached stem and potted whole plants of **P. radiata***

	Expt. 1		Expt. 2		Expt. 3		Expt. 4	
	% germination	No. spores examined	% germination	No. spores examined	% germination	No. spores examined	% germination	No. spores examined
Detached stems	61	2400	86	400	80	403	80	1498
Potted whole plants	9	2500	21	400	17	100	62	493
Slides	72	400	84	400	83	400	92	400

* All experiments were carried out during March and April 1973–74.
The numbers of plants used for each treatment were 10 and 14, 12, 12, and 15 in experiments 1, 2, 3, and 4 respectively.

** Chi-square value for detached **v.** potted whole plants = 63.5.

TABLE 3—**D. pinea** spore germination on host (**P. radiata**) and in drop-diffusates** at 25°C

Treatments	% germination*	% suppression***
Germination on host surface		
Potted whole plants (stem)	17.4	79
Stem epidermal strippings	66.4	20
Detached stem	88.8	—7
Germination on slides using:		
Drop-diffusate (boiled)	76.4	8
Drop-diffusate (not boiled)	77	7
Drop-diffusate (milipore filtered)	70	16
Drop-diffusate (Diplodia-challenged and boiled)	71	15
Drop-diffusate (Diplodia-challenged and not boiled)	76	9
Distilled water (control)	83	0

* Germination using spore concentration 15 000/ml, 6 h, 25°C; 200 spores counted for each treatment.

** Using either distilled water or spore suspension of 100 000/ml; droplets collected after 16 h of incubation at 25°C.

*** $\%\ \text{suppression} = \left(1 - \frac{\%\ \text{germination of treated}}{\%\ \text{germination of control (dist. } H_2O)}\right) \times 100$

host surface, or by preformed toxic substances in the cuticular layer (Martin *et al.* 1957), as then germination should also be suppressed on the detached plant parts. For the same reason it is dubious that epiphytic microflora played a major role. Since the suppressive effect appears to be dependent on the maintenance of whole plant integrity, active host metabolism is likely to play an important part in the process. The evidence for lack of phytoalexin activity is based on a single experiment, hence it cannot be considered as conclusive. Moreover, the drop-diffusate technique has its limitations. Without a major modification the technique is unable to detect volatile or labile host products involved in the suppressive effect. In view of the fungitoxicity of monoterpenes (Chou & Zabkiewicz 1976) this possibility deserves attention. Also, failure to detect inhibitory activity in the drop-diffusates does not necesarily mean the absence of such compound(s), because such compounds, if any, may be totally taken up by the spores and thus their activity will be largely tied up.

The significance of the observed phenomenon in host defense against *D. pinea* infection is not entirely clear and can only be speculated. Young shoots of *P. radiata* can be highly susceptible under apparently rather special natural conditions (Chou 1976a). Artificial inoculation studies showed that host susceptibility could be affected by pre-inoculation conditions (Chou, unpubl. data). Infection is affected by spore concentration (Chou 1976b), and only a small percentage of the germinating spores form infection structures (Chou 1978). These observations suggest that host surface suppression of spore germination, although not totally effective in preventing *D. pinea* infection, may nevertheless play a role in limiting the damage that this pathogen can cause under average conditions.

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REFERENCES

CHOU, C. K. S. 1976a: A shoot dieback in **Pinus radiata** caused by **Diplodia pinea** I. Symptoms, disease development, and isolation of pathogen. **New Zealand Journal of Forestry Science 6:** 72–9.

——— 1976b: A shoot dieback in **Pinus radiata** caused by **Diplodia pinea** II. Inoculation studies. **New Zealand Journal of Forestry Science 6:** 409–19.

——— 1978: Penetration of young stems of **Pinus radiata** by **Diplodia pinea**. **Physiological Plant Pathology 13:** 189–92.

CHOU, C. K. S.; ZABKIEWICZ, J. A. 1976: Toxicity of monoterpenes from **Pinus radiata** cortical oleoresin to **Diplodia pinea** spores. **European Journal of Forest Pathology 6:** 354–9.

CRUICKSHANK, I. A. M. 1963: Phytoalexins. **Annual Review of Phytopathology 1:** 351–74.

——— 1966: Defense mechanisms in plants. **World Review of Pest Control 5:** 161–76.

MARTIN, J. T.; BATT, R. F.; BURCHILL, R. T. 1957: Defense mechanisms of plants against fungi. **Nature (Lond.)180:** 796–7.