The results of this invesitgation indicate that fungi present in the seed of the two cultivars tested were not the cause of the poor survival, severe root rot, and plant weight reductions observed in field plantings. Ethanol and sodium hypochlorite treatments give control of most of the fungi which contaminate subterranean clover seed (M.J. Barbetti, unpublished data). However control of these fungi produced no beneficial effects in field sowings. Isolations from rot-affected roots confirmed that the seed treatments were in fact being tested against a typical range of potential pathogens. Metalaxyl and thiram were the only fungicides that showed any potential for development as possible commercial treatments for increasing seedling survival in susceptible cultivars being resown into areas affected by root rot. Metalaxyl was the most promising of the fungicides tested. This result is not unexpected as metalaxyl activity against Pythiaceous fungi is well recognised (3, 6, 9) and *Pythium irregulare* is important in root rot of subterranean clover in Western Australia (2).

Acknowledgement

I wish to thank Mr. N.R. Peterson for technical assistance with this work, and the Commonwealth Mycological Institute for assistance with identification of some fungal isolates.

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Use of a Paraquat-Diquat Herbicide for the Detection of *Phomopsis leptostromiformis* Infection in Lupins

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Studies on Phomopsis stem blight *(Phomopsis leptostromiformis* [Kuhn] Bubak ex Lind) of the narrow-leafed lupin *(Lupinus angustifolius* L.) have been hampered by the lack of a suitable test to detect infection. Normally, disease symptoms appear only at senescence. In the glasshouse, *L. angustifolius* plants remain symptomless when inoculated with spore suspensions of *P. leptostromiformis* (2). Cerkauskas and Sinclair (1), working on Phomopis pod and stem blight of soybeans, reported the use of paraquat to detect infection by *Phomopsis* spp. We have adapted the technique to lupins using a paraquat-diquat herbicide. Use of the technique will assist in studies of Phomopsis stem blight and the toxigenic disease (lupinosis) it causes in sheep grazing lupin stubble (3).

In glasshouse experiments, 27-day-old plants of *L. angustifolius* (cultivars Illyarrie, Chittick and Marri) were inoculated by misting with a pycnidiospore suspension of *P. leptostromiformis* (10^6 spores/ml in 0.5% gelatin) and were subjected to high humidity in small plastic tents for 48 hr. at 20 C day/15 C night. A control group of plants was sprayed with 0.5% gelatin only.

No disease symptoms appeared on plants, and growth of inoculated and control plants was similar. Twenty-one days following inoculation, 2-cm stem segments were either surface sterilised in 0.4% sodium hypochlorite or rinsed in distilled water for 1 min. Half the segments in each group were then dipped in 2% v/v Spray.Seed® (a mixture of 12.5% paraquat and 7.5 diquat) and the other half (controls) in distilled water for 1 min. After 12 days incubation on moist filter paper, stromata of *P. leptostromiformis* were visible in 20/30 Spray. Seed®-treated segments. This included 12/20 segments that were surface-sterilised and 8/10 that were not. The corresponding values for control segments were 0/20 and 0/10, respectively. Surface sterilisation did not affect the results, indicating that the origin of *P. leptostromiformis* in the segments exhibiting stromata was fungal hyphae within the stem. The three cultivars reacted similarly to the treatments.

The use of paraquat-diquat to detect infection of intact plants was investigated by inoculating 36-day-old Yandee lupins in a mist chamber, and spraying with 1% v/v Spray.Seed® at 10, 20, 30 and 40 days after inoculation. Stromata of *P. leptostromiformis* became visible on stems and petioles 7-10 days after herbicide treatment. The most extensive stromatal development occurred with herbicide application 20 or 30 days following inoculation.

The method was also tested in the field during winter 1983 with the cultivar Yandee. Infected lupin stubble was spread over alternating field plots leaving a stubble-free plot between each treated plot. Plants were selected at random in each plot and sprayed with 1% v/v Spray.Seed®. Fifteen days after herbicide treatment on June 21, every plant in the stubble-treated plots (34/34) displayed signs of *P. leptostromiformis,* compared with only 4/30 plants in the stubble-free areas.

The paraquat-diquat test overcomes the problems caused by the lack of symptoms of *P. leptostromiformis* infection in green lupin plants (2), and the low frequency of isolation of the fungus from symptomless infected green stems. It will be exploited in studies of the epidemiology of Phomopsis stem blight in Western Australia, and in the development of glasshouse screening tests for disease resistance. Application of the herbicide after a suitable incubation period should facilitate rapid progress in breeding lupin varieties resistant to the disease.

The authors thank Mr F. O'Donnell for valuable glasshouse and field assistance.

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Apple Fruit Rot Caused by *Trichoderma harzianum*

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Pome fruits are affected by many fungal rots during cold storage. The principal rots encountered in New South Wales are caused by *Penicillium expansum* Link ex Gray and *P. verrucosum* Dierckx (4). Many such rots arise because fruit are dipped in a solution of diphenylamine (DPA) to prevent physiological scald disfiguring the fruit. The solution used for dipping, quickly becomes fouled with rotted fruit, plant material, and soil from the bins into which the fruit is harvested and dipped. Dipping therefore provides an ideal environment for any fungi present to infect fruit through wounds.

During May 1983, a grower in the Bathurst district reported significant rotting of Granny Smith apple fruit (*Malus pumila*(L.) Mill.) which had been placed in cool store for about one month following dipping in DPA. Symptoms were of a firm, tan-brown rot, from 5 mm diameter to one encompassing the whole of the fruit. Infected fruit emitted a characteristic musty "coconut" odour. All rots appeared to be centred on a wound, the majority of which appeared to be due to the dimpling bug (*Campylomma livida* Reuter) stings which had occurred in the orchard.

After surface sterilization by flaming with alcohol, isolations were made from the margins of rot lesions onto acidified potato dextrose agar. Cultures were incubated at 25°C in the dark, and the fungal isolates identified.

The rotted areas yielded a number of fungi, of which by far the most common was *Trichoderma harzianum* Rifai aggr. Other fungi isolated included *P. expansum, P. verrucosum, Alternaria alternata* (Fr.) Keissler and *Rhizopus* sp.

Apple fruit (cv. Granny Smith) and pear fruit (*Pyrus communis*) (cv. Packham's Triumph) were surface sterilized by immersion for 1 min. in sodium hypochlorite (1 percent available chlorine). Mycelium and spores from cultures of *T. harzianum* were inoculated into wounds in the fruit made with a sterile scalpel. Rots, similar to those described above developed, and the fungus re-isolated from the margins of rotted areas after surface sterilization.

Trichoderma sp. has been reported in the United States of America as causing a pome fruit rot (3, 5) and Conway, (2) isolated *T. harzianum* from dump tanks used in flotation grading of apples in Pennsylvania. He showed the fungus was capable of causing a fruit rot, however, naturally infected fruit were not found.

This paper is the first report of *Trichoderma* sp. causing an apple rot in Australia, and the first report of *T. harzianum* causing a pome fruit rot anywhere in the world.