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DISEASE NOTES OR NEW RECORDS

First report of spring black stem and leaf spot in fenugreek (*Trigonella foenum-graecum*) caused by *Phoma pinodella* in Australia

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Abstract. A high incidence of black stem and leaf spot in a fenugreek (*Trigonella foenum-graecum*) crop was observed at Rupanyup in the Wimmera region of Victoria in 2004. *Phoma pinodella* was consistently isolated from diseased plants and shown to be the causal agent by Koch's postulates. This is the first record of *P. pinodella* in fenugreek in Australia.

Fenugreek (*Trigonella foenum-graecum*) is a minor legume field crop in Victoria, grown to produce seed for the spice market and also as a green manure crop. There appears to be considerable potential for expansion of the industry, especially following the recent selection of better genotypes with increased grain yield and higher biomass production (McCormick 2004). In September 2004, an unidentified disease occurred in isolated patches within a crop of fenugreek (accession 150118), which was undergoing seed multiplication at Rupanyup in the Wimmera region of Victoria. The disease seriously retarded plant growth and was estimated to have affected approximately 10% of the plants within the crop. Most infected plants were stunted with mild chlorosis and had elongated black lesions on the taproot. When the disease was severe, the lesions completely girdled and sometimes severed the taproot. On the leaves, petioles and stems, there were numerous small (3–4 mm long), irregularly shaped dark brown to black leaf lesions, sometimes with a small chlorotic area surrounding the lesion. Where the disease was severe, many leaves turned completely yellow and withered before falling to the ground.

To determine the causal organism, 40 plants (20 diseased and 20 apparently healthy) were removed from the affected crop on 30 September 2004 (approximately 12 weeks after emergence), when they were 15–20 cm tall and had developed four to five leaves. Individual plants were removed by digging to a depth of 20 cm, then washed to remove soil from the roots. To isolate directly from fenugreek plants, infected segments (approximately 1 cm long) of the stem and taproot were removed from each plant, split longitudinally, then surface sterilised (1 min in 0.5% NaOCl) and plated onto V8 agar. The same isolation procedure was used for infected leaf segments (approximately 1 cm^2 area). Apparently healthy segments were also taken from plants with no disease symptoms. Plates were incubated at room temperature ($20 \pm 2.5^{\circ}C$) for seven to ten days under continuous illumination provided by a Phillips TLD 36W/33 'white light'. Fungi growing from stem and leaf pieces were transferred to fresh plates of V8 agar and grown at room temperature under continuous illumination (as above), then identified.

The fungus most frequently isolated from diseased plants was *Phoma pinodella* (syn. *P. medicaginis* var. *pinodella*). Morphological characters agreed well with those given by Boerema *et al*. (2004): 'Pycnidia more or less globose, glabrous, 150–200 µm diam., with a single ostiole. Conidia hyaline, ovoid to ellipsoid, usually aseptate (approx. 6×2.5 µm), occasionally 1-septate (approx. 9×3 µm). Chlamydospores brown, globose to ellipsoidal, single or in short (1–4) chains, intercalary or terminal'. Dendritic crystals were formed on malt extract agar after seven days in the dark at 21°C. Three isolates have been lodged in the VPRI (Victorian Department of Primary Industries) herbarium under accessions 32171, 32172 and 32176. The rDNA ITS regions of two of these isolates were sequenced, along with a *P. pinodella* isolate from *Pisum sativum* (VPRI 32177). All three sequences were identical, and have been lodged in GenBank under accessions DQ087400–DQ087402. Blast searching of GenBank revealed the sequences to be most

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similar to *P. pinodella* sequences AY831562 and AY831556, differing by only a single base. There was a low incidence of *Fusarium* and *Pythium* species in diseased roots, and *Alternaria* and *Stemphylium* species were isolated from some diseased leaves and stems.

To determine the pathogenicity of the isolated fungi, fenugreek seedlings were inoculated with four isolates (including the three isolates deposited in VPRI) of *P. pinodella* under glasshouse conditions. All treatments were replicated four times. For foliar inoculation, each isolate was subcultured onto Coon's agar plates and incubated for 14 days in continuous light at room temperature. The cultures where then flooded with sterile distilled water for 30 min and gently rubbed with a sterile glass rod to dislodge spores. The resulting spore suspension was filtered through one layer of muslin cloth and the spore concentration adjusted to 10^5 conidia/mL. Plants were grown in a composted pine bark potting mix, in 10-cm-diameter plastic pots and inoculated 4 weeks after sowing when they were at the three to four leaf stage. Each pot contained four seedlings. The inoculum was atomised onto the plants with a 'Debliss' aerograph (5 ml/pot). Control plants were sprayed with sterile distilled water. Inoculated plants were transferred to a dew chamber for 48 h after which they were returned to a temperature-controlled glasshouse (15–25°C). Ten days after inoculation the disease severity on leaves and stems was recorded. For each isolate tested, foliar disease symptoms developed on all the inoculated plants but not on the non-inoculated control plants. The symptoms of disease in the artificially inoculated plants were the same as those observed in the fenugreek crop.

In a separate experiment, soil borne inoculum was prepared to determine whether the same isolates used for foliar inoculations were also capable of causing root rot in fenugreek. Inoculum was prepared by growing the isolates on autoclaved barley grain. To a 500 mL screw-top bottle, 100 g of whole barley and 300 mL of water was added, and allowed to soak for 24 h. Excess water was then drained off and the steeped barley autoclaved for 20 min, then allowed to stand overnight and autoclaved for another 20 min. The next day, two agar plugs (1 cm^2), colonised by *P. pinodella*, were added to each flask then incubated for 21 days, and shaken as often as necessary to prevent clumping of inoculum. The colonised barley grain was removed from the flasks and thoroughly mixed with field soil (sandy loam previously treated with aerated steam for 45 min at 80°C) at a ratio of 50 g inoculum/kg soil (5% w/w). Amended soil (500 g) was added to each 10-cm plastic pot and four fenugreek seeds were sown at a depth of 2 cm in each pot. Pots were placed in a glasshouse with a temperature range of 15–25°C and watered to field capacity daily. After 28 days, plants were removed from the pots, washed to remove soil and examined for symptoms of root rot.

All isolates tested caused root rot in fenugreek seedlings. The symptoms observed were similar to those found on diseased plants in the field. *P. pinodella* was readily re-isolated from infected tissues, therefore confirming Koch's postulates. This is the first published record of spring black stem and leaf spot in fenugreek caused by *P. pinodella* in Australia. *Phoma* spp. have been isolated from the seed of fenugreek from Egypt, India, Nepal, Pakistan, Sri Lanka, Sudan and Syria (Hashmi 1988), but there are no reports in the published literature of them causing serious damage to fenugreek crops.

Outside Australia, *Cercospora traversiana* (causal agent of leaf spot), *Fusarium* spp. and *Rhizoctonia solani* (causal agents of damping off and root rot), *Erysiphe* spp. (causal agent of powdery mildew) and *Pseudomonas syringae* (causal agent of bacterial blight) appear to be the most important pathogens of fenugreek. There is little information about diseases of fenugreek in Australia, other than bacterial blight caused by *P. syringae* pv. *syringae* (McCormick and Hollaway 1999) and leaf spot caused by *C. traversiana*, which was isolated from harvested seed in Queensland in 1986 (Ryley 1989).

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