CSIRO PUBLISHING

Australasian Plant Disease Notes, 2008, **3**, 8–9 www.publish.csiro.au/journals/apdn

Aphanomyces root rot on faba bean in northern NSW

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Abstract. *Aphanomyces euteiches* was found to be the causal agent of faba bean root rot in northern NSW, both in experimental and commercial fields. Resistance to Aphanomyces root rot appears to be common in the faba bean germplasm pool, but the susceptibility of some varieties justifies further research on the pathogen. This is the first report of *A. euteiches* as a faba bean pathogen in Australia.

Faba bean (*Vicia faba* L.) plants with root rot were found in 2000 at the Tamworth Agricultural Institute (TAI) in a paddock that had been under a close (alternating years) faba bean rotation since 1995. Symptomatic plants appeared to be clustered in waterlogged areas. The most affected plants within these clusters were severely stunted and dying, with complete rotting of primary and secondary roots. Plants pulled from the soil often retained only the centre stele of the top part of the root. Plants with less severe root rot were found outside these clusters and showed root discolouration and an absence of nodulation. Aboveground symptoms of less affected plants (yellowing, premature leaf abscission and earlier maturity) could be easily mistaken with those caused by nutrient deficiency, drought stress or virus infection. The large differences observed in root rot severity among faba bean genotypes growing next to each other in severely affected areas indicated large differences in resistance; some faba bean genotypes remained completely free of symptoms, both above and below ground (including nodulation). Similar symptoms were present during January 2001 in the summer faba bean increase plots at Hanging Rock (50 km south-east of Tamworth), a site used for faba bean trials since 1998. Also in this location most disease was found in low patches that had been flooded during heavy rain earlier in the season.

A bioassay was developed to screen soils for the presence of this root rot of unknown aetiology. Four faba bean genotypes were selected, based on differences observed in the field: Ac0805 (a germplasm accession originating from Yemen, highly root rot susceptible), Icarus (a variety selected from an Ecuadorian germplasm accession, susceptible), Fiord (a variety of Greek origin; moderately susceptible) and Ac1473 (a germplasm accession originating from Ethiopia, resistant). Plastic trays ($510 \times 350 \times 150$ mm) with perforated bases were filled with 25 L of sieved soil sampled from severely affected patches. To avoid cross-contamination during watering, individual trays were placed in a larger bottom tray ($670 \times 420 \times 75$ mm). Trays were sown with rows of the four test genotypes (20 seeds/genotype), completely saturated with water for 1 day and then drained. Ten days later (just after plant emergence) the soil was flooded for 1 week by filling the bottom trays completely with water. The trays were drained and for the remainder of the experiment watered without excess. Bioassays were carried out in a greenhouse with a night/day temperature of 18/25°C. Susceptible genotypes developed dark brown lesions on the stem bases within 4 weeks of sowing and these symptoms were quickly followed by root rot.

In March 2001, soils from three farmer fields in the Moree area, planted with faba bean in the preceding season, were bioassayed for root rot. Three composite soil samples (25 L of soil at 0–30 cm depth) were taken from points within a radius of 25 m from both low and high areas in each field. Trial paddocks of the northern faba bean breeding program at the Australian Cotton Research Institute (ACRI) at Narrabri were sampled in the same way as the commercial paddocks. The trial paddocks had been used for faba bean trials in 1996, 1998 and 2000 and sown to wheat in the alternating years. The commercial paddocks were rainfed, while flood irrigation was used at ACRI. Composite samples were tested in separate trays for the bioassay. Soil samples that failed to incite root rot were resown with the same four genotypes. Control trays containing sterilised soil were included in the bioassay to check for cross-contamination between soil samples and to rule out contaminated seed as a source for the disease. Control trays did not show any root rot even after being sown four successive times.

The bioassay indicated the presence of root rot inoculum in the soils of two of the three farmer fields. The first field had been cultivated with faba bean both in 1999 and 2000 and two of three samples reacted positive. The second field had been sown with faba bean in 2000 and with mungbean during the 2000–01 summer. In this field, root rot inoculum was detected in one sample, which was taken from a low and poorly drained area. The ACRI trial paddocks appeared to have a higher level of root rot inoculum than the commercial fields, with the three susceptible indicator lines in each sample showing root rot during the first test. In 2002, a very high root rot incidence was observed on emerging faba beans in parts of the sampled ACRI paddocks.

 10.1071/DN08004 1833-928X/08/010008

First attempts in 1999 and 2000 to isolate pathogens from affected roots resulted in a wide range of fungi, including *Fusarium* spp. (especially *F. solani*), *Rhizoctonia* and *Pythium* spp. Pathogenicity tests of these fungi failed to reproduce the differences between faba bean lines found in naturally infested soil. None of these fungi was therefore considered to be the causal agent of the observed root rot. After development of the bioassay, isolations were made from young lesions on test plants rather than from field samples. One-centimetre-long stem pieces were cut at the lesion edges, surface sterilised in 0.5% hypochlorite plus 20% ethanol for 1 min, followed by three rinses in sterile water and placed on one-third strength Potato Dextrose Agar (Difco) amended with 100 mg/L streptomycin sulfate. *Aphanomyces euteiches* Drechs was isolated from both Icarus and Ac0805 seedlings. A semi-selective medium was developed, based on the differential tolerance of *Aphanomyces* compared with other oomycetes to acylalanine fungicides (Pfender *et al*. 1984): half-strength Cornmeal Agar (CMA) amended with 150 mg/L streptomycin, 25 mg/L furalaxyl, 5 mg/L benomyl and 5 mg/L tolclofos-methyl. This semi-selective medium facilitated further isolations from seedlings grown in affected soil or in a mixture of chopped-up affected plants and sterile soil, but direct isolations from field samples remained problematic.

Purified cultures were examined to confirm their identity as *A. euteiches* as described by Stamps (1978). Oospores produced on CMA had uniformly thick walls and their diameters were in the range 18–25 µm. Sporangia were produced in sterile water cultures containing squares of grass leaf blades incubated at 25°C under 12 : 12 h dark : ultraviolet light. These formed elongated discharge tubes that released zoospores from their tips forming irregular clumps of spherical cysts, 9–10 µm in diameter. In addition, characteristic branching of hyphae at almost right angles (Papavizas and Ayres 1974) was observed in the mycelium cultures. The *A. euteiches* cultures were tested for pathogenicity by placing mycelial discs against the stem of 1-week-old seedlings grown in sterile soil. Despite the severity of this test, the four indicator lines reacted similarly to field and bioassay reactions, with Ac1473 not showing any symptoms. *A. euteiches* was readily reisolated from the susceptible lines, thereby confirming Koch's postulates.

In Australia, *A. euteiches* has been reported on peas in Tasmania (Geach 1936) and Queensland (Persley *et al*. 1989), on subterranean clover in Victoria (Greenhalgh *et al*. 1985) and on *Phaseolus* beans in NSW (Allen *et al*. 1987). Within Australia's northern grain region *A. euteiches* may be the cause for poor establishment of Phytophthora root rot-resistant lucerne cultivars (Abbo and Irwin 1990). Internationally, *A. euteiches* is a well described and major problem on field pea in most pea growing regions, but is also increasingly found to be of economic significance in other legume crops (Grau 2003). However, Aphanomyces root rot on faba bean under natural field conditions has only been reported as a problem in a Canadian research station (Lamari and Bernier 1985). Our report appears to be the first describing natural infection of faba bean by *A. euteiches* in Australia. So far we have not tested *A. euteiches* ex. faba bean for pathogenicity on other legumes nor expanded our surveys beyond northern NSW. However, it is surprising that Aphanomyces root rot has, to date, not been reported on field pea in major pea growing regions of south-east Australia. It is possible that this pathogen has been overlooked as many authors remark on the difficulty of isolating *A. euteiches*.

Our preliminary results indicate that *A. euteiches* is present in experimental and commercial faba bean crops in northern NSW and is not restricted to fields with a history of frequent faba bean cultivation. As faba bean cultivation in the region is relatively young, other hosts (like pasture legumes) are likely to have played a role in the establishment of the pathogen. Faba bean cultivation in the northern grain region is expanding, especially in heavy soils and high rainfall areas that are less suitable for other legume species. This is the type of environment where pathogens like *A. euteiches* can thrive. Although our preliminary screening results of faba bean germplasm indicated that resistance rather than susceptibility is common, a high level of susceptibility was found in some varieties (e.g. Icarus) used in the breeding program. A screening of parental material to avoid susceptibility in newly developed varieties is therefore warranted.

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Manuscript received 26 November 2007, accepted 14 January 2008