

Neocosmospora vasinfecta is pathogenic on peanut in Queensland

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Abstract. In March 2005, a high incidence of root rot and premature death was observed in a commercial peanut crop growing at Ban Ban Springs, Queensland. *Neocosmospora vasinfecta* is reported as pathogenic on peanut for the first time in Australia. Koch's postulates were fulfilled.

Most of the Australian peanut (*Arachis hypogaea*) crop is grown in Queensland and individual crops are sometimes affected by several root diseases such as cylindrocladium black rot (CBR) (causal agent *Cylindrocladium crotalariae*), diplodia blight/collar rot (causal agent *Lasiodiplodia theobromae*) and root rot (causal agent *Fusarium solani*). Another fungus, *Neocosmospora vasinfecta*, has been reported as the causal agent of foot rot of peanut (syn. groundnut) in South Africa (Baard and van Wyk 1985) and Taiwan (Huang *et al.* 1992). In Australia, *N. vasinfecta* has been isolated from peanut in New South Wales (DAR 76163) and in Queensland (BRIP 23244) but Koch's postulates were not fulfilled for either isolate (Australian Plant Pest Database, see <http://www.planthealthaustralia.com.au/APPD/queryForm.asp>, verified 17 January 2007).

In March 2005, irrigated plants of the peanut cultivars NC7 and Streeton were reported to be suffering severe root rot and premature death in an irrigated crop near Ban Ban Springs (25°40'54"S, 151°48'52"E) in the Burnett region of southern Queensland. Approximately 40% of plants within an area of 5 ha were affected, with plants of cv. NC7 exhibiting higher incidence and severity than plants of cv. Streeton. The symptoms of yellowing and wilt of aboveground parts, and root rot, were similar to those of CBR. Moreover, clusters of small, reddish-orange perithecia were found on the upper 5–6 cm of the diseased taproots close to the surface of the soil. The perithecia were ostiolate with necks up to 100 µm long. The asci within the perithecia were cylindrical, thin-walled and without any discernible apical thickening, and ascospores were uniseriate, globose, ellipsoidal, 10–16 × 7–12 µm, mostly aseptate with cerebriform ornamentation. The morphology of this fungus most closely resembles *N. vasinfecta* var. *africana* (Cannon and Hawksworth 1984).

Sections of diseased root tissue from cv. NC7 were washed thoroughly in running tap water then surface sterilised in a solution of 1% sodium hypochlorite for 2 min and blotted dry on sterile paper. Sections (5 mm) were transferred to half strength potato-dextrose agar amended with 0.01% streptomycin sulfate (1/2 PDAS) and incubated on a laboratory bench at 25°C. After 5 days, colonies of sparse, closely appressed, white mycelium

with young perithecia near the centre developed from most of the sections. Perithecia developed over the surface of the colony as it expanded, reaching maturity after 14 days. The morphology of the perithecia, asci and ascospores were identical to those described above. After 12 days, cultures of *N. vasinfecta* were transferred to fresh plates of half strength potato-dextrose agar (1/2 PDA). Isolates BRIP 47264a, 47265a and 47266a were lodged at BRIP (Department of Primary Industries & Fisheries, Queensland Plant Pathology Herbarium).

In a preliminary pathogenicity test, 1/2 PDA blocks (5 × 5 mm) containing actively growing mycelium were removed from a 10-day-old culture of *N. vasinfecta* (BRIP 47264a) and placed against the stem, just below soil level, of two 17-day-old peanut plants of cv. Conder growing in pasteurised (60°C for 30 min) potting mix (ferrosol/peat/sand, 1 : 1 : 1) in 15-cm-diameter pots. Two plants inoculated with agar plugs from non-colonised 1/2 PDA were used as controls. For all plants, a cut was made at the site of inoculation using a scalpel, and then covered with pasteurised potting mix. The pots were placed in a glasshouse at 18–30°C. After 11 days the inoculated stems developed a surface necrotic lesion that extended along the epicotyl to the crown, whereas the control plants remained healthy. *N. vasinfecta* was re-isolated from the necrotic tissue of both plants.

The pathogenicity of *N. vasinfecta* isolates was tested using two inoculation methods. In the first method, seed of peanut cv. NC7 were germinated on sterile germination paper in trays at 25°C for 7 days. After this time, the testae were removed and the seedlings were washed in deionised water. An ascospore suspension consisting of approximately equal proportions of isolates BRIP 47264a, 47265a and 47266a was prepared by scraping perithecia from 4-week-old 1/2 PDA cultures, then blending before quantification. Eight seedlings were either dipped in a suspension containing 2 × 10⁵ ascospores/mL of all isolates, or in distilled water, using a modification of the method described by Huang *et al.* (1992). After air-drying for 30 min, the seedlings were transplanted into pasteurised potting mix (1 plant per 15-cm-diameter pot), placed in a glasshouse maintained at 18–30°C and were watered when necessary. After 22 days, two stunted and wilted seedlings with brown-black, rotted epicotyls

were removed and assayed for *N. vasinfecta* using the isolation method previously described. Six sections from each plant were placed onto two plates of 1/2 PDAS. Within 14 days, perithecia identical to those *N. vasinfecta* were evident in colonies growing from 11 of the 12 epicotyl sections. Asci and ascospores within the perithecia were confirmed as *N. vasinfecta*. None of the control plants or the remaining six plants inoculated with the fungus developed symptoms of disease.

In the second method, the ascospore suspension detailed above was also diluted to 10^5 ascospores/mL and used to inoculate three bare-rooted 12-week-old peanut cv. D147-P3-6 (DPI&F breeding line) plants. After dipping the roots in the suspension for 30 s, the plants were repotted in pasteurised potting mixture and returned to the glasshouse (18–30°C) for observation, and were watered when necessary. Four control plants were dipped in deionised water only, then repotted. After eight days, all three plants inoculated with *N. vasinfecta* were wilted. After another 10 days, the inoculated and control plants were removed from the pots and washed in running water. The fine feeder roots of plants inoculated with *N. vasinfecta* were rotted or had extensive areas of discolouration. Although the taproots were intact, there was an overall slight discolouration extending into the cortex. Additionally, one peg was rotted. The roots of the control plants were not discoloured. Assays for *N. vasinfecta* were performed, as previously described, from diseased (taproot, feeder roots, and the rotten peg) and ostensibly healthy (control) tissue of all plants. Within 16 days, perithecia were evident in colonies growing from all sections of diseased tissue, whereas no colonies characteristic of *N. vasinfecta* developed from healthy (control) sections. The perithecia, asci and ascospores were identical with those of *N. vasinfecta* on the diseased host tissue from Ban Ban Springs.

Although *N. vasinfecta* is commonly isolated from peanut roots, neither its causal relationship to disease in peanut nor its epidemiology is well understood (Kucharek 2000). Overseas reports have demonstrated its pathogenicity on peanut (Baard and van Wyk 1985; Huang *et al.* 1992) and the results of the present investigation fulfilled Koch's postulates and demonstrated that *N. vasinfecta* was responsible for the severe root rot and premature death of peanut at Ban Ban Springs.

In 2006, *N. vasinfecta* was again isolated from peanut at that locality and also at Wooroolin (approx. 26°25'S, 151°49'E) in the Burnett region (BRIP 47601a). *N. vasinfecta* was previously recorded on peanut near Bowen in northern Qld in 1996 (BRIP 23244) (approx. 20°01'S, 148°13'E), and near Wee Waa, northern NSW (approx. 30°13'S, 149°25'E) in 2003 (DAR 76163). In Australia, *N. vasinfecta* has also been recorded on chickpea (*Cicer arietinum*), soybean (*Glycine max*), lucerne (*Medicago sativa*) and centro (*Centrosema pascuorum*), although the pathogen for the first three hosts is listed as *N. vasinfecta* var. *vasinfecta* in the Australian Plant Pest Database. Overseas, other hosts include pigeon pea (*Cajanus cajan*) (Haware and Nene 1976) and mungbean (*Vigna radiata*) (Rathore 1990). *N. vasinfecta* could pose a threat to all of these hosts which, apart from centro, are important crops in the diverse farming systems of southern Queensland and northern New South Wales. Peanut crops affected by *Neocosmospora* root rot may be misdiagnosed as CBR, so confirmation of the causal organism by laboratory examination is required.

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