Australasian Plant Dis. Notes (2012) 7:193–195
DOI 10.1007/s13314-012-0082-6

New weed hosts of *Macrophomina phaseolina* in Australia

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Received: 3 July 2012 / Accepted: 28 October 2012 / Published online: 10 November 2012

Abstract *Macrophomina phaseolina* was isolated from the roots of symptomless plants of 23 weed species found in Australian mungbean fields. Eight of these species are new host records for the world while 14 of the remaining 15 species are new reports in Australia. Isolates of *M. phaseolina* from all weeds were pathogenic on mungbean seedlings. These results suggest that apparently healthy weeds infected by *M. phaseolina* may serve as alternative hosts of the pathogen in Australian grain production regions.

Keywords Charcoal rot · *Macrophomina phaseolina* · Symptomless hosts · Weeds

Macrophomina phaseolina, the causal agent of charcoal rot and other diseases, has been recorded on over 500 monocotyledenous and dicotyledonous hosts species worldwide (Wyllie 1989), including the food crop mungbean (*Vigna radiata* and *V. mungo*) and many weed species (Bruton 1982; Dhingra and Sinclair 1978; Wyllie 1989; Young and Alcorn 1984). The wide host range and apparent lack of host specificity of *M. phaseolina* (Mihail and Taylor 1995), together with the longevity of its microsclerotia in soil, enable the fungus *M. phaseolina* to survive for many years in the absence of a host crop (Short et al. 1980).

In Australia, mungbean is grown predominantly as a dryland legume in the north-eastern grain region and is becoming increasingly important as a short-season cash crop. The presence of *M. phaseolina* is harmful for mungbean production and product quality (Fuhlbohm 2003). Many weeds have been reported as hosts of this *M. phaseolina* and may play a role in its survival between successive crops (Reichert and Hellinger 1947), but the role of weed hosts in Australia is unknown. Here we investigated if weeds common in the mungbean production areas would serve as alternative hosts.

In April 1996, six individual plants belonging to each of 24 common weed species were collected from two fields in which mungbean crops had been grown previously, one near Biloela (−24.400729, 150.512838) in the Dawson-Callide Valley of central Queensland, and the other near Brookstead (−27.754646, 151.448879) on the Darling Downs of southern Queensland, Australia. All individuals appeared healthy, except those of *Trianthema portulacastrum* which were wilted and had basal black stem lesions after being sprayed with a sublethal dose of glyphosate several weeks before.

The presence or absence of *M. phaseolina* in the plants was determined by the following method. Taproots or root segments adjacent to the rhizome (for *Sorghum halepense*) were rinsed in tap water for 5 min., dipped in 100 % ethanol for 10 s and then transferred to a solution of 2 % NaOCl for 3 min. After surface sterilisation, the roots were blot dried on sterile paper towel and cut into 10 mm long segments which were then placed in plates containing 2 % distilled water agar amended with 1 g L^{-1} streptomycin sulphate. The plates were incubated for 5 days in the dark at 32 °C,

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then examined for the presence of *M. phaseolina* colonies growing from the root segments. Isolates of *M. phaseolina*, identified by the presence of fluffy grey mycelium and dark microsclerotia immersed in the agar, were transferred to potato dextrose agar plates and hyphal-tipped to generate pure cultures. All purified isolates were deposited in the Biosecurity Queensland Plant Pathology Herbarium, Eco-Sciences Precinct, Dutton Park, Queensland (BRIP). One or two isolates from each weed host were used in the pathogenicity tests described below.

V8 broth was prepared by adding 10.3 g of $CaCO_3$ to 750 mL V8 juice, followed by centrifugation at 3,000 rpm for 20 min. The supernatant was diluted 1:4 with distilled water, and 40 mL was dispensed into 50 mL plastic tubes and autoclaved at 121 °C and 0.15 MPa for 20 min. For each isolate, a colonised agar block (5 mm×5 mm) was transferred to each of three tubes and placed on an orbital shaker (150 rpm) in the dark at 28±1 °C. After 5 days the mycelia were washed with sterile distilled water and collected by vacuum filtration onto Miracloth. Mycelium of each isolate was press-dried with paper towel, macerated in sterile water and adjusted to a concentration of 500 mg fresh weight of mycelium per 20 mL water.

Seeds of mungbean cv. Berken were germinated in moist, sterile vermiculite at 28±1 °C in darkness. After 4 days, 10 sprouts were immersed in a mycelial suspension of each isolate for 10 s then transplanted into a sterile substrate mix in 45 cm×30 cm×5 cm trays. The trays were covered with moist plastic bags and incubated at 28±1 °C in darkness. Sterile distilled water–treated seedlings served as an uninoculated control. After 5 days the expression of symptoms on the seedlings was noted. Six seedlings in total displaying charcoal rot symptoms were randomly selected from among the affected seedlings, and the presence or absence of *M. phaseolina* in the seedlings was determined using the methodology outlined above.

Macrophomina phaseolina was isolated from the roots of at least one plant of all weed species, except Mexican poppy (*Argemone ochroleuca*) (Table 1). All isolates of *M. phaseolina* obtained from the weed hosts were pathogenic on mungbean sprouts, causing a wet rot of the radicles. The pathogen was isolated from all six seedlings which displayed symptoms of charcoal rot infection, but was not isolated from mungbean sprouts which had been inoculated with SDW only.

Table 1 Weeds from which attempts at isolating *Macrophomina phaseolina* were performed during the current study

Scientific name	Family	Common name(s)[a]	BRIP accession[f]
Amaranthus macrocarpus[b]	Amaranthaceae	Dwarf amaranth	23500
Argemone ochroleuca[c]	Papaveraceae	Mexican poppy	
Asclepias physocarpa[d]	Apocynaceae	Balloon cotton bush	23501, 23502
Atriplex muelleri[d]	Chenopodiaceae	Annual saltbush, Mueller's saltbush	23487, 23488
Cassia spp.[b]	Caesalpinaceae		23495
Chamaesyce drummondii[b]	Euphorbiaceae	Caustic creeper	23496, 23497
Cissus opaca[d]	Vitaceae		23516, 23517
Corchorus trilocularis.[e]	Tiliaceae	Native jute	23511, 23512
Cullen tenax[d]	Fabaceae	Tough scurf pea	23498, 23499
Datura stramonium[e]	Solanaceae	Common thornapple, Datura	23493, 23494
Hibiscus trionum[b]	Malvaceae	Bladder ketmia, Wild cotton	23478, 23479
Macroptilium lathyroides[e]	Fabaceae	Phasey bean	23505, 23506
Malvastrum americanum[d]	Malvaceae	Spiked malvastrum, Malvastrum	23507, 23508
Neptunia gracilis[d]	Fabaceae	Native sensitive plant	23477
Physalis minima[b]	Solanaceae	Wild gooseberry	23480, 23481
Rapistrum rugosum[d]	Brassicaceae	Turnip weed, Giant mustard	23483, 23484
Salvia reflexa[b]	Lamiaceae	Mintweed	23509, 23510
Sesbania cannabina[e]	Fabaceae	Sesbania, Yellow pea bush	23489, 23490
Sonchus oleraceus[e]	Asteraceae	Sowthistle, Milkthistle	23482
Sorghum halepense[b]	Poaceae	Johnson grass	23485, 23486
Trianthema portulacastrum[e]	Aizoaceae	Giant pigweed, Black pigweed	23503, 23504
Tribulus terrestris[d]	Zygophyllaceae	Caltrop, Bullhead, Cat-head	23514, 23515
Verbena tenuisecta[b]	Verbenaceae	Moss verbena, Mayne's pest	23492
Wahlenbergia graniticola[b]	Campanulaceae	Bluebell	23513

[a]common names from Australian Plant Name Index (www.anbg.gov.au/apni/), and Weeds: The Ute Guide Northern Grain Belt Edition ISBN 0 7345 0078 5

[b]hosts in genus previously reported overseas

[c]*M. phaseolina* not isolated

[d]hosts not previously reported in Australia or overseas

[e]host species previously reported overseas

[f]Biosecurity Queensland Plant Pathology Herbarium, Eco Sciences Precinct, Dutton Park, Queensland 4102

In Australia, management of charcoal rot in crops such as sorghum, maize, sunflower, soybean and mungbean relies on agronomic practices such as optimising plant density, soil nutrition and planting time, because fungicides are ineffective and the current commercial hybrids or cultivars are susceptible to *M. phaseolina*. The discovery of the pathogen as extraordinarily on symptomless weeds and their role as inoculum sources merits being further investigated.

Acknowledgments This paper is in memory of Dr Michael Fuhlbohm, the senior author, a friend and colleague who died tragically on 27 April 2008 as a result of an accident whilst climbing on Mt. Cordeaux, southern Queensland. We thank the Australian Government for an Australian Postgraduate Research Award, and the Grains Research and Development Corporation, the former CRC for Tropical Plant Pathology and the former Botany Department, University of Queensland for financial support for Michael's research. He would also want us to thank his family, colleagues and friends for the moral and technical support they gave him during his studies.

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