Pythium/Rhizoctonia complex causing damping-off of cowpea in South Africa

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Pythium ultimum and *Rhizoctonia solani* were consistently isolated from cowpea seedlings with symptoms of damping-off in South Africa. Isolates of the two species were tested individually and in combination for their effect on cowpea seedlings in the greenhouse at 20, 26 and 32 °C. Damping-off induced by *R. solani* was the highest at 20 °C, and disease incidence decreased with increase in temperature. *P. ultimum* caused most disease at 26 °C and significantly less at 20 and 32 °C. Percentage damping-off induced by *P. ultimum* was higher than that induced by *R. solani* at all three temperatures. Disease incidence incited by *P. ultimum* was significantly reduced by *R. solani* when the two fungi were combined, suggesting an antagonistic interaction between them.

Key words: cowpea, damping-off, *Pythium ultimum, Rhizoctonia solani, Vigna unguiculata.*

Cowpea (*Vigna unguiculata* (L.) Walp) yields in Africa are low due to various pests and diseases, including pre- and post-emergence seedling damping-off, affecting production of the crop (Singh et al. 1997). Damping-off is caused mainly by *Pythium* spp., *Rhizoctonia solani* J G Kühn and *Sclerotium rolfsii* Sacc. (Emechebe 1981; Singh & Rachie 1985; Singh et al. 1997; Aveling & Adandonon 2000). The last two species, although occurring on cowpea in South Africa (Doidge 1924; Doidge & Bottomley 1931), have traditionally been regarded as unimportant in local plantings of the crop (Doidge et al. 1953). In a recent survey of cowpea fields in South Africa (Aveling & Adandonon 2000), *Pythium ultimum* Trow was consistently isolated from diseased seedlings. Infected seedlings that failed to emerge above the soil line exhibited water-soaked lesions girdling the hypocotyl. Infected hypocotyls had light brown lesions above the soil line and seedlings showed symptoms of wilting.

P. ultimum is known to cause disease in complexes with *R. solani* in leguminous crops such as bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) (Pieczarka & Abawi 1978; Xi et al. 1995). In view of the above and the lack of recent information on the occurrence of *R. solani* in local cowpea fields, a study was undertaken to ascertain the present status of *R. solani* as a cowpea damping-off pathogen in South Africa and to investigate its interaction with *P. ultimum.*

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Materials and methods

Approximately 30 cowpea seedlings with damping-off symptoms were collected from each of two fields in North-West Province and one field in KwaZulu-Natal. Tissue segments (c. 2 mm^2) of diseased hypocotyls were excised, surface-disinfested for 15 seconds in 0.5 % sodium hypochlorite (NaOCl), rinsed twice in sterile distilled water (SDW), blotted dry with sterile tissue paper and plated to potato-dextrose agar supplemented with 0.025 % chloramphenicol. After incubation for two days at room temperature, mycelium from emerging colonies was transferred to fresh PDA and water agar plates. Cultures were incubated for five days at 25 ± 1 °C under fluorescent light and identified. Voucher specimens of *P. ultimum* (PPRI 7100) and *R. solani* (PPRI 7101) were deposited in the National Collection of Fungi, Biosystematics Division, ARC-Plant Protection Research Institute, Pretoria.

Pathogenicity of 10 isolates of each of the two species was tested in the greenhouse using millet seed inoculum prepared as described by Weideman & Wehner (1993). Inoculum was incorporated at 50 g kg^{-1} into sandy loam soil pasteurised by aerated steam (60 °C for 30 minutes) 21 days prior to incorporation of the inoculum. Cowpea seeds (cv. Rhino) were surface-disinfested for two

Table 1. Effect of *Pythium ultimum* and *Rhizoctonia solani* singly and in combination on the incidence of cowpea damping-off in the greenhouse.

Treatment	Damping-off (%)[a]		
	20 °C	26 °C	32 °C
P. ultimum	56.3 h	68.8 i	26.0 d
R. solani	43.8 fg	31.3 de	9.3 b
P. ultimum + *R. solani*	37.5 ef	50.0 gh	18.8 c
Sterile millet seed	0 a	0 a	0 a
None	0 a	0 a	0 a

[a]Each value is the mean of two experiments each comprising four replicate pots with four plants in each. Means followed by the same letters do not differ significantly according to Fisher's least significant difference test ($P \leq 0.05$).

minutes in 1 % NaOCl, rinsed twice in SDW and planted in pots (14 cm diam., 18 cm high) filled with infested soil, using four seeds per pot and four pots per isolate. Control pots contained uninfested pasteurised soil. Pots were maintained at 25 ±1 °C in a greenhouse and were watered regularly with tap water. Reisolations from seedlings displaying damping-off were done to verify the presence of the inoculated fungal species.

To determine the interaction between *P. ultimum* and *R. solani,* millet seed inoculum of PPRI 7100 and PPRI 7101 were combined as follows (rates refer to millet seed inoculum kg^{-1} pasteurised soil): 50 g *R. solani*; 50 g *P. ultimum*; 25 g *R. solani* + 25 g *P. ultimum*; 50 g uncolonised steeped millet seed; no millet seed.

Cowpea seeds (cv. Rhino) were surface-disinfested and planted in pots as described above. Pots were incubated at 20, 26 and 32 °C in a greenhouse under fluorescent light and were watered daily with tap water. The experiment was designed as a randomised block with four replicates per treatment and was repeated once. The number of symptomatic seedlings was recorded three days after planting and then at two-day intervals for a further 30 days. Reisolations from seedlings displaying damping-off were done as described above. Data were arcsine (Y1/2) transformed and analysed by analysis of variance. Mean differences were separated using Fisher's least significant difference test.

Results and discussion

P. ultimum and *R. solani* were consistently isolated from diseased seedlings collected in the field. Pre- and post-emergence damping-off similar to that observed in the field was evident in cowpea seedlings artificially inoculated with *P. ultimum* and *R. solani* in the greenhouse, and the two fungi could readily be reisolated from diseased seedlings. Although symptoms caused by the two fungi were almost identical, lesions on the hypocotyls caused by *R. solani* were slightly darker in colour.

R. solani on its own caused the most damping-off at 20 °C and the disease incidence decreased with increase in temperature (Table 1). With *P. ultimum,* most disease occurred at 26 °C and significantly less at 20 and 32 °C. Infection by *P. ultimum* nevertheless resulted in a higher incidence of damping-off than was induced by *R. solani* at all three temperatures. Compared to *P. ultimum* on its own, disease was significantly less in the *P. ultimum* + *R. solani* treatment.

The difference in temperature preferences of the two pathogens is consistent with the optimal temperature for growth of 24–28 °C for *P. ultimum* (Singh 1964) and 16–25 °C for *R. solani* to cause disease (Domsch et al. 1980). The results also are in accordance with those of Pieczarka & Abawi (1978), who reported that *P. ultimum* caused more severe root rot in bean in the absence of *R. solani* than in its presence. Xi et al. (1995) similarly indicated that *P. ultimum* and *R. solani* in combination caused significantly less damping-off of pea than *P. ultimum* on its own. This is probably due to suppression of *P. ultimum* by *R. solani* as the latter species is known to actively parasitise members of the *Peronosporales* (Butler 1957) and to interact antagonistically with *Pythium* in soil (Flowers & Littrell 1973).

Although this is the first report of *P. ultimum* and *R. solani* in a complex causing damping-off of cowpea in South Africa, the extent to which the complex occurs in the country is not known. Growers should nevertheless take note of the unrelatedness of the two organisms that are

involved and adapt their control strategies accordingly.

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