A. pruni is reported for the first time from India on this host. No species of *Alternaria* has been known to occur on *Prunus amygdalis*[1-3]

Thanks are due to Mr J. David of CMI, Kew, England, for identification of the *Alternaria* species.

11 July 1988; Revised 8 September 1988

1. Bilgrami, K. S., Jamaluddin and Rizwi, M. A., *Fungi of India, Parts I & II*, Today & Tomorrow's Printers & Publishers, New Delhi, 1979.
2. Mukerji, K. G. and Bhasin, Jayanti, *Plant diseases of India, A source book*, Tata McGraw-Hill Publishing Company, New Delhi, 1986.
3. Sarbhoy, A. K., Agarwal, D. K. and Varshney, J. L., *Fungi of India (1977–81)*, Associated Publishing Company, New Delhi, 1986.

A NEW REPORT OF *RHIZOPUS* ROT OF GROUNDNUT FROM INDIA

R. B. GAUR, R. D. SINGH* and R. S. RATHORE**
Department of Plant Pathology, Agricultural Research Station, Sri Ganganagar 335 001, India.
*Present addresses: *Department of Plant Pathology. Agricultural Research Station, Durgapura 302 015, India.*
***Department of Plant Pathology, Agricultural Research Station, Mandore 342 304, India.*

DURING a survey in 1985 and 1986, a serious pre- and post-emergence seedling rot of groundnut (*Arachis hypogaea* L.) was observed in Sri Ganganagar district. Infected plants can be easily identified by the stunted growth, and on uprooting such plants necrosis is usually seen just below the cotyledonary axis. Mats of mycelium and a black mass of spores may be seen on necrotic tissues. If the plumule and cotyledonary laterals are completely destroyed, the seedling usually died. In some cases even older plants were found to bear disease symptoms. The disease was also observed on seeds sown in moist plots.

Samples from infected plants and seeds were collected and small bits of infected parts along with healthy areas were cut and surface-sterilized in mercuric chloride (0.1%) for one and a half min, washed thrice in sterile water, and transferred to petri dishes containing potato dextrose agar medium. Plates were incubated at 30°C for 5 days. Pathogenicity of the causal organism was tested successfully on groundnut cultivar M-13. Three kg of sterilized soil was inoculated with 150 g of soil maize medium containing 7-day-old culture. Healthy seeds of the same cultivar were also inoculated with a spore suspension containing 9×10^4 spores/ml from the same culture. Typical symptoms of the disease were observed on inoculated seeds two days after sowing. Reisolation from infected parts yielded the same pathogen. The pathogen was identified as *Rhizopus oryzae* Went and Prinsen Geerligs (IMI No. 299372).

R. nigricans and *R. arrhizus* were found to be associated with rot of groundnut in India[1,2] but *R. oryzae* causing pre- and post-emergence seedling rot has not been reported earlier.

The authors are grateful to Dr P. M. Kirk, CMI, England, for identification of the pathogen.

16 September 1987; Revised 7 October 1988

1. Chohan, J. S. and Gupta, V. K., *Indian J. Agric. Sci.*, 1968, **38**, 568.
2. Rao, V. G., *Mag. Coll. Agric. Dhulia*, 1963, **2**, 53.

SUSCEPTIBILITY OF RICE SHEATH BLIGHT PATHOGEN TO MYCOPARASITES

K. MANIBHUSHANRAO,
S. SREENIVASAPRASAD, U. I. BABY and
Y. JOE
Centre for Advanced Studies in Botany, University of Madras, Madras 600 025, India.

BIOCONTROL through the use of resident and introduced antagonists has of late gained importance and is poised for successful application in modern agriculture[1]. Among the many potentially antagonistic soil inhabitants, members of the genera *Gliocladium* and *Trichoderma* have gained considerable importance as biocontrol agents[2], and plant growth enhancement by *Trichoderma* spp. has also been reported[3]. Mycoparasitism of rice sheath blight incitant *Rhizoctonia solani* by *Trichoderma* spp.[4,5] and *Gliocladium* sp.[6] is known. The antagonistic potential of *G. virens* and *T. longibrachiatum* isolated from rice field soils to *R. solani*, their morphological interactions and the associated physiological mechanisms are reported here.

Soil samples (25 g) were air-dried, compacted and used (250 mg) for the isolation of antagonistic fungi on rose bengal agar medium[7] by the soil plate technique[8]. Fungal colonies, particularly *Gliocladium* and *Trichoderma* spp., were isolated and the stock cultures were maintained on potato dextrose agar (PDA).

The dual culture technique[9] was followed using PDA plates to screen the isolates, and their ability to inhibit pathogen growth and/or colonize the pathogen was taken to indicate antagonism. The effective antagonists were identified. For quantitative assessment, modified dual culture technique[10] using PDA and synthetic medium (SM-1)[11] plates and indirect assessment method[12] were followed. Periodic observations were made on the linear growth (in mm) of the antagonists colonizing the pathogen.

Specimens prepared using the slide culture technique and/or dual culture on water agar overlaid with cellophane[13] were used for photomicrography. Production of non-volatile antibiotic(s) was checked by the replacement culture technique and block bioassay[14]. In the replacement culture technique, radial growth (in mm) on malt agar medium was measured at 24 h intervals to calculate per cent inhibition, while in the block bioassay method, inhibition zones (in mm) were measured on PDA plates after 48 h.

The antagonists were grown in liquid media, namely PD broth, gliotoxin fermentation medium[15] and a synthetic medium (SM-II)[16], under static and shake conditions. The cell-free culture filtrate obtained from 7-day-old cultures was bioassayed at 10% with PDA, and uninoculated PDB at the required pH was used as control. Per cent inhibition was calculated by monitoring the radial growth of *R. solani*.

Antagonists were centrally inoculated on solidified SM-I amended with either chitin, laminarin, cellulose, casein or glucose (4 mg/ml), and SM-I devoid of any carbon source served as check. Growth and sporulation pattern were observed periodically. SM-I, and SM-I amended with cycloheximide[13] (10 and 25 ppm), were used for dual culture of the pathogen and the antagonist and observations were made on the mycoparasitic rate. Discs (5 mm) cut from 3-day-old and 6-day-old PDA cultures of the pathogen and antagonists respectively were used as inoculum.

Following the soil plating technique, 40 fungal isolates, mainly *Trichoderma* and *Gliocladium* spp. were obtained. Their screening revealed the positive antagonistic potential of 27 isolates, among which 22 were *T. harzianum*, 2 were *T. longibrachiatum* (Tl_1 and Tl_2) and 3 were *G. virens* (Gv_1, Gv_2 and Gv_3).

Both *T. longibrachiatum* and *G. virens* were strongly antagonistic to the rice sheath blight pathogen, *R. solani*. *T. longibrachiatum* directly attacks the hyphae and by the 8th day it colonizes even the sclerotia. On the other hand, *G. virens* arrests the pathogen's growth a few hours before invading it. Various stages of morphological interaction between the antagonists and the pathogen have been observed in our study (figure 1a, b) and the production of wall-degrading enzymes at the sites of interaction by *T. harzianum* has been reported using the calcofluor M2R[13].

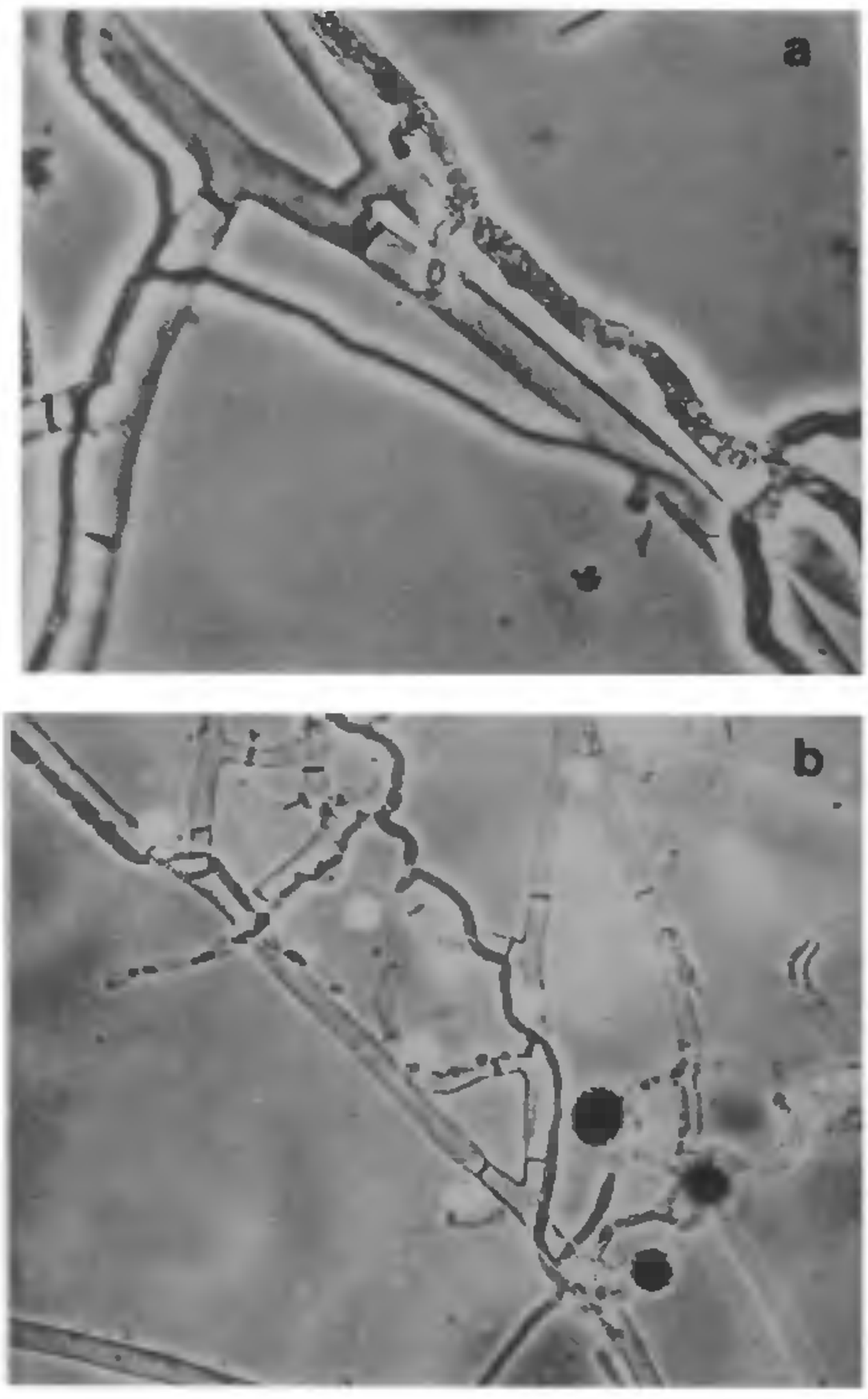

Figure 1a, b. Morphological interaction between *R. solani* and the antagonists. **a.** *T. longibrachiatum* hypha attached to the host by producing hooks/pegs (× 560); **b.** *G. virens* hyphae running along the host forming small appressoria-like intrusions (× 340).

Table 1 *Mycoparasitic potential of G. virens and T. longibrachiatum isolates and the effect of cycloheximide (CH)*

	Linear growth (mm) in dual culture								
	Time (h)								
	48		96		144		Linear growth† (mm)		
Antagonist	P*	S**	P	S	P	S	Control (SM-I)	SM-I with 10 ppm CH	SM-I with 25 ppm CH
Tl_1	36.5	28.5	47.0	50.5	60	63	71	54.0	40.0
Tl_2	37.0	30.0	50.0	54.0	60	61	69	41.5	25.5
Gv_1	28.0	24.0	62.5	60.0	73	72	78	58.0	36.5
Gv_2	28.0	23.0	60.0	62.0	71	70	76	53.5	32.0
Gv_3	17.0	20.0	48.0	51.0	65	69	67	46.0	29.0

*PDA; **SM-I; †Recorded on the day 8.

Quantitative assessment of the mycoparasitic potential of the two antagonists on PDA and SM-I did not reveal any preference for a particular nutritional source (medium). The two isolates of *T. longibrachiatum*, Tl_1 and Tl_2, did not vary much in their mycoparasitic ability, though Tl_2 sporulates sparsely. However, in the case of *G. virens*, the mycoparasitic potential of Gv_3 was markedly less than that of Gv_2 and Gv_1, which were almost comparable (table 1). The indirect assessment method[12] also confirmed the above observations (figure 2).

Cycloheximide considerably reduced the mycoparasitic activity of *T. longibrachiatum* . and *G. virens* at 10 ppm, and at 25 ppm the antagonists were unable to colonize the pathogen even by the 8th day (table 1). The two antagonists were able to utilize various cell wall components such as chitin, laminarin, cellulose, casein and glucose, with varying degrees of growth and sporulation.

Replacement culture technique and block bioassay revealed the stronger antibiosis of Tl_2, unlike Tl_1, and also the strong and very mild antibiosis of Gv_3 and Gv_1, and Gv_2 respectively (table 2). Further, bioassay of the culture filtrate revealed a preference for PDB and aeration for optimum production of antibiotic(s) by Gv_1 and Tl_2 (per cent inhibition of *R. solani* growth, 71 and 55 respectively).

There does not appear to be any specific correlation between antibiotic production and mycoparasitic potential of either of the antagonists, as Tl_2 which is slightly less mycoparasitic exhibited very strong antibiosis, in contrast to Tl_1. Production of antibiotic(s) such as trichodermin, suzukacillin and alamethicin by *T. harzianum* has been reported[14]. Antibiosis of *T. longibrachiatum* and also the preference for PDB and aeration for enhanced production of antibiotic(s) have been confirmed here. Indeed, antifungal compounds could be obtained in the chloroform and aqueous fractions of the *T. longibrachiatum* culture filtrate (unpublished). Similary Gv_3, which showed less mycoparasitism,

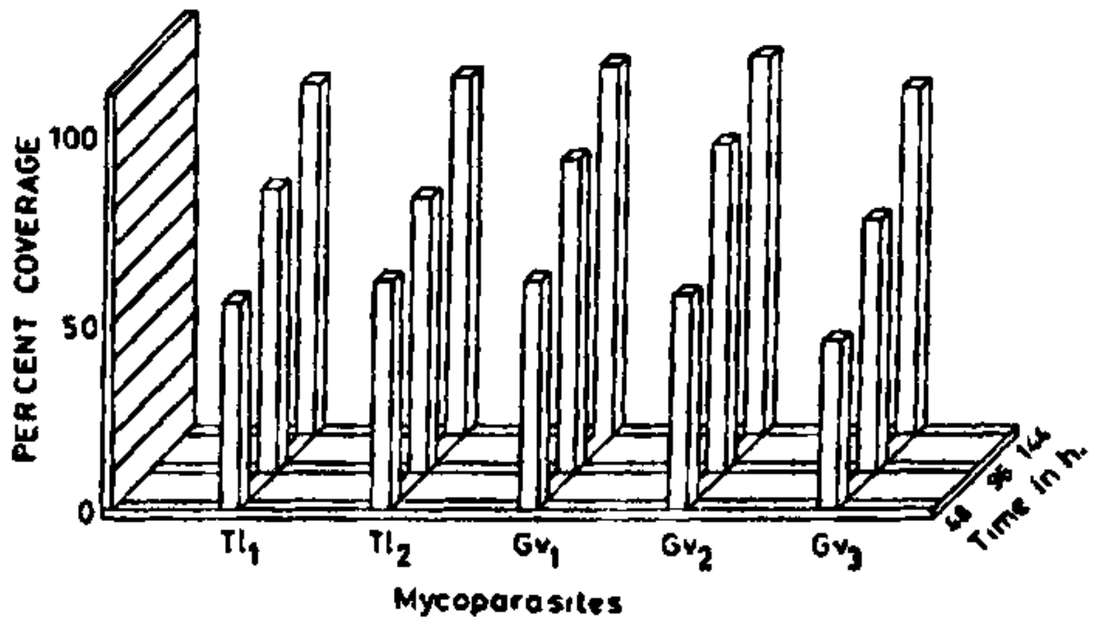

Figure 2. Mycoparasitic activity of *T. longibrachiatum* and *G. virens*.

Table 2 *Antibiotic potential of G. virens and T. longibrachiatum isolates*

	Per cent inhibition of *R. solani* radial growth*			
	Time (h)			Inhibition zone† (mm)
Antagonist	24	48	72	
Tl_1	—	—	—	—
Tl_2	69	66	59	7.0
Gv_1	100	100	100	10.5
Gv_2	50	28	—	2.0
Gv_3	100	100	100	10.0

*With replacement culture; †48 h after block bioassay.

exhibited strong antibiosis, equivalent to that of Gv_1, which was highly mycoparasitic, unlike Gv_2. It is pertinent to mention here that antibiotic production by *G. virens* was ascribed a more important role than mycoparasitism[17]. The ability of the two antagonists to utilize various components of fungal cell walls as carbon source, as well as the annulling of their mycoparasitic potential by cycloheximide, stress the importance of wall-lytic enzymes in mycoparasitism[13]. In fact, both *T. longibrachiatum* and *G. virens* produce wall-lytic enzymes at low level constitutively and production could be induced further (unpublished).

Thus, with *G. virens*, mycoparasitism does not seem to be the major mechanism in contrast to *T. longibrachiatum*. In view of the strain variation observed in the mycoparasitic and antibiotic potentials of these antagonists, it appears that antagonists possessing both these phenomena would be able to effectively combat plant pathogens such as *R. solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii*.

15 July 1988; Revised 4 October 1988

1. Cook, R. J., *Phytopathology*, 1985, **75**, 25.
2. Papavizas, G. C., *Annu. Rev. Phytopathol.*, 1985, **23**, 23.
3. Chang, Y. -C., Chang, Y. -C., Baker, R., Kleifeld, O. and Chet, I., *Plant Dis.*, 1986, **70**, 145.
4. Roy, A. K. and Sayre, R. M., *Indian Phytopathol.*, 1984, **37**, 710.
5. Hashioka, Y. and Fukita, T., *Rep. Tottori Mycol. Inst.*, 1969, **7**, 8.
6. Manibhushanrao, K., Sreenivasaprasad, S. and Baby, U. I., 1987, (Abstracts), *Workshop on biological control of plant diseases*, TNAU, Coimbatore, India, p. 11.
7. Martin, J. P., *Soil Sci.*, 1950, **69**, 215.
8. Warcup, J. H., *Nature (London)*, 1950, **166**, 117.
9. Tu, J. C., *Phytopathology*, 1980, **70**, 670.
10. Huang, H. C. and Hoes, J. A., *Can. J. Bot.*, 1976, **54**, 406.
11. Okon, Y., Chet, I. and Henis, Y., *J. Gen. Microbiol.*, 1973, **74**, 251.
12. Henis, Y., Lewis, J. A. and Papavizas, G. C., *Soil Biol. Biochem.*, 1984, **16**, 391.
13. Elad, Y., Chet, I., Boyle, P. and Henis, Y., *Phytopathology*, 1983, **73**, 85.
14. Dennis, C. and Webster, J., *Trans. Br. Mycol. Soc.*, 1971, **57**, 25.
15. Brian, P. W. and Hemming, H. G., *Ann. Appl. Biol.*, 1945, **32**, 214.
16. Ooka, T., Shimojima, Y., Akimoto, T., Tekeda, I., Senoh, S. and Abe, J., *Agric. Biol. Chem.*, 1966, **30**, 700.
17. Howell, C. R., *Phytopathology*, 1987, **77**, 992

EFFECT OF COPPER SULPHATE ALGICIDE ON GROWTH OF AND NITROGEN FIXATION IN *AZOLLA PINNATA* R.BR.

K. RAJARATHINAM*, M. JAYABALAN* and M. A. PADHYA
Department of Botany, Faculty of Science, M. S. University of Baroda, Baroda 390 002, India.
**Present address: Department of Botany, VHNSN College, Virudhunagar 626 001, India.*

THE most common method for the chemical control of free-living algae is the application of 1% copper sulphate solution[1]. Application of 10 ppm copper sulphate solution completely controlled snails, which affect the growth of *Azolla*, but the extent to which the copper sulphate affected growth of *Azolla* was not reported[2]. The present communication deals with the effect of copper sulphate on the growth, chlorophyll and nitrogen content, and acetylene reduction activity of the *Azolla–Anabaena* complex.

Azolla pinnata R. Br. plants were grown (3 g/l) on nitrogen-free liquid medium[3] with varying levels of copper (0, 2, 5, 10 and 20 ppm). Culture conditions were as described previously[4,5]. Plants were harvested after three weeks and their biomass was recorded. The chlorophyll content[6], total nitrogen content[7] and nitrogenase-catalysed acetylene reduction activity[8] were estimated as described earlier.

Increasing Cu concentration in the medium was associated with reduction in biomass, chlorophyll content, nitrogen content and acetylene reduction activity (table 1). The growth of *Azolla* in the presence of low concentration (2 ppm) of Cu was comparable to that of the control. At 5, 10 and 20 ppm Cu in the culture medium, dry matter accumulation showed 20, 32 and 63% reduction respectively. Chlorophyll and nitrogen content, and acetylene reduction activity also showed reduction at 5, 10 and 20 ppm Cu. A positive correlation exists between dry matter accumulation and nitrogen content ($r = 0.767$).