ACTA MYCOLOGICA
VOL. XIX (1): 115–119
1983

A possible mode of survival of Fusarium udum as a Mycoparasite

R. S. UPADHYAY, BHARAT RAI and R.C. GUPTA

Department of Botany, Banaras Hindu University
Varanasi 221005, India

Upadhyay R.S., Bharat Rai and Gupta R.C.: *A possible mode of survival of Fusarium udum as a Mycoparasite.* Acta Mycol. 19 (1) 115 - 119, 1983.

Survival of *Fusarium udum* Butler, the wilt pathogen of pigeon-pea, on other microfungi as a mycoparasite has been observed.

INTRODUCTION

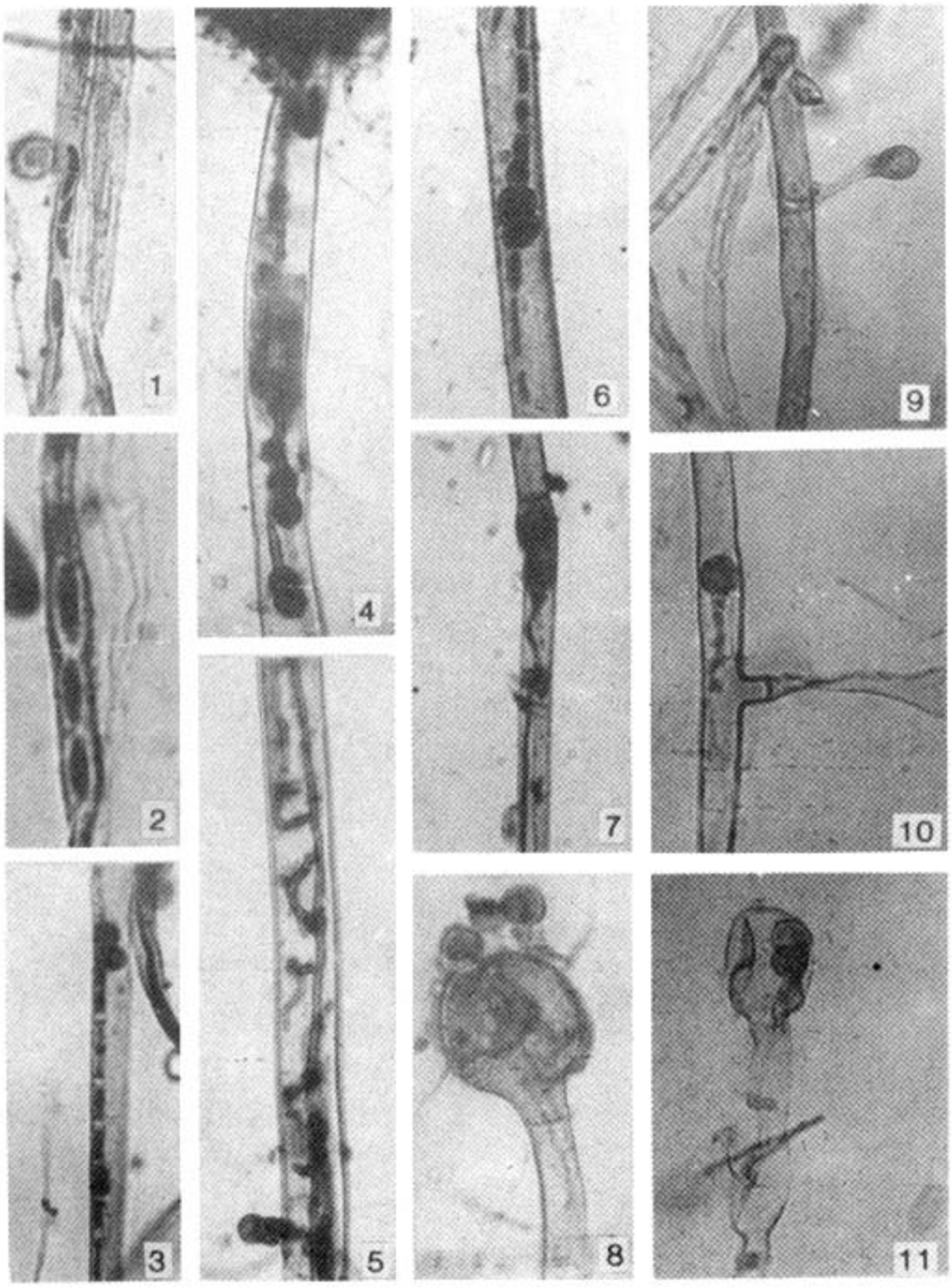
Fusaria as phytopathogens have been regarded as the most prevalent and devastating agent on crops all over the world (Booth 1971). The organic debris of host and non-host plants are the main sources of their survival and maintenance of inoculum potential which is of substantial importance in disease development (Garrett 1970). It has long been discussed that fungi may also survive and grow better for a longer period in soil in absence of recently added organic material. What factor acts in bridging this discontinuity in the environment is still unexplored. Attempts have been made by several workers, but they failed to provide direct evidence. However, Park (1965) speculated on the possibility that in soil an organism may continue to be active for a period in the absence of an external supply of nutrients by utilizing its own stored reserves. During our investigations on microbial interactions between *Fusarium* spp. and other saprophytic fungi of pigeon-pea rhizosphere it was frequently noted that *Fusarium udum* Butler penetrated and parasitized hyphae, conidiophores and sporangiophores of other fungi and formed conidia and chlamydospores inside the hosts as a consequence of mycoparasitism, providing evidence of survival of fungi on other fungal hosts. Further experiments were carried out to test whether such a phenomenon is of any importance under natural conditions.

MATERIALS AND METHODS

In vitro experiments were carried out in dual cultures following the method of H u a n g and H o e s (1979). The following fungus were isolated from the rhizosphere and non-rhizosphere soils of pigeon-pea field in order to study their interaction with *F. udum*: *Aspergillus luchuensis* Inui, *Cunninghamella echinulata* Thaxter, *Mortierella subtilissima* Oudemans, *Rhizopus nigricans* Ehrenb. and *Syncephalastrum racemosum* (Cohn) Schroet. The test fungi were inoculated over solid potato-dextrose agar (prepared from fresh potatoes) with or without cellophane covering (sterilized and deplasticised). The plates were incubated at 25 ± 1°C under flourescent light for 15 days and observations were made at 24-h intervals. Five small blocks were cut daily, one from each of 5 different replicate Petri dishes, from intermingling growth regions of test fungi. Observations were made directly under the microscope and also after staining the mycelium with cotton blue in lactophenol.

In a separate experiment, the inoculum of *F. udum* was amended individually with each of the following species separately: *Aspergillus flavus* Link ex Fr., *A. niger* v. Tiegh., *A. terreus* Thom, *Cunninghamella echinulata* Thaxter, *Penicillium citrinum* Thom, *Rhizopus nigricans* Ehrenb. and *Trichoderma viride* Pers. ex Gray. The pure soil inocula of *F. udum* as well as the test microorganisms were prepared by the following method. A soil sample was collected from a pigeon-pea field and was mixed well with washed sand (5 : 1) and 3% maize-meal. One hundred g samples were placed in 250 cm^3 conical flasks, sterilized and inoculated separately with cultures (3 blocks each of 10 mm diam.) of each individual test organism as well as *F. udum*. Three replicates were used for each organism and the flasks were incubated at 25 ± 2°C for 15 days. After incubation the soil-sand-inoculum of the test organisms were taken out of the flask aseptically and the population of each was adjusted to approximately 1×10^4 per gram dry soil by mixing in it and appropriate weight of washed sterilized sand. The ration of amendment (*F. udum*: test fungus) was 20 : 80; 50 : 50; 80 : 20 and 100 : 0. The mixed soil inocula were put in earthenware pots (8 cm diam) in three replicates and the moisture in each pot was maintained at 20% level without distrupting the soil. The samples were taken from the pots at monthly intervals and the population of *F. udum* per gram dry soil was determined by plating the soil on selective medium (N a s h, S n y d e r 1962) and by the soil plate method (W a r c u p 1979). The population of the test microorganisms per gram dry soil was also recorded by plating the soil on Martin's medium (M a r t i n 1950).

Plate I



Fusarium udum

1-2 – microconidia inside the lumen of *Cunninghamella echinulata* (× 280); *3* – chlamydospore inside the hypha of *C. echinulata* (× 480); *4* – chlamydospores inside the lumen of conidiophore of *Aspergillus luchuensis* (× 520); *5* – frequent branching of infection hypae of *F. udum* inside the conidiophore of *A. luchuensis* (× 448); *6* – chlamydospore inside the sporangiophore of *Rhizopus nigricans* (× 448); *7* – chlamydospore inside *Mortierella subtilissima* (× 656); *8* – chlamydospores on the infected head of *Syncephalastrum racemosum* (× 520); *9* – germ tube of a chlamydospore which coiled around a hypha of *S. racemosum* (× 520); *10* – chlamydospore inside a infected hypha of *S. racemosum* (× 520); *11* – chlamydospores inside the sporangial head of *S. racemosus* (× 520)

Table 1

The effect of microfungi[a] on the occurrence of Fusarium udum[a] g^{-1} dry soil /x 10^3/ in the successive months

Name of organism amended in soil with F. udum	One month[b]			Two months[c]			Three months[d]		
	Ratio of test fungus to F. udum								
	80 : 20	50 : 50	20 : 80	80 : 20	50 : 50	20 : 80	80 : 20	50 : 50	20 : 80
Fusarium udum Butler	5.50±0.6[e]	12.00±0.7	14.00±0.7	2.15±0.3	6.40±0.5	10.10±0.4	0.60±0.06	1.40±0.4	2.00±0.0
Aspergillus flavus Link	21.00±1.2[f]	22.55±1.0	11.50±1.0	22.50±2.0	20.50±1.4	14.70±0.3	21.50±1.5	19.50±1.0	18.00±1.0
F. udum	4.90±0.4	10.00±1.0	10.57±0.5	1.80±0.4	5.64±0.5	6.60±0.6	0.20±0.01	0.50±0.02	0.70±0.01
A. niger van Tieghem	46.00±1.1	17.81±0.8	20.00±0.5	44.17±1.0	22.80±1.0	18.00±1.3	42.50±1.5	26.00±2.0	18.00±0.7
F. udum	6.00±1.1	10.50±0.6	12.30±1.1	4.22± 0.4	8.10±1.0	10.12±1.0	1.60±0.4	1.60±0.3	1.60±0.3
A. terreus Thom	12.11±1.2	10.00±1.0	7.00±0.8	17.14±1.0	15.20±0.8	13.70±0.7	15.75±1.0	14.00±0.9	22.60±1.6
F. udum	20.00±1.5	21.00±1.3	36.00±1.8	18.10±1.0	18.78±1.1	27.15±0.5	17.7±±1.0	19.00±1.0	28.00±1.2
Cunninghamella echinulata Thaxter	4.50±0.5	3.00±0.3	1.10±0.2	3.29±0.6	2.11±0.3	0.80±0.1	0.80±0.1	0.60±0.05	0.50±0.2
F. udum	4.55±0.2	8.00±0.9	10.50±0.5	6.15±0.6	9.80±0.7	12.15±0.9	11.82±0.5	17.20±1.2	18.00±1.0
Penicillium citrinum Thom	30.80±2.1	12.00±1.1	5.00±0.5	20.22±1.2	13.27±0.0	2.55±0.2	12.00±0.4	5.00±0.4	1.00±0.4
F. udum	19.50±1.8	22.70±0.9	35.00±1.6	12.25±1.0	17.22±1.2	25.46±2.0	13.00±1.0	16.80±0.8	24.20±1.2
Rhizopus nigricans Ehrenberg	2.15±0.1	0.90±0.05	0.63±0.08	1.90±0.2	0.98±0.7	0.70±0.05	1.00±0.04	0.60±0.02	0.40±0.0
F. udum	7.00±1.4	12.00±0.8	15.00±0.9	8.12±0.6	11.11±0.8	13.21±1.0	10.00±0.4	13.00±0.8	24.00±1.0
Trichoderma viride Pers. ex Gray	15.50±1.8	12.40±0.5	3.00±0.5	15.75±0.9	12.00±0.8	2.78±0.04	16.00±1.2	12.00±0.8	1.50±0.8
Control /F. udum alone/	12.85±0.8	13.78±1.0	15.50±0.3	13.00±0.8	15.00±0.0	21.00±0.5	11.90±0.8	15.75±0.6	20.18±1.1

a - initial population 10 x 10^3 per g dry soil; b - 5th May, 1979; c - 5th June, 1979; d - 5th July, 1979;

e - population of F. udum in inocula mixture of test fungus and F. udum; f - population of test fungus in inocula mixture of test fungus and F. udum

RESULTS AND DISCUSSION

Fusarium udum was observed to penetrate the hyphae and conidiophores/sporangiophores of *A. luchuensis*, *C. echinulata*, *M. subtilissima*, *R. nigricans* and *S. racemosum* (U p a d h y a y 1979). In addition, *F. udum* formed conidia inside the lumen of the hyphae of *C. echinulata* (Figs 1, 2) and chlamydospores inside the host hyphae and conidiophores or sporangiophores of all the test fungi (Figs 3 - 10). Attempts were made to reisolate fungi from dual cultures after 15 days but only *F. udum* could be isolated which suggests necrotrophic parasitism.

Table 2

Analysis of variance /F/ and critical difference /C.D./ for the effect of soil amendments with microorganisms on occurrence of Fusarium udum in relation to different

A - inoculum ratios of the test microbes and F. udum /Reference Table 1/

Surface of variation	First sampling /One month/		Second sampling /Two months/		Third sampling /Three months/	
	F	C.D.	F	C.D.	F	C.D.
Amendment	21.50**	4.87	37.38**	3.18	27.22**	4.87
Inoculum ratio	18.70**	2.75	31.75**	1.69	9.49**	2.76

B - samplings /Reference Table 1/

Surface of variation	80:20 F.udum : microbe		50:50 F.udum : microbe		20:80 F.udum : microbe	
	F	C.D.	F	C.D.	F	C.D.
Amendment	22.60**	3.81	63.46**	2.12	18.10**	7.87
Sampling	1.60	-	12.50**	1.27	5.83*	3.80

* Significant at p = 0.05

** Significant at p = 0.01

The population of *F. udum* increased in the soil amended with *C. echinulata* and *R. nigricans* (in all ratios in all the samplings) apparently suppressing the population of the latter (cf. control, Table 1). Since the population of *C. echinulata* and *R. nigricans* varied in accordance with different percentages of the inoculum of *F. udum* it might be said that the effect was due to presence of the latter. The increase in the population of *F.udum* in soil amended individually with the inocula of *C. echinulata* and *R. nigricans* may, therefore, be correlated with the parasitic nature of *F. udum*. The non-host species *A. flavus*, *A. niger*, *A. terreus* and *P. citrinum* significently (p = 0.01) suppressed the population of *F. udum* (cf. control) in all the inoculum ratio (ref. C. D. for amendment, Table 2). *Trichoderma viride* also suppressed the population of *F. udum* but to a lesser extent. In a separate study these fungi were found to be antagonistic to *F. udum* (U p a d h y a y 1979). The above observation thus provides evidence for a possible mode of survival of *F. udum* on other microfungi as mycoparasite.

The authors are thankful to the Head of the Botany Department Banaras Hindu University for providing laboratory facilities. R.S.U. is grateful to the Council of Scientific and Industrial Research, New Delhi for financial assistance.

REFERENCES

B o o t h C., 1971, The genus *Fusarium*. C.M.I. Kew.

G a r r e t t S. D., 1970, Pathogenic root infecting fungi, Cambridge University Press.

M a r t i n J. P., 1950, Use of acid, rose bengal and streptomycin in the plate method for estimation of soil fungi, Soil Sci. 69: 215 - 232.

N a s h S. M. and W. C. S n y d e r, 1962, Quantitative estimation by plate counts for propagules of the bean root rot *Fusarium* in field soils, Phytopath. 52: 567 - 572.

P a r k D., 1965, Survival of microorganisms in soil. [In: Ecology of Soil-Borne Plant Pathogens, Ed. K. F. Baker, W. C. Snyder, pp. 88 - 97], J. Murry.

U p a d h y a y R. S., 1979, Ecological studies on *Fusarium udum* Butler causing wilt disease of pigeon-pea. Ph. D. Thesis, Banaras Hindu Univ.

W a r c u p J. H., 1979, The soil plate method for isolation of fungi from soil, Nature 166: 116 - 118.