

サトウキビ苗立枯病における病菌, *Curvulria lunata* と根こぶ線虫, *Meloidogyne javanica* との協合

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reaction for phloroglucinol-HCl reagent, whereas inoculated walls gave a positive reaction. Fig. 1 shows the site of tritiated phenylalanine incorporated in the parenchyma cell walls. Control slices did not produce silver grain (Fig. 1, A). In inoculated slices, noticeable silver grains were observed first in the inner layers of the cell wall and then in the middle lamella (Fig. 1, B, C, D). Since lignification was different from site to site in the infected parenchyma³⁾, various sites of incorporation were observed in the same material. However, there is no evidence for the presence of lignin deposition in the middle lamella alone and the absence of the deposition in the inner layers, the lignification seems to take place first in the inner layers. Also, this result seems to show that the silver grains were not due to any chemical fogging as artifacts. It is generally assumed that inner layers of a cell wall may be composed mainly of cellulose and a middle lamella may be of pectic substance. Therefore, we supposed that the lignification of the parenchyma cell walls induced by the infection was initiated first in the cellulose microfibrils of the inner layers and then in the pectic layer of the middle lamella.

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Interrelationship of a Fungus, *Curvularia lunata* with Root-Knot Nematode, *Meloidogyne javanica* in Sugarcane Seedling Blight**

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S.M. Paul Khurana* and Shatrughna Singh** : サトウキビ苗立枯病における病菌, *Curvularia lunata* と根こぶ線虫, *Meloidogyne javanica* との協合**

Disease complexes involving plant pathogenic nematodes and fungi have been well established in the past as reviewed by Goswami, *et al.*¹⁾ and Pitcher²⁾.

Recently a serious decline of Indo-American hybrid sugarcane seedlings (developed from crosses between Indian *Saccharum spontaneum* and Puerto Rican or Hawaiian sugarcane cultivars) due to blight was observed at this Institute**. Uprooting of these seedlings revealed that their root system was poor, discolored, rotting and had root-knots indicating infection with a fungus and nematode. In some cases collar rot was also observed. The fungus and nematode were isolated, tested for their pathogenicity and identified as *Curvularia lunata* (Walker) Boed. and *Meloidogyne javanica* (Treub.) Chitwood, respectively. Therefore, a study was undertaken to determine the relationship of the isolated fungus and nematode on infection to seedlings of sugarcane.

The experiment was conducted in a glasshouse. Healthy seedlings of hybrid sugarcane (PR.

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1) Goswami, B.K., Raychaudhuri, S.P., Gupta, J.N. and Singh, D.V. (1970). *Nematologica* 16. (in press) 2) Pitcher, R.S. (1965). *Helminth. Abstr.* 34 : 1-17.

1091×SES. 70), which were recorded to be most susceptible to the complex infection in nature, were transplanted to sterile 10 cm pots containing heat sterilized soil (1 kg/pot), at 3 months age. After a week's lapse, when the seedlings were established, they were inoculated to have six different treatments including control.

Single spore culture of the isolated pathogenic fungus was maintained on oat meal agar medium. The inoculum was prepared by suspending a heavy amount of conidia in sterile, distilled water. Two ml of this suspension was introduced in the rhizosphere of plants for inoculation. Nematode inoculum was prepared by placing few egg masses of the pathogenic nematode, from infected roots of sugarcane seedlings, in watch glasses having distilled water and incubated for 24 hours at room temperature. Two ml of the nematode-suspension containing 100 hatched larvae was used for inoculation by pouring it in the rhizosphere of each plant. Test plants were exposed to six different inoculation treatments, viz. fungus (F); nematode (N); fungus and nematode simultaneously (F+N); fungus followed by nematode after 10 days (F+n), nematode followed by fungus after 10 days (N+f) and control, inoculated with distilled water (C). Ten replicates/treatment were taken and the experiment was repeated at 7 days interval to have similar environmental conditions.

Pathogenicity and complex behaviour In plants of treatment F, where only fungus was inoculated, the roots were black, thin and dwarf as compared to nematode infected roots. Often the root ends also showed rotting. In the case of the seedling inoculated with nematode only (N) revealed little blackening, dwarfing as well as poor anchorage. The seedlings inoculated with fungus and nematode simultaneously (F+N) exhibited very detrimental effects, viz. their root system was very poor. Rootlets were badly discolored, rotting at several places and had blind-tips incited by the nematode. With the advancing age of infection, most of the F+N treatment plants had few dwarf, yellowing and withering leaves and such seedlings died soon. None of the symptoms described above developed in the control plants.

Interrelationship of the fungus and nematode The observations were recorded forty-five days after the last inoculations (F+n and N+f) with regards to the symptoms, plant mortality, height and weight of roots, number of green and withering leaves, number of tillers and the nematode population/500 g soil. Average of the data from both trials i.e. observations based on 20 plants/treatment are presented in Table 1.

It is clear from the Table 1 that there exists a significant synergistic effect of pathogens when inoculated simultaneously or at an interval of 10 days corresponding to the preceding infection. All the plants in treatments, viz. F+N and N+f, exhibited heavy response to the complex infection but there was a significant decrease in nematode population in soil as compared to N treatment. Collar rot was observed to be most frequent in plants of F, F+N and N+f treatments, however, maximum (30%) mortality of plants was observed in F+N treatment.

The nematode, *Meloidogyne javanica* infecting sugarcane is quite common but the pathogenic fungus, *Curvularia lunata*, infecting sugarcane roots is a new record, however, Singh³⁾ isolated it from leaves of many sugarcane varieties.

Distinct synergistic effects were observed when the seedlings were infected with fungus and nematode. Complex infection occurred when seedlings were inoculated simultaneously or at intervals by the corresponding pathogen to the preceding infection. Although both the pathogens, when inoculated singly, caused considerable damage to the host; pathogenic effects were enhanced and very detrimental in F+N treatment when both pathogens were inoculated simul-

3) Singh, S. (1969). Science and Culture 35 : 396.

Table 1. Effect of root-knot nematode and the fungus infection on sugarcane seedlings

Treatment	Average of 20 plants/treatment (10 plants per treatment in 2 trials)					
	L	Root Wt.	Leaves G/W	Tillers	Symptom, Mortality	Nematode Count ^{a)}
F	17.60	1.80	5.4/5.4	1.2	+++ 10%	0
N	22.80	2.20	5.2/3.2	1.4	++ 0%	1,600
F+N	18.50	1.26	3.8/5.0	0.6	++ 30%	920
F+n	21.80	1.28	4.6/3.4	2.0	+++ 15%	840
N+f	25.60	1.40	5.2/2.8	1.0	+++ 15%	840
Control	50.00	3.50	10.0/3.0	5.0	0%	0

L=Length; Wt.=Weight; G=Green; W=Withering; Symptoms: +++=Severe; ++=Moderate.

^{a)} Nematode population/500 g soil.

taneously. However, reduction in nematode population was observed as a result of complex infection. In contrast to the previous records of increase in nematode population due to simultaneous infection, we have found about 50% decrease in this case. This does not mean, of course, that the known synergistic effect of several fungi and nematode infection complexes where the nematode population is reported to be increased, does not occur. It merely indicates the complexity of different organisms which may behave differently in different combinations, as also evidenced by Bird⁴⁾.

Very recently, Santo and Holtzman⁵⁾ observed a similar synergistic interaction between lesion nematode, *Pratylenchus zeae* and root-rot fungus, *Pythium graminicola* on sugarcane, where the simultaneous infection had additive effects, however, nematode populations were much less when nematodes were inoculated to the roots in the presence of the fungus than when the fungus was absent. Also, Koike and Roman⁶⁾ found a similar reduction in nematode population while dealing with the same fungus against the nematode, *P. brachyurus* (pathogenic to sugarcane but not interacting with *Pythium*) on sugarcane. Though they found a distinct reduction in nematode population in roots as well as in rhizosphere-soil of plants with N+F treatment, but they presumed it to be due to sampling error and therefore, did not attach any significance to it.

The enhanced pathogenic effects resulting from interaction between fungus and nematode can be accounted for sharing and greater virulence developed for competition to survive in the available root-area for their pathogenesis and metabolic pools.

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4) Bird, A.F. (1969). Nematologica, 15: 201-209. 5) Santo, G.S. and Holtzman, O.V. (1970). Phytopathology 60: 1537. (abstr.) 6) Koike, H. and Roman, J. (1970). Ibid. 60: 1562-1565.