

The relationship between phenolic content and *Tylenchulus semipenetrans* populations in nitrogen-amended citrus plants

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SUMMARY

Treatment with nitrogenous compounds, viz., ammonium nitrate, ammonium sulphate and urea as well as fumigant DBCP on citrus seedlings showed an association between extensive synthesis of phenols in roots and the reduction in *Tylenchulus semipenetrans* in soil and roots. The content of phenols in treated roots was greater than in nematode infected untreated roots. The phenol content of nematode-infected roots was higher than untreated healthy roots. Higher levels of phenols in treated roots were associated with higher reduction in numbers of *T. semipenetrans* larvae and females and vice-versa. It was concluded that mineral nitrogenous amendments stimulated plants to synthesize extensive phenolic compounds and this, in turn, determined the nematode propagation inversely.

nature of resistance in plants. This study was conducted on citrus treated with inorganic amendments in search of such relationship.

Materials and methods

Six-month-old lime seedlings, *Citrus aurantiifolia* Swingle, were transplanted singly in 15-cm clay pots filled with autoclaved clay-loam soil. Following, the citrus nematode, *Tylenchulus semipenetrans* Cobb, was introduced into the rhizosphere of seedlings at about 5,000 larvae / pot. Two weeks after planting, the treatments were established : ammonium nitrate, ammonium sulphate or urea at two rates, viz., 0.2 and 0.4 g N /seedling. Another set was treated with the halogenated 1,2 dibromo-3-chloropropane (DBCP) known as Fumazone ® 75% EC at the rates of 24 and 36 liter /ha, while the fifth set was left untreated serving as controls. Each treatment was replicated four times and the whole experiment was grown in the greenhouse at $30 \pm 5^\circ$ for 60 days and then terminated. The nematode extraction from soil was

made by means of Oostenbrink's technique (1960), while root populations of the nematode were extracted with the waring blending technique of Fallis (1943) according to the modification of Taylor and Loegering (1953) procedure. Mono-, poly- and total-phenols were estimated in roots colorimetrically after extraction from 0.5 g ground root samples in 95% ethanol. Samples were left at 5° for 48 h then crude extracts were filtered through Whatmann No. 1 filter paper and transferred quantitatively into 100 ml volumetric flasks. Snell and Snell method (1953) with folin reagent was followed for the determination. Absorption was measured at 660 m μ with a photoelectric colorimeter and readings were compared with those obtained from pyrogallol standard solution. Phenolic values were expressed as $\mu\text{g/g}$ fresh weight.

Results

The phenolic fractions of citrus roots at the various treatments are given in Table 1. The inorganic nitrogenous fertilizers and DBCP treat-

Table 1
Effect of inorganic amendments and a fumigant nematicide (DBCP) on phenolic content of citrus roots as correlated with *T. semipenetrans* populations ($\mu\text{g/g}$ fresh weight).

Treatment	Plant weight (g)		Phenol content			Nematode population			Nematode reduction %
	shoot	root	mono-	poly-	total	soil	root	total	
P+N+amm. nitrate I	2.50	1.67	1,000	700	1,700	1,414	91	1,505	68.48
» II	6.00	1.83	1,430	990	2,420	322	0	322	93.26
P+N+amm. sulphate I	2.62	1.75	1,100	300	1,400	944	87	1,031	78.41
» II	5.62	2.00	1,800	900	2,700	200	40	240	94.97
P+N+urea I	3.83	1.35	1,300	300	1,600	306	66	372	92.21

tments assumed an increase in the phenol level present in root tissues up to 1,000-2,300 μg as compared with the untreated healthy roots, and 200-1 500 μg as compared with untreated nematode-infected roots. Data also showed that nematode infection increased phenol content of roots by 800 μg . Apparently, there was a consistent trend of phenolic content that was closely related to a higher suppression of nematodes by the different treatments. This emphasis is particularly evident with DBCP lower and higher rates treated plants where *T. semipenetrans* total populations were reduced by 98.95 and 97.15%, respectively. These plants contained the highest level of 2,500 and 2,300 μg phenols, respectively. On the other hand, decreased levels of phenolics were synthesized in the ammonium nitrate, ammonium sulphate and urea treatments, and meanwhile a slightly less nematode elimination was noted under these materials. A trend appeared that in all treatments and rates, increased phenols were associated with low counts of nematodes and vice-versa.

Host growth data presented in Table 1 showed that treatment with present nitrogenous fertilizers and DBCP improved seedlings stand significantly as compared to nontreated controls. These data were essentially indicative that phenol increment in tissues was not due to a plant damage caused by treatments. It was, however, noted that the double dose of urea induced a reverse effect on host growth, probably refers in part to the toxic biuret impurity in commercial urea.

Table 1 also points out to the observation that phenol synthesis in nematode infected roots was higher than those in healthy ones. Evidently, this increment was much less than the increased phenol values formed following inorganic nitrogenous amending.

Discussion

This study showed that phenolics synthesis could be stimulated in plants by application of nitrogenous compounds, nematocides and by nematodes themselves. One of the current host-parasite hypothesis is that the enzymatic oxida-

tion of phenolics gives rise to a hypersensitivity, which appears to be an important defense mechanism (Hung & Rohde, 1973). This hypersensitivity limits growth of invading pathogen in browning hypersensitive tissues (Allen, 1959). Any treatment which helps activation, builds up or stimulates the accumulation of phenolic compounds in tissues may suppress or inhibit expansion of parasitism. This work showed that nitrogenous treatment in the form of three mineral nitrogenous amendments was associated with synthesis of phenolics in treated plants which was closely correlated with a marked decline in nematode populations. This concept is supported by previous work on the significance of phenols for resistance in plants to nematodes and fungi. Biehn, Kuc and Williams (1968), Giebel (1970), Hung and Rohde (1973), Paxton (1975), Silvapalan and Shivanandara-jah (1974), Veech and McClure (1977) and Wakemoto and Yoshii (1958) came to the same conclusion that extensive composition of phenols has been an important feature of plants resistant to fungi and nematodes.

Phenol increment in infected roots was much less than the values formed after inorganic and DBCP treatments. These results are in agreement with past records of Alam *et al.* (1976), Balasubramanian and Purushothaman (1972), Davino *et al.* (1974), De Leo (1964), Epstein (1972, 1975) and Freidman and Rohde (1976). These workers and many others established that the amount of phenolics increased in infected plants, possibly due to breakdown of glucosides as a result of secreted glucosidase by the parasitizing microorganism, which eventually enriched infected tissues with phenolics.

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