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

Taysir BADRA (*) and Dawood M. ELGINDI

Nematology Research Centre, Faculty of Agriculture, Cairo University, Giza, Egypt.

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.

RÉSUMÉ

Relation entre le contenu phénolique de la plante et les populations de Tylenchulus semipenetrans *chez des citrus ayant reçu un amendement azoté*

De jeunes plants de citrus ont reçu trois engrais azotés : nitrate d'ammonium, sulfate d'ammonium et urée à la dose de 0,6, 0,2 et 0,4 g d'azote par plant et un nématicide fumigant, le D.B.C.P., à des doses équivalant à 24 et 36 litres à l'hectare. On a observé que ces produits stimulaient la synthèse par la plante de phénols, dont la teneur augmentait dans les racines. Cette augmentation du contenu en phénols des racines était dans une large mesure associée à une diminution du nombre de larves et de femelles de *Tylenchulus semipenetrans*. Des plus fortes doses de ces produits ont provoqué une synthèse plus intense des fractions phénoliques, apparemment liée à des niveaux plus bas des populations de nématodes. Il semble probable que la production de phénols, stimulée par ces produits, fait partie d'un mécanisme de défense de la plante.

Phenolic compounds have been recognized to play a major role in pathophysiological reactions in tissues inducing a defense mechanism and resitance to aggressive pathogens (Allen, 1959 ; Rohde, 1965). Ohkawa and Torikata (1972), Pi and Rohde (1967), Giebel (1970, 1971), Hung and Rohde (1973) and Rezk (1976) recorded that phenolic accumulation was high in fungus — and nematode — resistant plants. It appears, therefore, that the study of phenolics as an etiological agent is important to an understanding of host-parasite relationships and the

(*) Present address : IITA, Oyo road, PMB 5320, Ibadan, Nigeria.

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 aurantifolia* 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 ± 5° 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 colorimeterically 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 mμ with a photoelectric colorimeter and readings were compared with those obtained from pyrogallol standard solution. Phenolic values were expressed as μg /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 trea-

Table 1

Effect of inorganic amendments and a fumigant nematicide (DBCP) on phenolic content of citrus roots as correlated with *T. semipenetrans* populations (μg/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
» II	2.75	1.00	1,700	300	2,000	208	0	208	95.64
P+N+DBCP 24 liter/ha	2.54	1.13	1,200	1,300	2,500	0	50	50	98.95
» 36 liter/ha	2.95	1.16	1,700	600	2,300	96	40	136	97.15
P+N	1.87	0.50	800	400	1,200	4,618	157	4,775	—
P without N	2.00	0.75	300	100	400	—	—	—	—

P = Plant, N = Nematode, I and II = 0.2 and 0.4 g N/seedling, respectively.
Monophenols values were found by substracting polyphenols from total phenols.
Nematode reduction was computed with reference to total numbers of P+N.

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 oxidation 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 Shivanandarajah (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.

References

Alam, M. M., Ali, Q. G., Masood, A. & Khan, A. M. (1976). Studies on the chemical changes induced by the infection of the root-knot nematode *(Meloidogyne incognita)* in tomato and eggplant and the stunt nematode *(Tylenchorhynchus brassicae)* in cabbage and cauliflower roots. *Indian J. exper. Biol.*, 14 : 517-518.

Allen, P. J. (1959). Physiology and biochemistry of defense. In : Horsfall J. G. & Dimond, A. E. (Eds)

Plant Pathology, vol. I. *The diseased Plant.* New York and London, Academic Press, 435-469.

BALASUBRAMANIAN, M. & PURUSHOTHAMAN, D. (1972). Phenolic contents of root-knot affected tissues. *Indian J. Nematol.*, 2 : 77-79.

BIEHN, W. L., KUC, J. & WILLIAMS, E. B. (1968). Accumulation of phenols in resistant plant-fungi interactions. *Phytopathology*, 58 : 1255-1260.

DAVINO, M., CATARA, A., ROTTA, P. & GRASSO, G. (1974). Variation in the content of free phenolics in citrus plants inoculated with *Phoma tracheiphila. Revta Pat. veg.*, 10 : 123-136.

DE LEO, P. (1964). Variation in amounts of ortho-dihydroxy phenols after transplanting and infection of tomato varieties resistant and susceptible to *Fusarium oxysporum. Phytopath. Medit.*, 3: 95-108.

EPSTEIN, E. (1972). Biochemical changes in terminal root galls caused by an ectoparasite nematode, *Longidorus africanus. J. Nematol.*, 4 : 246-250.

EPSTEIN, E. & COHN, E. (1975). Physiological changes in *Longidorus africanus.* In : *Scientific activities 1971-1974, Div. Nematol.*, Inst. Pl. Prot., Bet Dagan, Israel, 128.

FALLIS, A. M. (1943). Use of the waring blendor to separate small parasites from tissues. *Can. J. publ, Hlth*, 34 : 44.

FRIEDMAN, P. A. & ROHDE, R. A. (1976). Phenol levels of tomato cultivars infected with *Pratylenchus penetrans. J. Nematol.*, 8 : 285.

GIEBEL, J. (1970). Phenolic content in roots of some Solanaceae and its influence on IAA-oxidase activity as an indicator of resistance to *Heterodera rostochiensis. Nematologica*, 16 : 22-32.

GIEBEL, J., KRENZ, J. & WILSKI, A. (1971). The formation of lignin-like substances in roots of resistant potato under the influence of *Heterodera rostochiensis* larvae. *Nematologica*, 17 : 601.

HUNG, C. & ROHDE, R. A. (1973). Phenol accumulation related to resistance in tomato infection by root-knot and lesion nematodes. *J. Nematol.*, 5 : 253-258.

OHKAWA, M. & TORIKATA, H. (1972). Studies on the resistance of Japanese pears to the black spot fungus, *Alternaria kikuchiona.* X. The relationship between phenolic compounds in Japanese pears and leaf browning by host specific toxin produced by the fungus. *J. Jap. Soc. hort. Sci.*, 41 : 119-126.

OOSTENBRINK, M. (1960). Estimating nematode populations by some selected methods. In : Sasser, J. N. & Jenkins, W. R. (Eds) *Nematology*, Chapel Hill, Univ. N. Carolina Press : 85-102.

PAXTON, J. D. (1975). Phytoalexins, phenolics and other antibiotics in roots resistant to soil-borne fungi. In : Bruelh, G. W. (Ed.) *Biology and control of soil-borne plant pathogens*, St. Paul, Minnesota, U.S.A., American Phytopath. Soc. : 185-192.

PI, C. L. & ROHDE, R. A. (1967). Phenolic compounds and host reaction in tomato to injury caused by root-knot and lesion nematodes. *Phytopathology*, 57 : 344.

REZK, M. A. (1976). *Studies on plant parasitic nematodes attacking Graminae.* Ph. D. Thesis, Alex. Univ., 159 p.

ROHDE, R. A. (1965). The nature of resistance in plants to nematodes. *Phytopathology*, 55 : 1159-1162.

SILVAPALAN, P. & SHIVANANDARAJAH, V. (1974). Polyphenol content in the feeder roots of nematode tolerant and susceptible tea clones in relation to infestation by *Pratylenchus loosi* Loof. *Tea Quart.*, 44 : 173-176.

SNELL, F. D. & SNELL, C. T. (1953). *Colorimetric methods of analysis including some turbimetric and nephalometric methods.* Vol. III, Princeton, N. J., Toronto, N. Y., London, D. van Nostrand, Inc., 606 p.

TAYLOR, A. L. & LOEGERING, W. Q. (1953). Nematodes associated with lesions in Abaca. *Turrialba*, 31 : 8-13.

VEECH, J. A. & MCCLURE, M. A. (1977). Terpenoid aldehydes in cotton roots susceptible and resistant to root-knot nematode, *Meloidogyne incognita. J. Nematol.*, 9 : 225-229.

WAKEMOTO, S. & YOSHII, H. (1958). Relations between polyphenols contained in plants and phytopathogenic fungi. I. Polyphenols contained in rice plants. *Ann. phytopath. Soc. Japan*, 23 : 79-84.

Accepté pour publication le 3 décembre 1978.