Accelerating the role of phenylalanine in shunting sex differentiation of *Rotylenchulus reniformis* by means of some nutrient formulae

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SUMMARY

Fertilization with nitrogen and phosphorus supplemented with several microelements influenced the multiplication and sex differentiation of *Rotylenchulus reniformis* associated with cowpeas. Phosphorus fertilization reduced nematode build up and increased percentages of non-feeding males, while fertilization with other elements either did not affect, or delayed sex differentiation.

Total amino acid content showed no significant increases or decreases in roots, but significant differences occurred among treatments with regard to the content of phenylalanine and tyrosine. Treatments induced slight variations in the phenylalanine content in tissues, which correlated well with alterations in sex differentiation of associated *R. reniformis*. When phenylalanine constituted 0.50-0.89 % of the total analyzed amino acids, sexual differentiation of the larvae to either sex was delayed or stopped (51-61 % larvae compared with 45 % in controls) ; when phenylalanine constituted 1.33-1.48 % of the total amino acid, larvae preferentially differentiated as males (53-79 % males compared with 25 % in controls). It is suggested that the increase in phenylalanine could be important due to its role as a precursor in the synthesis of phenylpropionic phenolics in cowpeas.

RÉSUMÉ

Renforcement de l'action de la phénylalanine dans la détermination du sexe chez Rotylenchulus reniformis *par le moyen de quelques formules de fumure*

La fumure azotée et phosphorée additionnée de plusieurs microéléments a modifié la multiplication et la détermination du sexe chez *Rotylenchulus reniformis* associé à *Vigna unguiculata*. La fumure phosphorée a réduit le développement des populations de nématodes et augmenté le pourcentage de mâles, alors que les autres fumures n'avaient aucun effet ou retardaient la différenciation sexuelle.

La teneur totale des racines en amino-acides est restée invariable mais des différences significatives ont été observées entre les traitements en ce qui concerne leur contenu en phénylalanine et en tyrosine. Les traitements ont provoqué des variations limitées du contenu des tissus en phénylalanine qui sont corrélées avec des altérations dans la différenciation sexuelle chez les *R. reniformis* associés à la plante. Quand la phénylalanine représentait 0,50-0,89 % des acides aminés totaux la différenciation sexuelle des larves vers l'un ou l'autre sexe était retardée ou stoppée (51-61 % des larves contre 45 % chez les témoins) ; quand la phénylalanine représentait 1,33 à 1,48 % des acides aminés totaux, les larves évoluaient préférentiellement en mâles (53-79 % de mâles contre 25 % chez les témoins). Les auteurs émettent l'hypothèse que la phénylalanine jouerait un rôle comme précurseur des composés phényl-propioniques chez *Vigna unguiculata*.

There is little information about the effect of the aromatic phenylalanine and tyrosine amino acids on parasitism and sex differentiation of nematodes. These aromatic compounds can act on nematodes directly, or indirectly through other by-products, restricting the growth of nematodes in tissues (Badra & Elgindi, 1979 ; Giebel, 1970). In this study we investigated the

influence of some inorganic fertilizers on sex differentiation in *Rotylenchulus reniformis* in cowpea roots.

Materials and methods

Cowpea plants, *Vigna unguiculata* L. Walp cv. Azmerely, were grown, in a greenhouse at 25-30°, in 15 cm clay pots containing 1 kg autoclaved clay loam soil, inoculated with 3 000 individuals of reniform nematodes, *Rotylenchulus reniformis* Linford & Oliveira, 1940. The nutrient solutions symbolized in Table 1 as N0, N2, N4, P0, P2, P4 and N1P1 including five microelement supplements (Cu, 0.025 ; MO, and 2 g of fresh roots, respectively extracted by Oostenbrink's (1960) and the fragmentation (Taylor & Loegering, 1953) techniques. Amino acids were extracted from 0.25 root tissues, separated by means of two-dimentional paper chromatography (Block, Durrum & Zweig, 1958), and characterized by their adsorption.
0.001 ; B, 0.793 ; Mn, 0.056 and Fe, 0.178 meq/l) were applied 200 ml/pot, ten days after nematode inoculation, at six weekly intervals. Each treatment and the control (no nutrients) were replicated six times ; the experiment lasted from August to October and was harvested in early November. At harvest nematode densities in each pot were estimated from 250 g of soil

Results

The fertilizers with nitrogen and phosphorus plus microelements significantly depressed soil and root populations of *R. reniformis*. The sex ratio and percent of the non-feeding males were drastically altered by P0, P2 and P4 formulae (Fig. 1). Development and sex differentiation of the larvae were markedly affected by the N1P1 formula. About 61% of the soil nematodes remained as sexually undifferentiated larvae compared with 45% larvae in the control.

The total amino acid content of treated roots varied with treatments, but was not significantly different from untreated controls (Tab. 2). There were differences in the relative concentrations of phenylalanine and tyrosine among treatments. Figure 1 shows the changes in percentage composition for these two amino acids in roots and sex differentiation of *R. reniformis*. The per-

Table 1
Macroelement composition of applied nutrient formulae

Compound and Treatment	*Milliequivalent/ liter*	*Compound and Treatment*	*Milliequivalent/ liter*
N0 :		*P0 :*	
K_2SO_4	4.50	KNO_3	5.17
$CaSO_4$	34.33	$Ca(NO_3)_2 \cdot 4H_2O$	47.11
$NaH_2PO_4 \cdot 2H_2O$	9.05	Na_2SO_4	8.03
$MgSO_4 \cdot 7H_2O$	30.61	$MgSO_4 \cdot 7H_2O$	30.61
N2 :		*P2 :*	
KNO_3	5.17	KNO_3	5.17
$Ca(NO_3)_2 \cdot 4H_2O$	47.11	$Ca(NO_3)_2 \cdot 4H_2O$	47.11
$NaH_2PO_4 \cdot 2H_2O$	9.05	$NaH_2PO_4 \cdot 2H_2O$	18.10
$Mg(NO_3)_2 \cdot 6H_2O$	31.59	$MgSO_4 \cdot 7H_2O$	30.61
$NaNO_3$	127.41	*P4 :*	
N4 :		KNO_3	5.17
KNO_3	5.17	$Ca(NO_3)_2 \cdot 4H_2O$	44.71
$Ca(NO_3)_2 \cdot 4H_2O$	47.11	$NaH_2PO_4 \cdot 2H_2O$	36.19
$NaH_2PO_4 \cdot 2H_2O$	9.05	$MgSO_4 \cdot 7H_2O$	30.61
$Mg(NO_3)_2 \cdot 6H_2O$	31.59		
$NaNO_3$	491.44		
N1 P1 :			
KNO_3	5.17		
$Ca(NO_3)_2 \cdot 4H_2O$	47.11		
$NaH_2PO_4 \cdot 2H_2O$	9.05		
$MgSO_4 \cdot 7H_2O$	30.61		

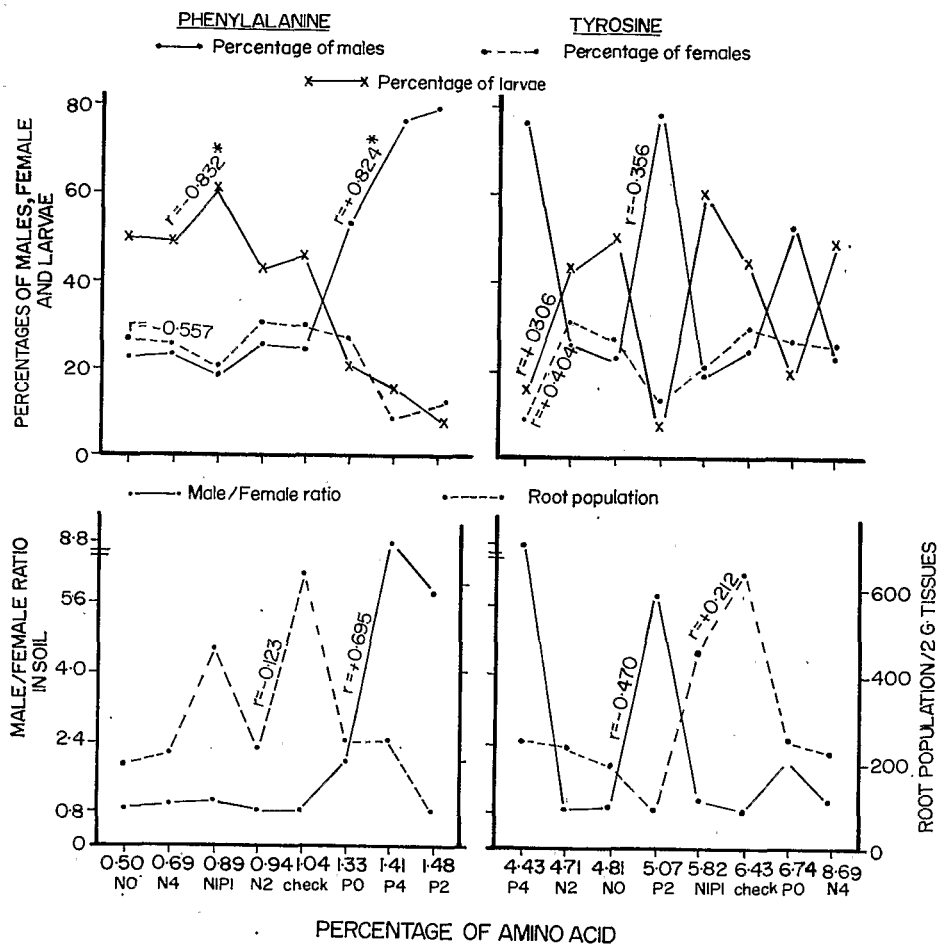

Fig. 1. Relationship between relative amount of phenylalanine or tyrosine and proportional development of *R. reniformis* stages in soil and/or roots. An * indicates a significant correlation, The correlation between phenylalanine and male/female ratio was only very strong over 1.04%.

centage composition of phenylalanine in roots had : *i*) significant positive and negative correlations with percentages of males and larvae in soil, respectively, *ii*) non significant negative correlations with percentage of females in soil and numbers on roots, respectively, and *iii*) non-significant positive correlation with the nematode sex ratio in soil.

Discussion

Present data suggest that percentage composition of phenylalanine and tyrosine in roots is linked to sexual differentiation of *R. reniformis*. We suggest the presence of a shunt level of phenylalanine (0.94-1.04%) below which, a large proportion of the larvae delay or stop their differentiation to either sex. Beyond that threshold, sexual differentiation is toward higher percentages of males. This assumption that phenylalanine might play a part in the present imbalance in sex differentiation is particularly important with reference to cowpeas. Phenylalanine is a primary metabolite in the biosynthesis pathways leading to the phenylpropionic phenolics (Street & Cockburn, 1972), and some phytotoxins and phytoalexins derived from phenylalanine have been detected in cowpeas, e.g. benzoic acid, phenylbutyric acid, phaseollin, phaseollidin and Kievitone (Toussoun *et al.*, 1968 ; Deverall, 1976).

Table 2

Absolute and relative amounts of phenylalanine and tyrosine in cowpea roots after the application of some nutrient formulae (µg/100 mg dry wt.)

Treatment	*Phenylalanine*	*Tyrosine*	*Total amount of 13 inclusive amino acids*
Nitrogen level :			
NO	66 **	637 **	13 243
	(0.50)	(4.81)	
N2	105	526 **	11 161
	(0.94)	(4.71)	
N4	100	1 261 **	14 507
	(0.69)	(8.69)	
Phosphorus level :			
PO	108	546 **	8 102
	(1.33)	(6.74)	
P2	148 **	509 **	10 034
	(1.48)	(5.07)	
P4	152 **	477 **	10 772
	(1.41)	(4.43)	
N1 P1	111	730	12 548
	(0.89)	(5.82)	
Untreated control	119	738	11 483
	(1.04)	(6.43)	
LSD 0.05	20	69	6 522
LSD 0.01	28	94	8 877

Values in parentheses represent percentage of each amino acid in the total composition.
** Significantly different from untreated control at 0.01.

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