

THE DEVELOPMENT OF ROTYLENCHULUS RENIFORMIS AND TYLENCHULUS SEMIPENETRANS ON GRAPE ROOTS .

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ملخص

تبين نتائج الدراسة ان كل من النيماتودا الكلوية ونيماتودا الحمضيات تضع بيضها داخل كيس جلاتيني خارج الجذر وان متوسط عدد البيض في الكيس الواحد بلغ ٨٤ بيضة للنيماتودا الكلوية و ٣٦ بيضة لنيماتودا الحمضيات وان مدة دورة حياة النيماتودا الكلوية ونيماتودا الحمضيات قد بلغت ٣٠ و ٤١ يوماً لكل منهما على التوالي . وأن دورة حياة كل منهما تنقسم الى طورين هما :-
الطور الاول : وهو الطور المتجول في التربة وهو غير متطفل في النيماتودا الكلوية ومتطفل في نيماتودا الحمضيات وتبلغ مدته ٢١ و ٣١ يوماً لكل منهما على التوالي
الطور الثاني : وهو الطور الساكن والمتطفل على جذور العنب في كل منهما وتبلغ مدته ٩ و ١٠ ايام لكل منهما على التوالي . وتبين الدراسة ان الاناث الصغيرة للنيماتودا الكلوية هي التي تقوم بالتطفل على جذور العنب فقط بينما تقوم جميع اطوار نيماتودا الحمضيات بالتطفل بها في ذلك الذكر

ABSTRACT

The results showed that the average numbers of eggs per an egg mass of R. reniformis and T. semipenetrans were 84 and 36 eggs , and the eggs hatched after 6 and 7 days respectively . The durations of the life cycle were 30 and 41 days , respectively . Life cycles were divided into migratory and sedentary phases . The migratory phase (soil phase) of R. reniformis was not parasitic and lasted for about 21 days ; while that of T. semipenetrans was parasitic and lasted for about 31 days . The sedentary phase of both nematodes was parasitic and lasted for about 9 and 10 days for R. reniformis and T. semipenetrans respectively . Only the young females of R. reniformis were the infective stages ; while all larval stages and young females of T. semipenetrans were able to penetrate root tissues .

INTRODUCTION

The development of R. reniformis and T. semipenetrans have been studied by several authors on various hosts . Their findings revealed that , the complete life cycle of R. reniformis duration ranged from 17 to 19 days (2,8,9) on Cowpea and 32 days on cotton (10) and 19 days on Soybean (11). The life cycle of T. semipenetrans required 6 – 8 weeks on citrus (15) , 14 weeks to be completed on trifoliolate orange and 7 weeks on sour orange and sweet lime (6) . The parasitic phase of R. reniformis was represented by the young females only (2,7,8,9,10 & 13) , while that of T. semipenetrans was represented by the young and larval stages (5,14 & 15) .

It was observed that the second and third stage larvae fed ectoparasitically on cells of citrus roots (14) . Later on the fourth stage larvae were observed with their heads penetrating three to four cells deep in the cortex (15) . Recent studies on the distribution of R. reniformis and T. semipenetrans in grapevine showed that , the grape roots were subjected to attack with these nematodes . Therefore the present work was undertaken to study the life histories and development of both nematodes on grape roots . Also histological studies were practiced to dig for their feeding sites in grape root tissues .

MATERIALS AND METHODS

A Egg hatching study :

In this study the nematode inoculum was originally obtained from cowpea roots and lime roots infected with R. reniformis and T. semipenetrans, respectively. The average number of eggs per an egg-mass of each nematode species was determined by averaging the number of eggs of 10 egg-masses. Egg hatching periodicity was determined as the following : Three egg-masses of each nematode species were placed singly in petri-dishes supplied with water and incubated at 30°C. Number of larvae hatched for each egg-mass was counted daily. The percentage of hatch was also estimated .

B Determination of the duration of life cycle(egg to egg) of R. reniformis and T. semipenetrans:

Grape vitis . vinifera VC AL Zeiny seeds were sown in sterilized loamy sand soil in trays. Tap water was used for watering the trays whenever necessary. Hundred seedling were transferred to pots of 10 cm in diameter at the top containing the same type of soil about 15 days after germination. Seedlings were inoculated after 55 days of germination with 10 egg–masses of either nematode species . Then, three seedling were uprooted at 2 and 3 days intervals for Rreniformis and T. semipenetrans , respectively. Roots were stained with cold lactophenol with acid Fuchsin and soaked in it for period of 24 hours in order to facilitate counting of the nematode stages per seedling . The experiment was terminated when the well developed egg–masses were detected. The number of immature and mature stages of each nematode species was counted .

RESULTS

The results of the egg hatching showed that some eggs hatched the second day and the larvae of both nematodes R. reniformis and T. semipenetrans , were detected (Table 1) . Most of eggs hatched within 5 and 6 da' s . Number of larvae decreased gradually after the seventh day till the 10th day where no newly hatched larvae were observed in both nematodes .

The average number of eggs per an egg–mass of each nematode species were 84 for R. reniformis and 36 for T. semipenetrans . On the other hand , the average percentages of the eggs hatch per an egg–mass were 73.8 and 77.5 in the same order .

The results of determination of life cycle of both nematodes showed that R. reniformis young females parasitized the roots 22 days after the addition of egg–masses (Table 2) . On the 24th day , some females became established with their kidney shape (Fig . 1) . The first eggs deposited in the gelatinous matrices were observed on the 26th day . The life cycle of R. reniformis , egg to egg , on grape roots took about 26 – 30 days .

TABLE 1

Average numbers of hatched larvae per egg-mass per day
of R. reniformis and T. semipenetrans

Time of examination (days)	Avg. Nos. of hatched larvae/ egg-mass / day	
	<u>R. reniformis</u>	<u>T. semipenetrans</u>
1	0	0
2	4.7	0.7
3	8.7	2.0
4	12.3	2.3
5	14.3	3.7
6	11.3	7.0
7	5.7	5.3
8	3.7	3.3
9	1.3	2.3
10	0	1.3
11	0	0
Total	62	27.9

TABLE 2

Average numbers of R. reniformis stages in grape roots
at two - day intervals following addition of 10
egg-masses / plant

Days after addition of egg - masses	Avg. nos. of nematode in all stages / plant
	u
22	17.7
24	41.0
26	113.3
28	187.7
30	202.3

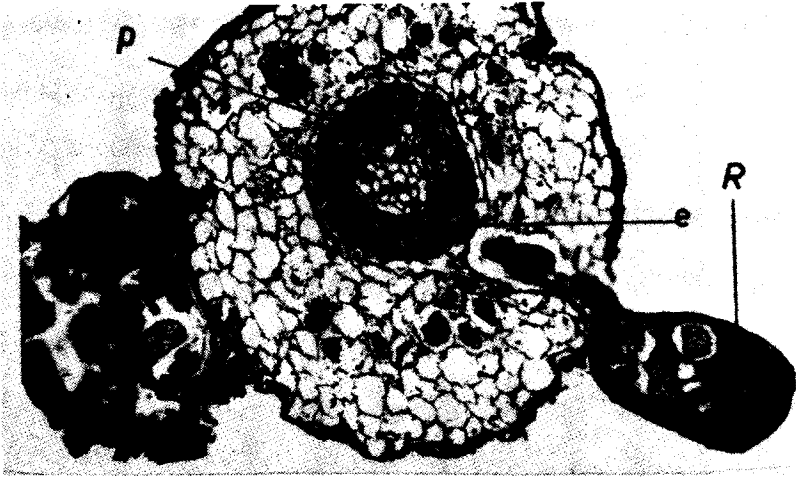


Fig. 1 – Cross section of R. reniformis infected grape roots showing the kidney shape matured female feeding on endodermal and pericycle cells .

TABLE 3

Average numbers of T. semipenetrans stages in grape roots
at three – day intervals following addition of 10
egg-masses / plant

Days after addition of egg-masses	Avg. nos.of nematode in all stages / plant
3 – 23	0
26	13.0
29	21.3
32	35.3
35	66.0
38	83.7
41	97.7
44	103.3

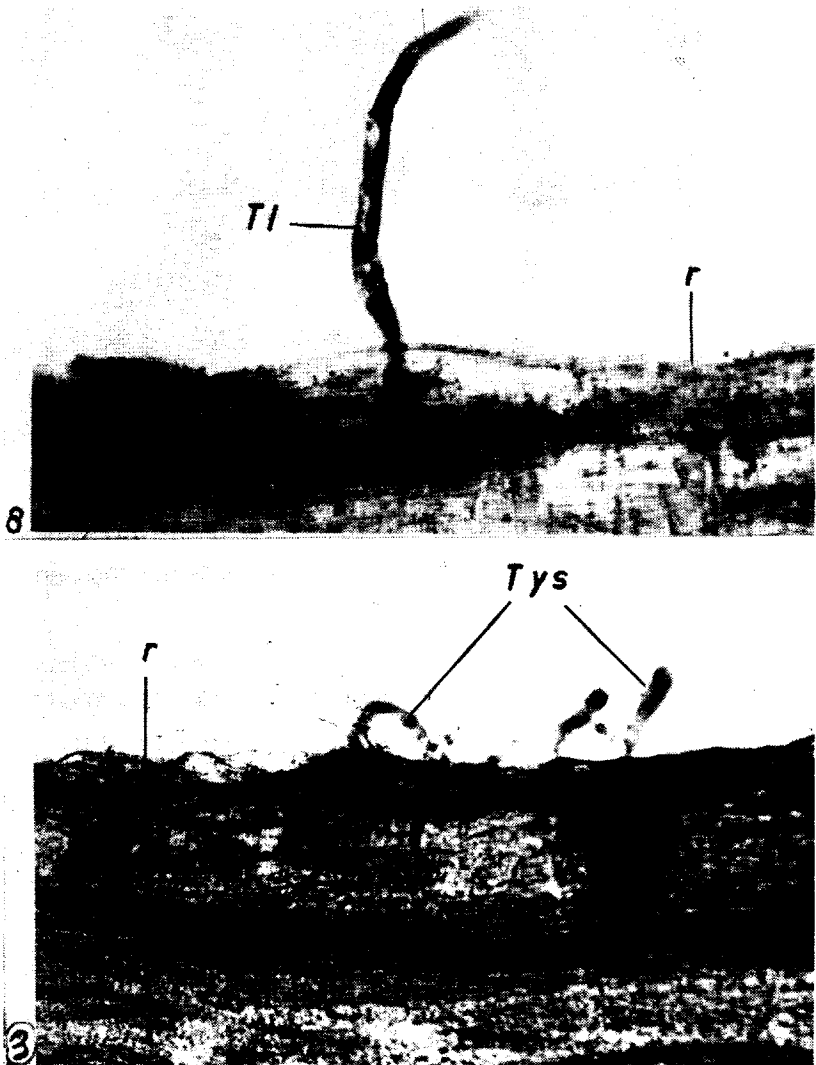


Fig. 2 & 3 – Grape root infected with different stages of T. semipenetrans
2nd stage , (Fig.2) 3rd larvae passed through epidermal cells ,
folded the anterior part in the cortical cells .

The results also showed that T. semipenetrans second – stage larvae parasitized the roots 26 days after the addition of egg–masses (Table 3) .

The second , third and fourth stage larvae were observed with the anterior portion of their bodies embedded in root tissues (Figs. 2&3)

In an unusual case , a male was found with its anterior end embedded in root tissues (Fig. 4). Young females were observed on the roots 32 days after the addition of egg–masses . It seems that they penetrated deeper in the cortical layers (Fig . 5) ; where they established their sedentary life .

No larvae of both nematodes were observed to attack the root tips of grape roots . On the other hand , all stages may occur singly or in groups (Figs.2&3&4) on the roots . Few eggs deposited in egg matrices were noticed on the 28th day . Well – developed egg–masses were formed 41 days after the addition of egg–masses . The complete life cycle of T. semipenetrans took 38 to 41 days (egg to egg) .

DISCUSSION

Adult females of R. reniformis and T. semipenetrans are obligate root parasites leading to a semiendoparasitic sedentary life , with a swollen body ends remain outside the root surface(2,3,4,5,6,14 & 15) . However , both species seem to show unusual behaviour in some cases where they remain entirely embedded in the cortex of the root (3,6 & 8) . In both cases the eggs were laid in masses of the root surface .

The results of this study have shown that the entire life cycle of R. reniformis and that of T. semipenetrans on grape roots were thirty and forty one days respectively . Our results seems to agree with the previous work (2,7,8,9,10 & 13) , and show that both species have variable lengths for the cycle depending on the host .

However , environmental conditions , soil factors and the structure of the nematode community also seems to have an influence on the duration of the cycle (2,6,9,10, & 15)

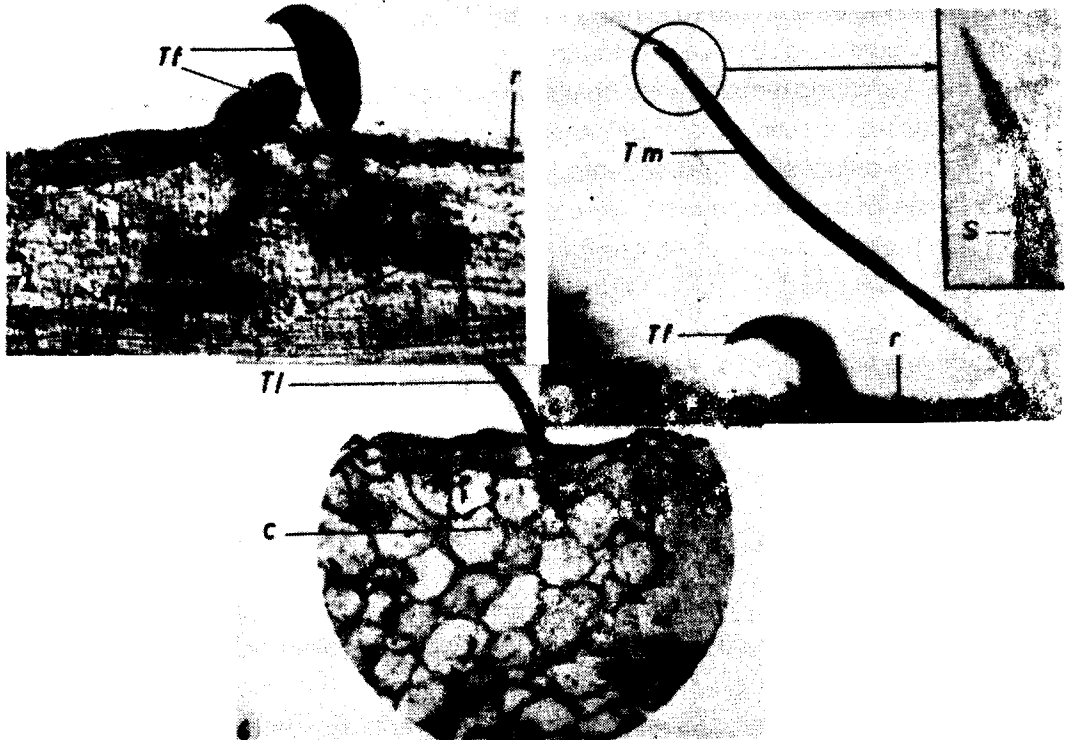


Fig. 4 & 5 – Grape root infected with *T. semipenetrans* females and one male attached to the root (fig. 4) and the altered cells (feeding sites) are heavily stained with acid fuchsin in lactophenol (Fig. 5) .

Fig. 6 – Cross section of grape root showing *T. semipenetrans* larvae passing through epidermal and cortical cells .

(C = cortex ; e = endodermis ; f = feeding sites ;

P = pericycle ; r = roots ; R = *R. reniformis* ;

S = specules ; TF = *T. semipenetrans* female ;

TL = *T. semipenetrans* larvae ; Tm = *T. semipenetrans*

male Tys = *T. semipenetrans* young stages) .

It is also clear that both species have the same developmental phases on grapes . The migratory phase (soil phase) for R. reniformis was not parasitic and took about twenty one days , while its sedentary phase was a parasitic and persisted for nine days . The behaviour of this species on grape roots is in agreement with its behaviour on other host plants (2,7,8,9,10 & 13) . On the other hand both phases of T. semipenetrans seems to be parasitic and were thirty one days for the migratory phase and ten days for the sedentary phase . The larval behaviour of this species seems to be similar to its behaviour on other host plants (4,5,6,14 & 15) where it seems to penetrate the surface layers of cortex .

It is worth noting that penetration does not take place before twenty five days , where the larvae feeds ectoparasitically up to this stage . This could explain the long duration phenomenon of the life cycle , which is mainly due to the ectoparasitically larval stage .

Previous work on the second stage males of T. semipenetrans (4,5,6,14 & 15) suggested that they develop within 7 days in soil without feeding . Our finding contradict with the previous work and show that the males attach themselves to the surface tissues of the root . This attachment seems to suggest a feeding role in males (Fig. 4) . The studies of (4) showed a similar behaviour for the males on roots of bindweed or climbing hempweed M. batatifolia .

Further studies on the behaviour of the males are required to explore in more details its relation with the surface tissues of the root .

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