

Relationship Between *Paratrichodorus* sp. Density, and Growth of Wheat in Pots

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Abstract: The effect of a *Paratrichodorus* sp. (close to *P. tunisiensis*) on the growth of wheat (*Triticum durum* Desf.) was investigated in pots containing different nematode densities and maintained in a growth chamber at 20 C for 40 days. The relation between fresh weight of tops and initial nematode density was according to the equation $y = m + (1 - m)z^{1-x}$. This suggests a tolerance limit of 1.4 nematodes/cm³ of soil under the conditions of the experiment; taking into account the effect of the great nematode mortality, it is estimated to be between 0.15 and 0.35 nematodes/cm³ soil. Models of the growth of the plants and the multiplication of the nematodes (assuming a constant mortality of the nematodes in the absence of roots) which explain the relation between initial and final nematodes densities at initial densities greater than 1 nematode/cm³ soil are described in an appendix. Sections of nematode infested roots showed disorganization of root structure due to abnormal proliferation of lateral roots. Nematode feeding on the root cap and apical meristem caused cessation of root elongation and induced abnormal production of lateral root primordia. **Key words:** stubby root nematode, cereal, tolerance limit, mathematical model, histopathology.

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A *Paratrichodorus* sp., morphologically very close to *P. tunisiensis* Siddiqi (7), has been found in association with durum wheat (*Triticum durum* Desf.) in Apulia, southern Italy. The nematode differs from *P. tunisiensis* in that adults have a shorter body, males have the third supplement closer to the cloacal opening, and females have a different tail shape and vaginal sclerotization with sperm distributed

throughout the uterus and not confined to a spermatheca.

A heavy infestation of the *Paratrichodorus* species was found on durum wheat in March 1978 in Apulia. Large patches of stunted plants were present in the crop. The damaged plants showed stubby roots (Fig. 1) and severe reduction in growth of tops and root systems. Similar symptoms were reported on wheat infested with *P. minor* (Colbran) Siddiqi (= *Trichodorus christiei* Allen) in the United States (2). Growth retardation of several crops by *Paratrichodorus* infestations has also been reported from other countries (8). Little information is available on the relation between population density of *Paratricho-*

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dorus sp. and the growth of their hosts (1). This paper gives the results of an experiment done in a growth chamber on the relationship between *Paratrichodorus* sp. population density and yield of wheat, and a description of the anatomical changes in host roots induced by nematode feeding.

MATERIALS AND METHODS

Plastic pots containing 160 cm³ steam pasteurized sandy loam were inoculated with *Paratrichodorus* sp. extracted from infested soil where durum wheat was growing. Inoculum consisting of adult and juvenile stages were added to soil in a geometric series of 0, 0.125, 0.25, . . . 256 per cm³ of soil (equivalent to 0, 20, 40, . . . 40,960 per pot). The nematodes in water suspension were poured into a cavity made in the soil in each pot. A pregerminated seed of durum wheat cv. Creso was then planted in each pot. Each inoculum level was replicated six times. Six pots containing naturally infested field soil were held without plants and served to calculate the nematode mortality rate. All pots were maintained in a growth chamber at 20 ± 1 C for 40 days when the plants were harvested and the fresh weights of the tops were recorded. Nematodes were extracted from each pot by decanting and sieving and counted.

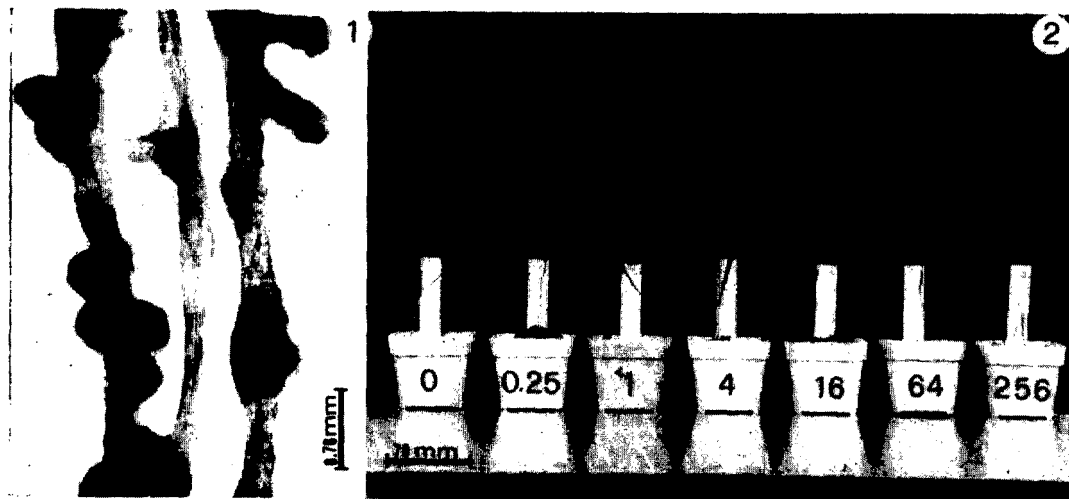
Stubby roots were selected from nematode infected wheat plants for histological

studies. Root segments were fixed in formalin, acetic acid, ethyl alcohol (FAA) for 48 h, dehydrated in tertiary butyl alcohol (TBA), and embedded in paraffin. Sections 10 and 15 μ m thick were stained in safranin-fast green and mounted in Permount.

RESULTS AND DISCUSSION

The relation between fresh weight of the tops of the wheat plants and nematode density at sowing is illustrated in Figure 2 and by the graph of Figure 3. A curve according to the equation $y = m + (1 - m)z^{P-T}$ for $P \geq T$ and $y = 1$ for $P \leq T$ (eq. i) (where y = relative yield; m = relative minimum yield; $z < 1$; P = initial nematode density and $z^T = 1.05$ [T = tolerance limit]) (3) was fitted to the observations. It suggests a tolerance limit (T) of wheat to *Paratrichodorus* sp. of 1.4 nematodes/cm³ of soil and a relative minimum yield $m = 0.25$ (Fig. 3). However, curves with $1 \leq z^T \leq 1.05$ would all fit about equally well to the data. For $z^T = 1$ ($T = 0$), z^P with $P = 1.4$ nematodes/cm³ soil z would be 0.95.

The relation between initial and final nematode densities is given in Figure 3. At all initial densities of 16–256 nematodes/cm³ soil, final densities were 0.0254 times the initial ones. The independence of this figure of nematode density suggests



Figs. 1–2. *Paratrichodorus* sp. on durum wheat cv. Creso. 1). Stubby adventitious roots, due to the nematode feeding activity, along the axis of a principal root of durum wheat. 2) Effect of nematode densities (number of nematodes/cm³ soil) on the growth of durum wheat, 30 days after sowing.

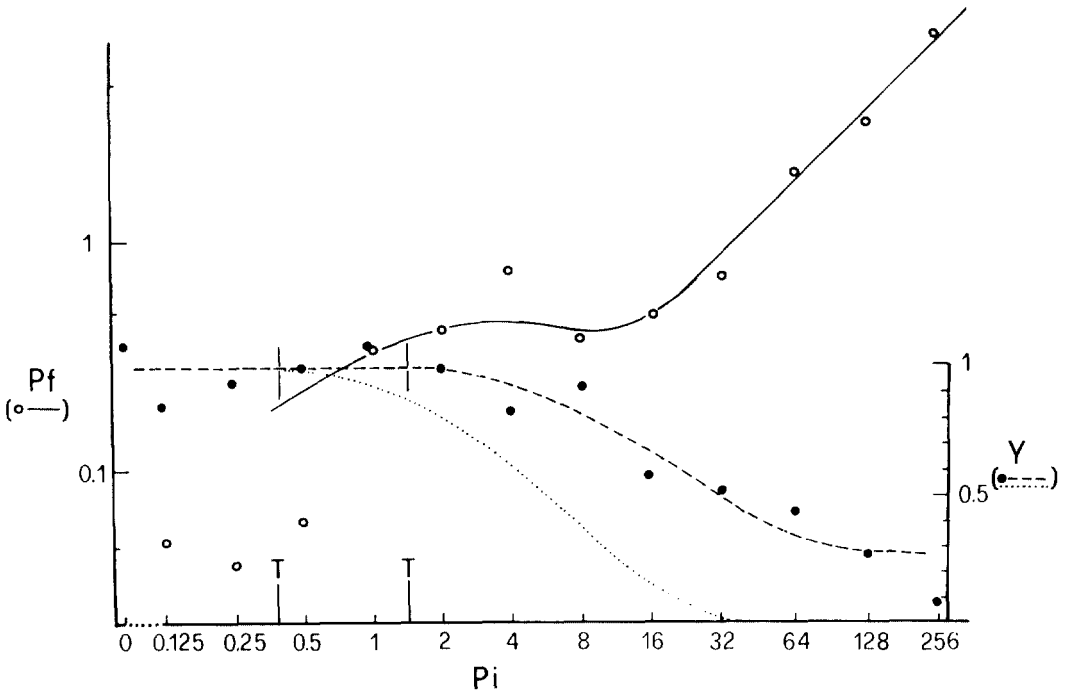


Fig. 3. Relation between initial population density (P_i) of *Paratrichodorus* sp. and relative weight of the tops of wheat plants y (●) and between P_i and final nematode density P_f (○).

----- graph according to $y = m + (1-m)z^{P-T}$ ($T = 1.4$, $m = 0.25$, $z^{-T} = 1.05$).

..... graph according to $y = z^{P-T}$ ($T = 0.35$, $z^{-T} = 1.05$) (presumably, the relation between P_i and y at the end of the experiment if nematode mortality had been small).

— relation between P_i and P_f according to model with continuous redistribution of nematodes on root system, $E = 0.5$ nematodes/cm³ soil, $e^r > 100$ and constant mortality rate of nematodes not feeding on roots (0.0254 times between inoculation and end of experiment).

mortality in the virtual absence of food, although the plants developed to a fair size. A reasonable fit to the observations was obtained by a curve according to the equation $P_f = (ya Mpi/[a P_i + M]) + b (1 - y) P_i$ (eq. ii), where y = relative size of the food source and equal to z^{P_i} (eq. iii) (4,5) with $y = 0.95$ for $P_i = 0.5$ nematodes/cm³ soil; a , the maximum rate of multiplication = 0.65, $M = 1$ nematode/cm³ soil ($M = P_f'$ for $P_i \rightarrow \infty$ and $P_f' =$ the final nematode density at initial nematode density P_i on a root mass equal to that of undamaged plants in 1 cm³ soil) and therefore = $aMP_i/(aP_i + M)$ (4), and b (= survival without food) = 0.0254. The latter figure (0.0254) is very similar to the survival rate of nematodes in pots with field soil without plants (0.032). Slightly different values of parameters a , M , and z give curves that fit about equally well to the data. The choice of the function $y = z^{P_i}$ (eq. iii) for the relative quantity of food available for the nematode

population is arbitrary, as there is no information on the effect of root attack by trichodurids on their food supply. The relation between nematode density and available food may be more complicated, as it is affected by the rate of destruction of root tips by the nematodes, the activation of root primordia on the same root, and the retardation of the growth of the plant caused by nematode attack. Because of the cumulative effect of the first and third effect, especially at relatively large nematode densities, the true relation might be represented by a steeper curve than that according to $y = z^{P_i}$ (eq. iii). This would result in a higher maximum of the sigmoid curve of Figure 3 and, therefore, a better fit to the data. However, a discrepancy remains between the supposed sizes of the food source and final plant weights at different initial nematode densities (T for the latter: 1.4 nematodes/cm³ soil, and virtual absence of food for the nematodes at plant weights

0.1 to 0.7 times those in the absence of nematodes). There could be two causes. First nematode attack could reduce the food supply to the nematode more than plant size. *Longidorus elongatus* (which also attacks and stops the growth of root tips) reduced the root system of *Lolium perenne* considerably without reduction of leaf weight (6). Or reduction of root weight might mean reduction of the quantity of food available to the nematodes. Second, the discrepancy may be explained by the strong decrease of the nematode population during the course of the experiment. The data do not reveal when most of the nematodes placed in pots died. If this was immediately after the inoculation, the true value of T would be $2 \times 0.0254 = 0.05$ nematodes/cm³ soil. The discrepancy between final plant size and final nematode density would remain unexplained. Growth curves were constructed for plants at dif-

ferent initial nematode densities according to Seinhorst's model (5), assuming that the mortality rate did not change during the experiment. It was further assumed that the growth of plants without nematodes was exponential: $dY/dt = r_0 Y$ in which Y is plant size at a given moment and r_0 is the growth rate, being a constant. Growth of the plants in the pots with nematodes is considered to be according to $dY/dt = r_{Pt} Y$, in which $r_{Pt} = r_0 z^{P_t}$, z a constant < 1 , P_t the nematode density in soil without roots at time t , and, further, $P_t = P_0 s^t$, P_0 = the nematode density at the start of the experiment, $s = 0.0254$ (the rate of decrease in 6 wk), and $t = 6 \text{ wk} = 1$. Growth curves must be constructed step by step. For Figure 4 this was done in steps of $1/8$ per week (rate of decrease of P_i from step to step 0.917 times). The relation between initial nematode density and plant weight remains according to equation (i) between

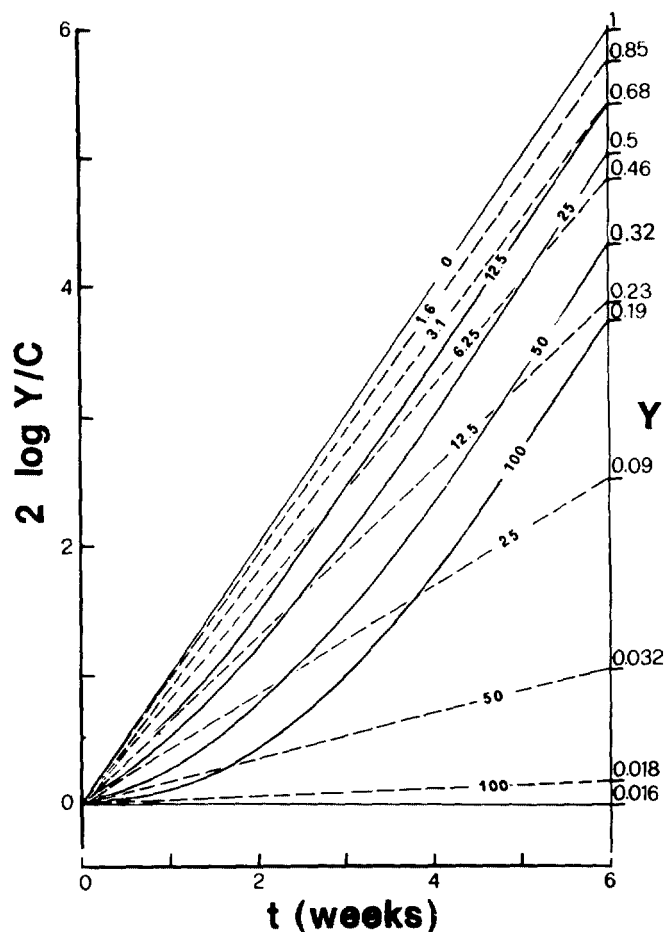


Fig. 4. Increase of growth rates due to decrease of nematode density according to $Y = k e^{r_{Pt}t}$ where k is the size of plant at sowing, r_p is the rate of growth at nematode density P (r_0 in absence of nematode), t = time in weeks from sowing, $e^{r_0} = 2$. Further $r_{Pt} = r_0 z^{P_t}$, drawn lines: growth with P decreasing by 50% each week, calculated in $1/8$ -week steps, assuming adaptation of growth to nematode density in soil without roots at end of each $1/8$ -week period.

T and 75 T , but T increases to four times the value at the beginning of the experiment and m from 0.016 to 0.15. At $P_i \geq 75$ times final T , final y decreases slowly from 0.15 to 0.016. This suggests a tolerance limit (T) without population decrease of one fourth of the 1.4 nematodes/cm³ soil of Figure 3 equals 0.35 nematodes/cm³ soil. A similar shift also would have occurred if population decrease had been less regular than assumed here, but a stronger decrease during the first week(s) than later would have resulted in a greater ratio between final and initial T a smaller decrease in a smaller ratio. In the center of a poor patch in a wheat field (plants about 1/5 normal size), nematode density was 6 nematodes/cm³ (\sim g) soil at the end of the growing season. With little food left for the nematodes because of severe damage to the root system, the initial population could not have increased much. Then, according to equation (i) (with $z^r = 1.05$), T would be of the order 1/40 to 1/20 times 6 = 0.15 to 0.3 nematodes/cm³ soil in the field, which is a reasonable accordance with the 0.35 nematodes/cm³ soil derived by assuming a constant mortality of the nematodes in the absence of host roots.

A further analysis of the data requires the estimation of a maximum rate of multiplication of the nematodes on host roots and of the associated equilibrium density (the maximum density that could be maintained on root mass of a certain size; e.g., of a plant without nematodes at the end of the experiment). This is discussed later, and we concluded that the relation between final population densities and final plant weights is in agreement with a high but constant mortality rate of the nematodes when not feeding on host roots.

Symptoms and anatomy of deformed root tissue: In the experiment all the wheat plants inoculated with more than four nematodes/cm³ soil (640 nematodes/pot) showed stubby root symptoms with abnormal production of lateral roots (Fig. 1). These new lateral roots were in turn attacked by the nematode and consequently were stunted and with blunt tips. They were similar in appearance to the *Rhizobium* nodules of leguminous plants and occurred in clusters along the root axis

(Fig. 1). Brown lesions were also noted in the swollen root tips.

Transverse (Fig. 5) and longitudinal (Fig. 6) sections of nematode infested wheat root showed disorder of the root structure due to abnormal proliferation of lateral roots. The nematode fed on the elongation zone of the root, behind the root tip, and also on the apical region, causing collapse of the root cap, necrosis of calyptrogen (Fig. 6), and stimulation of the production of adventitious root primordia (Figs. 5-6). Cell necrosis also was seen in epidermal, cortical, and vascular tissues (Figs. 5-6).

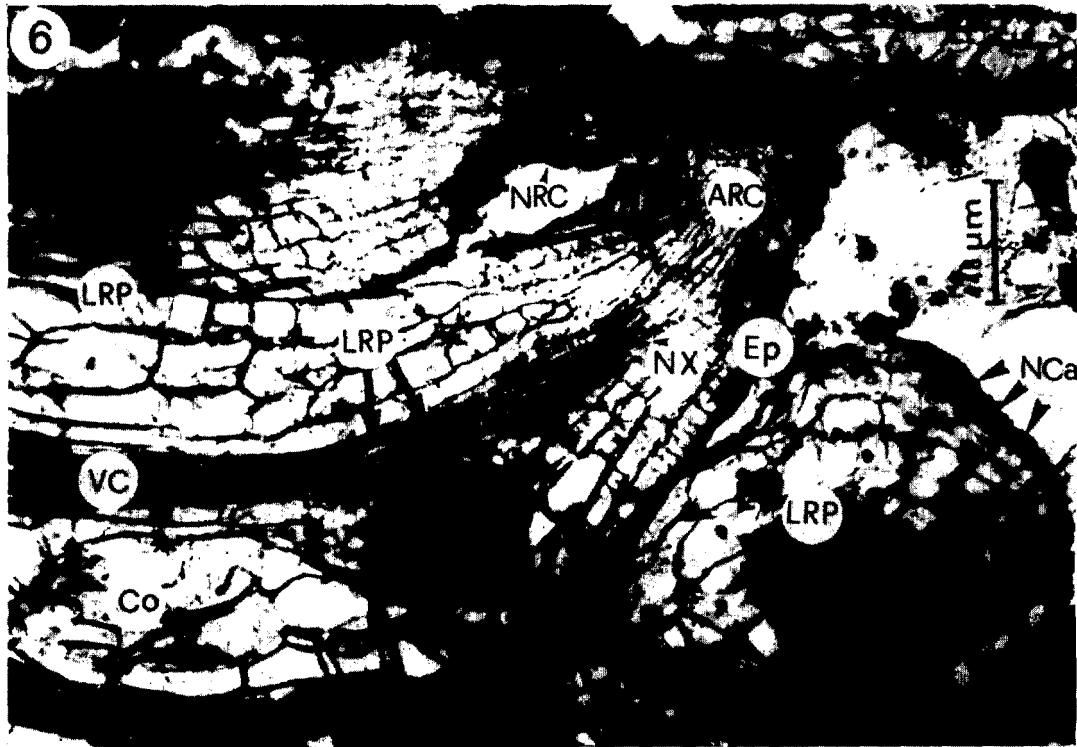
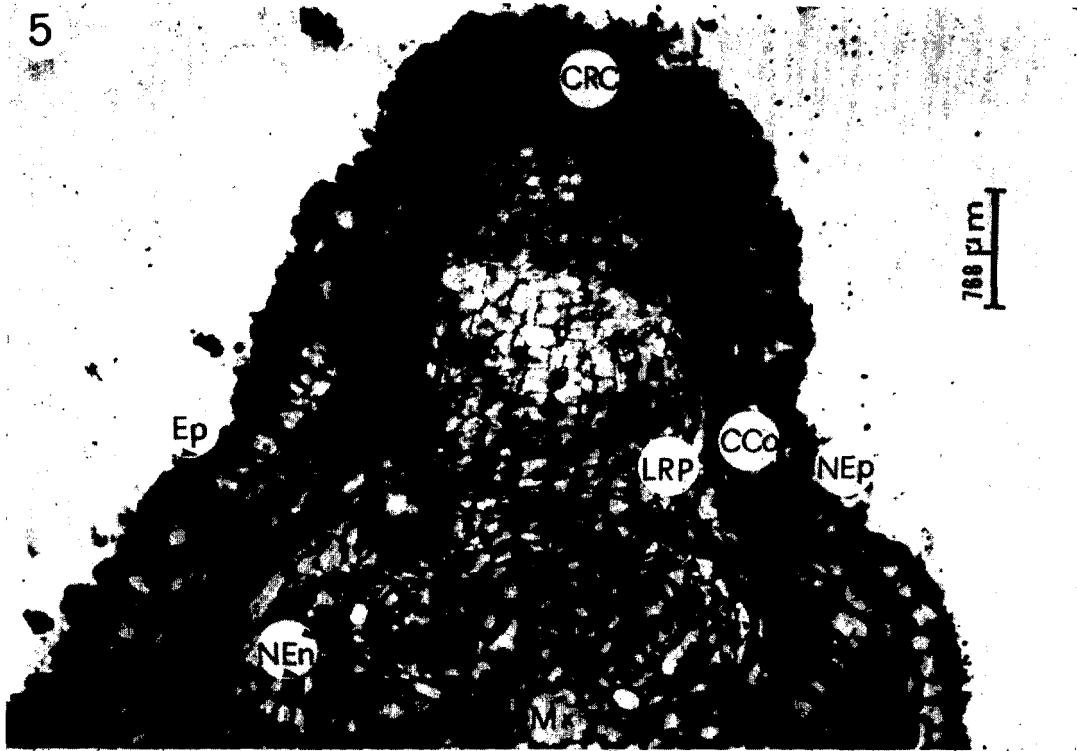
Many aspects of the parasitic habit as well as the damage of this *Paratrichodorus* sp. to the root cap and apical meristem are common to other *Paratrichodorus* species (2). In Italy this species has more frequently been detected in light soils (sandy or sandy loam) than in clay soils.

CALCULATIONS

A discussion of the methods of calculation of maximum rates of multiplication, equilibrium densities, and ways to deal with the effects of strong decrease of nematode population density on the results of experiments are in order.

A repetition of the experiment described above, although desirable, does not warrant a less rapid decrease of nematode numbers in the absence of roots of a host plant. Therefore, an effort is made to cope with the problems arising from it by investigating the consequences for final nematode numbers found, making certain assumptions on growth of the host plant and behavior of the nematodes.

At very small nematode densities, the rate of increase of a population P is $dP/dt = rP$ (eq. iv) and, therefore, $P = Ce^{rt}$ (eq. v). If, further, the following assumptions are made: 1) the nematodes were distributed randomly through the soil at the start of the experiment and were not attracted from great distances by root tips; 2) nematodes, which did not feed died at a constant rate (survival after a time $t = s^t$. In the calculations $t = 1$ for 40 days and $s = 0.0254$); 3) the volume of soil from which nematodes fed on roots was proportional to the size of the plants; 4) the size of the nematode food source (Yt) increased ex-



ponentially being k at $t = 0$ and 1 at $t = 1$. Then $Y_t = ke^{pt}$ (eq. vi) (p is the growth constant). It is assumed that $e^p = 64$ (doubling of food source each week). Then $k = 0.0156$ and $p = 4.159$. During the very short period Δt between times t and $t + \Delta t$ from the start of the experiment, the increase was $\Delta Y = pY \Delta t = pke^{pt}\Delta t$, (eq. vii), which attracted a proportion $pke^{pt}s^t\Delta t$ (eq. viii) of the initial nematode population P_i (density at time $t = s^tP_i$). This has multiplied by the end of the experiment ($t = 1$) to $e^{r(1-t)}P_i pke^{pt}s^t\Delta t$ (eq. ix). If Δt is infinitely small, then at time $t = 1$ the initial nematode density P_i in the part of the soil from which nematodes were feed-

ing on roots had become $Pf = \int_{t=0}^1 e^{r(1-t)}$

$$P_i pke^{pt}s^t dt = pk P_i e^r \int_{t=0}^1 (e^{p-rs})^t dt$$

(eq. x). Therefore, $Pf/P_i = pke^r(se^{p-r})^{(1-\log e^{p-r})^{-1}}$ (eq. xi). If, at the end of the experiment a proportion u of the nematodes had found roots to feed on, then the total population was $u Pf + (1-u)sP_i$, which, according to an earlier suggestion was $0.65 P_i$. Substituting the values of the various constants given above results in $0.65-s(1-u) = \times 4.159 \times 0.0156e^r(0.0254 \times 64e^{-r}-1) \times [e^r \log (0.0254 \times 64e^{-r})]^{-1} = u \times 0.0649e^r (1.626e^{-r}-1) (-0.486r)^{-1}$. For $u = 1$, $e^r = 32$ fits well to this equation. For $u = 0.5$, e^r becomes 80.

This means that the part of the population feeding on wheat roots increased in number, despite the great mortality in the absence of food, which may or may not have affected the feeding nematodes as well.

To calculate multiplication rates and final nematode numbers at the higher initial nematode densities, logistic population growth ($dP/dt = rP(1-P/E)$) (eq. xii) can

be assumed, in which E is the nematode number that can maintain itself on the roots present in a unit volume of soil. However, nematode density on the root system could develop in two ways: either the nematodes stay on or near the tips of the roots on which they started to feed or they redistribute continuously, thus maintaining a random distribution on the root system. For the following calculations it is assumed that the nematodes remain distributed randomly in the soil until root tips come in close proximity. The nematode density in the soil without roots at time $t = P_i s^t$. If the volume of the pot is taken as the unit volume of soil and, at the end of the experiment, a proportion u of the pot soil contains roots at very small initial nematode densities (no reduction of plant size by nematode attack), then $u\Delta Y_{P_i,t}P_i s^t$ nematodes start to feed between time t and $t + \Delta t$ if $u\Delta Y_{P_i,t}$ is the proportion of the soil that is occupied by new roots between time t and $t + \Delta t$. If there is no redistribution, then these nematodes increase during the time $1-t$ until the end of the experiment to:

$$u\Delta Y_{P_i,t}Pf = u\Delta Y_{P_i,t}e^{r(1-t)}EP_i s^t \{ (e^{r(1-t)}-1)P_i s^t \}^{-1} \text{ (eq. xiii).}$$

The final population on the root system is $u\Delta Y_{P_i,t}Pf$ and the total population in the pot $u(\Delta Y_{P_i,t}Pf) + (1-u\Delta Y_{P_i,t})P_i s$ (eq. xiv). At small P_i , $\Delta Y_{P_i,t}$ becomes 1 and, therefore, the total population in the pot $u\Delta Y_{P_i,t}Pf + (1-u)P_i s$ (eq. xv), which at still smaller initial densities reduces to equation xi. If it is assumed that proportions of the soil occupied by roots are the same as u times the ratios between plant sizes at time t and at the end of the experiment, then the successive values of $\Delta Y_{P_i,t}$ can be derived from Figure 3.

If there is a continuous redistribution of the nematodes on the root system (probably closer to reality than no redistribution at all), the relation between numbers of



Figs. 5-6. Anatomical changes induced in durum wheat roots by *Paratrichodorus* sp. infestations. 5) Nematode infested durum wheat root cross section showing collapsed cells of cortex (CCo) and root cap (CRC), along with necrosis of epidermis (NEp) and endodermis (NEn). Differentiation of lateral root primordium (LRP) is evident at the periphery of the stellar area (Mx = metaxylem). 6) Cluster of durum wheat lateral root longitudinal section showing necrosis of calyptrogen (NCa) and xylem elements (NX), abnormal growth (ARC) and necrosis (NRC) of root cap, and formation of lateral root primordia (LRP) in consequence of the nematode feeding activity (Co = cortex; Ep = epidermis; VC = vascular cylinder).

nematodes on root systems at time $t - \Delta t$ and t ($Y_{Pi,t-\Delta t}P_{t-\Delta t}$ and $Y_{Pi,t}P_t$, respectively) is given by the equation:

$$uY_{Pi,t}P = uY_{Pi,t-\Delta t}e^{r\Delta t}EP_{t-\Delta t}((e^{r\Delta t}-1)P_{t-\Delta t} + E)^{-1} + Y_{Pi,t-\Delta t}Pi s^t \text{ (eq. xvi).}$$

The first term of the right hand part of this equation reflects the multiplication of the nematodes present on the whole root system between time $t - \Delta t$ and t . The second term represents the number of nematodes that start to feed during that period. The increase $\Delta Y_{Pi,t}$ of the size of a plant between times $t - \Delta t$ and t depends on P_t (if $> T$), which in its turn depends on $t - \Delta t$. Therefore to calculate final population density at a given P_i , plant sizes $Y_{Pi,t}$ and nematode densities P_t on the root system must be calculated in successive small steps Δt .

Final population density according to equation xiii on the portion of a plant grown during one week calculated in one step (mid-week relative size of plant \times mid-week nematode population in soil \times multiplication rate mid-week to end of experiment) hardly differed from the final population calculated in eight steps. Calculations of final nematode densities at different initial nematode densities according to equation xiii were made, therefore, in weekly steps, deriving the weekly increases of plant size from Figure 4. A good fit of calculated to observed final densities was obtained at $P_i \geq 1$ with $e^r = 120$, $E = 1$ nematode/cm³ soil and $u = 1$ but at the cost of a great overestimation of P_f compared to observed values at $P_i \leq 0.5$ nematode/cm³ soil. Calculations according to equation xiv required at least several hundred steps to avoid a gross overestimation of e^r . From some calculations in 40 steps ($\Delta t = 0.025$) and comparing one such step to ten with $\Delta t = 0.0025$, probable values of E of 0.5 nematodes/cm³ soil and of e^r between 100 and 150 were derived for a reasonable fit of calculated to observed P_f at $P_i \geq 1$ nematode/cm³ soil for $\mu = 1$ (Fig. 3). At smaller values of u , this would require larger values of E and of e^r . Calculations at $P_i \geq 4$ nematodes/cm³ soil are simplified considerably by the fact that nematode densities in the soil and on the roots are larger than E during the first three or more weeks

of the experiment and that the growth of the plant is hardly or not reduced afterwards. P_f values according to equation xiv at $P_i \leq 0.5$ nematodes/cm³ soil are certainly much larger than those observed. As the species involved is bisexual, underpopulation could be the cause of the discrepancy.

Both models suggest a large maximum multiplication rate and a much smaller equilibrium density than in the field, where it was at least six nematodes/cm³ soil. The difference could be due to smaller numbers of root tips per unit volume of soil in the pots than with much larger plants in the field and, also, to a greater mortality of the feeding nematodes than in the field, where the population did not decrease very much in the absence of a host crop. However, this would mean that the maximum multiplication rate of the nematode in the absence of this mortality would still have been much larger than the 100 to 150 times in 6 wk estimated from the results of this experiment.

The nematodes were not mixed through the soil after the inoculation. Therefore, most probably they were not distributed randomly in the soil during the first weeks of the experiment, as was assumed in the calculations made above. As growth was almost stopped at the largest initial densities, all root tips must have been attacked at these densities from the start of the experiment. Assuming very large degrees of aggregation of the nematodes (e.g., all nematodes in a tenth of the volume of the pots during the whole or most of the duration of the experiment) leads to improbably large estimated T values. Lesser degrees of aggregation would have resulted in either more or fewer than the average number of nematodes attacking roots during the first week(s) of the experiment (depending on where the nematodes were placed in the pots relative to the seeds) resulting in slower or faster growth of the plants, respectively, than at a more uniform distribution. Probably the situation would be different in different pots. This would result in an increase of pot to pot variability of growth. The situation would be reversed in due time, but due to the rapid decrease

of nematode numbers this would not compensate the effects of the differences in growth in the first week(s).

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