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GROWTH, INVASION AND ROOT DIFFUSATE PRODUCTION IN TOMATO AND BLACK NIGHTSHADE INOCULATED WITH POTATO-ROOT EELWORM *)

BY

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In the summer of 1951 and again in 1953, attempts were made to compare the variations in growth, root diffusate production and extent of root invasion by the potato-root eelworm, *Heterodera rostochiensis*, Woll. in a favourable and an unfavourable host subjected to varying rates of nematode inoculation. The hosts chosen were tomato, *Lycopersicon esculentum*, Mill. and black nightshade, *Solanum nigrum*, L. Tomato is a satisfactory host and is known to produce an active root diffusate; potato-root eelworms will penetrate the roots and produce mature males and females. Black nightshade, on the other hand, is an unsatisfactory host in that invading larvae normally fail to mature beyond the third stage (DONCASTER, 1953) but like tomato, it produces an active root diffusate (RUSSEL, TODD & WARING, 1949).

METHODS

Seedlings of black nightshade and of tomato were planted in pots of an unsterilized 3:1 loam-sand mixture. For each species of plant, sixteen pots of this mixture were put up with no eelworm infestation (rate 0), sixteen were inoculated with cysts of the potato-root eelworm equivalent to 5 eggs and larvae per gram of soil (rate 1), sixteen with an inoculation equivalent to 30 eggs and larvae per gram of soil (rate 2) and sixteen with an equivalent of 180 eggs and larvae per gram of soil (rate 3). In each series of sixteen randomised pots four replicates were marked for treatment at the end of each of the four periods, one, two, four and eight weeks. A further set of sixteen pots of loam-sand mixture with no inoculation or plant was set aside as a control series for the root diffusate tests. All pots were sunk in a shady gravel plunge at the beginning of the experiment.

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The treatments at the end of each period consisted of leaching the appropriate pots with 50 ml. of tap water, the leachings being poured through each pot four times, collected and stored at a temperature of 4° C. The plants were then very carefully washed free from the soil, and fixed and stored in formal-acetic alcohol. Each sample of root diffusate was tested on duplicate batches of about 200 cysts obtained by weighing (FENWICK & REID 1951), the hatched larvae being removed weekly for counting.

The plants were partially dried by gently pressing them between towels, the root systems were cut off at the crown and then weighed immediately. Following this they were stained in hot acid fuchsin lactophenol and the larvae within them were counted. In 1951 the counts were done on the stained larvae in the entire root systems which were spread out in a petri dish under the binocular microscope. However, the roots of many of the eight-week old plants inoculated at the light and the intermediate rates were so large that estimation of the larvae which they contained could not be attempted. In 1953, however, the plants from the 1951 experiment, in which the eelworms had been counted, were used in a test of the accuracy of the "Waring Blendor" technique for assessing eelworm numbers within roots (TAYLOR & LOEGERING, 1953, and WILLIAMS & WINSLOW, 1955). The stained complete roots were rinsed in acidified water, cut into short pieces with scissors and put into the "Waring Blendor" flask with about 60 ml. of acidified water. It was found that the eelworms suffered little damage even after five minutes treatment in the "Blendor" running at full speed, but in spite of this rather long processing a few nematodes still remained inside fragments of root. In order to minimise damage to the nematodes and at the same time to ensure that the majority were liberated, a time of three minutes was considered the most satisfactory.

Unlike the methods employed by TAYLOR & LOEGERING and by WILLIAMS & WINSLOW the suspension of nematodes and macerated root tissue was not sieved as it was found that some eelworms were lost on the coarse sieve through not having been liberated from the larger root fragments and an appreciable number of second stage larvae were lost through the fine sieve, which in this case was bolting silk of about 160 meshes to the inch. The complete suspensions were therefore made up to 100 ml. and eelworm counts from aliquot samples agreed very closely in every case with the counts made in 1951. In the 1953 repetition of the experiment, therefore, estimation of the

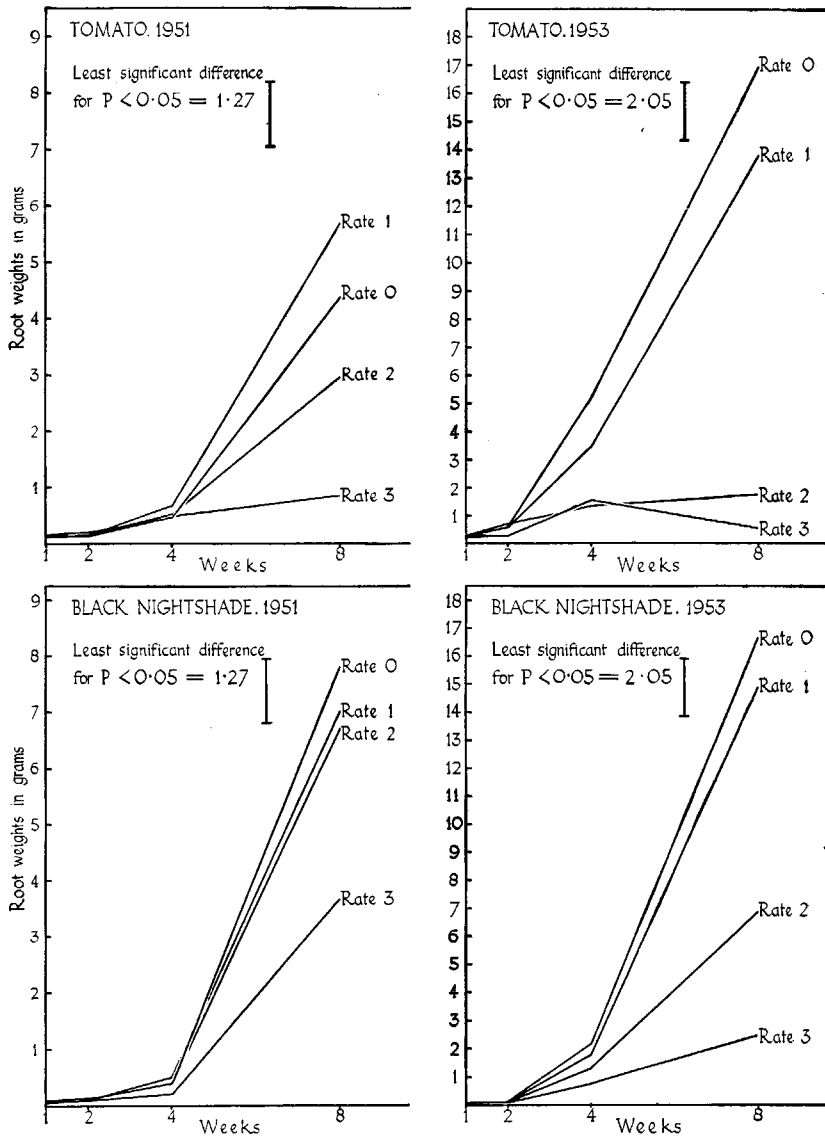


Fig. 1. The effect of inoculation on root development.

number of larvae within the host plant was made by the "Waring Blendor" technique. Where the root systems weighed as much as five grams the suspensions were washed into a measuring cylinder, made up to 100 ml. and aliquot samples were withdrawn for counting.

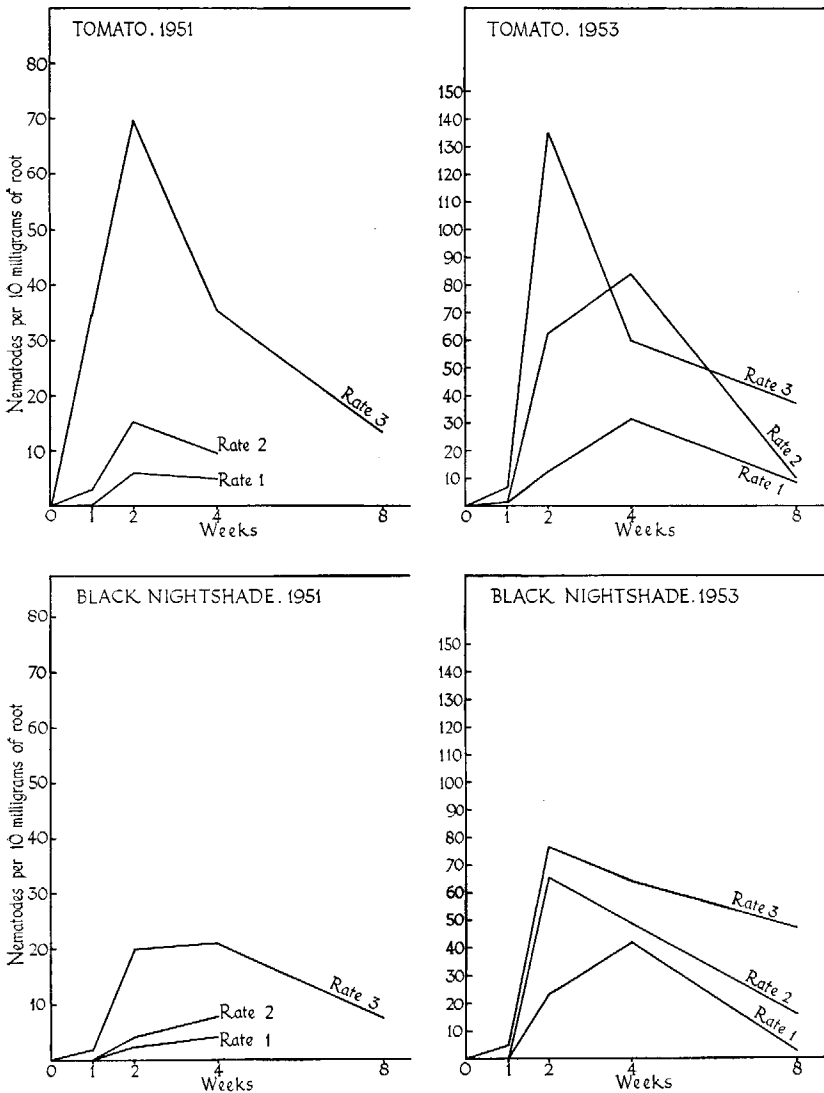


Fig. 2. Factors influencing the population density of *H. rostochiensis* in roots.

Where the root systems were smaller, however, the suspensions were centrifuged down to a convenient volume and aliquots of constant proportion were used for counting. In every case one tenth by volume of the root system was examined in this way.

RESULTS

In the experiment of 1951 the plants made noticeably poor growth, possibly due to the use of three-inch pots which were undoubtedly too small. Four-inch pots were therefore used in 1953 and development of the uninoculated plants was considered normal. The results of the experiments are presented in four figures.

Root development

Figure 1 shows root development at the four rates of inoculation. Analysis of variance on the data on root weights was carried out in arithmetic form and it is therefore possible to indicate by linear measurement on the figure the least significant differences between points. It is seen that in neither year was black nightshade as seriously affected by inoculation as tomato. In both years black nightshade showed significant effects of inoculation only by the eighth week and in 1951, when growth was generally poor, only the heavy rate of inoculation had any appreciable effect. In 1953 however, the intermediate rate was enough to cause noticeable stunting. At the end of the experiment all heavily inoculated nightshade and tomato plants appeared quite incapable of recovery and the tomato plants inoculated at the intermediate rate also reached a similar condition.

In 1953, when growing conditions were better, inoculation had a quicker and more pronounced effect on the tomato plants; stunting of the root systems due to the higher inoculation rates became apparent at four weeks and the light rate was sufficient to cause definite stunting by the eighth week.

Nematode populations within roots

Of the two host species, tomato was generally the more susceptible to invasion and bore not only the greater total number, but the greater density of parasites. Figure 2 shows that the maximum number of larvae in unit weight of root of each host species was more or less proportional to the inoculation rate, and in every case the peak lay between the second and fourth week.

If the numbers of nematodes in the entire root systems are considered, it is seen in figure 3 that whereas in nightshade the total number progressively increased, in tomato it fell off sharply between the fourth and the eighth week.

In 1953 records were kept of the numbers of potato-root eelworms

in the various life cycle stages which were found in the roots. From black nightshade a total number of 1013 larvae were counted and of these only 6 had reached the third stage. In the tomato plants a total of 1361 larvae were counted and of these 58 had reached stages between the fourth and the adult stage by the fourth week and a further 115 by the eighth week. There was a decline in the number of second and third stage larvae from 698 at the fourth week to 273 at the eighth.

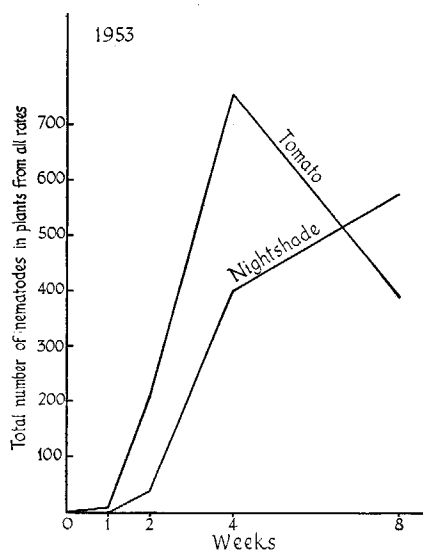


Fig. 3. Total numbers of *H. rostochiensis* in host plants.

Root diffusate production

As in the case of root development, root diffusate production appeared to be more affected by inoculation in 1953 than it was in 1951, when growing conditions were less favourable (fig. 4). In 1951 the tomatoes showed little reaction to inoculation until the eighth week when root diffusate production from the heavily inoculated plants was less than from the others. At the end of the first week's growth in this experiment, the tomatoes inoculated at the heavy rate gave a relatively high root diffusate output: it is possible that this was a genuine stimulatory effect, and perhaps comparable with it was the fact that at the second week in the 1953 experiment the lightly inoculated tomatoes were producing the most active root diffusate.

Under the improved growing conditions in 1953, tomato-root diffusate production was considerably affected by variations in inocula-

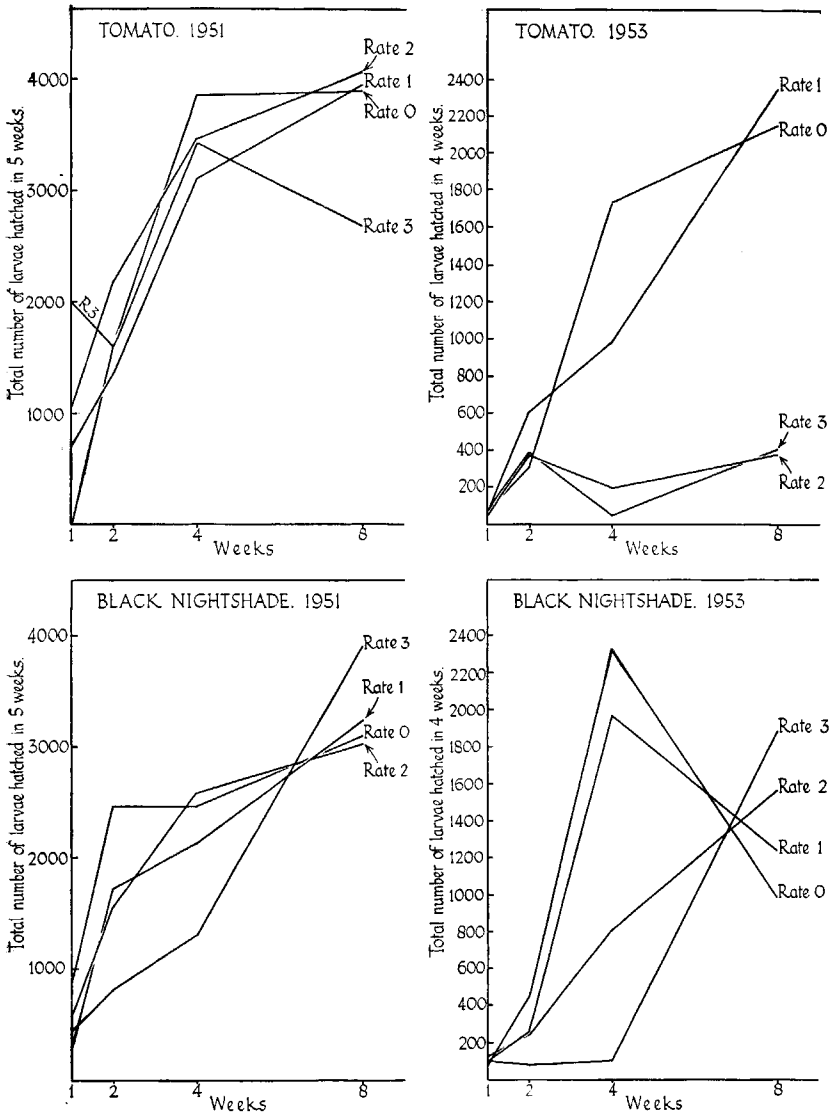


Fig. 4. The effect of inoculation on root diffusate production.

tion rate. There was a suggestion that the light inoculation rate caused some check between the second and fourth week and that there was a subsequent recovery. The intermediate and heavy rates caused a very severe check between the second and fourth week and this was followed by a slight but definite recovery.

From black nightshade, there was a tendency for increased inoculation merely to delay root diffusate production. In 1953 this was particularly marked and whereas the peak production of root diffusate from plants with light and with no inoculation was reached about the fourth week, the peak from those in the intermediate and heavy rates was reached at sometime about the eighth week. In both years, between the fourth and the eighth week, there was an apparent inversion of the order of root diffusate activity from the different rates: plants lightly or uninoculated giving a more active root diffusate than the others at the fourth week, and those more heavily inoculated giving the most active root diffusate of any at the eighth week. In neither year, however, did the differences at the eighth week achieve statistical significance.

CONCLUSIONS

The results of the experiments show a logical relationship between hosts and parasite: in each year, plant growth was reduced by eelworm attack, but to a lesser degree in the unfavourable host plant. Invasion of nightshade roots was evidently continuous throughout the experiment to an extent which exceeded the loss of eelworms due to death and disintegration or to other causes. It is reasonable to assume that a number of eelworms were lost from the tomato roots as a result of maturation, and to account for the significant reduction in the total number of parasites within the tomato roots it is probable that the extent of invasion became reduced during the second month of the experiment.

I wish to thank my colleagues, particularly Dr. D. W. FENWICK and Mr. F. G. W. JONES for much helpful advice during the course of the work and Miss S. COX for assistance with the statistical computations involved.

ZUSAMMENFASSUNG

Vergleichende Untersuchungen wurden über Wachstum, Wurzeldiffusatmenge und Befallshöhe durch den Kartoffelnematoden, *Heterodera rostochiensis*, bei Tomate (*Lycopersicon esculentum*) und Nachtschatten (*Solanum nigrum*) angestellt. Bei der Tomate wirkte sich der Nematodenbefall auf die Wurzelentwicklung stärker als bei Nachtschatten aus. Beide Pflanzenarten wurden von L II des Kartoffelnematoden befallen, aber während sich die Nematoden in den Tomatenwurzeln bis zur Reife entwickelten, erreichten sie bei Nachtschatten nur in wenigen Fällen das dritte Stadium. Bis zum Ende des ersten Monats war bei Tomatenwurzeln eine grössere Dichte und eine grössere Gesamtzahl an Nematoden zu beobachten. Dann aber trat ein Absinken ein, das wahrscheinlich durch die Reife der Parasiten veranlasst wurde. Demgegenüber nahm die Nematoden-

zahl in den Nachtschattenwurzeln progressiv zu. Starker Nematodenbefall wirkt auf die Ausscheidung von Tomatenwurzel diffusat anscheinend reduzierend ein, während er bei Nachtschatten nur die Spitzenproduktion des Wurzel diffusats behindert.

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