

\*CSIRO Division of Horticultural Research  
Box 350, GPO, Adelaide, S.A. 5001

+CSIRO Division of Horticultural Research  
Merbein, Vic. 3505

## THE INFLUENCE OF LIGHT QUALITY ON THE GROWTH AND FECUNDITY OF *MELOIDOGYNE* INFESTING TOMATO

by

A. F. BIRD\*, M. R. SAUER<sup>+</sup>, R. N. CHAPMAN<sup>+</sup> and B. R. LOVEYS\*

Loveys (pers. comm.) showed that xylem sap from tomato plants grown under fluorescent light contained more cytokinin than from plants illuminated by tungsten filament lamps. In his experiments there was no difference in either the temperature or the amount of photosynthetically active radiation (400-700 nm).

At present there is some conflict expressed in the literature about the influence of cytokinins on nematode growth in susceptible plants. On the one hand Brueske and Bergeson (1972) have shown that root-knot nematodes can cause a decrease in the amount of cytokinin in the root tissues and xylem exudates of tomatoes, and on the other, van Staden and Dimalla (1977) have shown that root-knot nematodes increase the amounts of cytokinins in roots, but do not appear to alter the cytokinin complement of the xylem sap. They thought the change to cytokinin concentrations in infected roots was due to accumulation of cytokinins by the nematodes themselves (Dimalla and van Staden, 1977).

There appears to be a relationship between the degree of resistance to nematodes in plants and the presence of cytokinins. Thus Dropkin *et al.* (1969) and Kochba and Samish (1971) have shown that exogenously supplied cytokinins make resistant tomato plants more susceptible, and Kochba and Samish (1972) have shown that cytokinin levels are lower in resistant peach rootstocks than in susceptible ones. However, Skene and Antcliff (1972) did not find any difference in the cytokinin content of the sap from the nematode-resistant rootstock Salt Creek compared with the susceptible Sultana rootstock.

In the investigation described here we observed the growth of *Meloidogyne javanica* (Treub) Chitw. in plants grown under conditions known to lead to changes in the cytokinin levels of the sap.

## MATERIALS AND METHODS

Freshly hatched second stage larvae (L<sub>2</sub>) of *Meloidogyne javanica* were used in the experiments.

Tomato plants (*Lycopersicon esculentum* Mill. cv. Tatura Dwarf) were grown singly in 15 cm diameter pots in sand containing complete nutrients. When the plants were approximately 15 cm high they were placed in growth cabinets for a week and then the plants to be infected were each inoculated with approximately 2,000 L<sub>2</sub>.

Each of the two growth cabinets used was operated with a regime of 16 hr daylight and 8 hr darkness. In the red-enriched (R) cabinet, light was provided by ten 140 W Philips TLMF white fluorescent tubes and five Philips TL 40 red fluorescent tubes. In the far-red (FR) cabinet, light was provided by two 140 W white fluorescent tubes and ten 150 W tungsten lamps, heat from which was reduced by a 10 cm thick water filter. The ratio of energy at 660 nm to that at 730 nm was 9.20 in the R cabinet and 0.95 in the FR cabinet. Photosynthetically active radiation (400-700 nm) was maintained at 100  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in both cabinets throughout the experimental period.

Records of air and soil temperature in both cabinets showed that the soil temperature was depressed by 2 to 3° C for a period of several hours after watering. The soil temperature in the far-red (FR) cabinet was 22.6° C and in the red (R) cabinet it was 23.1° C. The air temperature in each cabinet was between 1° C and 2° C higher than the respective soil temperatures.

Forty plants were used in the experiment; 24 were inoculated with L<sub>2</sub>s and 16 served as nematode-free controls. Each cabinet contained 12 infected plants and 8 controls.

After a week, half of the infected plants, six from each cabinet, were harvested and weighed. The roots were fixed in F.A.A. (Formalin 30 ml, absolute ethanol 100 ml, glacial acetic acid 5 ml and water 200 ml) and the numbers of galls and larvae counted in each of the six replicates. The cross-sectional area of representative samples of the larvae was also measured using a planimetric technique.

The remaining plants (6 infected and 8 controls from each cabinet) were harvested five weeks from the commencement of the experiment. The tops of the plants were removed at the cotyledonary node and both fresh and dry weights were obtained. Sap was collected from the stumps over a period of two days by connecting them to a vial via a latex tube. The roots were then removed and weighed fresh. Dry weights were obtained for the controls and the infected roots were placed on paper tissue over funnels in a misting apparatus and the larvae emerging from eggs counted over a two day period. Ten egg masses were dissected from each root and the L<sub>2</sub>s that hatched from these were measured to detect any differences in length. The state of embryogenesis of eggs from ten egg masses per plant produced by these females was also determined.

Sap was assayed for cytokinins by the following technique. It was filtered, adjusted to pH 8.5 and extracted three times with equal volumes of n-butanol. The butanol extract was evaporated to dryness *in vacuo*, redissolved in methanol and chromatographed on Whatman 3 MM paper with sec butanol: 25% ammonium hydroxide (4:1). Each chromatogram was divided transversely into 10 equal portions. Each piece was then incorporated into 30 ml of the appropriate agar medium and assayed with the soybean callus assay (Miller, 1965).

The infected roots were fixed in 4% buffered paraformaldehyde and females were dissected from the roots and their cross-sectional area measured.

## RESULTS

The difference in quality of light did not significantly influence the ability of L<sub>2</sub> larvae of *M. javanica* to invade and establish themselves in roots (Table I) although more parasitic L<sub>2</sub>s were usually found in the roots of plants grown under FR than under R and the number of galls formed under FR was significantly greater ( $P < .01$ ) than under R (Table I). Furthermore, these larvae dissected from the roots a week after inoculation were significantly larger in plants under FR than under R. The mean cross-sectional area of larvae grown under FR was  $1.58 \pm 0.05$  (s.e.) sq  $\mu\text{m} \times 10^4$  compared with  $0.79 \pm 0.01$  (s.e.) sq  $\mu\text{m} \times 10^4$  under R.

The plants harvested after one week under FR were significantly

( $P=0.01$ ) larger than those under R. These FR plants had a mean fresh weight for the six plants of 23.28 g compared with 18.77 for the R plants.

This difference in size of whole plants grown under light of different quality was not significant after five weeks although there was a significant ( $P=0.05$ ) difference in the mean fresh root weight of infected plants: 8.31 g compared with uninfected plants 5.72 g. This difference did not appear to be associated with light quality, but there was a tendency for FR plants to have larger tops than R plants although this was not significant.

The nematodes reacted to these differences in the quality of light by growing more rapidly, not only in the pre-moult stages, as mentioned above, but they also reached maturity and laid eggs earlier. When the plants were harvested after five weeks the female nematodes from both light regimes had attained maximum size, reached maturity and were laying eggs; no significant differences were observed in their size. However, there was a highly significant difference in the numbers of L<sub>2</sub>s hatching over a 40 hr period. No larvae from roots of plants exposed to R illumination hatched whereas a mean of 5,252 per plant (range 2,050-8,510) hatched from roots of plants exposed to FR illumination.

After 39 days the proportion of eggs which contained a recognizable larva was significantly greater from FR plants than from R plants (Table II).

The results show that the quality of light to which the plant is exposed has a marked effect on the rate of development of the nematode feeding within the roots. No differences were detected in the

Table I - *Mean numbers of larvae and galls in roots of tomato plants grown with a preponderance of red (R) light in one instance and a preponderance of far-red (FR) light in the other.*

Treatments	Mean no. of nematodes dissected from roots <sup>1</sup>	Mean no. of galls
R (n = 6)	589.8 (s.e. $\pm$ 64.2)	167.8 (s.e. $\pm$ 20.9)
FR (n = 6)	746.7 (s.e. $\pm$ 61.7)	289.2 (s.e. $\pm$ 27.4)
L.S.D. 1%	n.s.	109

(<sup>1</sup>) The plants were harvested one week after infection with approximately 2,000 L<sub>2</sub> of *M. javanica*.

length of L<sub>2</sub>s hatched from egg masses dissected from either of the treatments. The mean length of larvae from the R treatment was 441.90±2.1 µm (s.e.) (585 measurements) and that from the FR treatment 444.08±2.0 µm (s.e.) (596 measurements).

The soybean callus assay on the sap from the control and infected plants of the two treatments revealed three zones of activity with Rfs of 0-0.3, 0.3-0.4 and 0.6-0.9.

Our results on cv. Tatura Dwarf support those of Loveys (pers. comm.) on xylem sap from tomato plants cvs Grosse Lisse and Tatura Dwarf. More cytokinin-like substances were found in the sap from plants grown under R illumination than from plants grown under FR illumination. The same results was obtained from infected and non-infected plants. Within the light treatments there did not appear to be any major difference between infected and non-infected plants. However, because only small amounts of sap could be collected (R cont=17 ml, FR cont=27 ml, R inf=13 ml and FR inf=22 ml) we were unable to include enough replicates for statistical analysis and so the effect of nematodes on cytokinin levels could not be determined with certainty. Our results suggest that the presence of nematodes has little effect. The total activity from the three chromatogram zones (kinetin equivalents: ng/ml sap) was 12.8 and 14.6 from the control and infected plants under R and 4.9 and 4.4 from the control and infected plants under FR respectively.

Table II - Comparison between rates of embryogenesis of eggs laid by nematodes from plants under red (R) and far-red (FR) illumination.

Eggs counted <sup>1</sup>	R (n = 6)	FR (n = 6)
Eggs with larvae L.S.D. 1%	mean = 321.7 (s.e. ± 113) 457	mean = 1057.5 (s.e. ± 89.2)
Total no. of eggs L.S.D. 1%	mean = 2130.8 (s.e. ± 484.3) 1809	mean = 3454.7 (s.e. ± 302.1)
Percentage of eggs with larvae L.S.D. 1%	mean = 12.92 (s.e. ± 3.0) 9.69	mean = 30.7 (s.e. ± 0.62)

(<sup>1</sup>) Eggs from 10 egg masses per treatment 39 days after infection.

## DISCUSSION

Differences in published opinion concerning the influence of cytokinins on nematodes may only be a matter of differences in experimental design and interpretation. For instance, van Staden and Dimalla (1977) placed their seedlings in nematode infested soil under natural summer conditions for a period of three months before collecting xylem sap, whereas Brueske and Bergeson (1972) placed theirs in pots of sandy loam to which chopped galled roots were added and collected the xylem sap from the plants eight weeks later. Although soil temperatures were not recorded in either case, it would seem that there could well be great differences in the stages of host-parasite relationships between these two sets of experiments. In the case of tomato plants exposed to infection for twelve weeks, a second generation of nematodes would have had time to develop and their syncytia would be close to their peak of activity, whereas after eight weeks the second generation of nematodes would be close to moulting. It is established that syncytia go through a physiological cycle of growth and decay (Bird, 1975) and it seems reasonable to suggest that the quantity of cytokinins in xylem sap may be related to this cycle.

In our experiments we have not observed any differences in cytokinin-like materials in the xylem exudates of plants infected with nematodes as compared with the controls. These measurements were made 39 days after infection and after the peak of syncytial activity which corresponds with the commencement of egg laying. The amounts of cytokinin-like materials moving through the xylem of infected plants could vary with the stage of syncytial development and this might explain the anomalies in the literature; the concentrations of cytokinins can vary during development in the uninfected plant (Davey and van Staden, 1976).

We do not know why the growth and fecundity of *M. javanica* is accelerated in plants grown under light containing a preponderance of the far-red. It is not a temperature effect and may have nothing to do with cytokinins. Gillard and van den Brande (1956) have observed that *M. arenaria* in tomatoes growing under red light contained more galls and female nematodes than in plants growing under green, blue or white light, although exposed to the same temperatures and inoculation densities. However, it is unlikely that the photosynthetically active radiation was maintained at a constant level in their

experiments. Gillard and van den Brande (1956) suggested that the differences that they observed were due either to an increase in the rate of development of the nematode in its host under red light, or to the effect of red light on the attractant properties of the host's roots to the invading L<sub>2</sub> larvae. Our observations give stronger support to their first hypothesis, namely, that development of the root-knot nematode is influenced by the quality of the light under which its host is grown.

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#### S U M M A R Y

The quality of light to which tomato plants were exposed had a marked effect on the rate of development of nematodes feeding within their roots. *Meloidogyne javanica* (Treb) Chitw. developed, laid eggs which underwent embryogenesis and hatched earlier in plants grown under artificial lighting with a preponderance of light in the far-red (tungsten filament lamps) compared with plants grown under similar temperatures, day length and photosynthetically active radiation but lacking the far-red component (fluorescent lamps). Plants grew more rapidly initially in the far-red and contained less cytokinin-like materials in their xylem exudates than plants in the red. The presence or absence of nematodes did not appear to affect the amounts of cytokinin-like materials in the xylem exudates of either treatments collected 39 days after infection.

#### R I A S S U N T O

*Influenza della qualità della luce su sviluppo e fecondità di Meloidogyne in radici di Pomodoro.*

La qualità della luce alla quale sono state esposte le piante di Pomodoro ha avuto un effetto marcato sullo sviluppo dei nematodi nelle radici di tali piante. *Meloidogyne javanica* (Treb) Chitw. si è sviluppata, ha deposto uova, ha compiuto lo sviluppo embrionale ed ha schiuso le uova in tempi più brevi quando è stata allevata su piante esposte a luce artificiale con preponderanza di luce del visibile (lampade al tungsteno) che non in radici di piante esposte alla stessa temperatura, ma a luce in cui tale componente era assente (lampade fluorescenti). Le piante si sono sviluppate più rapidamente all'inizio, sotto luce visibile e contenevano negli essudati xilematici meno sostanze del tipo citochinina delle piante allevate a luce rossa. La presenza o meno dei nematodi non sembra invece aver alterato il contenuto di queste sostanze citochinino-simili.

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