THE INFLUENCE OF SOME METABOLIC INHIBITORS ON THE 
RESPONSE OF SUSCEPTIBLE/RESISTANT CULTIVARS OF TOMATO TO 
MELOIDOGYNE INCognITA

BY

RAMMA SAWHNEY * and J. M. WEBSTER

Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

The effect of cycloheximide and puromycin (protein synthesis inhibitors) and actinomycin D (RNA synthesis inhibitor) was studied on host parasite interaction between Meloidogyne incognita and susceptible and resistant cultivars of tomato, Lycopersicon esculentum. The concentrations of cycloheximide (1 ppm) and puromycin (40 ppm) which caused great inhibition of root growth, did not affect the galling response or nematode establishment and development in the infected, susceptible cultivar. Thus, a high level of protein synthesis may not be required for initiation of susceptible response to M. incognita infection. However, relatively high concentrations of cycloheximide (2.5 ppm) and puromycin (55 ppm) did inhibit galling and larval development, suggesting that, a certain minimum level of protein synthesis is necessary for establishment of the nematode within the susceptible host. The concentrations of Actinomycin D that were inhibitory to root growth were also inhibitory to galling. In the resistant cultivar, cycloheximide treated infected roots did not exhibit any browning (hypersensitive reaction, HR) of cells surrounding the nematodes within the roots. Our results suggest that lack of HR in these roots many have led to exit of the nematodes from the roots. Hence, inhibition of HR does not confer susceptibility to the resistant plants. Some other factor seems to be necessary for the plants to develop susceptibility to M. incognita. Alternatively, some mechanism of resistance other than HR may be operative within the resistant plants. Puromycin does not show any inhibitory effect on the visible browning of the root tips in the resistant plants.

Giant cell or syncytium formation has been shown to be essential for the normal development and reproduction of the root-knot nematode, Meloidogyne ip., and there is a high level of protein, RNA and DNA synthesis during its formation (Owens & Novotny, 1960; Owens & Specht, 1966; Owens & Rubinstein, 1966; Endo & Veech, 1970; Veech & Endo, 1970). Antimetabolites, such as 6-azauridine and 5-bromo - 2' – deoxycytidine (Bird & McGuire, 1966), azauracil (Endo & Schaeffer, 1967), morphactin (Orion & Minz, 1971) and maleic hydrazide (Nusbaum, 1958) inhibit RNA, DNA or protein synthesis and also inhibit giant cell formation and subsequent nematode development. It was concluded that active protein, RNA and DNA synthesis is essential for the maintenance of the giant cells and associated nematode development.

When M. incognita enters resistant cultivars of tomato, there is no giant cell formation but there is browning and necrosis of cells around the nematodes (hypersensitive reaction). Presumably this mechanism prevents the development of

* Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.
the invading nematodes within the resistant host. Not much is known regarding protein or RNA synthesis in relation to the hypersensitive reaction. We tested the effect of (1) puromycin and cycloheximide, both of which inhibit protein synthesis through their effect at the site of transfer of amino-acyl-tRNA to ribosome (Nathans, 1964; Siegel & Sisler, 1964) and (2) actinomycin D, which complexes with DNA and prevents the activity of RNA polymerase, subsequently inhibiting RNA synthesis (Sobell, 1974) on susceptible and resistant cultivars of tomato infected with Meloidogyne incognita.

MATERIALS AND METHODS

Two cultivars of tomato, Lycopersicon esculentum Mill. were used in the investigation. The susceptible cultivar, Bonny Best (BB), produces giant cells and galls, whereas the resistant cultivar Nematex (Ntex) develops a hypersensitive reaction (HR), in response to infection with M. incognita (Paulson & Webster, 1970 and 1972).

Seeds of both tomato cultivars were surface sterilized with 5% sodium hypochlorite for 15 min, washed with distilled water and then germinated in Petri dishes containing potato-dextrose-agar medium. The Petri dishes were maintained in darkness. Three-day-old seedlings were transferred to 6 cm diameter Petri dishes containing a 1.5% agar medium prepared in Hoagland's nutrient solution, sucrose (1.5%) and the different inhibitors to be tested. Sucrose was added to the medium because the plants were maintained in continuous darkness. The seedlings were inoculated with nematode larvae 24 hr after planting on the agar medium.

Six to 7 days before inoculating the seedlings, egg sacs of M. incognita were collected from stock plants and surface sterilized by immersing sequentially in a solution of penicillin (0.1%) and streptomycin (0.1%) for 45 min, of Cetavelon (0.05%) for 1 minute and of Hibitane (0.4%) for 6 min (Aist & Riggs, 1969). They were rinsed with sterile water and maintained in aerated water at 27°. The hatched, second stage larvae were surface sterilized with Hibitane (0.3%) for 15 min, washed with sterile water and 60-70 larvae per plant were transferred, under aseptic conditions, close to the root tips of the 4-day old seedlings on agar. The plants were maintained in darkness at 27° for 15 days following which observations were made and the experiments terminated.

Roots were examined with a dissecting microscope for the formation of root galls and swellings or browning (necrosis) of the root tips. Absence of galls or swellings on the roots and the presence of external browning was taken as a measure of resistance. The results are expressed as 1) total number of galls and swellings per ten plants, 2) percentage of plants that show these symptoms, and 3) presence or absence of necrosis of the root tips.

Larval development was followed by staining batches of seedling roots with 0.1% cotton blue in lactophenol (Hooper, 1970) at different times after inoculation. The degree of larval development was taken as one of the measures of susceptibility of the host.