THE EFFECT OF TEMPERATURE AND NEMATODE SPECIES ON INTERACTIONS BETWEEN THE NEMATOPHAGOUS FUNGUS VERTICILLIUM CHLAMYDOSPORIUM AND ROOT-KNOT NEMATODES (MELOIDOGYNE SPP.)

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Optimal growth and sporulation on agar of an isolate of Verticillium chlamydosporium occurred at 32°C and 22°C, respectively. Differences in the numbers of colony-forming units isolated from the root surface of tomato plants grown at different temperatures reflected the rate of hyphal growth on agar. However, the numbers of colony-forming units in soil at different temperatures was related to the effect of temperature on sporulation in vitro; the fungus was most abundant in soil at 22°C, which was the optimal temperature for sporulation. After one nematode generation the most extensive root colonisation occurred at 20°C followed by 25 and 30°C. As a result, egg-masses exposed on the root surface were most extensively colonised at 20°C followed by 25 and 30°C. Total egg and juvenile counts showed that greatest control of Meloidogyne was achieved at 25°C (>90%), while control at 20 and 30°C was usually 60-70%. The lower level of control by V. chlamydosporium at 20°C may result from a reduced proportion of nematode egg-masses exposed on the root surface at this temperature. Eggs of M. arenaria, M. incognita and M. javanica were equally susceptible to parasitism by V. chlamydosporium. Of these three species, M. arenaria induced large galls and formed large egg-masses while M. incognita and M. javanica induced small galls and formed small egg-masses at 20°C. The end result was that control with V. chlamydosporium was approximately the same for the three nematode species.

Keywords: Verticillium chlamydosporium, Meloidogyne spp., sporulation, temperature, root colonisation, biological control.

Verticillium chlamydosporium Goddard is a facultative parasite of eggs and females of cyst and root-knot nematodes (Willcox & Tribe, 1974; Kerry, 1975; Morgan-Jones et al., 1981; Freire & Bridge, 1985). De Leij & Kerry (1991) showed that an isolate of V. chlamydosporium effective against Meloidogyne arenaria Neal was able to establish and proliferate in non-sterile soil, and colonise the rhizosphere of tomato plants, especially those sites where gall formation as a result of nematode invasion had occurred. This enabled the fungus to colonise the egg-masses of M. arenaria when they appeared on the root-surface. At this stage the nematode eggs were still immature and, therefore, more susceptible to infection by V. chlamydosporium than eggs containing second-stage juveniles (Irving & Kerry, 1986).

Temperature is known to affect the rate of production, development and hatching of Meloidogyne eggs as well as the rate of development from juvenile
to egg-laying female (Tyler, 1933; Wallace, 1971; Bird, 1972; Ferris et al., 1978). In general, there are lower and upper threshold temperatures, between which the rate of development is usually linearly correlated with temperature (Allee, et al., 1949). The rate at which nematode eggs are laid and develop and the rate at which V. chlamydosporium can infect immature eggs depend, therefore, on the growth rate of the fungus in relation to the rate of development of the nematode species involved. Stirling (1979) found that the egg-parasite Dactylella oviparasitica Stirling & Mankau was more effective in parasitising eggs inside egg-masses of M. incognita Kofoid & White on agar at temperatures which were sub-optimal for egg-production, egg-development and hatch.

Meloidogyne arenaria, M. incognita and M. javanica Treub are regarded as thermophiles and are widespread in the warmer regions of the world (Van Gundy, 1985). The temperature requirements of these three species are almost the same (de Guiran & Ritter, 1979). It is, however, not known whether the species differ in their susceptibility to particular isolates of V. chlamydosporium. When 103 V. chlamydosporium isolates were tested for their capacity to infect eggs of Heterodera avenae Woll., Globodera rostochiensis (Woll.) and M. incognita on water agar, isolates differed greatly in virulence, but the three nematode species did not differ in susceptibility (Kerry, 1990). Although susceptibility of nematode eggs to infection by V. chlamydosporium greatly influences the efficacy of the fungus as a biological control agent, little is known about nematode-host plant interactions in terms of gall-size and fecundity, both of which can have implications for the efficacy of nematophagous fungi (Stirling et al., 1974; de Leij & Kerry, 1991; de Leij et al., 1992).

The experiments presented in this paper include in vitro tests and pot tests designed to study the effect of temperature on the interactions between V. chlamydosporium and Meloidogyne species on tomato plants.

MATERIALS AND METHODS

**Fungal isolate:** An isolate of V. chlamydosporium (CMI cc 334168) originally from M. incognita eggs and effective against M. arenaria and M. incognita in pot tests was used (de Leij & Kerry, 1991; de Leij et al., 1992). The isolate was stored at 5°C on silica gel (Smith & Onions, 1983). A few silica gel crystals were sprinkled onto a Petri dish containing water agar (0.8%) and incubated at 25°C for 2wk, allowing the fungus to grow away from the crystals onto the agar. Plugs taken from the fungal colonies that developed were used for experiments.

In vitro experiments

**Growth-rate on agar at different temperatures:** Agar plugs (7 mm diam) were placed in the middle of 9 cm diam Petri dishes containing corn meal agar