Verticillium chlamydosporium multiplied in peaty-sand from inoculum rates of 500, 1,000, 5,000 or 10,000 chlamydospores/g soil to a maximum number of $5.5 \times 10^4$ cfu/g soil and survived for at least 8 wk in pots planted with tomato plants inoculated with 1,000 second stage juveniles of Meloidogyne incognita. The fungus survived, but did not multiply, in loamy sand or sand. Establishment of V. chlamydosporium on the rhizoplane of tomato plants was greater in peaty sand than in loamy sand or sand. Nematode control was in general greater in peaty sand (average 59% control) than in the other two soil types (average control in loamy sand 51% and in sand 39%). In a microplot experiment on sandy loam, V. chlamydosporium controlled populations of M. hapla on tomato plants by more than 90%. The fungus multiplied and survived in soil for at least 123 days. More fungus was found in rhizosphere soil than in non-rhizosphere soil. Combining V. chlamydosporium with an aldicarb treatment equivalent to 2.8 kg a.i./ha did not affect the activity of the fungus, and gave better control (98%) than a treatment with V. chlamydosporium or aldicarb alone (90%).

Keywords: Verticillium chlamydosporium, biological control, Meloidogyne incognita, Meloidogyne hapla, egg-masses, soil type, inoculum

INTRODUCTION

An isolate of Verticillium chlamydosporium Goddard controlled four different Meloidogyne spp. when the fungus was mixed into a non-sterilised sand-peat potting compost (de Leij & Kerry, 1990, 1991). The fungus is a parasite of cyst nematodes (Heterodera spp. and Globodera spp.) and root-knot nematodes (Meloidogyne spp.), (Willcox & Tribe, 1974; Kerry, 1975; Morgan-Jones, Godoy & Rodriguez-Kábana, 1981; Freire & Bridge, 1985), but can survive as a saprophyte in the absence of a nematode host. In order to control Meloidogyne spp., fungal inoculum applied to soil must grow and colonise the rhizosphere, particularly root-galls induced by the nematode (de Leij & Kerry, 1991). Here the fungus infects eggs laid by root-knot nematodes in egg-masses laid on the root surface. Although growth of the fungus on the rhizoplane can, to some extent, compensate for a low inoculum density in soil (de Leij, Davies & Kerry, 1992), the soil environment may be important for the initial establishment of the fungus on the rhizoplane.

In this paper the effects of three soil types on the establishment of V. chlamydosporium in soil and on the rhizoplane were tested. Five inoculum rates
and the resulting population control of *Meloidogyne incognita* (Kofoid & White) were examined in a pot test. The fungus was also tested against *Meloidogyne hapla* Chitwood in a microplot experiment in a plastic tunnel-house.

**MATERIALS AND METHODS**

*Fungal isolate and production of inoculum:* An isolate of *V. chlamydosporium* (CMI cc 334 168) originally isolated from *M. incognita* eggs was used because it previously controlled *Meloidogyne arenaria* (Neal), *M. incognita*, *Meloidogyne javanica* (Treub) and *M. hapla* (de Leij & Kerry, 1990). The isolate was stored at 5°C on silica gel (Smith & Onions, 1983). For experimental use, a few silica gel crystals were sprinkled onto a Petri dish containing water agar (0.8%) and were incubated at 25°C for 2 wk. Two plugs (7 mm diam) taken from the fungal colonies that developed were used to inoculate 250 ml conical flasks, each containing 150 g of a moist, autoclaved mixture of sand and milled barley grain (1:1 v/v). After 2 wk at 25°C the cultures were washed on a 50 μm aperture sieve with a fine water spray to retain the sand and grain, and fungal propagules were collected on a 10 μm aperture sieve held beneath. The residue on this sieve was further washed to remove conidia and small hyphal fragments, leaving mainly chlamydospores and some larger hyphal fragments. The chlamydospores were counted in a haemocytometer. Inoculum was prepared by mixing the residue from the 10 μm sieve with fine sand to give a concentration of 10⁷ chlamydospores/g sand. This inoculum was then further mixed with the soils used for the different experiments.

**Pot experiment**

*Soil types and inoculum rates:* Three soil types were selected: an unsterilised peat-based potting compost (37% peat, 19% fine sand, 44% coarse sand), a loamy sand from Woburn, England, mixed with 25% coarse sand (22% silt, 47% fine sand, 31% coarse sand) and sand mixed with 25% Woburn soil (7% silt, 53% fine sand, 40% coarse sand). Pots (12.5 diam) were filled with 750 ml of the different soil-types, and each of four replicate pots were inoculated with 0, 500, 1,000, 5,000 or 10,000 chlamydospores/g soil. Each pot was planted with one 4 wk old tomato seedling (cv. Pixie) that was allowed to establish for 2 wk before 1,000 second stage *M. incognita* juveniles (< 1 wk old) suspended in 10 ml water were pipetted into three holes around the roots. The pots were placed in a glasshouse at 25-30°C in four completely randomized blocks. Each plant received 0.75 g general fertiliser (Phostrogen; N:10%, P:10%, K:27%, Mg:1.3%, Fe:0.4%, Mn:0.02%) at planting, and was watered daily according to need.

*Fungal establishment:* Three of the four replicates of each treatment were sampled immediately after inoculation with chlamydospores and at 2 wk intervals throughout the experiment. At each sampling, two cores (7 mm diam) were