**CROP PROTECTION PROGRAMME**

# Integrated management of root-knot nematodes on vegetables in Kenya

**R 7472 (ZA 0324)**

**FINAL TECHNICAL REPORT**

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## Executive Summary

Root-knot nematodes are one of several pest and disease constraints that can severely limit productivity of vegetable crops. They are a particularly difficult problem because of their wide range of host plants, including weeds. In addition, nematodes become more damaging where land is cropped continuously. The situation in the Peri-urban zones of vegetable production in Kenya is therefore conducive to the increase of this pest. Appropriate methods to combat nematodes in vegetables are needed urgently and this project has sought to develop management strategies based on naturally occurring biological control agents (bcas), traditional cultural and management practices and rotation of tomato a high-value susceptible crop with kale and cabbage, leaf vegetables less susceptible to root-knot nematodes.

Tomatoes are the most valuable of the crops grown by the peri-urban farmers in Kenya. The gross margins can range from £1000-6000 per crop per hectare (compared to £260-2700 for other vegetables). Farmers are mindful of the need to use healthy transplants. The project has addressed the concept of treating nursery seedbeds with biocontrol agents. These have been successful but no more so than the traditional practice of burning trash to kill soil pathogens. The decision as to whether farmers will apply bcas hangs on cost. The use of plants as toxic green manures was not outstanding in efficacy and was not acceptable to farmers on account of cost of irrigating a non-crop species prior to seeding. Bcas can be applied to the field with transplanted tomatoes, applying them to seedbeds could be means of delivery but results would suggest that greater quantities of the "product" are needed with the transplant. Future work should explore the feasibility of specialist production systems for transplants and incorporation of bcas within the root-ball at planting.

Interest in using bcas was shown by an organic producer group in the Thika district. Through budget participatory exercises insights into the potential uptake of nematode management strategies were gained. The major success is the acceptance of the principle, the questions of bca production and cost now remain the challenges.

The project activities are of interest to the export vegetable and flower producers. These companies supplying overseas markets have to meet the demands of the importers who are demanding lower pesticide inputs. In response to this, one company Dudutech (Kenya) Ltd is mass-producing a range of predators, parasitoids and microbial products for use by commercial farms and contracted out-growers. The presence of such companies would appear to give promise to the expectation that production of bcas is possible in Kenya and that through this products will become available to all sectors of the farming community.

## Background

Root-knot nematodes are a global problem particularly in the warmer soils and where land is cropped intensively. Their presence and importance may be unrecognised as the direct symptoms of attack occur in the root systems although indirect effects are manifest above ground as poor growth and yield which can be attributed to a number of causes. An immediate problem for a research programme is to evaluate farmers' perceptions of these pests and in many cases draw their attention to them.

Management of root-knot nematodes is difficult because of the lack of economically valuable non-host crops that can be grown in crop rotations and the lack of appropriate resistance characters in varieties that farmers (in Kenya) might wish to grow. Other cultural or management practices such as use of soil solarisation do not provide long-term solutions to the problem, largely because of the capacity of root-knot nematodes to multiply several thousand-fold in a crop cycle.

Estimates of losses attributed to root-knot nematodes can vary; 20 % crop loss is usual but total crop failure is not uncommon. On vegetables in Kenya, yield reductions caused by root-knot nematodes may be up to 50% (Hollis, 1962) and these are principally due to *M. incognita* and *M. javanica* (Kanyagia, 1980). These nematodes can also exacerbate diseases particularly vascular wilts.

This project has sought to build upon earlier research (R6611 done on the biological control agents *Pasteuria penetrans* and *Verticillium chlamydosporium* (now renamed as *Pochonia chlamydosporia)*. *P penetrans* (Pp) is an obligate parasite of root-knot nematodes that exists in the soil as a spore, which attaches to the infective stage of the nematode when it is free-living in the soil. The parasitism progresses after a nematode has entered a root and infected nematodes develop normally but fail to produce eggs. Parasitised female nematodes become full of spores and these eventually enter the soil as they (and root systems containing them) disintegrate. The important issues concerning this biological control agent are its mass production, the distribution and density (concentration) of spores in soil and the specificity of the particular spore isolate to populations of root-knot nematodes.

*Pochonia chlamydosporia* (Pc) on the other hand is an egg parasite. The fungus colonises the rhizosphere of plants and infects the egg masses of root-knot nematodes that are extruded by female nematodes to the root surface. Pc may also have some host specificity indeed some isolates are more effective than others in root colonisation and egg infection (Bourne *et al*, 1996) but the fungus is not able to significantly reduce damaging nematode populations on highly susceptible crops. The strategy to exploit *P. chlamydosporia* combines applications of the fungus with poor host crops for the nematode, which significantly decrease infestations of the nematode in soil before a fully susceptible crop is grown in the rotation. This strategy has been successful in small plots (Kerry, 1995) and in the field in Cuba (Atkins *et al*., 2002) and will be further evaluated in Kenya, using local isolates of the fungus.

The fungus can be grown *in vitro* on a wide range of media but cheap methods are required to provide sufficient inoculum for field-testing. *Pochonia chlamydosporia* produces chlamydospores, which are robust, easy to handle and can be added to soil in simple aqueous suspensions to establish the fungus in the rhizosphere. However, they are not produced efficiently in liquid fermentation and a biphasic system is needed in which fungal biomass is produced in liquid fermentation and used to seed a solid medium on which the fungus sporulates. Few commercial companies have the capability of such production methods, which are expensive and to evaluate the efficacy of the agent in a number of field trials simple and cheap methods of mass production are required. Solid media are favoured for the simple production of fungal biological control agents because they do not require the significant investment of sterile, liquid fermenters but they are difficult to keep free of contamination.

Mass production of inoculum is an important constraint for scaling up evaluation trials and grower involvement. There is a need to reduce inoculum rates by reducing broadcast to in row treatments and to understand factors affecting the survival of inoculum between crops to estimate the need for repeated applications to the same area. Although the fungus may remain active for several months in soil, the long-term survival between crops is unknown.

The biological control agents used in this project are complementary in that Pp can influence numbers of nematodes entering roots and the reproduction of those that do establish in root systems and Pc can parasitise the eggs of those nematodes that may have escaped infection by Pp.

The project has been based on the premises that Pp will become established in soil at sufficient spore densities to cause root-knot nematode populations to decline but that Pc may require repeated application at unknown frequencies. The epidemiology of both bcas is influenced by the host crop and rotation is therefore an important component of a management strategy. At the project inception it was not known how farmers perceived root-knot nematodes as a problem. An objective of the project has been to assess farmer awareness of this pest. Demand for this research came from KARI which recognises nematodes as pests and that there is little remedial treatment other than use of nematicides which have environmental and human toxicological concerns. Need for more nematological (and IPM) research has been expressed by various donor agencies in East Africa.

**Project Purpose**

The project purpose is to develop and promote improved methods of nematode control in market gardening and horticultural enterprises through the development of bio-management strategies that integrate the use of selected biological control agents with other control methods. The research will lead to environmentally benign methods of nematode management and improve the yield and quality of crops produced by resource-poor farmers in Kenya. The local production of indigenous, selected biological control agents will provide employment opportunities in rural communities.

**Research Activities**

**1.1 - 1.2 Training**:

Two Kenyan scientists were trained in the identification, handling, production, application and evaluation of *P. chlamydosporia* and *P. penetrans.* For *P. chlamydosporia* a workshop manual produced in a EU project, which describes all standard operating procedures used and the background to the practical exploitation of the fungus was provided. A large bibliography of references on *Pasteuria* and biocontrol methods in general was provided. The scientists visited the laboratories at Reading and Rothamsted to gain practical experience at the inception of the project

**1.3 - 1.4 Establishment of trials in Kenya to integrate bcas with other cropping practices.**

The field trials work in Kenya investigated two questions. First, it is already established that farmers will give attention to the preparation of their seedbeds in order to produce healthy plants for transplanting in the field. The burning of trash on the seedbed prior to seeding is practised by some farmers (Oruko and Ndun'gu 2001) [Vegetable Cluster Projects Output]. A question asked for this project was "is it possible to develop a system of controlling nematodes with biological control agents in the seedbed and will this also provide a means of introducing bcas into the field through the transplants?" These nursery bed trials were conducted on farmers' fields near Thika and in Mwea.

The long-term evaluation of the bcas was done at the KARI Horticultural station at Thika. In this experiment bcas were applied to plots which were then planted with tomato (highly susceptible to root-knot) or cabbage (less susceptible/tolerant). In the following crop cycles the plots were planted with either of these crops. This experiment is a key component of the project but due to the severe drought at Thika in 2000 the trial could not be established until April 2001 (Appendix CABI-KARI Folder : File Rotation Trial layout).

A fourth crop cycle (tomato) was planted in May 2002. The results of this will not be evaluated until after submission of this report in September 2002.

**1.5 - 1.7**
**Characterisation of local strains of bcas**
**Factors influencing proliferation and survival**
**Comparison of pathogenicity of Kenyan bcas against standards held in UK**

POCHONIA CHLAMYDOSPORIA

**Characterisation of isolates**

**Identification of *P. chlamydosporia* by β-tubulin PCR**

Cultures of all (85) *P. chlamydosporia* isolates were maintained on Czapek Dox agar at 4°C prior to use. 10 ml Czapek Dox liquid medium (30 g $l^{-1}$ sucrose, 3 g $l^{-1}$ $NaNO_3$, 0.5 g $l^{-1}$ $MgSO_4$, 0.5 g $l^{-1}$ KCl, 0.5 g $l^{-1}$ yeast extract (Difco), 1 g $l^{-1}$ $K_2HPO_4$, 0.01 g $l^{-1}$ $FeSO_4$) was inoculated with 3 mm diameter agar plugs taken from the colony margin of plates in 25 ml flasks. Flasks were shaken at 100 rpm and incubated at 28 °C for 7 days. After this time, mycelium was harvested on Whatman no. 1 filter paper (UK) under vacuum filtration.

Genomic DNA was extracted from dried mycelium using the method of Klimyuk *et al*. 1993 for extraction of genomic DNA from plant tissues. Fungal samples were tested with a primer set based on the *P. chlamydosporia* β-tubulin gene (tub1f + tub1r; Hirsch *et al.,* 2001), specific for this species and utilised to verify whether a sample was *P. chlamydosporia*. β-tubulin primer sequences were: tub 1f 5'-TTT GCA GTA TCT CAG TGT TC-3; tub 1r 5'-ATG CAA GAA AGC CTT GCG AC-3'.

Fungal samples were tested with a primer set designed to amplify a fragment of the *P. chlamydosporia* β tubulin gene (Hirsch et al., 2001). β-tubulin PCR reactions of 20 μl, contained: 1μl sample DNA; 0.1 μM of each primer; 1 × PCR buffer (Roche Diagnostics, Lewes, UK; 1.5 mM $Mg^{2+}$); 1 mM $MgCl_2$; 0.2 mM of each dNTP (Roche) and 1 U Taq polymerase (Roche). Thermocycling conditions were 95°C for 1 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and a final extension of 72°C for 5 min.

A total of 15 RAPD primers were tested (Operon technologies Inc, USA) for suitability for discrimination of the 38 isolates of *P. chlamydosporia* selected for further screening of their biological control potential as described below.

**RAPD Primer sequences.**

| *Primer Name* | *Primer Sequence 5' to 3'* |
|---|---|
| ***OPA-02*** | ***TCCCGAGCTG*** |
| ***OPA-07*** | ***GAAACGGGTG*** |
| ***OPE-06*** | ***AAGACCCCTC*** |
| ***OPG-06*** | ***GTCCCTAACC*** |
| ***OPG-16*** | ***AGCGTCCTCC*** |
| ***OPG-19*** | ***GTCAGGGCAA*** |
| ***OPI-13*** | ***CTGGGGCTGA*** |
| ***OPJ-19*** | ***GGACACCACT*** |
| ***OPK-04*** | ***CCGCCCAAAC*** |
| ***OPN-11*** | ***TCGCCGCAAA*** |
| ***OPN-13*** | ***AGCGTCACTC*** |
| ***OPN-18*** | ***GGTGAGGTCA*** |
| ***OPV-01*** | ***TGACGCATGG*** |
| ***OPV-16*** | ***ACACCCCACA*** |
| ***OPV-17*** | ***ACCGGCTTGT*** |

RAPD PCR reactions of 20μl were set up containing 16.7μM primer, 1 x PCR buffer (Roche), 100μM each dNTP (Roche), 0.4U Taq polymerase (Roche), 0.5μl fungal DNA (10 – 100ng) and 1mM $MgCl_2$. RAPD PCR thermocycling conditions were 39 cycles of: 92°C for 2 min; 35 °C for 1 min; 72°C for 1 min followed by 1 cycle of 92°C for 1 min, 35°C and a final extension of 72°C for 5 min.

All PCR products were run on 1.5% agarose gels (Helena Bioscience, Sunderland, UK), stained with ethidium bromide (0.5μg $ml^{-1}$) and illuminated with UV light (320nm). Product sizes were estimated by comparison with a 123 base pair DNA ladder (Gibco BRL ®, Paisley, UK). Gels were photographed using the Eagle Eye Imaging System (Stratagene).

**Laboratory screening of selected isolates of *P. chlamydosporia***

Isolates were obtained from soil samples as described by Hidalgo-Diaz *et al*. (2000) and stored on corn meal agar slopes (1.7%) at $4^oC$. The isolates were then screened to determine their potential as biological control agents. The fungus must be able to colonise the rhizosphere, parasitise eggs of root-knot nematodes and produce chlamydospores, which are the preferred inoculum, if they are to be considered as potential biological control agents. Of the 85 isolates collected, 38 were selected for screening on the basis of the climatic conditions, soil types and different crops from which they were collected.

To assess the ability of the isolates to colonise the rhizosphere, maize seeds (cv. Katumani) were surface sterilised in 7% calcium hypochlorite for 45 minutes on a wrist action shaker and washed in sterile distilled water. The seeds were aseptically transferred to a seed germinating medium (1L distilled water, 12g technical agar (Oxoid) 10g glucose, 0.1g peptone, 0.1g yeast extract) and incubated at $25^oC$ to test that all surface contaminants had been removed (Kerry *et al*., 1984). Test tubes (15 x 2.5 cm) were two thirds filled with vermiculite, moistened, stoppered with cotton wool and autoclaved. When cool, four 2mm plugs of the fungus on CMA was inserted just below the surface of the vermiculite and a surface sterile, germinated maize seed placed on top of the fungus. Aluminium foil was wrapped around the outside of each tube to the level of the vermiculite. After 3 weeks at room temperature, the roots were shaken free of vermiculite, cut into 1cm sections and placed on water agar (containing 50mg $1^{-1}$ of each of streptomycin sulphate, chloramphenicol and chlortetracycline). After 4 days incubation at $25^oC$ the number of segments colonised by the fungus was counted. There were two replicates of each isolate.

The number of eggs of *M. incognita* parasitised by each isolate was estimated by washing the fungus growing on CMA in 2-5ml of sterile distilled water. The surface of the Petri dish

was scraped with a sterile glass rod to suspend the fungus. A 0.2ml aliquot of the fungal suspension was then spread on a 9cm Petri dish containing water agar with antibiotics (50 mg $l^{-1}$ streptomycin sulphate, chloramphenicol, chlortetracycline) and incubated at 25$^{o}$C for 2 days. The roots of an eggplant (cv. Long Purple) infected with *M. incognita* (R2 1135) were washed and several hundred egg masses removed by hand. These were crushed mechanically to separate the eggs from the gelatinous matrix of the egg mass. About 200 eggs were added to each plate in 0.5 ml of water and plates were incubated at 25$^{o}$C for 3 days after which time the percentage of eggs parasitised was estimated. Eggs were also placed on water agar with no surface growth of fungus to estimate the numbers of infected eggs within the nematode populations. Egg infection was also compared with isolate Vc10, the standard used at Rothamsted.

Chlamydospores were produced on a sand:milled barley medium (Leij De & Kerry, 1991). There were two flasks per isolate from each of which two 1g subsamples were removed after thorough mixing. The number of chlamydospores in each 1g subsample of the colonised medium was estimated using a haemocytometer. The viability of the chlamydospores was checked by plating 0.2ml of a suspension of chlamydospores onto sorbose agar amended with antibiotics (50mg $l^{-1}$ streptomycin sulphate, chloramphenicol, chlortetracycline) and assessing the percentage germination after 48 hours. The standard isolate Vc10 was not included in this test as it is not permitted to bring exotic isolates into Kenya. The rhizosphere colonisation and chlamydospore production screen were done at CAB International, Nairobi as part of the collaborative project.

**Glasshouse screening of selected isolates of *P. chlamydosporia*.**

From the results of the laboratory screen, six isolates were selected on the basis of their ability to parasitise eggs and produce chlamydospores (9A, 12T2, 20A and 23A). All the isolates tested proved to be equally able to colonise the rhizosphere so this criteria could not be used in the selection of isolates for glasshouse screening. The standard isolate Vc10, held at Rothamsted, was also included and control pots with no fungal treatment. Chlamydospores were produced as described (Leij De & Kerry, 1991) and unsterilised compost (peat: coarse sand 3 : 1 v/v) inoculated with fungus at an application rate of 5000 viable chlamydospores $g^{-1}$ soil. A 3-week old tomato seedling (cv. Tiny Tim) was transplanted into each pot (13cm diameter) filled with this growth medium, and after a week, 3000 second stage juveniles of *M. incognita* were added in suspension in 10ml water to the compost around each plant. The pots were placed in the glasshouse at 20$^{o}$C for 52 days and watered and fertilised as required. There were 5 replicates of each treatment (isolate).

The plants were harvested, the roots washed in water, blotted dry and the fresh root weight recorded. The roots of each plant were cut into 1cm sections, mixed thoroughly and two 1g subsamples were removed. One subsample was used to estimate colonisation of the rhizosphere (colony forming units - CFU $g^{-1}$ root) by dilution plating on a semi-selective medium (Bourne *et al*., 1996). Egg masses were counted and removed from the other subsample and the number of eggs per egg mass estimated by dissolving the gelatinous matrix of 10 egg masses in 5% sodium hypochlorite for 2-5 minutes and counting the numbers of eggs using standard techniques (Hooper, 1986). The number of eggs infected by *P. chlamydosporia* was estimated by mechanically crushing about 60 egg masses to release the eggs. About 200-300 eggs (in 0.5ml water) were then spread onto a water agar amended with antibiotics and incubated at 25$^{o}$C, after which the eggs were viewed using a microscope (x400) for evidence of infection by the fungus. However, in this experiment there were few infected eggs, possibly because the experiment had been harvested too early, so only the growth of the fungus from the egg mass was recorded.

The amount of fungus in the soil in terms of CFU $g^{-1}$ soil was estimated both at the beginning of the experiment immediately after inoculation (initial CFU) and at the time of harvest (final CFU). This was done using a 1g subsample of soil and dilution plating onto semi-selective medium as described by Leij De & Kerry (1991).

**Studies on the proliferation and survival of *P. chlamydosporia* in soil**

**Survival of *P. chlamydosporia* in two soils following growth on cabbage, bean and tomato plant roots in the presence and absence of *M. incognita*.**

Chlamydospores of the fungus were produced on the sand-bran medium as described and added in an aqueous suspension at a rate of 5000 spores $g^{-1}$ soil to pots (12.5 diam.) containing a peat or sand-based compost. A 4-wk old tomato seedling cv. Tiny Tim or a 2-wk old seedling of cabbage cv. Durham Early or a local line of Phaseolus bean from Malawi was transplanted into each pot. There were 48 pots for each plant cultivar in each soil (96 pots) and a similar number of pots were left unplanted. One week later, 3000 second stage juveniles of *Meloidogyne incognita* were added to half the pots. The nematodes were extracted from cultures maintained on Aubergine cv. Purple Ruby in the glasshouse and the eggs hatched using standard techniques (Hooper, 1986). Each treatment combination was replicated four times and the experiment was conducted in a completely randomised design on a glasshouse bench. The pots were watered as required and treated with a slow release fertiliser.

After 9 weeks, the plant shoots were removed and the fungal densities in soil estimated in four replicates of each treatment selected at random using the dilution plate method on a selective medium (Kerry *et al*., 1993). Thereafter, to determine the survival of the fungus in soil, similar samples were processed by the same method at monthly intervals on five occasions. Throughout the experiment the pots were kept moist and soil temperatures were maintained above 20$^{o}$C.

**The effect of chlamydospore size on the establishment of *P. chlamydosporia* in soil.**

As the extraction of small chlamydospores from the sand-bran medium is difficult and labour intensive, a comparison was made of the ability of spores of different size ranges to establish the fungus in the soil and rhizosphere. If small spores were ineffective as a soil inoculum, the extraction of chlamydospores from the medium would be greatly eased by the use of coarser sieves than the 10µm aperture ones currently used, which retain all chlamydospores. Spores washed from the sand-bran medium were collected on nested sieves that divided them into three size classes; >30µm, 20-30µm and 10-20µm. The viability of each group was checked by plating 0.2ml of the suspension of chlamydospores onto sorbose dextrose agar amended with antibiotics (Leij De *et al*, 1993). After 2 days at 25$^{o}$C, the proportion of spores germinated was determined. Each class of spores was added to a sand-based compost at a rate of 5000 spores $g^{-1}$, as before, and a tomato seedling cv. Tiny Tim planted in each pot (12.5cm diam.) of treated soil. Six replicates of each size class and a further 6 pots untreated with the fungus were set up in a randomised block in the glasshouse and watered and supplied with slow release fertiliser, as necessary. After one week, 2500 second-stage juveniles of *M. incognita* were added to all pots.

The experiment was harvested after 8 weeks, the fresh weight of the tomato shoots and roots measured, and the density of the fungus on roots and in soil estimated using standard techniques (Leij De & Kerry, 1991). The roots were cut into 1cm sections, thoroughly mixed and a sample (1g) taken at random to estimate the number of nematode egg masses, which were counted at x 50 magnification using a dissecting microscope. The egg masses were broken up mechanically and the eggs released in a suspension of water and counted (Leij De & Kerry, 1991). At least 200 eggs were plated onto 0.2% water agar (Oxoid) and incubated at 25$^{o}$C for 3 days to estimate those colonised by the fungus.

**Survival of *P. chlamydosporia* in soil at a range of temperatures and water potentials**

Chlamydospores were produced as described and soil (peat : coarse sand 3 : 1v/v) was inoculated with the fungus at an application rate of 5,000 chlamydospores/g soil. Three week old tomato seedlings (cv. Pixie) were transplanted into 13cm diameter pots containing inoculated soil and after a further week 3,000 J2 of *M. incognita* were added to the soil around

each plant. After 6 weeks, the plants were removed, the roots cut in 1cm portions and reincorporated into the soil. The roots and soil from all pots were thoroughly mixed together in a large polythene bag. This initial stage in the experiment allowed the fungus to establish and proliferate in the soil and rhizosphere before being subjected to adverse conditions.

Water potential was adjusted by placing soil and a sodium chloride solution in close proximity in a sealed container, the water potential of the soil can be altered so as to equilibrate with the water potential of the sodium chloride solution. A 30g subsample of the soil was spread in the base of a 9cm diameter Petri dish and five of those in covered dishes placed in each large plastic freezer box (30 x 22 x 14cm). Survival of the fungus was monitored at temperatures of 25, 30 and 40$^{o}$C and four water potentials, with one freezer box for each treatment combination. Water potentials were achieved by placing 5 open Petri dishes (9cm diameter) of different molarities of sodium chloride in the boxes as follows:-

| | | |
|---|---|---|
| 0.05 | Molal. equivalent to | -2 bar |
| 0.5 | | -23 |
| 1.7 | | -82 |
| 2.0 | | -97 |

(where 1 molal $\equiv$ 58.5 g NaCl + 1000 g $H_2O$)

Boxes were sealed and one of each water potential placed in temperature-controlled incubators at each of the experimental temperatures. At two weekly intervals, a subsample of soil was removed and assessed for abundance of the fungus and moisture content (estimated by air drying a known weight of soil for 1 week). The water potential generated by the sodium chloride solutions does differ with temperature but over the range 25-40$^{o}$C, differences were negligible (Lang, 1967).

**Competitive saprophytic ability of *P. chlamydosporia***

The spread of the fungus in soil and in the rhizosphere will depend, in part, on the ability of specific isolates to compete with the residual microflora. This experiment aims simply to compare the growth of the fungus in composts with different levels of microbial activity. Chlamydospores were produced and extracted as described. Moist compost was put into a sterile 250 ml flask in different proportions of autoclaved to unautoclaved compost; 0, 20, 40, 60, 80, and 100% to give a total of 150g compost/flask. There were 3 replicates (flasks) of each combination. In aseptic conditions, chlamydospores were mixed with the compost by shaking the flasks, at a rate of 5,000 chlamydospores/g compost. Flasks were incubated at 25$^{o}$C. Subsamples were taken (aseptically) on day 0, weeks 1-5, 7, 20, 50 and 70 after inoculation with the fungus and assessed for the amount of fungus in terms of CFU.

**To determine the proliferation of *P. chlamydosporia* in sand at different application rates of the fungus**

Sand was inoculated with *P. chlamydosporia* at application rates of 50, 500, 5000, 15000, 30000 and 50000 chlamydospores/g sand. A 3 week old seedling of tomato (cv. Pixie) was transplanted into each 13cm diameter pot of inoculated sand. After a further week, 1000 J2 of *M. incognita* were added to the soil in each pot. There were 4 replicates (pots) of each treatment. After 6 weeks, plants were harvested and colonisation of the soil and rhizosphere by the fungus was estimated and the numbers of nematodes in the roots counted.

**Measurement of survival of *P. chlamydosporia* in a field trial in Kenya**

The rotational trial at the National Horticulture Research Centre, Thika District, has been described elsewhere in this report. Here, only the work on the measurement of the survival of the fungus in soil is reported. In April 2001, 5000 chlamydospores $g^{-1}$ soil were added to a litre of soil around the roots of the transplanted cabbage and tomato plants, which were harvested in July and August, respectively. The abundance of the fungus was estimated after the first harvest (cycle 1) and after the subsequent cabbage cycle 2 (December, 2001)

and tomato crops using standard techniques (cycle 3, April, 2002) (Kerry *et al.*, 1993). Hence, the survival of the fungus from a single application was measured over three crops.

**Studies on proliferation and application of *Pochonia chlamydosporia***

The experiment was laid out in a split plot design with the whole plot treatments being the 2 crops, tomato and cabbage (Plate 1). The sub-plot treatments were (1) application methods (2) type of seedling i.e. bare rooted or with root plugs. The whole plot treatments had 4 replications, to give a total of 48 experimental units, i.e. 2 whole plot treatments x 4 replications x 2 seedling types x 3 treatments of application of fungal inoculum.

**Plate1.** Experiment on proliferation and application of *Pochonia* in the polytunnel

(1) Application methods included root and soil treatments. For the root treatments, the bare roots of the seedling was dipped in a clay slurry containing spores of *Pochonia* and the root plugs in a water suspension of the same. The soil treatment consisted of spores incorporated into soil by spraying the spore suspension on the soil surface and turning the soil over to a depth of 15 cm. In all cases the targeted inoculum level of 5000 spores per gram of soil was maintained. The control plots had no fungal inoculum.
(2) The bare rooted seedlings were raised in plain trays while the root-plugs were raised in modular trays.

The seedlings were transplanted into planter bags of 30x30x100 cm, to simulate mini field plots (Plate 1). Each bag (an experimental unit) accommodated 4 plants of either tomato or cabbage. Root-knot nematode was cultured on tomato plants grown in similar bags, the soil from which used as the source of inoculum; 1.5l of soil containing 3500 nematodes (estimated with a bio-assay) were added into each bag and incorporated thoroughly. The crops were harvested after 90 days and assessed for the number of colony forming units of *Pochonia* on roots, and percentage of eggs infected.

PASTEURIA PENETRANS

A strain of *Pasteuria penetrans* was found at the Thika Horticultural station (in roots of tomato plants grown by Kenya Seed Company on plots adjacent to the experimental site). This isolate was found to parasitise the local root-knot nematode population and also that from Mwea.

**Observations on infected females in roots**

The basic materials used for working with *P. penetrans* are root systems that contain the spore-filled root-knot nematode females. Until *in vitro* production techniques are discovered,

mass-production of *P. penetrans* will be through the *in vivo* system originally described by Stirling and Wachtel (1980).

The method for adding this type of inoculum under field conditions will be direct application of infested roots to soil. The recommendation will be that roots are dried before use in order to kill any nematode eggs that may be contained in them (because there is always likely to be a proportion of uninfected nematodes in any root-system and farmers will not wish to inoculate live nematode to their plots). There remains an issue that plant roots may contain other pests or pathogens and this concern must be addressed before the practice becomes a recommendation. Where natural Pp infection is known to occur farmers will be advised to leave root systems in the plots.

Questions posed in this study relate to the release of spores from females embedded in roots and to the efficiency of spore assessment from root samples which will be an essential component of evaluating spore yields from mass production studies. For spore concentration assessments in roots, dried roots are milled into a powder and spore counts are made from water suspensions derived from the powder.

In 2000, samples of dry roots were collected from a site used by the Kenya Seed Company. Dry roots from a harvested tomato crop were collected off the ground from a site adjacent to the field plots at the KARI (Kenya Agriculture Research Institute) Field Station, Thika. It was established that these roots contained *Pasteuria*-infected female cadavers. Root samples were re-hydrated in water for 1-2 hours. After re-hydration it was possible to dissect females using a dissecting microscope (25x magnification) from the galled tissue (Pembroke and Gowen, 1992). Parasitised females are distinct in appearance, dense and pearly white (Plate 8). Estimates of spore concentrations were made following the method of Stirling and Wachtel (1980), the first estimations of these spore concentrations indicated *circa* 20,000 spores/mg, this would be considered a low count however there were several hundreds of grams of infected material.

Re-calibration of this spore powder at Reading was proving difficult, with some samples yielding no spores at all. Because of the low concentrations it was difficult to undertake attachment studies, or mass production of the Thika Pp population on Kenyan root-knot nematodes. In Petri dishes containing nematode suspension of the Kenyan root-knot and Thika Pp spore powder inoculum that were left for many days it was observed that spore attachment did occur. This confirmed that there were spores present in the samples although at very low densities. The need to achieve a higher concentration of spores was becoming more urgent.

## Methods

With the knowledge that infected females in root tissue will re-hydrate in water (Plate 8) it was decided to make direct observations on the Thika ground root material. Samples of approximately 1g were placed in Petri dishes and allowed to re-hydrate in water. The samples were then examined under a dissecting microscope 25x magnification. The roots had clearly suffered a large root-knot nematode infestation in the field, dead females, egg masses and galls were readily observed. Only those females that had not laid their eggs, or Pp-infected females showed white when observed with overhead illumination. A few whole infected females were quickly discovered, and infection was confirmed by the presence of mature endospores in the female cadavers when viewed under high-power magnification (400x).

The Thika root material had not been finely ground >2mm (Thomas Scientific Laboratory Mill Model 4). Subsequent observations made on other samples of powder produced at Reading that were more finely ground (<1mm) were also found to contain entire spore-filled female cadavers. Dry and re-hydrated root powders were again studied under a dissecting microscope (Plate 7). Even if cuticles of the females were damaged in the milling process, upon re-hydration, large aggregations of spores still remain.

## Production per female

Mass-producing *Pasteuria* using the *in vivo* system is likely to be used in smallholder and commercial enterprises as it can be done without specialist laboratory facilities. Refinements in the system may be possible. Earlier work has demonstrated that spore rich powders can be derived from roots containing female root-knot nematodes that had been infected with *P. penetrans*. Hitherto, no systematic attempt has been attempted to define the conditions under which maximum spore production might be achieved. In this study plants were inoculated with spore-encumbered juveniles and the plants were grown for twelve weeks at temperatures favourable for *P penetrans* development - in a glasshouse with variable temperatures (20-35 $^{o}$C) and in a growth room (25-28$^{o}$C). Individual infected females were sampled, weighed and numbers of spores per nematode were counted.

## Studies on proliferation, survival and epidemiology of *Pasteuria penetrans*

An important characteristic of *P. penetrans* is the nature of the resting spore usually known as the endospore. These spores can remain viable for many years but their persistence and movement in soil is little understood.

A feature of immediate significance is the time required for spore-filled cadavers of root-knot nematodes to degrade and for the spores to be released into the soil. (see Figure )

In a controlled experiment, a single spore-filled nematode containing *c* 2 million spores was placed in soil in pots and the soil was kept moist for periods of 1, 2 or 3 weeks to allow the female cadaver to decay after which 2000 freshly hatched juveniles were added to each pot. Two days later 4-week old tomato seedlings were planted in the pots which were then left in a growth room at 25-28$^{o}$C.

The plants were evaluated for infection by root-knot nematodes and the presence of *P. penetrans* on the nematodes after 6 and 12 weeks. This was done by counting the numbers of infected and non-infected females in the roots and the numbers of egg masses that were produced. The estimate of numbers of egg masses and infected females provided an indication of the efficiency of *P. penetrans.* Greater infection would be assumed to occur in those pots where the dispersal *P. penetrans* spores had been greatest.

**The effect of the time that juveniles are spore-encumbered before root invasion; levels of spore attachment and the subsequent infection by *P. penetrans* in the root-knot nematode females**

The epidemiological significance of the numbers of *P. penetrans* spores that are attached to the free-living juvenile stage of root-knot nematodes is not fully understood. This is partly because the infection process that eventually leads to the body contents of the female nematode being consumed by the developing parasite will only occur after the juvenile nematode has entered the plant.

It is not certain that all spores that have become attached to a nematode will actually germinate and penetrate the nematode after it has invaded a root.

Root invasion may be influenced by the number of spores attached to the nematode but can also be affected by the vigour of the nematode. The longer that juveniles spend searching for a host root the greater is the chance to acquire spores but nematode vigour may decline. Experiments were conducted to compare infection in three cohorts of spore attachment (low, medium and high) (Table 10) inoculated on tomato plants on the same day and after 24 and 48 hours. About 100 nematodes were inoculated on each tomato seedling and the seedlings were then grown for 42 days in a growth room at 28$^{o}$C. Plants were then washed from their pots and roots were examined for numbers of nematodes and incidence of *P. penetrans* infection.

**2.1. Develop methods of on-farm or small-scale commercial production of bcas**

**Mass production of inoculum of *P. chlamydosporia***

**Evaluation of media for mass production of the fungus**

Although the fungus is able to proliferate in the soil and rhizosphere after addition to soil, application rates of 5000 chlamydospores g soil$^{-1}$ are usually required to cause significant reductions in *Meloidogyne* populations. Current methods of production based on sand:cereal bran media usually produce only $10^{6}$ chlamydospores g$^{-1}$ medium and there is a need to improve the efficacy of culturing methods and the extraction of inocula from the media.

Several materials, including crop residues were evaluated as media to support the production of chlamydospores. Approximately 250ml of aubergine leaves, Napier grass, Neem (leaves, cake and seed), maize leaves, bagasse, and barley, maize, sorghum, rice and soybean seeds were used. The material was milled in a coffee grinder for 3 minutes and mixed with an equal volume of moist, coarse sand. The media were each divided equally into five 3mm plugs taken from the edge of cultures of P. chlamydosporia. The cultures were inoculated for 3 wks at 25$^{o}$C before a 1g sample was removed at random and the chlamydospores counted using standard methods. Duplicate estimates of the numbers of chlamydospores were made from each flask.

- A simple biphasic method of production of *P. chlamydosporia* on cracked rice grain developed by L. Hidalgo-Diaz (unpublished) was evaluated. Briefly, the method involves:

- 40g of rice is heated in 1 litre of water in a bath for 45mins, filtered and the filtrate dispensed in 100ml aliquots into 250ml conical flasks and autoclaved (121$^{o}$C for 15min).

- The liquid medium is inoculated with a 1cm plug taken from the edge of a *P. chlamydosporia* colony and incubated on an orbital shaker (150 rpm) for 3 days at 25$^{o}$C.

- Rice is soaked for 30 minutes and pre-cooked at 100$^{o}$C for 12 minutes before dispensing 100ml aliquots into conical flasks to be autoclaved.

- 10ml of the colonised liquid medium is added to each flask and incubated for 21 days at 25$^{o}$C.

- Chlamydospores are recovered directly from the colonised grain by washing, using standard methods (Leij De & Kerry, 1991) or it is dried for 2 days and the spores are collected using a MycoHarvester (CABI, UK).

**Mass production of inoculum of *P. penetrans***

The mass production of *P. penetrans* can be done on root-knot nematodes on a host plant. A field plot was established in which *P. penetrans* spores in the form of a ground root-powder (a standard technique) was applied to tomatoes in land infested with the root-knot nematodes (Appendix CABI-KARI Data Folder :Pp Production layout).

This experiment was conducted at the National Horticulture Research Centre in Thika District, Central Province of Kenya, to explore the possibility of on-farm production of *P. penetrans* on tomato plants at different inoculum densities. The experiment was laid out in a randomised complete block design, with four treatments: 0g (Control), 1g, 2g and 4g of *P. penetrans* endospore laden root powder (2000 spores mg$^{-1}$) per plant, replicated in three blocks (11.4 x 5.4m). For each treatment, apart from control, approximately 1000cm$^{3}$ of soil was treated with the required amount of *P. penetrans* inoculum, which gave the application rates of 0, 2 x 10$^{4}$, 4 x 10$^{4}$ and 8 x 10$^{4}$ spores g$^{-1}$ soil. Each treatment was applied on plots consisting of 35 tomato (cv Cal-J) plants, planted in 7 single rows at the recommended spacing of 90 x 60cm, with a 2m path between the blocks. Four weeks old tomato seedlings were transplanted on 19 April 2001 to the field, immediately after treatment application. A sub-sample of dried root was taken for determination of the concentration of *P. penetrans* endospores in each plot. This trial has undergone three crop cycles and a fourth is still in progress.

**3.1 Identify promotion pathways for research outputs**

Interest in the project was shown by a group of producers who are members of the Kenya Organic Farmers Association in the Thika district, an assemblage of commercial growers (supplying local markets) in Mwea and Dudutech (Kenya) Ltd from the commercial sector (see below)

**Plate 2:** Demonstration of the nursery seedbed trial at Karigu-ini

**4.1- 4.2 Identify different target groups, variations in resource bases and perceptions of crop problems/ Review uptake and attitudes of farmers in different target and /or resource groups**

A socio-economic survey done by the project in Thika, Kiambu, Machakos and Kajiado (Oruko and Nungu, 2001) identified that farmers in the Thika area are concerned about root-knot nematode and so this would seem to be an appropriate target area. The study identified tomato growers with moderate inputs and outputs ("lowland") and high inputs and outputs (Kiambu), likely to be influenced by market access. A rapid appraisal carried out by the project in Mwea (draft report made by Ndungu, 2001) found it to be an appropriate region to represent the high-input, high-output system since tomatoes are an important cash crop, and nematode problems appear to influence crop production practices such as choice of rotation

Organic farmers were also identified as an interesting and appropriate group. At present they represent the moderate-input, moderate-output category, and they are likely to be especially interested in bcm methods. There are between 11,000 and 24,000 farmers in Kenya trained in organic methods (T. Kiroga, KOFA, pers. comm.) although considerably fewer practising them wholeheartedly. Investigations have begun into certification and sale of organic produce to Switzerland, and marketing prospects in Nairobi, so the resource base of this group may shift in time, but their commitment to biological control methods will remain.

The project decided to conduct on-farm work with high-input, high-output farmers in Mwea and organic farmers in Karigu-ini (near Thika) to assess their interest in the bca technology. The participatory budgets developed with both target groups (section 3) confirmed that both are interested in the bcas and potential target groups but might have different uptake requirements.

**Evaluation of technology**

It is hard to provide an immediate and convincing demonstration of the effect of *Pochonia* and *Pasteuria* on root-knot nematodes because the nematode is invisible, the bcas cannot be seen and their impact on plant growth is slow. Therefore, it was important to find an evaluation process that would encourage dialogue between farmers and researchers. Reports from other crop protection projects (e.g. Little *et al.*, 2000) suggested the value of participatory approaches and so the decision was made to carry out an on-farm participatory evaluation.

**Methods**

Trial sites

On-farm evaluation of root-knot nematode (rkn) control using the two bcas developed by the project was carried out at two sites. Karigu-ini near Thika represented a moderate-input, moderate-output organic system. Mwea represented a high-input, high-output system.

One farmer group from each system was identified which appeared to be representative of the system and was willing to work with the project and provide a field site. In each group, the group members chose one of their number to act as host to the trial.

Record keeping

Each group attended meetings at their trial site during preparation of the seedbed, care of seedlings and transplanting. The seedlings were planted in a randomised block design with six replicates and six treatments: control; soil fumigation; combined *Pasteuria* and *Pochonia;* incorporation of *Crotalaria* seedlings; incorporation of Mexican marigold (*Tagetes minuta*) seedlings*;* trash burning. Transplanting was completed in December and the groups again visited the trial sites in mid February to observe progress. In March group representatives were involved in conducting and recording the harvest.

The farmers were fully involved in record keeping. Records were kept on flip charts and once they understood the process a farmer from each group volunteered to facilitate the recording. The farmers were enthusiastic about keeping accurate records and timed the activities as done by men and women (they decided to record the results as an average) The layout of the inputs sheets was as follows:

INPUTS

| Date | Activity | Input | Quantity | Cost (if farmer provided) |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |

OUTPUTS

| Treatment | | |
|---|---|---|
| Plot | Grade | Yield and units |
| 1 | Good | |
| | Medium | |
| | Poor | |
| 2 | Good | |
| | Medium | |
| | Poor | |

**Partial budget**

The partial budget was constructed in three phases:

1. input amounts
2. input costs
3. outputs and comparison

The first phase was done immediately after the tomatoes were transplanted. The researchers went through the list of the inputs with the farmers and any activities or inputs that had been omitted from the records were added. This proved to be a useful step as a few things had been left out but group recall was very good.

The partial budget concept was then explained to the farmers using four piles of mangoes to represent inputs and outputs for imaginary “control” and “treatment” plots. The differences between the inputs for control and treatment plots were estimated by counting the differences in mangoes, and the same was done with the outputs. The difference in in-puts was then compared to the difference in outputs. The farmers understood the general concept but one group was uneasy about projecting outputs for a crop that had only just been transplanted.

The input sheets from the trial were then displayed for all the farmers to see. They were asked to indicate those inputs that were used at the same level for all treatments and the control. Because their cost was not affected by the treatments, these inputs were crossed out and excluded from the rest of the analysis. The remaining inputs (those that differed between treatments and control) were then transferred into a summary sheet.

At this point, farmers were asked to make an initial evaluation: If yields for all treatments were the same but more than the control, which one would they choose to use?

After the meeting, the researchers calculated the amount of each input used for each seedling plot for the entire trial.

The second phase was done two weeks after the tomatoes were transplanted. One of the farmers reminded the others what had been done in the first phase and the conclusions that were made. No new inputs were added in the second phase because the inputs used later were the same across treatments. The researchers explained the calculations that they had made to estimate inputs on a per plot basis.

The unit prices of all of the inputs were determined. In the case of labour, it was estimated at KSh 100 based on the market price. A casual worker in Mwea is paid KSh 100 for a day's work while at Karigu-ini a days labour is paid KSh 80 and the labourer given lunch. The prices for other inputs were suggested by the researchers (who had purchased most of the inputs) and then discussed. The price for *Crotalaria* and *Tagetes* seeds were hard to estimate. They were provided by the researchers, but farmers said that they are locally available and the labour cost for collecting the seeds would be negligible. The cost of the *Pochonia* and *Pasteuria* agents could not be provided because no conclusions had been reached about the commercial production method and costs, so this cost had to be left out.

The total cost of inputs for each treatment was then estimated from the input volumes derived in Phase 1 and the unit prices. The farmers were asked to suggest which treatment they might prefer if all yielded the same amount of crop. They stated some preferences but were unable to make a final decision because the full cost of the bcs was unknown.

The third phase was done after the harvest. The group assembled and the output sheet was displayed and explained to those who had not taken part in the harvest. The plot yield averages (in Kg) for each treatment and the control were transferred to an outputs table. The farmers determined the unit price for each of the plot averages based on the price at

which they had sold the tomatoes and this was used to calculate the output value, which was entered into outputs tables.

Finally, comparison tables were constructed by the researchers in the presence of the farmers and the results were discussed in detail.

### 5.1 Successful collaboration with other CPP IPM projects and other organisations

Where appropriate resources for managing field work at Thika were shared. Contacts with the commercial biological control company Dudutech (Kenya) Ltd led to this company entering into agreements with CPP and the inclusion of this company as a collaborator within the peri-urban vegetable cluster. Collaboration with the organic farmer group at Thika has been mentioned elsewhere in this report.

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**Outputs**

**1. Field Trials**

**Nursery bed trials**

A principle already accepted by farmers is the need to have healthy plants to transplant in to the field. In a nursery trial established on a farm at Karigu-ini (Thika District) seedbeds were prepared with the following treatments: incorporation of spores of Pc and Pp, pregrowth and subsequent incorporation of *Crotalaria grahamiana* and/or *Tagetes minuta*, burning of trash (to kill pathogens with heat), treatment with dazomet (Basamid) which releases a fumigant methylisothiocyanate and an untreated control (Plate 3)) (Appendix CABI-KARI Folder: Folder Nurserybed: Nurserybed layout)

Assessments
Soil samples were collected from both the nursery beds and the main field before and after treatment applications for assessment of the nematode population (second stage juveniles) and *P. chlamydosporia* and *P. penetrans* levels, using standard techniques. Before transplanting and at the end of the harvesting period ten sample plants were randomly selected from each plot for assessment of gall index, shoot and root length and fresh weight of roots. The tomato yield from each plot was also recorded at each harvest.
Basamid and *Crotalaria* treatments had an effect on numbers of nematodes in soil and roots of the tomato transplants and the bca treatments had a significant effect on numbers of nematodes in the roots (Table 1). After transplanting, there was a significant treatment effect on the numbers of egg masses on the roots of the tomato at the end of the crop (Table 2) but there was no significant yield benefit (Figure 1) from this demonstration that some nematode control was achieved.

**Table 1:** The effect of different treatments on the number of nematodes in soil and roots of tomato seedlings in nursery beds at Karigu-ini, 4 weeks after planting (means of 5 replicates)

| Treatment | Final number of nematodes (J2s) per 200ml of soil[1] | Mean gall index on roots | Number of nematodes (eggs, J2, J3, J4 & females) $g^{-1}$ root[1] | Number of females and juveniles (minus eggs) $g^{-1}$ root[1] |
|---|---|---|---|---|
| *Pochonia* + *Pasteuria* | 40 (3.05) | 0.54 | 4 (0.91) | 4 (0.91) |
| *Crotalaria grahamiana* | 16 (2.44) | 0.50 | 7(1.16) | 7 (1.16) |
| *Tagetes minuta* | 44 (3.74) | 1.46 | 52 (2.28) | 20 (1.94) |
| Basamid | 4 (0.61) | 0.70 | 30 (2.35) | 16 (1.73) |
| Trash burning | 40 (2.52) | 0.82 | 74 (2.02) | 26 (1.67) |
| Control | 96 (4.44) | 1.62 | 141 (3.34) | 33 (2.74) |
| P-value (Overall) | p < 0.01 (SED = 0.83) | p = 0.45 SED = 0.69 | p = 0.19 (SED = 0.97) | p = 0.27 (SED = 0.77) |

[1] *ln (x + 1) transformed data used in ANOVA in parenthesis*

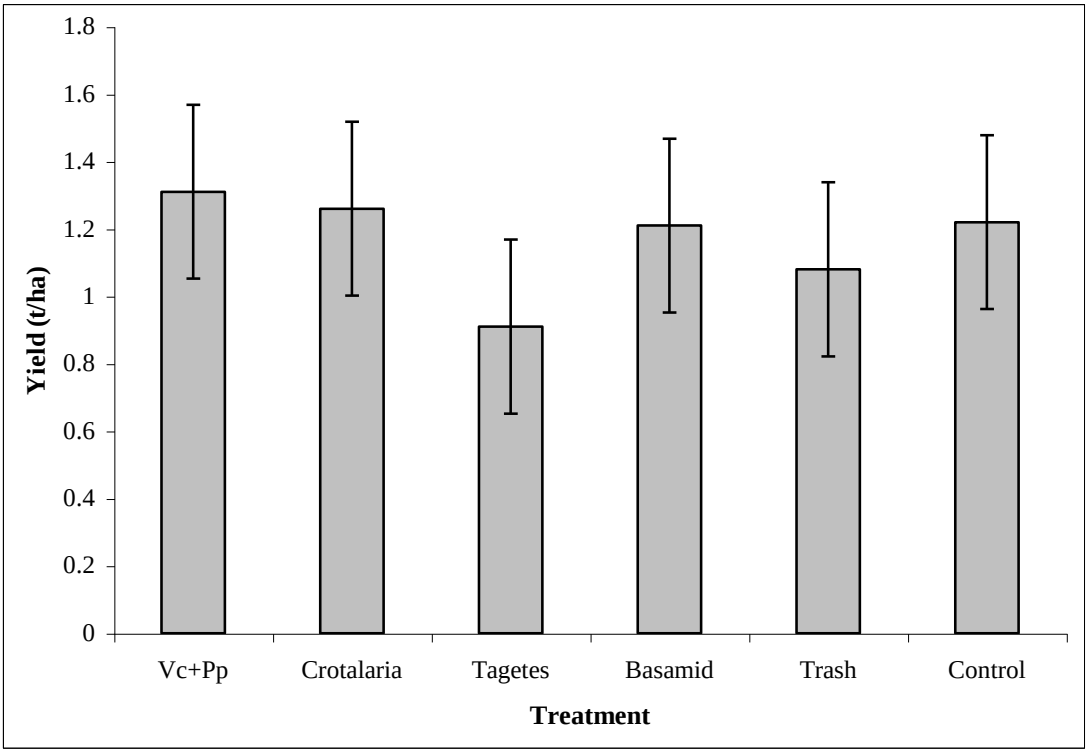
**Table 2:** The effect of different nursery bed treatments on the number of nematodes in soil and roots of tomato plants at Karigu-ini at the end of harvesting period (means of 5 replicates)

| Treatment | Final number of nematodes (J2s) per 200ml of soil[1] | Mean gall index on roots | Number of nematodes (eggs, J2, J3, J4 & females) $g^{-1}$ root[2] | Number of females and juveniles (minus eggs) $g^{-1}$ root[2] | Number of egg mass $g^{-1}$ root |
|---|---|---|---|---|---|
| *Pochonia* + *Pasteuria* | 292 (5.11) | 3.6 | 2200 (7.42) | 332 (5.79) | 0.0 |
| *Crotalaria grahamiana* | 276 (5.23) | 3.3 | 2460 (7.55) | 396 (5.88) | 0.6 |
| *Tagetes minuta* | 224 (5.17) | 3.4 | 4672 (8.25) | 476 (5.75) | 1.0 |
| Basamid | 481 (5.88) | 3.4 | 2888 (7.88) | 604 (6.20) | 1.6 |
| Trash burning | 560 (6.02) | 3.4 | 2212 (7.63) | 592 (6.06) | 0.6 |
| Control | 668 (5.86) | 3.9 | 2692 (7.75) | 544 (6.16) | 2.8 |
| P-Value (Overall) | p = 0.54 (SED = 0.64) | p = 0.82 SED = 0.48 | p = 0.24 (SED = 0.34) | p = 0.67 (SED = 0.34) | p = 0.02 SED = 0.76 |

[1] *ln (x + 1) transformed data used in ANOVA in parenthesis*
[2] *ln transformed data used in ANOVA in parenthesis*

**Plate 3:** The nursery bed trial at Karigu-ini during the first season

**Figure 1:** Effect of nursery bed treatments on marketable tomato yield at Karigu-ini




## Conclusion

Some of the treatments applied to the nursery beds did have an effect on the numbers of infective stage root-knot nematodes in the soil which was reflected by the numbers in the root systems and in the subsequent crop transplanted in the field (Tables 1 & 2).
The treatment effects on nematode numbers are marginally significant but ultimately these treatments did not have a significant effect on the marketable yield of the crop (Figure 1).

However from the farmers' perceptions of spending time and resources on growing *Crotalaria* and *Tagetes* for 4 weeks prior to their incorporation before sowing seed was that it was not worthwhile. Farmers prefer the traditional technique of burning trash to clean the seedbed but

would consider using the bcas if the cost of treatment was acceptable. It is felt that the work on nursery seed beds has achieved its objective and that this work would not proceed further in another phase. However, development of techniques for applying bcas with transplants is worthy of further research (Appendix CABI-KARI Folder: Sub-folder: Nurserybed; Powerpoint)

### 1.2 Rotation trial

**Investigation into the use of crop rotation and combined application of *Pochonia chlamydosporia*, and *Pasteuria penetrans* for control of root-knot nematodes in naturally infested soil**

This trial was established on the KARI National Horticulture Research Centre (NHRC) in Thika district, Central Province in April 2001 (Plate 4). This trial is still in progress and is intended to continue through a next phase of the work.

**Plate 4:** First cycle of the rotation trial at Thika (April-September 2001)

This experiment was laid out in a randomised complete block design with five replicates. The treatments in the first rotation cycle were: 1) Tomato + BCAs, 2) Cabbage +BCAs, 3) Tomato (Untreated) and 4) Cabbage (Untreated).

Each of the respective treatment plots, 4.5 x 3.6m in size, consisted of forty-two cabbage (Gloria $F_1$ = Victoria) and fifty-six tomato (Cal-J VF) plants. The seedlings were raised in nursery beds and transplanted, four weeks after sowing, into the nematode infested field on 18 April 2001. Planting was done at the recommended spacing of 90 x 60cm and 60 x 60cm for tomato and cabbage, respectively. Before transplanting, approximately 1000$cm^3$ of soil from each plant was treated with *P. chlamydosporia* and *P. penetrans* at the rate of 5000 chlamydospores and 2 x 10 endospores per gram of soil, respectively. Diammonium phosphate fertiliser was applied to all plots at the rate of 2g per plant.

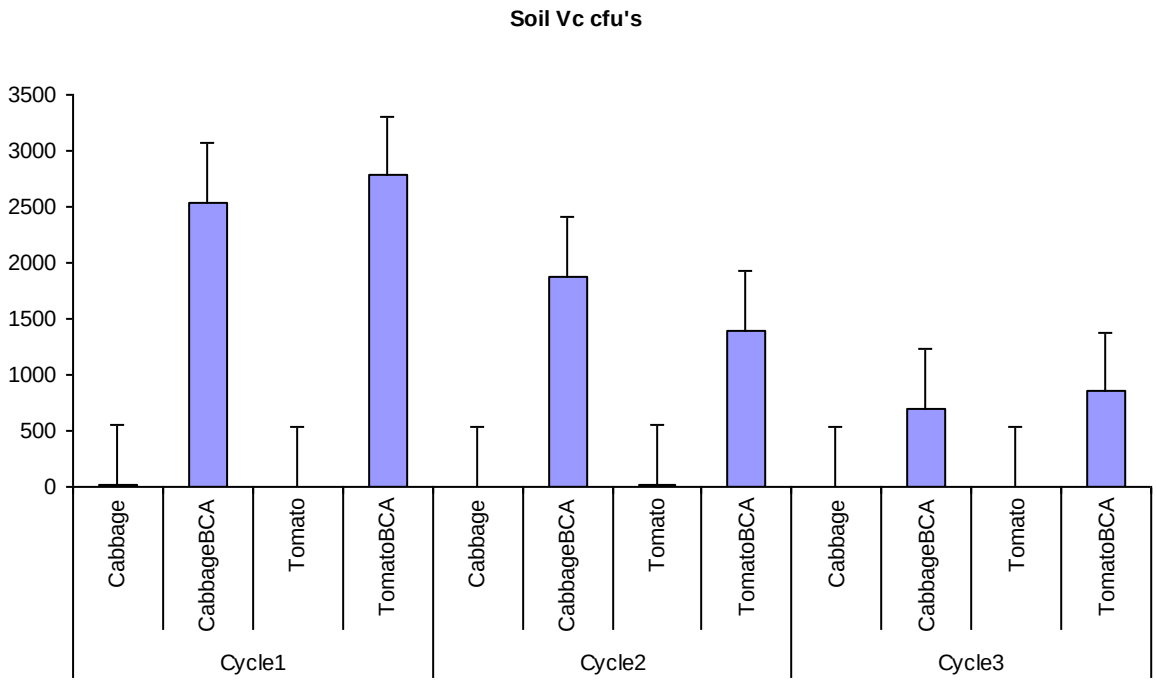
The cabbages were all harvested on 16 July 2001, whereas the tomatoes were harvested repeatedly from 6 July to 24 August 2001. Both marketable and non-marketable yields were recorded. Ten sample plants were randomly selected from each plot for sampling. The parameters recorded included gall index, shoot height, root length, fresh and dry weight of shoot, fresh weight of roots, number of egg masses and nematodes in the roots. Additionally, numbers of second stage juveniles (J2s) in the soil and populations of BCAs in the soil before and after crop harvest, were estimated.

The second crop cycle entirely of cabbage was planted on 2 October 2001 and harvested 15 January 2002, and a third crop cycle (tomato) was planted 18 January and harvested 3 May 2002. No further applications of bca had been made.

The data collected from each crop cycle are presented in Appendix CABI-KARI Folder : Sub-folder; Rotation Trial: Files; Cycles 1,2 and 3. There was no significant effect on the crop yields. The most interesting data are the counts of second stage (infective) root-knot nematode juveniles in the soil after each crop (Figures 3,4 & 5). These data show a consistent trend in decline of nematodes in the plots that received the bcas. Also very encouraging were the counts for the colony forming units of *P. chlamydosporia* (Figure 2).

A fourth crop (tomato) was planted in April 2002 and a reapplication of *P. chlamydosporia* was made. The results of this crop cycle are not yet available as harvesting has continued until September.

**Figure 2:** Numbers of colony forming units per g of soil taken at the end of each crop. *Verticillium* was applied only to cabbage and tomato at planting (cycle 1) thereafter, cycle 2 was cabbage throughout and cycle 3 was tomato throughout – the integrity of the original plots was maintained throughout (April 2001-May 2002).




Although there was a general decline in the amount of *P. chlamydosporia* in soil over the three cropping cycles (Fig. 2), the data suggest that the fungus proliferated in soil and remained in appreciable quantities despite the considerable amount of soil mixing that occurred during cultivations. There was no significant effect of the crop (cabbage or tomato) on the abundance of the fungus in any of the crop cycles.

It is probable that repeated application of the fungus would enable it to build up in soil to densities that would suppress nematode multiplications.

**Figure 3:** Root-knot nematode juveniles (J2) in BCA and no BCA treatments after third crop cycle (May 2002) (from 200ml soil samples)

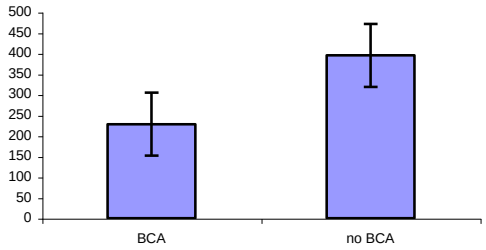



**Figure 4:** Juveniles in 200ml soil samples at end of the third crop cycle (tomato) in May 2002 following initial bca treatments at planting of first cycle (tomato and cabbage) in April 2001.

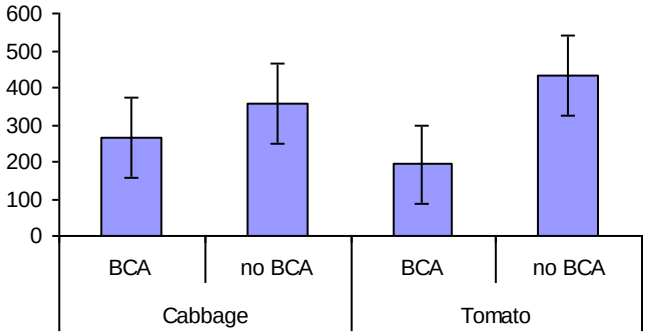
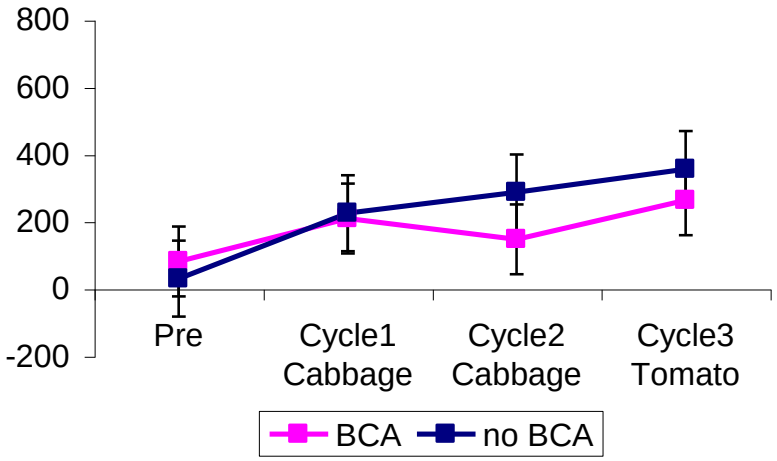


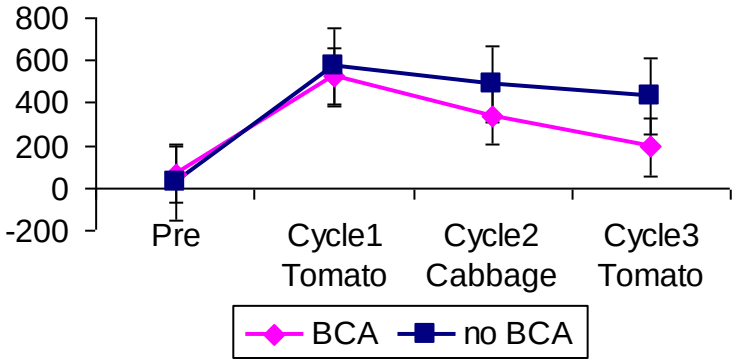

**Figure 5. A and B** Number of root-knot nematode juveniles extracted from 200ml soil samples pre-planting and at the end of each successive crop (means of 5 replicates)

**A:** Cabbage, cabbage, tomato
**B:** Tomato, cabbage, tomato.

**A**



**B**




**Conclusions**

- Following one initial application of the biological control agents in April 2001, there was a progressive decline in the numbers of second stage juveniles (J2) in the soil. By May 2002 J2 numbers in the treated plots were 228 compared with 395 per 200ml soil in the untreated (s.e.d. 76.4 P = 0.05).
- Counts of colony forming units of *P chlamydosporia* indicated that the fungus persisted in the plots for the duration. Some juveniles with spore attachment were found in 50% of plots originally treated with *Pasteuria penetrans* (Appendix CABI-KARI Folder : Sub Folder: Rotation Trial; Pc cfu counts).
- Although the occurrence of Pp spore encumbered juveniles recovered from soil is low. However, results of the pot experiment Figure 12d would indicate infection is present in some of the females. This could be epidemiologically significant.
- The benefit of applying the bcas had not yet extended to showing any significant improvement in crop yield (all other data presented in Appendices).
- The objective of developing a suppressive soil has been partially achieved and the need to continue the rotation treatment is evident. It was always intended that this rotation trial would by its nature be required to continue for at least 3 years.

**2. Characterisation of bcas**
**Identification of *P. chlamydosporia* by- β tubulin PCR**

The identification of the fungus as *P. chlamydosporia* was confirmed by PCR using β-tubulin primers and RAPD-PCR was used to distinguish differences between the isolates.

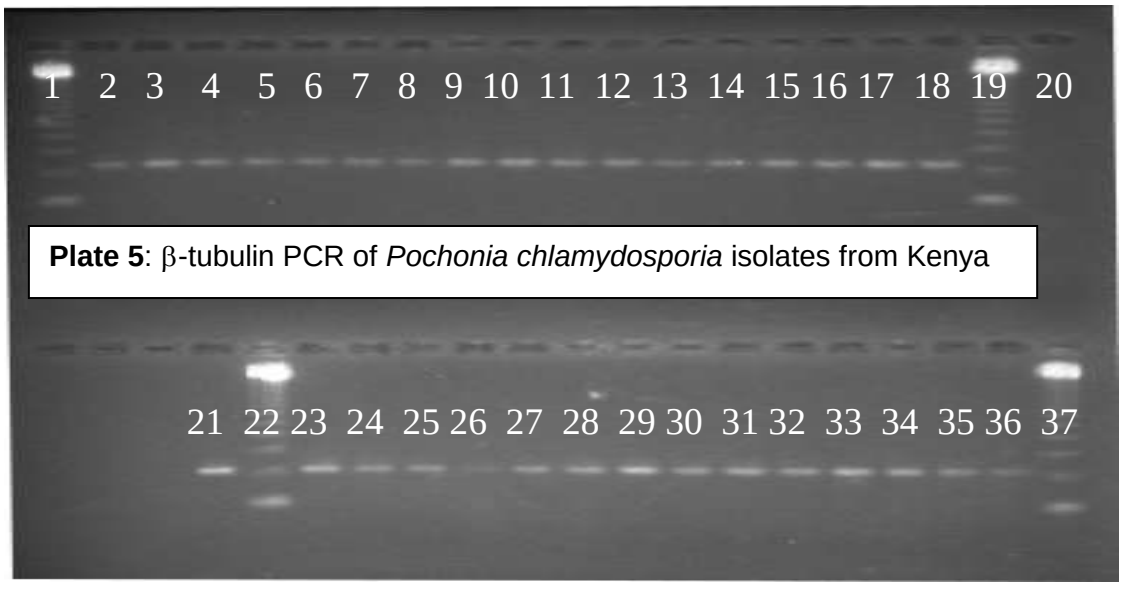



**Plate 5**: β-tubulin PCR of *Pochonia chlamydosporia* isolates from Kenya

Lane 1, 19, 22 and 37: 123Bp marker

Lane 2: 23B
Lane 3: 22
Lane 4: 21A
Lane 5: 20A
Lane 6: 19B
Lane 7: 17B
Lane 8: 17A
Lane 9: 15T5
Lane 10: 13T3
Lane 11: 12T2
Lane 12: 11T1
Lane 13: 10B

Lane 25: 23(2)
Lane 26: 21(2)
Lane 27: 20(2)
Lane 28: 18(2)
Lane 29: 15(2)
Lane 30: 13(2)
Lane 31: 12(2)
Lane 32: 10(2)
Lane 33: 26A
Lane 34: 25B
Lane 35: 24T4
Lane 36: 24T2

Lane 14: 9A
Lane 15: 7B Lane 16: 6B
Lane 17: 6A
Lane 18: 4A
Lane 20: -ve control
Lane 21: +ve control VC10
Lane 23: 25(2)
Lane 24: 24(2)

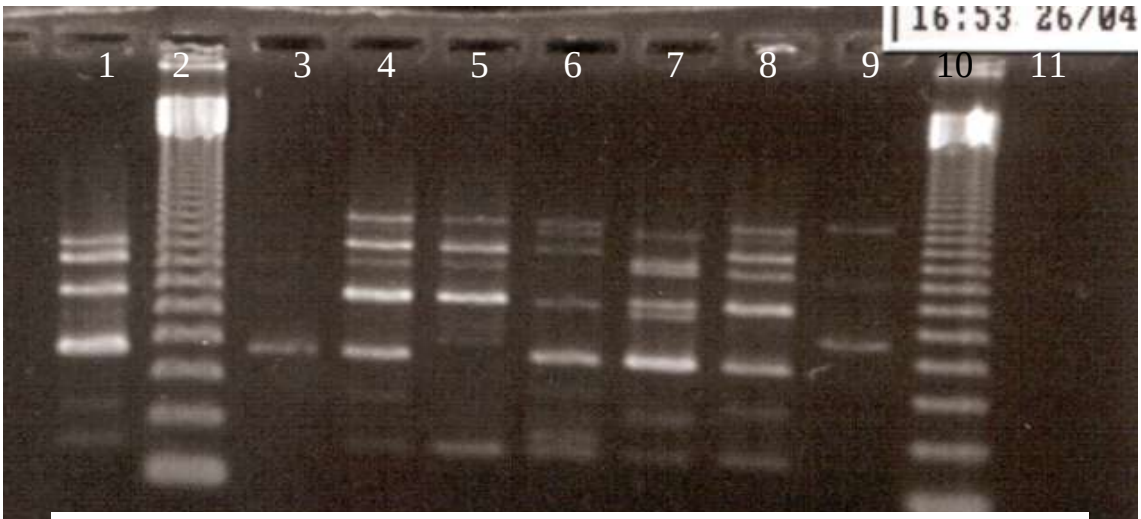



**Plate: 6** RAPD PCR of *P. chlamydosporia* isolates from Kenya.

Lane 1: VC10
Lane 2 and 10 : 123Bp markers.
Lane 3: 12 (2)
Lane 4: 23A
Lane 5: 20A
Lane 6: 12T2
Lane 7: 9A
Lane 8: 24 (2)
Lane 9: 6A
Lane 11: -ve control

Eighty two isolates of *Pochonia* species are held in the culture collection at Rothamsted of which 75% were *P. chlamydosporia* as diagnosed with the β - tubulin primers (Plate 5). RAPD – PCR detected differences between a selection of these isolates as has been observed in surveys of the fungus elsewhere (Plate 6). Hence, a range of isolates of the fungus have been verified as *P. chlamydosporia* but it is clear that these isolates include several genetic polymorphisms. The importance of this variation in the regulation of nematode host populations is unknown but this is the topic of a BBSRC-funded project, which will begin at Rothamsted in October, 2002. Thirty seven isolates of *P. chlamydosporia* were selected as representative of all the sites in the 1998/99 survey and characterised in a range of bioassays to select those with most potential as biological control agents.

**Laboratory screening of selected isolates of *P. chlamydosporia***

Isolates from both surveys showed great variation in their ability to produce chlamydospores, from 1 x $10^5$ to 44.5 x $10^5$ chlamydospores $g^{-1}$ medium (Table 1). Although it was not possible to include the standard isolate, Vc10, in this screen, this isolate generally yields about 10 x $10^5$ chlamydospores $g^{-1}$ medium, thus some of these Kenyan isolates are particularly prolific chlamydospore producers.

The egg parasitism test was done in England, so Vc10 was included, and only 30% of the tested isolates were able to infect a greater percentage of eggs than Vc10 (Appendix Rothamsted Data; Table 1). In all cases, overall egg parasitism was uncharacteristically low compared to that obtained in other screens (Hidalgo-Diaz *et al*., 2000). All the isolates were equally effective at colonising the rhizosphere of maize roots, so this test gave no information other than that all the isolates could potentially effectively colonise the surface of roots in non-sterile conditions. On the basis of these results, the isolates used in the glasshouse screen were selected (9A, 12T2, 20A, 23A, 12 and Vc10); being able to produce relatively large numbers of chlamydospores and parasitise relatively large numbers of eggs.

**Glasshouse screening of selected isolates of *P. chlamydosporia***

The Kenyan isolates tested in the glasshouse screen differed in their ability to colonise the soil and root surface, with Vc10 being the least prolific isolate compared to the Kenyan isolates selected (Table 3). None had a detrimental effect on plant growth (Table 4). Generally, isolates that were abundant in the rhizosphere were also abundant in the soil, such as 12T2 and 12, although the amount of fungus in the soil, estimated as CFU, immediately after application of the chlamydospores was greater than that of the other isolates (Table 3).

**Table 3:** Colonisation of soil and roots of tomato plants by isolates of *P. chlamydosporia* from Kenya, in pot tests. Means of 5 replicates

| Isolate | CFU $g^{-1}$ soil initial ($log_{10}$) | CFU $g^{-1}$ soil Final ($log_{10}$+1) | CFU $g^{-1}$ root ($log_{10}$+1) |
|---|---|---|---|
| Vc10 | 3959 (3.55) | 2568 (2.11) | 499 (1.62) |
| 20A | 10295 (3.99) | 72360 (4.84) | 4418 (3.51) |
| 23A | 8913 (3.88) | 100660 (5.00) | 4600 (2.85) |
| 9A | 15831 (4.14) | 13904 (4.11) | 1754 (2.42) |
| 12T2 | 17360 (4.20) | 101520 (4.91) | 9160 (3.89) |
| 12 (2) | 17317 (4.21) | 118040 (5.03) | 11420 (3.90) |
| | P < 0.001 (SED = 0.137) | P < 0.001 (SED = 0.515) | P < 0.05 (SED = 0.661) |

**Table 4:** Effect of different isolates of *P. chlamydosporia* on tomato plant growth and numbers of nematodes in the roots. Means of 5 replicates

| Isolate | Shoot Weight (g) | Root Weight (g) | Egg masses per g root | Eggs per egg mass | Number of nematodes $g^{-1}$ root ($log_{10}$) (excluding eggs on the root surface) |
|---|---|---|---|---|---|
| Vc10 | 111.9 | 13.1 | 19 | 273 | (3.91) |
| 20A | 99.1 | 15.2 | 22 | 409 | (3.78) |
| 23A | 106.8 | 14.8 | 9 | 381 | (3.63) |
| 9A | 117.2 | 13.3 | 15 | 378 | (3.69) |
| 12T2 | 112.0 | 13.9 | 22 | 314 | 11040 (4.03) |
| 12 (2) | 108.3 | 12.6 | 24 | 322 | (3.85) |
| Control | 109.9 | 14.3 | 31 | 392 | (3.87) |
| | n.s. | n.s. | n.s. | n.s. | P < 0.05 (SED = 0.10) |

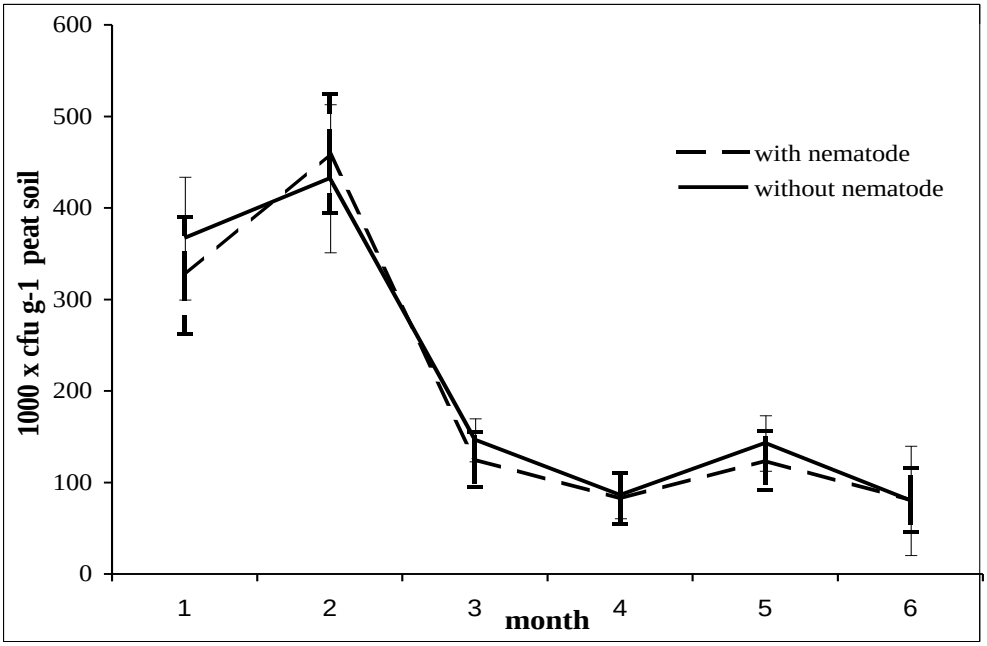
Isolates 23A and 9A established well in the soil but did not substantially colonise the rhizosphere compared to the other Kenyan isolates tested (Table 3), but despite this the greatest reduction in nematodes occurred where those isolates had been applied (Table 4). The total numbers of nematodes in the roots of all stages (Table 4) did not appear to have any correlation to the numbers of eggs parasitised in the laboratory screen (see Appendix RES Isolates Table 4) Although it was not possible to estimate the percentage of eggs infected by the fungus, all isolates had colonised the egg mass gelatinous matrix and

presumably would eventually have colonised a proportion of the eggs within it. In general, the isolates that proliferated in the soil to a greater extent (Table 3) were also those that produced greatest number of chlamydospores on the sand-bran medium (see Appendix RES Isolates Table 4). From these results, there seems to be little association between the amount of fungus in the rhizosphere (Table 3) and the reduction in nematodes in the roots (Table 4), however, all were considered good root colonisers.

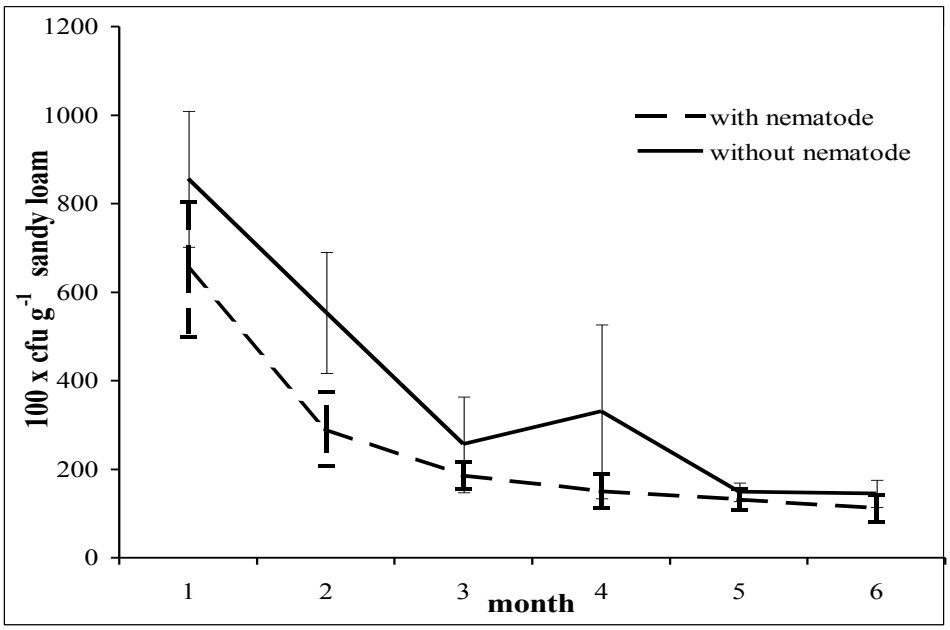
The survival of the fungus was similar on all three plant cultivars tested and the data presented are overall means. *Pochonia chlamydosporia* was significantly more abundant in the peat based compost than in the one based on sand but the presence of the nematode host had no significant effect on the abundance or survival of the fungus (Fig. 6A, B). In general, populations of the fungus in soil declined for the first 2 months after the plants were harvested and then stabilised or declined more slowly for the remaining 3 months of observations. At the end of the experiment, the fungus was still more prevalent in both soils than at the time immediately after its application. On several of the sampling occasions the standard errors associated with the estimates of fungal densities were large, which suggests that the fungal distribution in soil was aggregated even in a pot experiment in which the initial inoculum was thoroughly mixed with the contents of the pot.

**Figure 6:** Changes in the density of *P. chlamydosporia* in peat (A) and sandy loam (B) following healthy and *M. incognita* infested crops (means of 12 replicates).

**A**

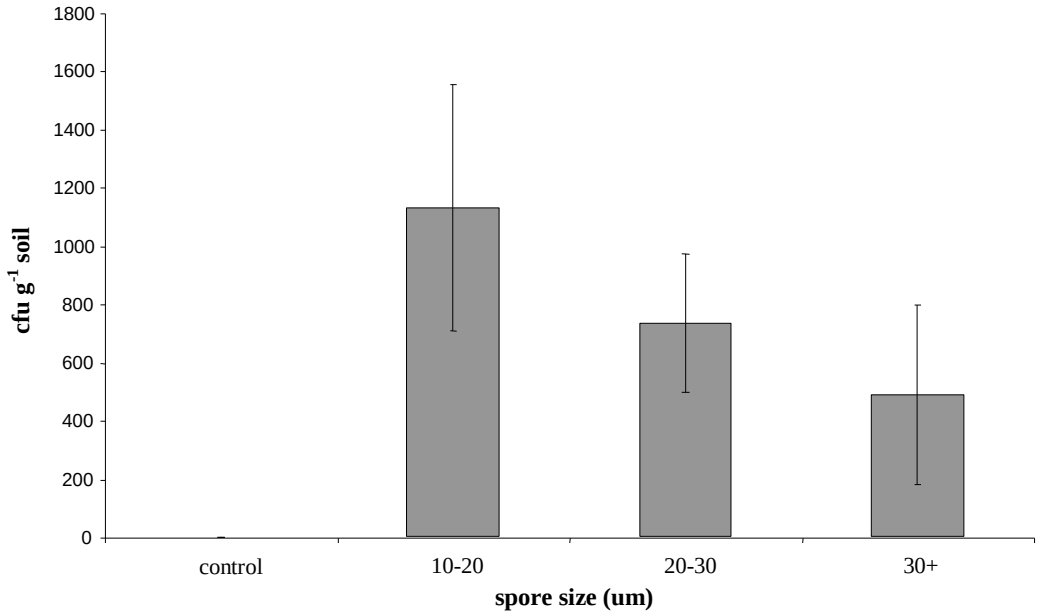


**B**




Although there is a trend that smaller chlamydospores are more effective than large ones for establishing the fungus in the rhizosphere (Fig. 7), the differences are not statistically significant and there were no significant differences in the numbers of nematode eggs produced on the tomato roots or on the proportion infected.

**Figure 7:** Effect of chlamydospore size on the establishment of *P. chlamydosporia* on tomato roots after 9 weeks.



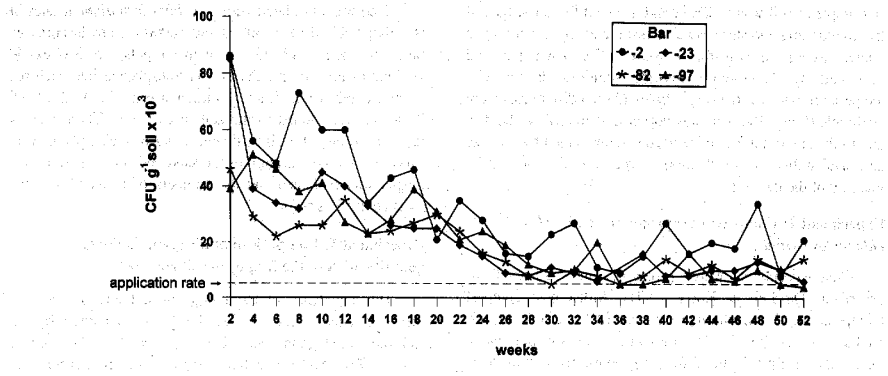
Survival of *P. chlamydosporia* at a range of temperatures and water potentials

*P. chlamydosporia* was able to survive at a range of water potentials at both 25 and 30°C (Figs. 8a and 8b). Although the fungus did not proliferate, in terms of number of CFU, by the end of the experiment, there was a larger amount of fungus than has been found in naturally suppressive soils (Kerry *et al*., 1993). As the abundance of the fungus decreased over the experiment, it suggested that it will not proliferate in the absence of a host plant and nematodes, but will proliferate extensively in their presence (Bourne *et al*., 1996). The fungus survived in larger numbers of CFU at water potentials of -2 bar (0.05 molal.). At 40°C, no fungus was re-isolated from the soil on the selective medium after 10 weeks at a water potential of -97 bar (2.0 molal.) and 34 weeks at -82 bar (1.7 molal.) (Fig. 8c).

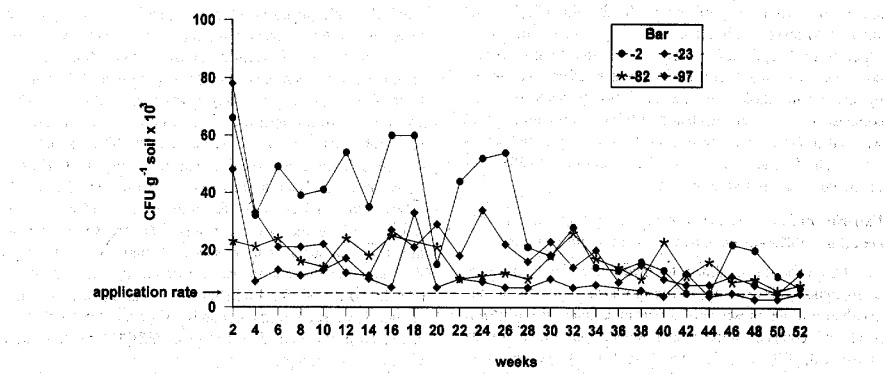
The water content of the soil samples was relatively consistent after 2-4 weeks by which time, water content would have equilibrated with the potential generated by the sodium chloride solution. Opening the boxes to remove soil samples did not affect the water content of the soil and so was assumed not to cause imbalance in the equilibrium. However, Figs. 8a-c show large differences in the amount of fungus between 2 and 4 weeks suggesting that the temperature was initially an important factor determining numbers of CFU.

**Figure 8:** The abundance of *P. chlamydosporia* in soil at different water potentials and at a) 25°C, b) 30°C and c) 4°C

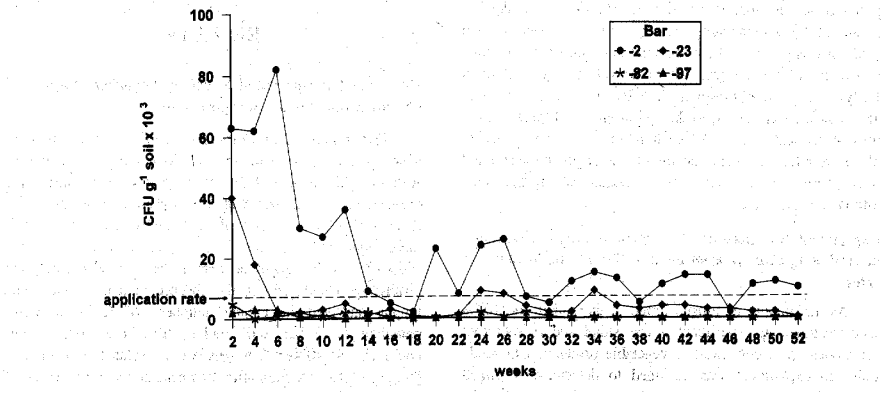
**a.**




**b.**




**c.**



**Competitive saprophytic ability of *P. chlamydosporia***

*Pochonia chlamydosporia* was able to proliferate in compost amended with 0-80% autoclaved compost to similar extents (Table 5). The amount of fungus increased until 20 weeks after the start of the experiment and by 50 weeks had decreased to numbers of CFU similar to the initial application rate. In 100% autoclaved compost the fungus proliferated rapidly suggesting that in the presence of competition from other micro-organisms in the soil, abundance of *P. chlamydosporia* is reduced, irrespective of the relative proportion of sterile and non-sterile compost.

**Table 5:** Proliferation of *Pochonia chlamydosporia* over time in different proportions of sterilised and non-sterilised compost. Means of 3 replicates (CFU $g^{-1}$ soil x $10^3$).

| | Weeks | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % autoclaved compost | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 20 | 50 | 70 | Mean | SED |
| 0 | 3 | 28 | 39 | 39 | 33 | 23 | 30 | 95 | 2 | 0.3 | 29 | |
| 20 | 4 | 21 | 27 | 30 | 16 | 33 | 19 | 143 | 2 | 0.1 | 30 | |
| 40 | 3 | 21 | 43 | 37 | 38 | 36 | 28 | 99 | 3 | 0.2 | 31 | 688 P<0.001 |
| 60 | 3 | 37 | 40 | 66 | 41 | 41 | 34 | 86 | 1 | 0.1 | 35 | |
| 80 | 2 | 49 | 83 | 65 | 77 | 57 | 36 | 114 | 2 | 0 | 49 | |
| 100 | 2 | 2272 | 4939 | 11194 | 9101 | 29749 | 7288 | 17233 | 107 | 10 | 8190 | |
| Mean | 3 | 405 | 862 | 1905 | 1551 | 4990 | 1239 | 2962 | 20 | 2 | | |
| SED | | | | | | 888 | P<0.001 | | | | | |

Interaction effect P<0.001 SED = 2176 x $10^3$

**To determine the proliferation of *P. chlamydosporia* in sand at different application rates of the fungus**

Increasing the application rate of the fungus in a sand soil, resulted a greater abundance of the fungus in both soil and rhizosphere (Table 6) At the recommended application rate of 5,000 chlamydospores/g soil, (Leij De & Kerry, 1991), the amount of fungus in the soil at the end of the experiment was greater than that commonly found in naturally suppressive soils (Kerry *et al*., 1993). At all application rates, the fungus proliferated to a greater (P <0.01) extent on the root surface than in soil, the increase being greater at the smaller application rates. The reduced multiplication in the soil at the largest application rate (50,000 chlamydospores $g^{-1}$ soil) suggests that the carrying capacity of the soil for the fungus occurs at these densities but that the root surface can support larger densities than in the soil. There were no significant differences in the numbers of nematodes in the root systems of the tomato plants (Table 6); thus, the density of *P. chlamydosporia* does not appear to affect invasion of the nematode in the first generation.

**Table 6:** Effect of application rate of *Pochonia chlamydosporia* in sand on fungal proliferation and nematode populations in tomato roots. Means of 4 replicates.

| Application rate (chlamydospores $g^{-1}$ sand) | CFU $g^{-1}$ sand at start of experiment | CFU $g^{-1}$ sand at harvest | CFU $g^{-1}$ root | Total number of nematodes $g^{-1}$ root (excluding eggs) |
|---|---|---|---|---|
| 50 | 63 | 1658 | 2435 | 307 |
| 500 | 398 | 1832 | 6228 | 289 |
| 5000 | 4509 | 13195 | 28177 | 423 |
| 15000 | 9145 | 32770 | 50707 | 147 |
| 30000 | 20130 | 125763 | 193479 | 421 |
| 50000 | 50206 | 76683 | 346088 | 299 |
| | P<0.01 SED = 11476 | P<0.01 SED = 32606 | P<0.01 SED = 74162 | n.s |

**Measurement of survival of *P. chlamydosporia* in a field trial in Kenya**

Although there was a general decline in the amount of *P. chlamydosporia* in soil over the three cropping cycles (Fig. 2), the data suggest that the fungus proliferated in soil and remained in appreciable quantities despite the considerable amount of soil mixing that occurred during cultivations. There was no significant effect of the crop (cabbage or tomato) on the abundance of the fungus in any of the crop cycles.

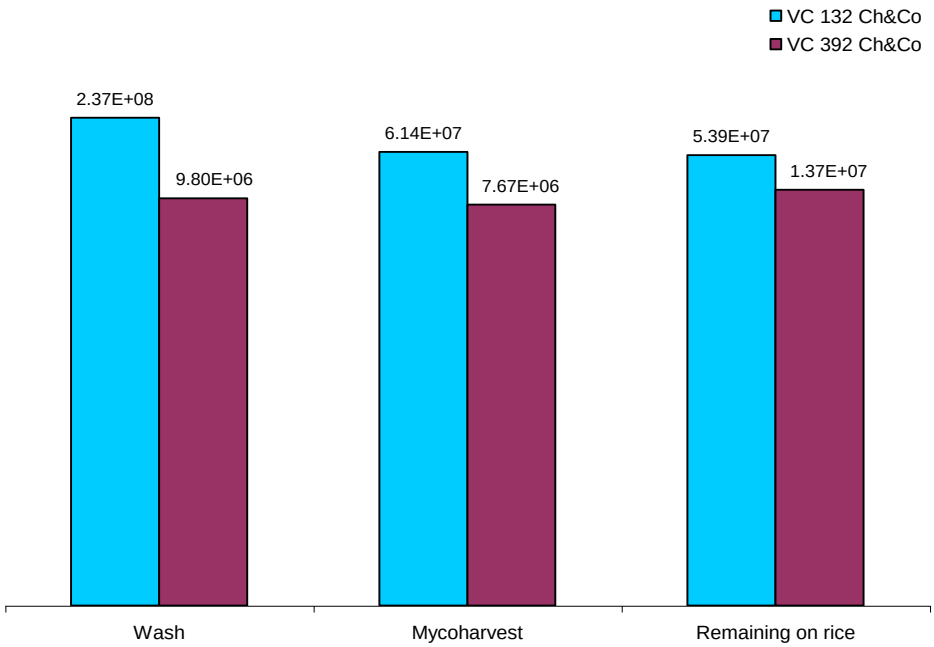
It is probable that repeated application of the fungus would enable it to build up in soil to densities that would suppress nematode multiplications.

**Evaluation of media for mass production of the fungus**

None of the materials tested improved significantly on the standard method of inoculum production (barley bran: sand mixture), which produced between 1.7 – 2.4 x $10^6$ chlamydospores $g^{-1}$ medium. However, the maize bran: sand medium was similarly effective.

The biphasic system of production using a growth phase in rice both followed by a sporulation phase on rice grain resulted in a 10-fold improvement in production with 1.2 x $10^7$ chlamydospores $g^{-1}$ medium produced by selected isolates of *P. chlamydosporia*. More spores were washed from the fresh medium than were collected using the Mycoharvester and considerable numbers of spores were left on the dried rice after treatment (Fig. 9). The efficiency of recovery with the Mycoharvester would increase of the moisture content of the dried, colonised medium was reduced from 10% to 5% moisture. However, the impact of this level of drying on the viability of the spores needs testing. In a non-formulated state the viability of chlamydospores stored dry at room temperature decreased from 90-70% after one week but decreased little after that for at least 2 months storage.

**Figure 9:** Total amount of chlamydospores $g^{-1}$ rice at 10% humidity of isolates 132 and 392 before and after harvesting using the Mycoharvester.




**Studies on proliferation and application of *Pochonia chlamydosporia***

**Colonization of roots by *Pochonia*, measured as colony forming units per gram of root.**

Method: 1g of washed and crushed root is taken up in 0.05% water agar, and a serial dilution from a tenth to a hundredth and to a thousandth concentration is made. 0.2ml of the suspension is plated on semi-selective medium and incubated at 25°C for 3 weeks. Colony forming units appear as white cottony growths, which were counted.

**Table 7:** Means of numbers of colony forming units (with log-transformed data)

| Item | Bare-rooted seedlings | | | Seedlings with root plugs | | | Mean |
|---|---|---|---|---|---|---|---|
| | Root appl | Soil appl | No appl | Root appl | Soil appl | No appl | |
| Tomato | | | | | | | |
| CFU-Log(10) | 3.815 | 3.996 | 3.547 | 3.694 | 3.624 | 3.081 | 3.626 |
| CFU counts | 14763 | 12125 | 2913 | 5288 | 5312 | 688 | 6848 |
| Cabbage | | | | | | | |
| CFU-Log(10) | 3.655 | 3.213 | 2.530 | 3.166 | 3.647 | 3.121 | 3.222 |
| CFU counts | 12938 | 2225 | 675 | 4820 | 12363 | 738 | 5626 |
| Means | | | | | | | |
| Seedling type | 3.459 | | | 3.389 | | | |
| Application | | | | 3.583 | 3.620 | 3.070 * | |
| l.s.d. | | | | | | 0.3998 | |
| s.e.d | | | | | | 0.1941 | |

* Significant (p=0.015)

The absence of a significant difference in the interaction between seedling types and the methods of application indicates that all methods tried produce comparable colonisation,

**Assessment of egg infection by *Pochonia chlamydosporia***

Method: A fixed number of egg masses were picked from the roots, and mixed with a mechanical stirrer with flexible blades to break the protective gelatinous material around the egg mass; 0.2 ml of egg suspension was collected and plated on to selective medium and incubated at $25^0$C for 4 days. Infected eggs were identified by the fungal growth emerging from the individual eggs. The total number of infected eggs per plate was expressed as a percentage of the total.

The estimate of infection of eggs is expressed in percentage and therefore it could be said that about 70% of eggs may not hatch when treated with *Pochonia chlamydosporia* But this may not translate to 70% control because of other factors like cultural practices, follow-on crop and selection pressure which would contribute to subsequent build up of nematode populations from the expected 30% hatch.

**Table 8:** Infection of nematode eggs by *Pochonia* (in percentage)

| Item | Bare-rooted seedlings | | | Seedlings with root plugs | | | Mean |
|---|---|---|---|---|---|---|---|
| | Root appl | Soil appl | No appl | Root appl | Soil appl | No appl | |
| Tomato | | | | | | | |
| Egg Infection% | 64.4 | 61.6 | 20.5 | 56.3 | 54.2 | 17.2 | 45.7 |
| Cabbage | | | | | | | |
| Egg Infection% | 75.5 | 84.6 | 6.1 | 80.0 | 84.4 | 12.9 | 57.3 |
| Means | | | | | | | |
| Effect of Appln | (s.e.d.=3.55, l.s.d.=7.32) | | | 69.0 | 71.2 | 14.2* | |
| Effect of Crop | (s.e.d.=5.89,l.s.d.=13.89) | | | 60.4 | 57.9 | 18.8 | |
| X appln | | | | 77.7 | 84.5 | 9.5 | |

* Significant (p=<0.001)

A significant difference (p=<0.001) is seen in the infection of eggs between treated and untreated plots although there is no significant difference at the whole plot level between the crops. The interaction between whole plot treatments and the presence or absence of *Pochonia chlamydosporia* is highly significant (p=<0.001)

PASTEURIA PENETRANS

**Observations on infected root-knot nematode females in roots**

Milling roots does not break-up and disperse infected females. Aggregations of spores may still be found in the powder when dry or after rehydration (Plate 7). This unexpected observation could account for inaccuracies in spore-powder calibration for deciding application rates.

**Plate 7: Above** Dry (arrowed) and **below** re-hydrated Pp infected females from roots dried and ground for two minutes using a Glen Creston Hammer Mill (1mm mesh) 6x magnification.

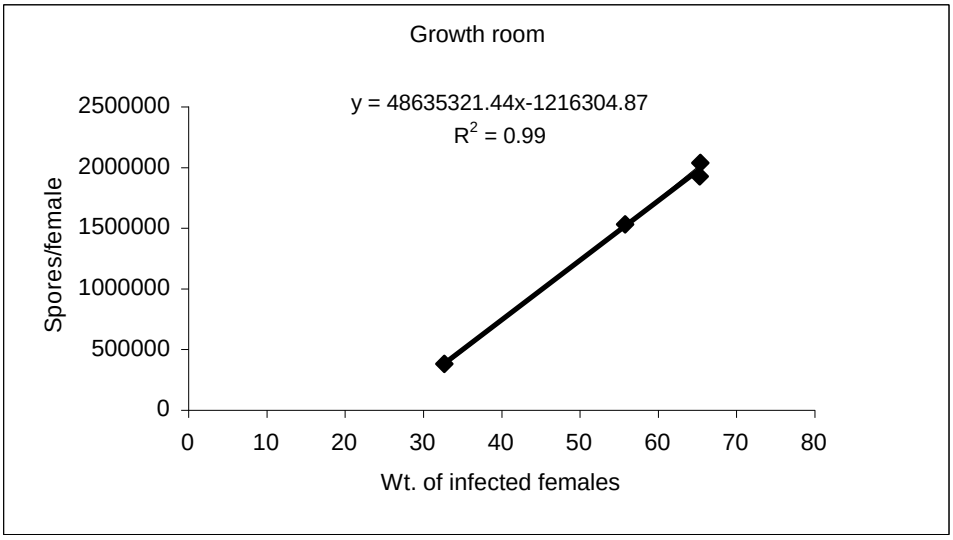
**Plate 8:** Infected root-knot nematode female dissected from re-hydrated root 150x magnification

**Spore production per female**

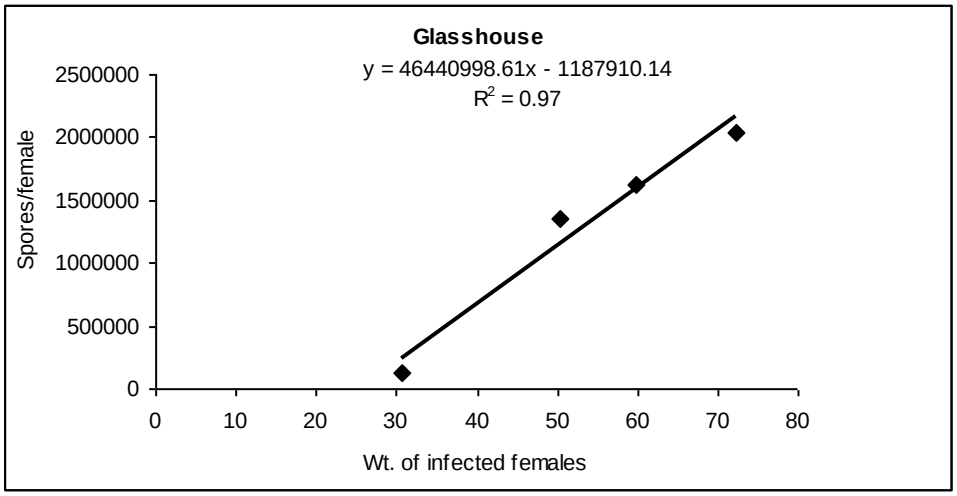
In the growth room females reached their maximum weight at 80 days (Fig.10a) whereas in the glasshouse the weights were still increasing at the time of the final harvest (88 days)(Fig.10b). This suggests that spore production in females was still increasing in the glasshouse treatment and that under both regimes 2 million spores or more may develop in a female but these amounts will be dependent upon body size which will increase with time. This data confirms that production of >2 million spores per infected female is possible but more importantly the duration of in vivo production systems in tropical locations where soil temperatures are at 25-3$^{o}$C should be at least 12 weeks if maximum production is to be achieved.

**Figure 10:a and b** Correlation between the weight (μg) of infected females and the production of endospores of Pasteuria penetrans per female. Values are means of 5 replicates.

**a.** In growth room at 25-28$^{0}$C




**b.** In glasshouse at 20-35$^{0}$C



**Studies on proliferation, survival and epidemiology of *Pasteuria penetrans***

This experiment has shown the importance of the degradation time necessary to release spores into the rhizosphere. The promising outcome is the high proportion of infected females (38% [s.e.d 3.1] of the 286 females [s.e.d. 63] in the week 3 cohort) after the second harvest considering that the original "inoculum" was only one spore-filled female (Fig. 11). Estimations of the spore concentrations in samples of dried root powder from these plants (Table 9) show the amounts of spores that might be returned to the system (Appendix: Reading Data Folder: Fig 11).

**Figure 11:** Total number of females and proportions infected with *Pasteuria penetrans* after 6 and 12 weeks (harvests 1 and 2 respectively).

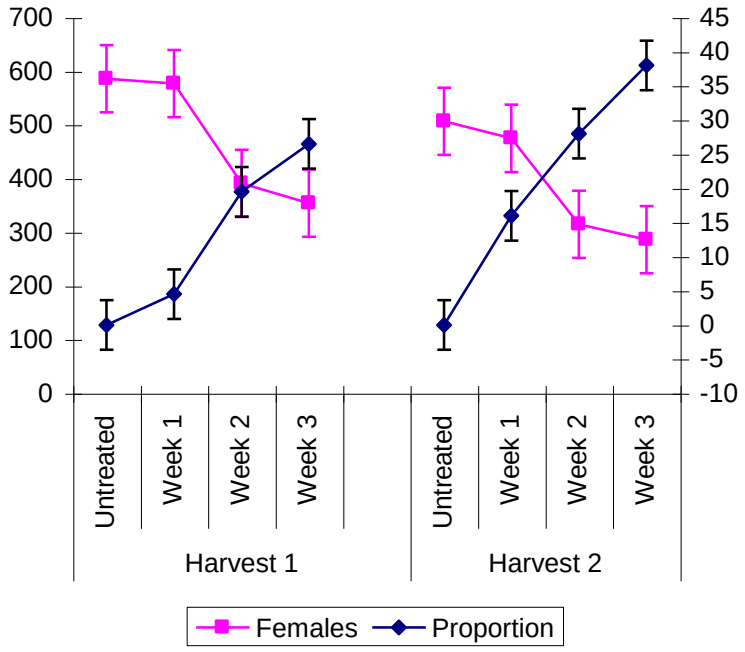



**Table 9:** Effect of degradation of *P. penetrans* infected females over different time intervals on spores produced per mg dried root powder (first harvest after 42days, second harvest 84 days).

| | Endospores/mg dried root (x$10^4$) Harvest 1 | Endospores/mg dried root (x$10^4$) Harvest 2 |
|---|---|---|
| 1 Week | 6 | 14 |
| 2 Weeks | 29 | 49 |
| 3 Weeks | 37 | 69 |
| Control | - | - |
| SED. | 2.1 | 3.2 |
| P value. | <0.01 | <0.01 |

Data are means of 5 replicates.

**The effect of the time that juveniles are spore-encumbered before root invasion; levels of spore attachment and the subsequent infection by *P. penetrans* in the root-knot nematode females**

The objective of this experiment was to see whether the likelihood of a female becoming infected by the parasite was influenced by the time between spore attachment to the juvenile and the time of root invasion. Additionally this affected by the level of spore attachment.

The number of females in the control (non-*Pasteuria* treatment) was lower than expected (Figure 12a) a result that cannot be explained, however the trend was for the number of females to decline over the storage time and there were significantly more (P=0.05) females in the Day 1 and 2 treatments than Day 3 (Appendix: Reading Data Folder: Fig 12). This would confirm the assumption that younger nematodes are more active and have a greater infection potential. When the percentage of infected females is compared the affect of delayed invasion was not significant. It can be concluded that the delay in invasion by *P penetrans*-encumbered juveniles does not influence the percentage infection.

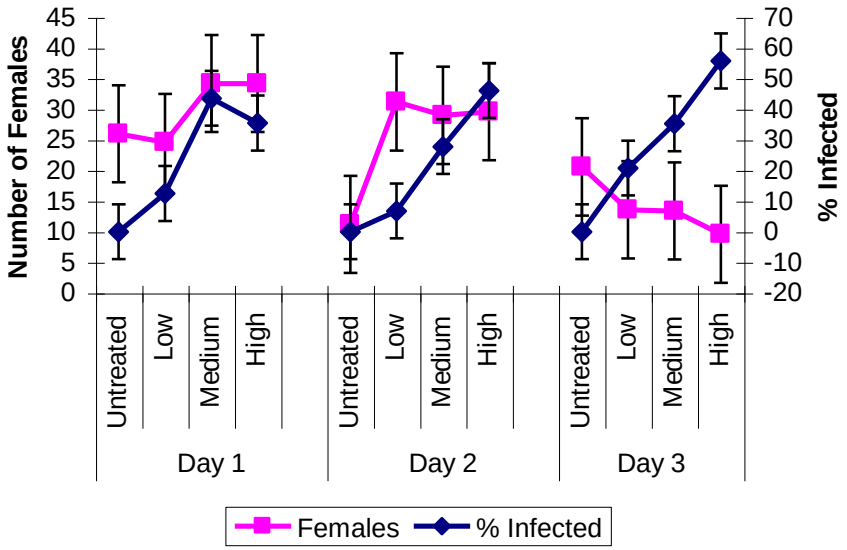
This result is confirmed when comparing numbers of egg masses which showed a similar trend to the females (Figure 12b). If the hypothesis was that infection would increase with delayed invasion it would be expected that the number of egg masses would decline more in the Day 3 treatment but this was not the case (P=0.05).

If greater levels of attachment lead to higher incidence of infection it would be expected that the numbers of egg masses would decline as the level of spore attachment increased; this is borne out by the data (Figure 12c). The correlation between percentage infection and spore attachment on infective juveniles is clearly demonstrated in Figure 12d.

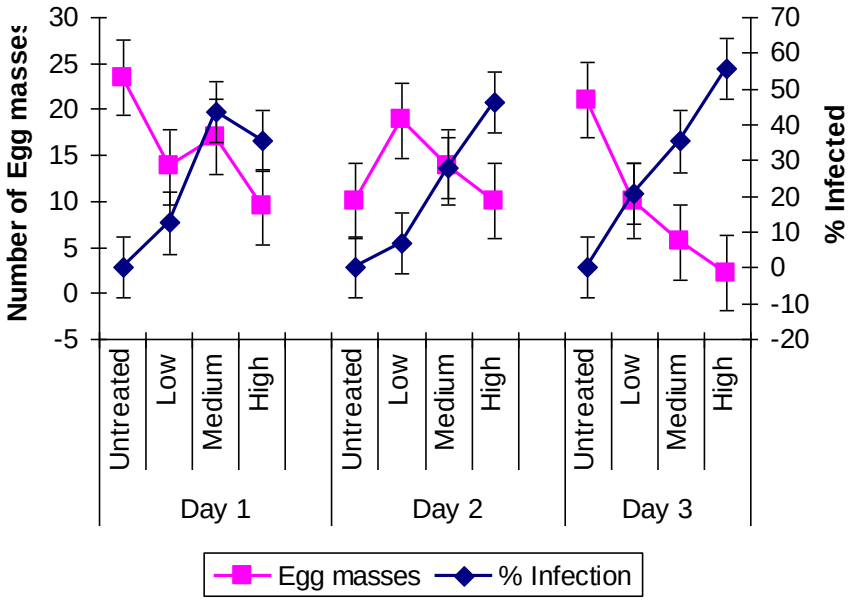
All spore attachment levels, female and egg mass data are summarised in Table 10.

**Figure 12: a,b,c and d.** The relationships between three different levels of spore attachment on juveniles and resultant numbers of females, egg masses and percentages infected females following inoculation of tomato plants with cohorts of 100 nematodes that had been spore encumbered for 1,2, and 3 days (means of five replicates)

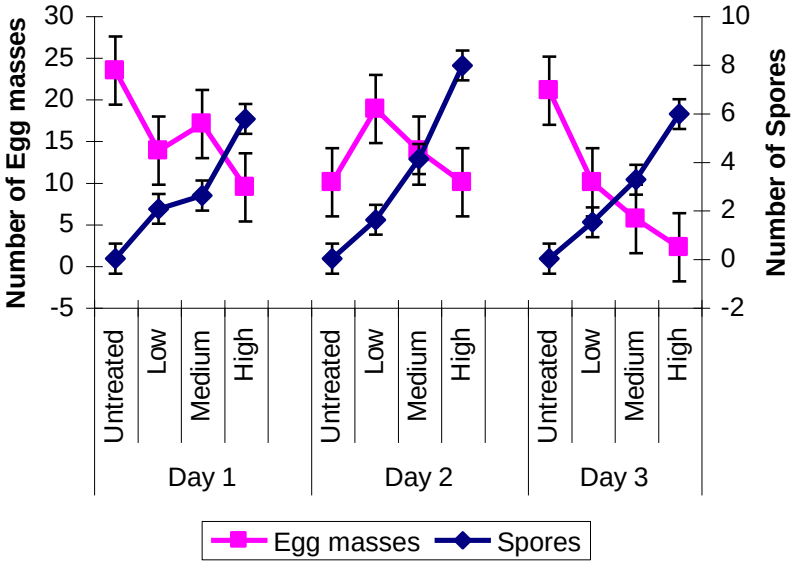
**a.** Number of females and percentage infected by *P. penetrans*



**b.** Numbers of egg masses in relation to percentage of females infected by *P.penetrans*




**c.** Numbers of egg masses in relation to spore attachment level on juveniles



**d.** Percentage of *P. penetrans* infected females in relation spore attachment level on juveniles

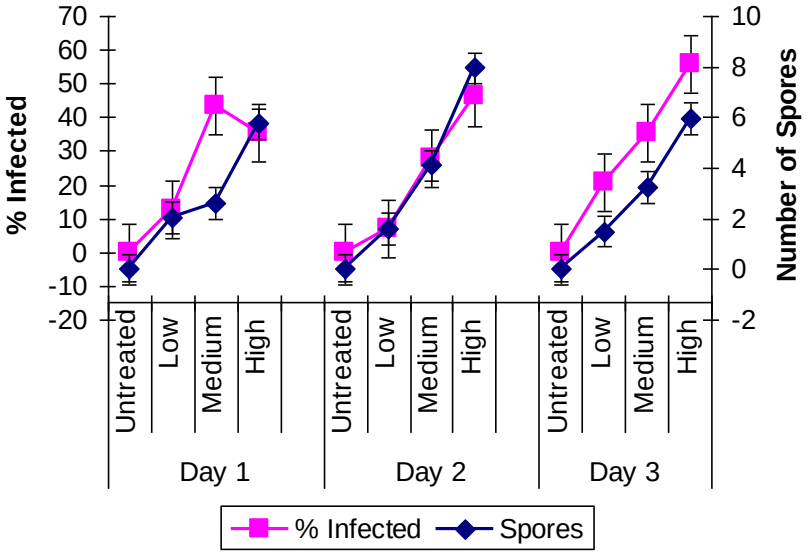


**Table 10:** Summary data of means for each treatment; attachment, frequency (range of attachment) cumulative percentage and total number of nematode females, egg masses, and infected females.

| Days | Treatment | Attachment* | Frequency (range of attachment) | Cumulative Percentage** | Total No. Eggmasses (Means of 5 replicates) | Total No. Females (Means of 5 replicates) | Infected Females (Means of 5 replicates) | % of Infected Females (Means of 5 replicates) |
|---|---|---|---|---|---|---|---|---|
| **Day 1** | Control | | | | 23.5 (±2.36) | 26.0 (±2.38) | | |
| | Low | 2.0 (±0.352) | 0 1 2 3 (6)† | 15 35 75 85 (100)† | 13.8 (±3.693) | 14.6 (±7.18) | 2.8 (±0.8) | 12.48 (±3.395) |
| | Medium | 2.6 (±0.294) | 1 2 3 4 (5) | 20 50 70 95 (100) | 17.0 (±3.33) | 34.2 (±5.25) | 14.0 (±1.304) | 43.61 (±6.18) |
| | High | 5.8 (±0.688) | 3 4 5 (13) | 20 35 65 (100) | 9.4 (±2.926) | 34.2 (±9.39) | 10.8 (±2.267) | 35.45 (±5.15) |
| **Day 2** | Control | | | | 10.0 (±2.881) | 11.2 (±3.47) | | |
| | Low | 1.6 (±0.265) | 0 1 2 (4) | 10 60 85 (100) | 18.8 (±3.25) | 31.2 (±2.69) | 2.2 (±0.663) | 6.82 (±2.111) |
| | Medium | 4.1 (±0.481) | 1 2 3 4 5 (9) | 15 25 35 60 80 (100) | 13.8 (±1.497) | 29.0 (±4.82) | 9.0 (±3.271) | 27.77 (±6.26) |
| | High | 8.0 (±0.851) | 4 5 6 7 9 (20) | 15 25 35 60 80 (100) | 10.0 (±1.304) | 29.6 (±8.77) | 13.4 (±4.654) | 46.08 (±4.38) |
| **Day 3** | Control | | | | 21.0 (±5) | 20.6 (±5.32) | | |
| | Low | 1.5 (±0.312) | 0 1 2 (4) | 20 65 85 (100) | 10.0 (±3.256) | 13.6 (±3.97) | 3.0 (±1.517) | 20.79 (±7.73) |
| | Medium | 3.2 (±0.464) | 1 2 3 4 5 (9) | 15 35 70 80 90 (100) | 5.6(±2.159) | 13.4 ±5.297) | 3.75(±1.548) | 35.33 (±12.42) |
| | High | 6 0 (±0.545) | 2 4 5 6 7 8 (10) | 10 35 45 55 70 80 (100) | 2.2(±0.97) | 9.6 ±3.868) | 4.6 (±1.833) | 55.76 (±11.33) |

* Means of 20 juveniles

** Values with frequency less than 1 juvenile not included, for example, data in top row shows numbers of J2s with 0,1,2 or 3 spores attached

† Numbers in parenthesis show maximum numbers of spores recorded

**Field mass production of *P. penetrans* at Thika**

This objective has not yet been achieved. High spore concentrations in tomato roots have not been recovered (Appendix: CABI-KARI Folder: Subfolder; Pp Production trial). The reasons for this could be low initial nematode densities in the plots and perhaps more importantly, underestimates of the spore concentration in the inoculum applied at the start. Observations made on spore powder (Plate 7) could explain this. It is encouraging that spore encumbered juveniles are now being recovered from 6 of the 24 plots.

## Socio-economic assessments

**Identify promotion pathways for research outputs** (Appendix: Reading Data Folder; Participatory budgets)

### Inputs (Tables 11a-b and 12a-b)

Immediately after preparation of the inputs tables, the reaction of both groups was that they preferred trash burning because it seemed to have less purchased inputs than the other treatments. The Karigu-ini group (the organic farmers) commented that they prefer methods that do not involve chemical use. Basamid required a lot of separate activities and precision to apply it, and the Karigu-ini group did not like it because it was poisonous. The initial impression of bcas was that they did not take a lot of inputs, but the farmers were uncertain about them because they did not know where they would obtain them.

**Table 11a. Karigui-ini. Input table constructed with farmers for tomato seedbed treatments against root-knot nematode**

| INPUTS | CONTROL | BASAMID | BCAS | CROTA-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|
| Labour to collect water | | 40 mins a day for 13 days | | 40 mins a day for 4 weeks | 40 mins a day for 6 weeks | |
| Polythene bags | | 2 sq metres | | | | |
| Labour | | | | | | |
| Preparing before application | | 6 m per plot | | | | |
| Application | | 5 m per plot | | | | |
| Covering with plastic | | 8 m per plot | | | | |
| Uncovering & mixing | | 5 m per plot | | | | |
| Planting beans | | 1 m per plot | | | | |
| Putting bcas onto seedbed | | | 7 m per plot | | | |
| Sowing | | | | 3 m per plot | 3 m per plot | |
| Incorporating into soil | | | | 5 m per plot | 3 m per plot | |
| Cutting and collecting trash | | | | | | 30 m for 6 plots |
| Burning | | | | | | 2 m per plot |
| Seeds | | | | | | |
| *Crotalaria* | | | | 366 per plot | | |
| Tagetes | | | | | 180 gms per plot | |
| Bcas | | | | | | |
| Vc | | | 75 gms per plot | | | |
| Pp | | | 5 gms per plot | | | |
| Basamid | | 50 gms per plot | | | | |

**Table 12a. Mwea Input table constructed with farmers for tomato seedbed treatments against root-knot nematode**

| INPUTS | CONTROL | BASAMID | BCAS | CROTA-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|
| Watering labour (6 plots) | | 40 m a day, 13 days | | 40 m a day for 28 days | 40 m a day for 42 days | |
| Polythene bags | | 2 sq metres | | | | |
| Labour (m=minutes) | | | | | | |
| Preparing before application | | 5 m per plot | | | | |
| Application | | 3 m per plot | | | | |
| Covering with plastic | | 3 m per plot | | | | |
| Uncovering & mixing | | 3 m per plot | | | | |
| Planting beans | | 3 m per plot | | | | |
| Putting bcas onto seedbed | | | 6 m per plot | | | |
| Sowing | | | | 2 m per plot | 3 m per plot | |
| Incorporating into soil | | | | 5 m per plot | 3 m per plot | |
| Cutting and collecting trash | | | | | | 25 m per plot |
| Burning | | | | | | 1 m per plot |
| Seeds | | | | | | |
| *Crotalaria* | | | | 366 per plot | | |
| *Tagetes* | | | | | 180 gms per plot | |
| Bcas | as for Karigu-ini | | | | | |
| Basamid | as for Karigu-ini | | | | | |

**Table 11b. Karigu-ini. Summary of inputs per plot**

| INPUTS | Input units | CONTROL | BASAMID | BCAS | CROTAL-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|---|
| Labour to collect water | labour days (1) | | 0.289 | | 0.622 | 0.933 | |
| Polythene bags | sq m polythene | | 0.6666667 | | | | |
| Labour | labour days (1) | | 0.083 | 0.023 | 0.027 | 0.020 | 0.023 |
| Seeds | | | | | | | |
| *Crotalaria* (2) | labour days (1) | | | | 0.017 | | |
| *Tagetes* (2) | labour days (1) | | | | 0.017 | | |
| Bcas | | | | | | | |
| | Vc gms | | | 75 | | | |
| | Pp gms | | | 5 | | | |
| Basamid | 50 gms | | | 1 | | | |
| notes | (1) Based on 5 hour working day | | | (2) Cost based on time to collect | | | |

**Table 12b. Mwea. Summary of inputs per plot**

| INPUTS | Input units | CONTROL | BASAMID | BCAS | CROTAL-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|---|
| Watering | labour days (1) | | 0.289 | | 0.622 | 0.933 | |
| Polythene sheet | sq m polythene | | 2 | | | | |
| Labour | labour days (1) | | 0.057 | 0.020 | 0.023 | 0.020 | 0.017 |
| Seeds | | | | | | | |
| *Crotalaria* (2) | labour days (1) | | | | 0.017 | | |
| *Tagetes* (2) | labour days (1) | | | | 0.017 | | |
| Bcas | | | | | | | |
| | Vc gms | | | 75 | | | |
| | Pp gms | | | 5 | | | |
| Basamid | gms | | 50 | | | | |
| notes | (1) Based on 5 hour working day | | | (2) Cost based on time to collect | | | |

## Costs (Table 11c and 12c) Kenya Shillings (KSh)

The highest total cost was for Basamid treatment. The highest individual input cost was for watering the *Tagetes* plot. *Crotalaria* was also watered, but not for as many days as *Tagetes*. Farmers did not pay for the actual water at either site. The farmers saw that the high labour costs for both treatments could be reduced by planting during the rainy period, possibly in another seedbed closer to the house, and then harvesting the seedlings and incorporating them into the tomato seedbed.

In Mwea, farmers remarked that the Basamid treatment was quite complicated to carry out.

The lowest cost was for trash burning, which the farmers regarded as a simple and convenient seedbed treatment. They did not appear to be concerned that the crop residues and other material burned might have a value for manure or livestock. The amounts required for seedbed treatment on small plots were quite small.

The farmers expressed a concern that the costs for the bcas were not available and this made it difficult to complete the evaluation. It seemed to be a simpler and less labour intensive treatment than *Tagetes*, *Crotalaria* or Basamid.

**Table 11c. Karigu-ini. Costs per plot KSh**

| INPUTS | Unit price KSh | CONTROL | BASAMID | BCAS | CROTAL-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|---|
| Labour to collect water (1) | 100 | | 28.9 | | 62.2 | 93.3 | |
| Polythene sheet (2) | 50 | | 33.3 | | | | |
| Labour for seedbed trtments | 100 | | 8.3 | 2.3 | 2.7 | 2.0 | 2.3 |
| Seeds | | | | | | | |
| *Crotalaria* collection | 100 | | | | 1.7 | | |
| *Tagetes* collection | 100 | | | | 1.7 | | |
| Bcas | | | | | | | |
| Vc | | | | 0.0 | | | |
| Pp | | | | 0.0 | | | |
| Basamid | 33.4 | | 33.4 | | | | |
| Total cost | | 0.0 | 104.0 | 2.3 | 68.2 | 95.3 | 2.3 |
| notes | (1) a labourer is paid KSh 80 per day and given lunch | | | | (2) assumed to last 3 years | | |

**Table 12c. Mwea. Costs per plot KSh**

| INPUTS | Unit price KSh | CONTROL | BASAMID | BCAS | CROTAL-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|---|
| Labour to collect water (1) | 100 | | 28.9 | | 62.2 | 93.3 | |
| Polythene sheet (2) | 50 | | 33.3 | | | | |
| Labour for seedbed trtmnts | 100 | | 5.7 | 2.0 | 2.3 | 2.0 | 1.7 |
| Seeds | | | | | | | |
| *Crotalaria* collection | 100 | | | | 1.7 | | |
| *Tagetes* collection | 100 | | | | 1.7 | | |
| Bcas | | | | | | | |
| Vc | | | | 0.0 | | | |
| Pp | | | | 0.0 | | | |
| Basamid | 33.4 | | 33.4 | | | | |
| Total cost | | 0.0 | 101.3 | 2.0 | 67.9 | 95.3 | 1.7 |
| notes | (1) casual labour is paid KSh 100 a day | | | | (2) assumed to last 3 years | | |

## Outputs (Tables 11d and 12d)

In Karigu-ini, both the total output and the output of higher grade tomatoes were greater from Basamid, bcas and trash burning than the control and so the value of yield was 60% higher or more in all of these treatments. However, the field in which the trial was carried out experienced a wilt problem unrelated to root-knot nematodes and this affected the treatments unevenly. Statistical analysis later performed by the biophysical scientists suggested that the trial had had a minimal impact on outputs. In Mwea, the control out-performed all of the treatments and this made it difficult to appreciate the value of root-knot nematodes control or to have a full discussion about different treatments.

**Table 11d. Karigu-ini. Value of outputs per plot**

| | | | Average yield per plot | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | CONTROL | BASAMID | BCAS | CROTAL-LARIA | TAGETES | TRASH BURNING |
| Grade 1 | kg | | 0.1 | 0.2 | 0.2 | 0.03 | 0.1 | 0.2 |
| Grade 2 | kg | | 0.8 | 1.8 | 2.1 | 0.8 | 0.7 | 2 |
| Grade 3 | kg | | 1.8 | 4.3 | 4.5 | 2.4 | 2.6 | 4.8 |
| Grade 4 | kg | | 5.7 | 8.2 | 8.4 | 5.2 | 5.9 | 9 |
| Grade 5 | kg | | 3.2 | 3.2 | 3.3 | 3.4 | 2.6 | 1.8 |
| | | | 11.6 | 17.7 | 18.5 | 11.83 | 11.9 | 17.8 |
| | Price KSh | | Value of output per plot | | | | | |
| Grade 1 | | 13 | 1.3 | 2.6 | 2.6 | 0.39 | 1.3 | 2.6 |
| Grade 2 | | 12 | 9.6 | 21.6 | 25.2 | 9.6 | 8.4 | 24 |
| Grade 3 | | 10 | 18 | 43 | 45 | 24 | 26 | 48 |
| Grade 4 | | 8 | 45.6 | 65.6 | 67.2 | 41.6 | 47.2 | 72 |
| Grade 5 | | 7 | 22.4 | 22.4 | 23.1 | 23.8 | 18.2 | 12.6 |
| | Total | | 96.9 | 155.2 | 163.1 | 99.39 | 101.1 | 159.2 |

**Table 12d. Mwea. Value of outputs per plot**

| | | | Average yield per plot | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | CONTROL | BASAMID | BCAS | CROTAL-LARIA | *I* | TRASH BURNING |
| Grade 1 | kg | | 1.2 | 2.3 | 1.4 | 1 | 1.8 | 2.4 |
| Grade 2 | kg | | 2.3 | 2.8 | 3.6 | 2 | 2.2 | 4.1 |
| Grade 3 | kg | | 13.8 | 4.7 | 5.2 | 3.9 | 4.2 | 6 |
| Grade 4 | kg | | 5 | 5.9 | 3.7 | 4.8 | 5.8 | 6.9 |
| Grade 5 | kg | | 9.5 | 11 | 9.2 | 8.7 | 9.7 | 11.2 |
| | | | 31.8 | 26.7 | 23.1 | 20.4 | 23.7 | 30.6 |
| | Price KSh | | Value of output per plot | | | | | |
| Grade 1 | | 13 | 15.6 | 29.9 | 18.2 | 13 | 23.4 | 31.2 |
| Grade 2 | | 12 | 27.6 | 33.6 | 43.2 | 24 | 26.4 | 49.2 |
| Grade 3 | | 10 | 138 | 47 | 52 | 39 | 42 | 60 |
| Grade 4 | | 8 | 40 | 47.2 | 29.6 | 38.4 | 46.4 | 55.2 |
| Grade 5 | | 7 | 66.5 | 77 | 64.4 | 60.9 | 67.9 | 78.4 |
| | Total | | 287.7 | 234.7 | 207.4 | 175.3 | 206.1 | 274 |

## Comparison of outputs and costs (Tables 11e and 12e)

In Mwea, the control outperformed all of the treatments. Trash burning showed the smallest net loss, and bcas were next.

In Karigu-ini, *Crotalaria*, *Tagetes* and Basamid showed a net loss while trash burning and bcas showed a net benefit. This did not include the cost of the bcas themselves. Break-even estimates were made against the control and against trash burning, showing that if the differences from this trial are accurate, bcas would need to be very cheap to outperform trash burning as a seedbed treatment. However, it is possible that the labour for trash burning has been underestimated, and the trash may have an opportunity cost.

The farmers concluded that trash burning seems the most cost effective method for root-knot nematodes treatment. They indicated that bca may also be a good control but they stressed the need to know the bca costs. They said that they would prefer the bca to Basamid since bca is a biological control. When asked what they would be willing to pay for it, they insisted that it should be cheaper than Basamid and some of them suggested that for the same unit, the price of bca should about half that of Basamid. The bcas should be packed in small quantities if they are to be applied in the nursery bed.

The statistical analysis later carried out by the biophysical scientists suggested that the yield advantage in Karigu-ini from the bcas was lower than originally suspected. Their assessment was that it would be necessary to run the trial for more than one season or use a different method of bca incorporation to obtain full impact.

**Table 11e. Karigu-ini. Comparison of treatments with control per plot**

| | CONTROL | BASAMID | BCAS | CROTAL-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|
| Additional output KSh | | 0 | 58.3 | 66.2 | 2.49 | 4.2 | 62.3 |
| Additional costs KSh | | 0 | 104 | 2 | 68 | 95 | 2 |
| Net difference output - cost KSh | | 0 | -46 | 64 | -66 | -91 | 60 |

(1) full cost of bcas not estimated because production costs not known

**Break-even cost for bcas per plot against control** **64**

**Break-even cost for bcas per plot against Trash burning** **4**

**Table 12e. Mwea. Comparison of treatments with control per plot**

| | CONTROL | BASAMID | BCAS | CROTAL-LARIA | TAGETES | TRASH BURNING |
|---|---|---|---|---|---|---|
| Additional output KSh | | 0 | -53 | -80.3 | -112.4 | -81.6 | -13.7 |
| Additional costs KSh | | 0 | 101 | 2 | 68 | 95 | 2 |
| Net difference output - cost KSh | | 0 | -154 | -82 | -180 | -177 | -15 |

(1) full cost of bcas not estimated because production costs not known

## Further research needed

It would be valuable to run trials for longer to provide a better estimate of long term impact – this need not necessarily be done on farm provided that there was sufficient farmer input to the evaluation process. Further participatory budgeting work would make it possible to take into account the variety of rotations practised by farmers and the limitations imposed by renting land – a common practice in Mwea.

More work needs to be done in determining what form the commercial production of the bcas might take and its eventual cost to farmers. The target for high-output high-input farmers should be to undercut the total cost of Basamid. More needs to be done to ascertain a target for moderate-output moderate-input farmers, and it is likely that the convenience of the delivery channel and a simple and clearly explained application method

with a good “fit” to their other farming practices would be very important in reaching this group.

As a seedbed treatment, the bcas would be in competition with trash burning. It would be useful not only to examine effects over the long term but also to investigate effects of using bcas in the field in combination with trash burning on the seedbed.

**Contributions of Outputs to developmental impact**

Asaba (2000) suggests that farmers ranked extension services highly as a source of information - however, she mentioned that the service was often inaccessible and mentioned a wide variety of sources.

- geographic distribution
- accessibility
- reliability of information
- extensiveness or versatility of the communication methods

All of these factors will need to be taken into account in considering promotion and uptake pathways for the root-knot nematode bcas. It is unlikely that any one promotion pathway will satisfy all criteria and a combination of approaches will be needed. This seems particularly important in view of the fact that bcas need to be well understood and applied correctly. Since their effect may not be immediately visible, farmers may be tempted to cut corners or give up too early. The most common source of information generally seems to be other farmers, who may not be the best providers of advice on bca unless that have been well trained.

The following possibilities might be appropriate:

- The importance of either involving local KARI stations or briefing them very well on the application of the technology

- Direct training of contact farmers, working through marketing groups (where they exist) or church groups, or Farmer training centres (such as the one in Mwea) or Farmer Field Schools (which seem to work well for bca although they may be expensive to establish), including participatory budgeting;

- Training of local extension agents in areas where they are active

- Use of “visual” training material such as videos, slides, theatre and participatory techniques

None of these additional training activities (apart from the involvement of KARI) were budgeted in this project, promotion would need to form a more significant part of any follow on project.

Developmental impact will depend on the ability to mass produce the agents and supply them at a cost that is attractive to growers. Farmers have indicated their interest and keenness to use bcas if the cost of production is acceptable.

The methods developed in this project do not produce sufficient *P. chlamydosporia* to be practical as one gramme of medium is still required to treat only one kilo of soil. Research conducted at Rothamsted is targeted at the improvement of mass production methods:

- DFID have provided funds to build a pilot production plant in the CENSA laboratories in Havana, Cuba and a product, KlamiC, based on *P. chlamydosporia* is being developed and is going through toxicity testing.

- Biological Control Products Ltd. have entered an agreement to produce South African isolates of *P. chlamydosporia*.

Although much can be learnt from the approaches being taken in these two projects, they are unlikely to produce methods that could be exploited in Kenya.

It has already been mentioned that the supply routes for *Pasteuria* and *Pochonia* have not yet been determined. Two questions remain unanswered: what will they cost, and where will farmers go to obtain them (which has both cost and exclusivity implications)?

For the *Pasteuria*, which is an obligate parasite that cannot be mass-produced rapidly on a defined medium, production through on-farm techniques is possible. Nevertheless, there remains a requirement for laboratory involvement (high power microscope) to verify the presence of *P. penetrans* spores. This aspect is being addressed in a new project with Dudutech (Kenya) Ltd.

For the *Pochonia* chlamydospores, at present the production process is complex, involving several stages and autoclaving, all of which would be beyond the ability of a farmer, farmer group or local supplier. The possibility of commercial production within Kenya must therefore be considered, with the final product probably being a packet of mixed sand, cereal flour and spores to be incorporated into the soil of the seedbed. Provided that the product has a reasonable shelf life, this might prove to be an acceptable formulation and application process. However, since it has only been produced on a very small scale, there are no data within CABI to suggest what the ultimate production cost and hence the supply cost to the distributor and the cost to the farmer would be.

There is also a larger issue to consider. The technical and socio-economic potential for a number of bcas in East Africa may be very promising, yet if there is no reliable production site for the agents the questions of supply route and market price will recur in every bca project. Some of these issues concerning *Pasteuria* will be met through the Dudtech project there is a need for a further project to evaluate, quality control and go wider on cross cutting issues with partners in Kenya.

Negotiations have begun with Dudutech, a producer of biocontrol agents in Kenya, for them to produce indigenous isolates of the bcas which would be available to their outreach growers for evaluation. This will be widened to any other producers and/or promoters who may wish to develop and promote these technologies.

Other added benefits from the programme at Rothamsted include EU funded research aimed to measure the environmental impact of applications of the fungus. The impacts on crop plants, rhizosphere bacteria, mycorrhiza, rhizobia and the free-living nematode community have been assessed and no detrimental effects have been observed. Hence, if methods of production can be developed for widespread application of the fungus in vegetable producing areas, such treatments would be environmentally benign.

Before *P. chlamydosporia* and *P. penetrans* can be exploited effectively there is a need for further development to:

- Conduct trials on a range of soils and crops to determine the best time in the cropping cycle to apply selected isolates of *P. chlamydosporia* and to determine the most effective methods and rates of application.

- Develop methods to monitor the quality of *P. chlamydosporia* inoculum produced in successive batches from the fermenters.

- Develop mixtures of *P penetrans* collected from within Kenya to broaden the pathogenicity and overcome the problem of specificity that could arise within mixed nematode populations in the field.

- Develop rapid soil assays to assess the need to apply *P. chlamydosporia* and *P. penetrans*.

- Develop predictive models for infection of nematodes by *P. penetrans* based on soil attachment assays.

- Investigate the practical and economic feasibility of introducing bcas with seedling transplants through the development of a specialised transplant production system.

- Expand socio-economic inputs to determine the most cost effective method of production and to assess the likely uptake of such new technologies.

A project focussing on commercial production cannot reasonably be expected to make an objective assessment of its own impact on small farmers. It would, therefore, be advisable to carry out additional research to evaluate the uptake by different types of farmer (with different levels of input; with and without out-grower relationships with an input supplier). The objective of the research would be to explore an acceptable balance between the value chain of the producer/supplier (i.e. costs and capabilities required to produce and supply the technology to smallholders, possibly using a network of commercial and non-commercial organisations) and the transaction costs (costs of learning about, obtaining and testing technology) experienced by small farmers in using bcas for crop protection.

Such areas of research will form the basis of a further application for funding**.**

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