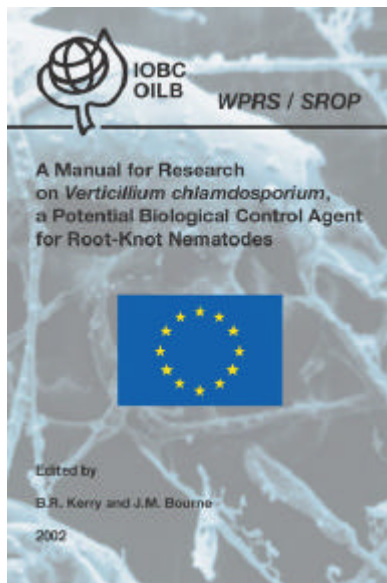


## BYk '€6 7 #k dfg'Di V]WU]cbg

K Y'UFY[ 'UX'hc 'Ubbci bW'Ub'ja dcfhUbhbYk '€6 7 #k dfg'Vcc\_



B.R. KERRY & J.M. BOURNE (2002):

A Manual for Research on *Verticillium chlamydosporium*, a Potential Biological Control Agent for Root-Knot Nematodes.

XIII + 84 pp., 14 fig., 4 fig. in colour, Gent, IOBC/wprs, ISBN 92-9067-138-2. Price: € 25,00. You may order the book by letter, fax or e-mail from: Dr. Annette Herz, Federal Research Centre for Cultivated Plants, Julius Kuehn Institute, Heinrichstraße 243, D-64287 Darmstadt, GERMANY, email: Annette.Herz@jki.bund.de, Fax: ++ 49 (0) 6151-407-290

: fca 'h Y'DfYZJW

This Manual was produced following a Workshop Meeting at the University of Reading, UK, in 1998, which marked the beginning of a European Community funded project FAIR5-PL97-3444. The project is entitled "Development of a sustainable strategy for the management of root-knot nematodes in vegetable crops in southern Europe – an alternative to the use of methyl bromide". The research aims to incorporate applications of the nematophagous fungus, *Verticillium chlamydosporium* Goddard, with other control measures to manage root-knot nematodes on vegetable crops. The control of these nematodes will become a major problem in southern Europe and elsewhere when the general soil sterilant, methyl bromide, is withdrawn from the market in 2005, because of its effects as a depleter of ozone. In southern Europe, root-knot nematodes are major pests in field vegetable crops and in protected crops. These nematodes cannot be eradicated and growers apply large amounts of methyl bromide to manage populations below damaging levels. There is, therefore, an urgent need in southern Europe to find alternative methods to the widespread use of methyl bromide, which are practical and sustainable. A biomanagement strategy, which combines the use of a biological agent with cultural methods, including the use of partially resistant/non-host cultivars, may provide such an alternative, which will reduce nematicide applications. The strategy is being evaluated in commercial situations in southern Europe, where pests are damaging a wide range of crops throughout the Mediterranean region. The project has three specific objectives:

- To evaluate the efficacy of a biomanagement strategy in commercial production systems in southern Europe. This strategy involves the application of *V. chlamydosporium* applied to soil before planting poor hosts for root-knot nematodes in the cropping cycle, to reduce populations before susceptible tomatoes are grown. This strategy has been compared with conventional nematode control (use of methyl bromide) and an integrated management strategy that replaces methyl bromide with other pesticides, including oxamyl for nematode control:
- To develop immunological and molecular methods to monitor the fungus after its release.
- To measure the impact of the fungus on soil microbial biodiversity and non-target organisms.

The project provides a model for the practical application of a soil applied biological control agent and the development of a rational policy relating to such releases. The methods described for *V. chlamydosporium* could be used for a range of fungi isolated from nematode females and eggs that have a similar mode of action. The work described has addressed a number of the problems involved in the scale-up of testing of biological control agents from experiments in controlled conditions to small-scale field trials. Information has been collected on the distribution and variation in isolates of the fungus collected in southern Europe and other parts of the world. The mass-production of chlamydo spores for inoculation remains a problem.

The project has brought together a group of nematologists with interests in the management of nematodes and molecular biologists developing new methods to study microorganisms in the rhizosphere (their names and addresses are listed in Appendix III). The leaders of the participating research teams have written different sections of this Manual and their names and addresses are listed overleaf. I have much enjoyed the interactions involved in coordinating the programme and gratefully acknowledge the hard work, research innovation and friendship of my colleagues involved in the project and the production of this Manual.

Brian Kerry

## Contents

The use of <i>Verticillium chlamydosporium</i> as a biological control agent .....	1
Isolation of <i>V. chlamydosporium</i> from soil, roots and nematodes .....	15
Estimation of growth of <i>V. chlamydosporium</i> isolates on semi-selective medium .....	17
Extraction of chlamydospores of <i>V. chlamydosporium</i> from soil .....	18
Storage.....	19
Identification of <i>Verticillium</i> species from nematode eggs .....	20
Production of inoculum .....	22
Inoculation of soil with <i>V. chlamydosporium</i> .....	24
Screening <i>V. chlamydosporium</i> isolates for their potential as control agents .....	25
Analysis .....	28
Estimating populations of nematodes in soil .....	30
Estimating populations of nematodes in roots .....	31
Visualisation of the fungus in the rhizosphere .....	33
Light microscopy .....	34
Electron microscopy .....	37
Impact of <i>Verticillium chlamydosporium</i> on plant symbiotic micro-organisms.....	44
Nematode culturing and extraction .....	47
<i>Verticillium chlamydosporium</i> isolate selection and pot tests .....	48
Field trials for evaluation of <i>V. chlamydosporium</i> as a control agent of <i>Meloidogyne</i> spp. in vegetable crops .....	57
Identification of root-knot nematodes .....	60
Electrophoresis of enzymes by the automated miniaturized system ( <i>Phast System, Pharmacia</i> ).....	64
The development of molecular markers and the polymerase chain reaction to identify specific isolates of <i>V. chlamydosporium</i> .....	66
Appendices	
I Stock solutions for polyacrylamide gel preparation; electrophoresis and staining .....	75
II Media used .....	77
III Project participants .....	78
IV Selected bibliography for <i>Verticillium chlamydosporium</i> .....	80