New Strategies for the Management of Plant Parasitic Nematodes with Especial Emphasis on Biological Control

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Abstract

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Since 1950s nematicides have provided effective control of a wide range of nematode pests on many crops throughout the world. However, their high cost and environmental and health hazards associated with their use have tended to limit their application. Frequently, alternatives to chemicals have proved impractical or inadequate. Hence, the development of sustainable methods of nematode control is dependent upon the integration of several control techniques, which usually rely on the accurate identification and qualification of the pest species and on a detailed knowledge of their ecology and biology. Even for the most extensively studied nematode pests, such information is often lacking. New methods based on immunoassays appear to have great potential for the identification of specific nematode pests and their quantification in soil. Molecular techniques for the production of transgenic plants with novel resistances to both sedentary and migratory nematodes are well advanced and some nematicidal genes have been identified. However, experience with resistant plants has indicated that their use requires careful management to prevent the development of virulent nematode populations capable of overcoming such resistance. Soils that suppress the multiplication of several nematode pests have been identified throughout the world and have been associated with the build up of antagonists. Such natural control has proved difficult to manipulate but studies on suppressive soils have led to the identification of a range of microbial agents which may have potential for biological control. Two parasites, *Verticillium chlamydosporium* and *Pasteuria penetrans*, are being studied at Rothamsted for their potential as biological control agents for root-knot nematodes in vegetable crops. Key factors affecting the efficacy of these organisms have been identified and their application at a specific cropping cycle may provide consistent and effective control. Clearly, there are exciting new prospects for the management of nematode pests but there is a need for much research and development before many of the new approachers are available to the grower.

Key words: Nematodes, biological control, suppressive soil, IPM, transgenic resistance.

Introduction

Since the 1950s, nematicides have provided effective control of a wide range of nematode pests on many crops throughout the world. However, their high cost has tended to limit their use and, in some cases, environmental and health hazards have led to the withdrawal of products from the market. Within Europe some countries, such as the Netherlands, have legislated to reduce significantly treatments of soil applied pesticides and by 2000 the use of methyl bromide, which is widely used in horticulture for nematode control, will be banned in the USA. Frequently, alternatives to the application of chemicals have proved impractical or inadequate. Hence, the development of sustainable methods of nematode control is dependent upon the integration of several control techniques which often rely on the accurate identification and quantification of the pest species and on a detailed knowledge of their ecology and biology. Even for the most extensively studied nematode pests such information is often lacking. In this brief review, three topics which may make significant contributions to the development of new strategies for nematode control are discussed with especial reference to cyst (*Globodera* and *Heterodera*) and root-knot (*Meloidogyne*) nematodes which are the most important nematode crop pests worldwide.

New methods for the identification of nematode pests

In many countries, including those with a long tradition of nematological research, there is shortage of skilled nematode taxonomists able to accurately identify pest species. Sustainable methods of nematode management, unlike the use of nematicides, are likely to be more specific to particular pest groups or species and so their effective deployment depends on the accurate identification and quantification of the pest species targeted. Therefore, there is an urgent need to increase the number of trained taxonomists and to develop simple biochemical, immunological or molecular based tests for the identification of nematodes. To this end there have

been important technical developments in recent years. The separation of proteins on isoelectric focusing gels has led to the identification of esterase and malate dehydrogenase phenotypes which distinguish the major root-knot species (8). Diagnostic proteins have been identified for other nematode groups, including the potato cyst nematodes (10), but the methods need careful standardisation, are relatively insensitive and only qualitative, and are laboratory based.

Monoclonal antibodies (MAbs) have been produced which identify several cyst and root-knot nematodes (6, 26). In the simplest form of enzyme-linked immunosorbent assay (ELISA), the MAb which identifies the antigen of interest is linked to an enzyme which gives a colour reaction in the conditions of the assay; the antigen is immobilised on a suitable surface, such as the well of a microtitre plate, and is recognised by the diagnostic MAb and the intensity of the colour reaction is indicative of the amount of antigen present. Hence, MAbs can be used to quantify nematode populations and methods have been used to estimate the densities of *Meloidogyne* spp. in roots (5) and potato cyst nematodes in soil extracts (9). These methods are relatively cheap and can be partially automated to allow the analysis of large numbers of samples. Assays based on MAbs are potentially more sensitive than protein analyses and can detect populations of nematodes at densities of about 1-5 eggs/g soil (K. Evans pers. comm.). Although ELISA is a laboratory based analysis, simple dipstick tests using MAbs could be used to assess the presence of specific pests in the field.

A range of techniques to analyse differences in DNA sequences between nematode species have been developed (4). These techniques are less well advanced than those using MAbs but some, such as the polymerase chain reaction (PCR), enable the DNA from a single nematode to be multiplied in the laboratory for analysis. Such methods are very sensitive and could be developed for the detection of nematodes in support of quarantine and certification schemes. Although DNA probes which separate nematode pathotypes/races have been reported (3), none has been tested widely on defined populations to determine the utility of probes for the characterisation of field populations. Clearly, if the probe is closely linked to a virulence gene it is likely to have great importance for the detection of specific pathotypes/races and for studies on their population genetics in the field. These technologies are expanding rapidly and they will undoubtedly contribute much to nematode diagnosis at all taxonomic levels.

Molecular approaches for the development of plants with novel resistance to nematodes

Most plant parasitic nematodes are pathogenic and kill the plant cells on which they feed before migrating to a new feeding site. Those nematodes which have sedentary stages in roots must cause the formation of complex feeding cells from which they derive nutrition for their development. Although the feeding cells of cyst and root-knot nematodes differ in their structure (the former nematodes produce syncytia, the latter giant cells), they have similar functions and if they are not produced the nematodes die. The feeding cells are metabolically very active and are able to transfer nutrients rapidly from the conducting tissue in the root to the developing nematode. These cells are similar to transfer cells which are produced in healthy plants where nutrients must be transported rapidly, e.g. they are commonly found between the developing embryo and the storage tissue in seeds. Hence, to support their development, these nematodes appear to regulate plant genes (1). The application of molecular biological techniques to the understanding of the mechanism for this gene regulation is underway in many laboratories.

Molecular techniques are also being used to produce transgenic plants which express foreign genes that are toxic to nematodes or inhibit their development. Four broad approaches can be identified: (a) the introduction of nematicidal genes such as Bt toxins or key enzyme inhibitors; (b) the characterisation of known resistance genes which are then introduced into susceptible plants; (c) the expression of selected antibodies within plants to inhibit the function of key proteins in the host parasite relationship; and (d) the use of antisense techniques to interfere with the regulation of genes in the feeding cells of sedentary nematodes.

Specific promoters have been identified which can limit the expression of introduced genes to roots or even individual nematode feeding cells (11). The expression of nematicidal genes or antibodies in root tissue may provide opportunities to develop plants with resistance to migratory nematodes. The characterisation of the Mi resistance gene to root-knot nematodes (34) and the H_1 gene against *Globodera rostochiensis* is well advanced (24). Transgenic plants with resistance to root-knot nematodes have been produced (21) and field tested. At present there is a general lack of nematicidal genes to insert into plants but molecular approaches to nematode resistance will undoubtedly play a major role in the development of future management strategies. If selected genes can be inserted without altering other plant characteristics, these methods will be especially important for transferring nematode resistance to preferred local cultivars but, unless more than one gene is transferred, transgenic resistant plants may need to be carefully integrated with other control measures to prevent the selection of virulent nematode populations.

The role of biological agents in the integrated control of nematode pests

Under perennial crops or crops grown in monocultures nematophagous fungi and bacteria have been demonstrated to increase to densities which eventually control specific pest nematodes including root-knot and cyst nematodes (15). In northern Europe, the cereal cyst nematode has been effectively controlled for the past 20 years in many soils by parasitic fungi which kill developing female nematodes on the roots of susceptible crops. Thus the nematophagous fungi, *Nematophthora gynophila* and *Verticillium chlamydosporium* responsible for this natural regulation provide sustainable control in an intensive cropping system. However, the eventual control of the nematode is dependent on an initial period when the nematode host is prevalent and able to support the increase of the biological control agents. This increase is usually slow and it may take 4-5 crops before the nematode is regulated to non-damaging population densities. During this initial phase, other control methods would be required to prevent major yield losses. However, treatments which reduce the numbers of female nematodes on roots would decrease the build up of the natural enemies and it has proved difficult to manage natural control in practice. The addition of organic matter to soil as green manure crops or selected amendments such as chitin may enhance the activity of the nematophagous microflora in soils and increase nematode control (25).

Several species of bacteria and fungi have been investigated for their potential as biological agents for application to soil for the control of specific nematode pests. Although some organisms have been commercialised, none has proved successful as the control achieved has been inconsistent. The type of agent selected depends on the nematode pest to be controlled. Thus, fungi and bacteria used against migratory nematodes which remain active throughout their life must produce adhesive spores or specialist traps to infect their host. Sedentary stages of nematodes may be parasitised by non-specialist opportunistic fungi that develop in the rhizosphere. Some endophytic fungi which colonise plant roots may produce toxins or compete for space with endoparasitic nematodes and significantly reduce nematode infestations (27). These fungi appear to be non-pathogenic to the plant and are often numerous in root tissue, but little is known of their interactions with nematodes. Certain bacteria in the rhizosphere may reduce the attractiveness of roots to nematodes by the alteration of root exudates, by the production of toxins which are directly nematicidal, or by the induction of resistance in roots to nematode invasion (13). Bacteria such as *Pseudomonas fluorescens* selected from the rhizosphere have been applied to sugar beet seed and significantly reduced invasion by the beet cyst nematode, *Heterodera schachtii* (23). Similar bacteria have been demonstrated to reduce the invasion of the roots of several crops by root-knot nematodes (28) but protection has tended to be short-lived and none has prevented nematode populations increasing. Therefore, their use would be to protect susceptible plants from damaging nematode infestations whereas parasitic organisms may reduce both damage and nematode multiplication but tend to be slower acting. Two parasites which have been studied in some detail, *Pasteuria penetrans* and *V. chlamydosporium*, are briefly discussed below to illustrate the major considerations in the development of biological control agents for nematodes. Several more comprehensive reviews are available (14, 15, 27, 30).

Pasteuria penetrans is an obligate bacterial parasite that produces spores which adhere to the cuticle of root-knot nematodes in particular; different *Pasteuria* spp. infect other nematode taxa. The spores germinate when the infective second-stage juveniles of *Meloidogyne* invade the root and begin feeding but infected nematodes are not killed until they have become adult females. At this time the female reproductive tract has been destroyed, usually no eggs are produced and the body is filled with about 2×10^6 spores. As infected females and roots degenerate, the spores are released into the soil and the cycle is repeated. The spores of this bacterium are particularly resistant, can be stored dry without loss of viability and survive several years after their introduction into soil. This ease of handling greatly facilitates the use of *P. penetrans* as a biological control agent. However, the bacterium has not been cultured *in vitro* (2, 33). Spores can be produced *in vivo* (31) but the methods are labour intensive and unsatisfactory for widespread application. Also, populations of *P. penetrans* differ in their specificity and virulence and careful selection of the bacterium is essential to ensure that appropriate strains are used against particular pests (7). Spores applied at rates of $2\text{-}5 \times 10^4$ spores/g soil have given significant control of *M. javanica* on tomatoes (29). Control of *Meloidogyne* spp. on cucumbers was enhanced by integrating the use of *P. penetrans* with applications of the nematicide, oxamyl, and pre-treatment of the soil by solarisation (32). *Pasteuria penetrans* provided effective natural control of *M. arenaria* on peanuts in Florida (22). Clearly, *P. penetrans* has considerable potential as a biological control agent but commercial development will largely depend on the development of a simple method of mass production.

Verticillium chlamydosporium is a facultative parasite of nematode eggs and females and is widely distributed in both tropical and temperate soils. All stages of the fungus (hyphae, conidia and chlamydospores) occur in

soil. The fungus can be readily cultured on both solid and liquid media but few chlamydospores, the most effective propagules for establishment of the fungus in soil, are produced in the latter (18). As with *P. penetrans*, improved methods for the mass production of inoculum are required. *Verticillium chlamydosporium* proliferates in the rhizosphere of a wide range of plant species but does not colonise the root cortex, cause lesions or reduce root growth (19). Cyst nematode females or the egg masses of root-knot nematodes developing in the rhizosphere are colonised by the fungus and their eggs parasitised. An appressorium is produced from which an infection peg penetrates the eggshell and gives rise to a mycelium which destroys the egg contents. Different isolates of the fungus differ greatly in their virulence, ability to produce chlamydospores and rhizosphere competence (16). Only selected isolates which are able to colonise the rhizosphere have provided significant control of *Meloidogyne* spp. in laboratory tests (19). Plant species differ in their ability to support the fungus on their roots and growth in the rhizosphere appears to be much affected by root exudates. The fungus is more abundant on the surface of nematode damaged roots than on healthy ones (19). In general, the amount of fungus on the roots is directly related to the level of nematode control. However, on very susceptible plants and at large nematode densities, large galls are produced in response to nematode attack and these prevent the egg masses being exposed to fungal parasitism in the rhizosphere. In such conditions, a significant proportion of eggs escape attack and control is poor. Hence, effective control with *V. chlamydosporium* is largely dependent on the size of galls produced in response to nematode attack. The combined use of the fungus with nematicides or with tolerant or poor hosts for the nematode which will limit gall size should maximise the number of egg masses exposed to the fungus and the control achieved. In small plot tests with *V. chlamydosporium*, applications of 5 x 10^3 chlamydospores/g soil have provided effective control of small infestations of *M. hapla* on tomato plants and a single application at planting was sufficient for the growing season (20). *Verticillium chlamydosporium* needs more widespread field testing to assess the conditions in which it is effective and its potential as a commercial biological control agent.

Paecilomyces lilacinus is a nematophagous fungus similar to *V. chlamydosporium* but has been extensively field tested and is now commercially produced as Bioact in the Philippines. In general, large dose rates (1 x 10^6 spores/g soil) are required (12) and control has been variable and often difficult to measure (17). Clearly, the fungus is affected by environmental conditions and it remains to be seen whether this fungus or those with similar modes of action can be integrated into farming systems to provide consistent and effective control of nematode pests.

Nematologists should learn from their entomologist and plant pathologist colleagues and begin to test the application of biological control agents in horticulture rather than attempt to apply agents to field crops. Horticultural crops are usually grown in limited areas which would require much less inoculum than field crops and many are transplanted, which would allow the application of the agent in bare root dips, in peat blocks or in potting compost. Such applications would enable the introduction of inoculum to be made close to the root system and are likely to be more effective than broadcast treatments prior to drilling. Experience gained in the practical use of biological control agents in such situations could then be applied to the more demanding conditions with field and perennial crops.

There are, therefore, several possibilities for improving the management of nematode pests but much more research and development is necessary before such approaches will be available to the grower. For the foreseeable future, the management of plant parasitic nematodes will continue to rely on the integration of existing control methods including the judicious use of nematicides where appropriate.

الملخص

كيري، بريان. 1995. استراتيجيات جديدة لإدارة النيماتودا النباتية المتطفلة مع تركيز خاص على المكافحة الأحيائية. مجلة وقاية النبات العربية. 13(1): 47-52

أتاح استخدام مبيدات النيماتودا، منذ مطلع الخمسينات، مكافحة فاعلة لمدى واسع من الآفات النيماتودية، على عديد من المحاصيل، وفي أرجاء مختلفة من العالم. على أن التكلفة العالية لهذه المبيدات، ومايرافق استخدامها من مخاطر بيئية وصحية أدت إلى الحدّ من استخدامها. وقد تبين، في كثير من الأحيان، أن البدائل غير الكيميائية للمكافحة غير عملية أو غير كافية. وعليه فإن تطوير طرائق ثابتة لمكافحة النيماتودا تعتمد على تكامل تقاني مكافحة معقدة، تتوقف عادة على التشخيص الدقيق للأنواع، وكثافة مجتمعاتها، وعلى معلومات مفصلة عن بيئياتها وحياتياتها. وهناك افتقار لهذه المعلومات حتى في أنواع النيماتودا الأكثر دراسة. ويبدو أن للتقاني الحديثة في التعريف، المستندة إلى اختبارات مناعية، إمكانية عظيمة في تعريف آفات معينة وتحديد أعدادها في التربة. فالتقاني الجزيئية لكل من النيماتودا المستقرة والمهاجرة متطورة، وتم تعريف بعض المورثات التي تمارس فعلاً مميتاً للنيماتودا. وتشير التجارب التي نفذت على النباتات المقاومة، أن استخدامها يتطلب إدارة فائقة لاجتناب تطوير عشائر نيماتودية شرسة تستطيع كسر هذه المقاومة. وقد تم تعريف كثير من الأتربة المعيقة لنشاط النيماتودا من مناطق مختلفة من العالم، وكانت الإعاقة التي تمارسها هذه الأتربة مرتبطة

بارتفاع مجتمعات الكائنات المضادة. وقد تبيّن أنه من الصعب التحكّم بهذا النوع من المكافحة الأحيائية، ولو أن الدراسات المنفذة على الأتربة المعيقة للنيماتودا قد سمحت بتعريف مدى من العوامل الميكروبية التي قد يكون لها إمكانية واعدة في المكافحة الأحيائية. وقد تم، في محطة روث أمستد، دراسة طفيلين هما فطر *Verticillium chlamydosporium* وبكتريا *Pasteuria penetrans* كعاملي مكافحة لنيماتودا تعقد الجذور في محاصيل الخضروات. وتم تحديد العوامل الرئيسة التي تؤثر في فاعلية هذه الكائنات واستخدامها في أوقات محددة من فصل النمو. ويبدو واضحاً أن هناك إمكانيات جديدة لإدارة الآفات النيماتودية، ولكن هناك حاجة أيضاً لمزيد من البحوث قبل أن تصبح هذه الطرائق متاحة للمزارع.

كلمات مفتاحية: نيماتودا، مكافحة أحيائية، أتربة معيقة، مكافحة متكاملة، مقاومة محورة وراثياً.

References

1. **Bird, D.McK.** 1992. Mechanisms of the *Meloidogyne* - host interaction. *In:* Nematology from molecule to ecosystem. Eds: F.J. Gommers and P.W.Th. Maas. Dekker and Huisman, Wildervank, 51-59.
2. **Bishop, A.H. and D.J. Ellar.** 1991. Attempts to culture Pasteuria penetrans in vitro. Biocontrol Sci. and Tech. 1:101-114.
3. **Burrows, P.R.** 1990. DNA hybridisation probes to identify pathotypes of *Globodera rostochiensis* and *Globodera pallida* second stage juveniles. Nematologica 36:336-337 (Abstract).
4. **Curran, J.** 1992. Molecular taxonomy of nematodes. *In:* Nematology from molecule to ecosystem. Eds: F.J. Gommers and P.W.Th. Maas. Dekker and Huisman, Wildervank, 83-91.
5. **Curran, J. and M.P. Robinson.** 1993. Molecular aids to nematode diagnosis. *In:* Plant parasitic nematodes in temperate agriculture. Eds: K. Evans, D.L. Trudgill and J.M. Webster. CABI, Wallingford, 545-564.
6. **Davies, K.G. and E.B. Lander.** 1991. Immunological differentiation of root-knot nematodes (*Meloidogyne* spp.) using monoclonal and polyclonal antibodies. Nematologica 38:353-366.
7. **Davies, K.G., de F.A.A.M. Leij and B.R. Kerry.** 1991. Microbial agents for the biological control of plant-parasitic nematodes in tropical agriculture. Tropical Pest Management 37:303-320.
8. **Esbenshade, P.R. and A.C. Triantaphyllou.** 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. J. Nemato. 22:10-15.
9. **Evans, K., R.H. Curtis, M.P. Robinson and M. Yeung.** 1995. The use of monoclonal antibodies for the identification and quantification of potato cyst nematodes. EPPO Bulletin (in press).
10. **Fleming, C.C. and R.J. Marks.** 1983. The identification of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* by isoelectric focusing of proteins on polyacrylamide gels. Ann. Appl. Biol. 103:277-287.
11. **Goddijn, J.M.O., K. Lindsey, F.M. Lee van der, J.C. Klap and P.C. Sijmons.** 1993. Differential gene expression in nematode-induced feeding structures of transgenic plants harbouring promoter -gusA fusion constructs. The Plant Journal 4:863-873.
12. **Gomes, C.R.M.D. and J.C. Cayrol.** 1991. Relationship between inoculum density of the nematophagous fungus *Paecilomyces lilacinus* and control of *Meloidogyne arenaria* on tomato. Revue de Nematologie 14:629-634.
13. **Hasky, K. and R.A. Sikora.** 1994. Induced resistance - a mechanism induced systemically throughout the root system by rhizosphere bacteria towards the potato cyst nematode *Globodera pallida*. Proceedings 22nd International Symposium of the European Society of Nematologists, 7-12 August 1994, Gent, Belgium, 67, Abstract.
14. **Jatala, P.** 1986. Biological control of plant-parasitic nematodes. Ann. Rev. Phytopath. 24:453-489.
15. **Kerry, B.R.** 1987. Biological control. *In:* Principles and practice of nematode control in crops, Eds: R.H. Brown and B.R. Kerry. Academic Press, Sydney, 233-263.
16. **Kerry, B.R.** 1990a. Selection of exploitable biological control agents for plant-parasitic nematodes. Aspects of Appl. Biol. 24:1-9.
17. **Kerry, B.R.** 1990b. An assessment of progress toward microbial control of plant-parasitic nematodes. Ann. Appl. Nemato. 22:621-631.
18. **Kerry, B.R., F. Irving and J.C. Hornsey.** 1986. Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium* Goddard. I. Factors affecting growth *in vitro*. Nematologica 32:461-473.
19. **Leij, de F.A.A.M. and B.R. Kerry.** 1991. The nematophagous fungus, *Verticillium chlamydosporium* Goddard, as a potential biological control agent for *Meloidogyne arenaria* (Neal) Chitwood. Revue de Nematologie 14:157-164.

20. **Leij, de F.A.A.M., B.R. Kerry and J.A. Dennehy.** 1993. *Verticillium chlamydosporium* as a biological control agent for *Meloidogyne incognita* and *M. hapla* in pot and micro-plot tests. Nematologica 39:115-126.
21. **Opperman, C.H., C.G. Taylor and M.A. Conkling.** 1994. Root-knot nematode-directed expression of a plant root-specific gene. Science 263:221-223.
22. **Oostendorp, M., D.W. Dickson and D.J. Mitchell.** 1991. Population development of *Pasteuria penetrans* on *Meloidogyne arenaria*. J. Nemato. 23:58-64.
23. **Oostendorp, M. and R.A. Sikora.** 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. Revue de Nematologie 12:77-83.
24. **Pineda, O., M.W. Bonierbale, R.L. Plaisted, B.B. Brodie and S.D. Tanksley.** 1993. Identification of RFLP markers linked to the H_1 gene conferring resistance to the potato cyst nematode (*Globodera rostochiensis*). Genome 36:152-156.
25. **Rodriguez-Kabana, R., G. Morgan-Jones and I. Chet.** 1987. Biological control of nematodes : soil amendments and microbial antagonists. Plant and Soil 100:237-247.
26. **Schots, A., F.J. Gommers, J. Bakker and E. Egberts.** 1990. Serological differentiation of plant-parasitic nematode species with polyclonal and monoclonal antibodies. J. Nemato. 22:16-23.
27. **Sikora, R.A.** 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. Ann. Rev. Phytopath. 30:245-270.
28. **Spiegel, Y., E. Cohn, S. Galper, E. Sharon and I. Chet.** 1991. Evaluation of a newly isolated bacterium, *Pseudomonas chitinolytica* sp. nov., for controlling the root-knot nematode *Meloidogyne javanica*. Biocontrol Sci. and Techn. 1:115-125.
29. **Stirling, G.R.** 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology 74:55-60.
30. **Stirling, G.R.** 1991. Biological Control of Plant Parasitic Nematodes. CABI, Wallingford, pp. 282.
31. **Stirling, G.R. and M.F. Wachtel.** 1980. Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica 26:308-312.
32. **Tzoetzakakis, E.A. and S.R. Gowen, S.R.** 1994. Evaluation of *Pasteuria penetrans* alone and in conjunction with oxamyl, plant resistance and solarisation for the control of *Meloidogyne* spp. on vegetables grown in greenhouses in Crete. Crop Protection 13:455-462.
33. **Williams, A.B., G.R. Stirling, A.C. Hayward and J. Perry.** 1989. Properties and attempted culture of *Pasteuria penetrans*, a bacterial parasite of root-knot nematode (*Meloidogyne javanica*). J. Appl. Bacteriol. 67:145-156.
34. **Williamson, V.M., J-Y, HO and H.M. MA.** 1992. Molecular transfer of nematode resistance genes. J. Nemato. 24:234-241.