Individual progress report

Title page

FAIR CT97 3444

"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."

from 1 September 2000 to 28 February 2001

Type of contract: Shared-cost research project

Total cost:	1,198,172 ECU	**EC contribution:** 889,000ECU (75%)
Participant no. 05 total cost	122,000 ECU	**EC contribution to partner no. 05:** 122,000 ECU (100%)

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Participant no. 05: Institut de Recerca i Tecnologia Agroalimentàries. Contractor, established in Spain.

INDIVIDUAL PROGRESS REPORT

Participant No. 05: Departament de Protecció Vegetal. IRTA. Spain.

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Objectives: To evaluate the efficacy of the biomanagement strategy in commercial production systems for vegetable crops in southern Europe.

Actions in the project:

Task 1.1. **Collection and selection of isolates of *V. chlamydosporium***
Subtask 1.1.1. **Collection and characterisation of isolates.**
1.1.2. **Selection of isolates.**

Task 1.2. **Evaluation of the biomanagement strategy.**
Subtask 1.2.2. **Evaluation of the strategy in plastic tunnel houses**.

Task 1.1. Collection and selection of isolates of *V. chlamydosporium.*

Task 1.2. Evaluation of the biomanagement strategy.

The biomanagement strategy consisted of the application of *V. chlamydosporium* at the time of planting a poor host plant for the nematode to allow fungal establishment and development in the soil before planting a nematode susceptible crop. This strategy combined to management tactics: the use of a nematode antagonist and crop rotation. The effectiveness of the fungus was compared to an untreated control, oxamyl alone or in combination with *V. chlamydosporium*, and fumigation with methyl bromide. The trials included five treatments:
- *Verticillium chlamydosporium* (F) (biomanagement strategy).
- Oxamyl: Two applications of a liquid formulation of Oxamyl three and 21 days after planting a susceptible host plant (tomato) for the nematode.
- Fungus + oxamyl: A combination of the two previous treatments (integrated strategy).
- Methyl bromide (conventional strategy)
- Untreated control: Plots infested with *Meloidogyne javanica*.

Subtask 1.2.2. Evaluation of the strategy in plastic tunnel houses.

The unheated plastic houses were located at Centre de Cabrils, Institut de Recerca i Tecnologia Agroalimentàries, Cabrils, Barcelona (site Q21), and at Experimental Farm Can Comas, Consell Comarcal del Baix Llobregat, El Prat, Barcelona, Spain (site CC). Soil was infested by *Meloidogyne javanica* (Treub) Chitwood in both sites. The main characteristics and history of the sites and the physical properties of the soil are described in Table 1. Each experiment involved two crops. Lettuce, *Lactuca sativa* L. type Maravilla cv Arena that was cultivated in autumn-

winter, and tomato, *Lycopersicon esculentum* L. cv Durinta, cultivated in spring-summer. Two experiments were conducted in each site at commercial scale for two consecutive growing seasons starting in November 1998 through July 2000. Experiments were conducted in a similar way unless otherwise stated. Nematode sampling, fungal inoculation, nematicide applications, and yield assessment for each experiment and site was done when indicated in Tables 2 and 3.

Procedure. Densities of *M. javanica* were increased at both sites by planting a root-knot nematode-susceptible tomato in spring 1998. Plants were harvested at the beginning of October and soil was disked and prepared for planting. Plots of 12.5 or 9 m^2 were marked at Q21 and CC, respectively, and they were sampled individually to determine the number of second-stage juveniles (J2) in soil before application of the treatments. The experimental design was randomized incomplete blocks due to the patchy distribution of *M. javanica* in both sites. Plots with similar population densities were grouped in blocks by nematode level and five levels were established at each site. Pre-treatment nematode densities ranged from 250 to 1240 J2 / 250 cm^3 soil ($x = 685 \pm 390$) (mean ± standard deviation) at site Q21, and from 42 to 1750 J2 / 250 cm^3 soil ($x= 510 \pm 410$) at site CC. There was no statistical difference in densities of *M. javanica* among treatments in either site.

Methyl bromide (98% methyl bromide + 2% chloropicrine) was applied at a rate of 75 g /m^2 before starting the trials in October 1998 to plots receiving the conventional strategy. A liquid formulation of oxamyl (Vydate L 24% a.i.) was applied as a soil drench using a watering can 3 and 21 days after planting tomatoes at a rate of 7.5 litres per ha (Table 2 and 3). Plants were immediately irrigated after application of the nematicide. Isolate 10 of *V. chlamydosporium,* was provided by partner 01, and partner 04 prepared the inoculum needed for the experiments. The inoculum consisted mainly of fungal chlamydospores that were obtained on a corn flour-coarse sand (3-4 mm particles) mixture (30/30) (v/v) in 250-cm^3 conical flasks after incubation at 25 ºC for 4 weeks following the procedure described in the technical annex. The chlamydospores were added to sterile sand used as a carrier to bulk up the inoculum. In Exp. 1, the fungus was inoculated at a rate of 2.4×10^6 chlamydospores per plant by adding 60 cm^3 of the sand - chlamydospores mixture into the planting hole at the time of transplanting lettuce. In Exp, 2, the fungus was inoculated at a rate of 1.3×10^6 chlamydospores per plant by removing the first 15 cm of soil from the planting row, mixed thoroughly with the inoculum in a concrete mixer, and returned to the planting row. A second application of *V. chlamydosporium* (F2X) was done at the time of planting tomato in the second experiment at a rate of 2×10^7 chlamydospores per plant. Only half of the tomato plants per plot received the fungus that was applied into the planting hole.

Assessment of population densities of *M. javanica*, and nematode damage. Composite soil samples were collected before and after lettuce and tomato in each experiment and site. Individual samples consisted of nine soil cores taken from the first 30 cm of soil with a soil auger (2.5-cm diameter). Soil cores were mixed thoroughly and nematodes were extracted from 500-cm^3-soil subsample using Baermann trays. Juveniles migrating to the water were collected one week later, concentrated in a 25 µm sieve, and counted. The number of J2 was expressed per 250 cm^3 of soil.

Nematode damage was evaluated at harvest using a gall index. Following sampling for final nematode densities, ten (Exp. 1) or eight (Exp. 2) randomly selected plants per plot of lettuce or tomato were dug and rated for galling on a scale of 0 to 10,

where 0 = complete and healthy root system and 10 = plants and roots were dead. Roots from each plot were then bulked, chopped, and used for egg extraction. Eggs were extracted from three 5-gram (lettuce) or 10-gram (tomato) root subsamples by blender maceration in a 0.5% NaOCL solution for 10 minutes. The number of eggs was expressed per gram of root.

Assessment of fungal densities and egg parasitism. Fungal colonization was assessed by determining the number of colony forming units (CFU) four weeks after fungal inoculation (soil), after lettuce (soil and root), before planting (soil), and after tomato (soil and root). One-gram soil or root subsample from individual plots treated with the fungus or fungus + oxamyl was use for dilution plating. Aliquots of 200 µl were plated on a semi-selective medium and fungal colonies were counted after incubation of the plates at 25ºC for 3 weeks.

Since nematode reproduction did not occur on lettuce, parasitism of nematode eggs was only assessed on tomato. Galled tomato roots were chopped, mixed, and 30-40 egg masses handpicked with the aid of a dissecting scope from a 10-gram root subsample. Eggs were dispersed from egg masses with ca. 0.2 ml of sterile water using a pestle in an eppendorff tube. Eggs were spread on a restrictive growth medium in three replicated Petri dishes. Parasitized eggs were readily identified after incubation at 25ºC for 48 hour because fungal hyphae were growing out from the eggs.

Yield assessment. The effect of *M. javanica* on lettuce yield was assessed by weighing ten (Exp. 1) or eight (Exp. 2) randomly selected lettuce heads (the same ones used for root gall index). To determine tomato yield, fruits produced per ten (Exp. 1) or eight plants (Exp. 2) from each plot were harvested as they matured. Tomatoes were harvested once a week for 6 weeks. Fruits were counted, weighed and expressed as kilogram per m^2.

Plant maintenance. Plants were irrigated by a drip irrigation system as needed, and they were fertilized following standard practices in the area for lettuce and tomato. The fertilizer was delivered through the irrigation system except for Exp. 1 in site CC that was broadcast. Soil preparation was done by hand hoeing the plots individually to prevent cross contamination from treatments. Tomato plants were vertically trained using canes, and plants used for yield assessment were marked 4 weeks after planting. The pollination of tomatoes was achieved by placing a colony of *Bombus* bees into each plastic house at the time the first set of fruits was in blossom. After the final tomato harvest, plants were cut at grown level and allowed to dry before they were removed from the plastic house. Soil temperatures in each site were recorded daily at 30-minute intervals with temperature probes places at 15-cm depth during the duration of the experiments. Maximum, minimum, and average soil temperature are provided in Fig. 1.

Statistical analysis. The methyl bromide treatment was excluded from nematode analyses because *M. javanica* was not detected in fumigated plots during the study. Data on number of nematodes in soil, and eggs per gram root were transformed to [log (x+1)], and subjected to analysis of variance using the GLM procedure of SAS version 8 (SAS Institute Inc., Cary, NC). When the overall *F* test was significant, means were separated by the Least Significant Difference (LSD) method ($P< 0.05$). Data on yield of lettuce and tomato were subjected to analysis of variance and they were analyzed for experiment and site. When the overall *F* test was significant, means were separated by LSD procedure ($P < 0.05$).

RESULTS

Densities of *M. javanica* and evaluation of nematode damage. The nematode was not detected in methyl bromide treated plots at the end of this 2-year study in sites Q21 and CC (Table 4 and 5). Initial and final soil population densities of *M. javanica* did not differ among treatments (methyl bromide excluded) before or after the cultivation of lettuce in any of the experiments conducted at either plastic house (Tables 4, and 5). Galled roots were present on lettuce roots in Exp. 2 but not in Exp. 1 in both sites. Eggs were not recovered from lettuce roots in any of the experiments or sites (Tables 4, and 5).

In site Q21, tomatoes treated with oxamyl alone had the lowest ($P<0.05$) gall rating followed by those treated with F + oxamyl. Eggs production in F + oxamyl or untreated plots was lower ($P<0.05$) than in plots treated with the fungus alone. In Exp. 2, initial densities in untreated plots were lower ($P<0.05$) than in treated plots (methyl bromide excluded) (Table 4), but final densities did not differ between treated or untreated plots. Tomatoes in F + oxamyl treated plots showed the lowest ($P<0.05$) gall rating followed by those treated with the fungus alone. None of the treatments reduced eggs production on tomato plants in Exp. 2 in site Q21 (Table 4).

In site CC, final soil densities in F + oxamyl treated plots were lower ($P<0.05$) than in oxamyl or untreated plots. Tomato plants in untreated plots showed the highest ($P<0.05$) gall rating followed by those treated with *V. chlamydosporium* alone whereas the lowest ($P<0.05$) rating was recorded from F + oxamyl treated plots (Table 5). Egg production in F + oxamyl treated plots was lower ($P<0.05$) than in the remaining infested plots (Table 5). In Exp. 2, *M. javanica* was not detected before planting tomato but was recovered at the end of the crop cycle (Table 5). Although final soil densities were lowest ($P<0.05$) in oxamyl treated plots, they did not differ from those in F + oxamyl or untreated plots. The lowest ($P<0.05$) gall rating was recorded from F + oxamyl followed by oxamyl treated plots. Eggs per gram of root in plots treated with oxamyl alone or in combination with *V. chlamydosporium* were lower ($P<0.05$) than plots treated with the fungus or left untreated (Table 5).

In general, soil temperatures followed a similar pattern of fluctuations in both plastic houses and growing seasons (Fig. 1), although they tended to be slightly higher in site Q21 than site CC. The number of heat units accumulated by *M. javanica* during the autumn-winter crop was insufficient for completion of one nematode generation on lettuce (343º degree-days from J2 to J2, base 13ºC) although more heat units were accumulated when lettuce was planted in October 1999 than in November 1998. The nematode accumulated similar number of heat units during the spring-summer crop irrespective of the site or year, and three generations of *M. javanica* were estimated to occur in tomato.

Densities of *V. chlamydosporium* and egg parasitism. Native strains of *V. chlamydosporium* were not detected in soil samples collected in either plastic house before starting the study in October 1998. Chamydospore germination from the inoculum prepared for both experiments was greater than 80 %, and thus the inoculum was considered viable. The numbers of colony forming units (cfu) per gram of soil four

weeks after application was about half the number of chlamydospores applied in Exp. 1, and much lower in Exp. 2, and their numbers decreased progressively until the end of the study (Table 6). The fungus was re-isolated from root samples nine and eight months after its application to the preceding crop in Exp. 1 and Exp. 2, respectively in site Q21. In contrast, *V. chlamydosporium* did not establish in site CC, and was not re-isolated from root samples at the end of the experiments (Table 6). The fungus parasitized 1% of the egg population in Exp. 1 in site Q21 (Table 7), whereas percent parasitism ranged from 3 to 5.3% in Exp. 2. In site CC, parasitized eggs were not found in Exp. 1, and 5.2% of the eggs were parasitized in plots treated with the fungus alone in Exp. 2. A second application of the fungus (F2X) to tomato in Exp. 2 did not affect final densities or egg production of *M. javanica* in site Q21 (Table 4). The number of cfu and egg parasitism did not increase after a second fungal application (Table 6 and 7). In site CC, two applications of *V. chlamydosporium* reduced egg production compared to one application (Table 5), and tended to increase percent egg parasitism (Table 7), but it has no effect on the number of cfu recovered from the soil or root. Egg parasitism in F + oxamyl treated plots was difficult to assess because only a few egg masses were present on tomato roots in these plots.

Crop yield. Lettuce head weight did not differ among treatments in either plastic house (data not shown). Methyl bromide or oxamyl had no effect on yield of tomato in any of the experiments conducted in site CC (Table 8). Yield in F + oxamyl treated plots was lower ($P<0.05$) than in plots treated with methyl bromide, oxamyl, or left untreated in Exp. 1, but there was no difference among treatments in Exp. 2. In site Q21, methyl bromide increased ($P<0.05$) accumulated tomato yield in both experiments (Table 8). Significant differences were shown from the first harvest in Exp. 1, and after the third one in Exp. 2. Oxamyl alone had no effect on tomato yield in any of the experiments (Table 8). Yield in plots treated with the fungus alone was lower ($P<0.05$) than in untreated plots in Exp. 1 and in Exp. 2. Plots treated with the fungus in combination with oxamyl showed lower ($P<0.05$) yield than untreated plots in Exp. 2 but not in Exp. 1.

DISCUSSION

Verticillium chlamydosporium survived in the soil throughout the growing season, and eggs parasitized by the fungus were recorded from infected tomato roots eight and nine months after fungal application in Exp. 2 and Exp. 1, respectively. However, percent parasitism was low after two fungal applications over four crops. Effective establishment of the fungus in the field will require most probably several fungal applications because numerous factors affect the saprophytic phase of its life cycle. The repeated application of the fungus tended to increase percent parasitism of *M. javanica* eggs, and fungal distribution in the soil in Exp. 2 respect to Exp. 1 (Tables 6 and 7). Still, the level of parasitism reached after two growing seasons was insufficient to affect nematode densities. The application rates of the fungus used in this study were lower than the recommended rate of 5000 chlamydospores per gram of soil because production of the large quantities of inoculum needed for the experiments at field scale turned to be difficult due to chlamydospores variability between individual flasks.

In both growing seasons, the population dynamics of *M. javanica* followed a similar pattern of fluctuation; densities decreased after the autumn-winter lettuce and

increased after the spring-summer tomato. This pattern was independent of treatment, experiment or site, and was regulated by changes in soil temperature, which greatly affected the activity of both the nematode and its fungal antagonist. The nematode was unable to complete its life cycle on lettuce cultivated in autumn-winter. The presence of galled roots on lettuce in Exp. 2, and their absence in Exp. 1 could be explained by differences in soil temperatures due to a change in planting date of the experiments (from November to October), and hence in the number of accumulated degree-days. In contrast, the nematode reached very high population densities, produced profuse root galling, and yield losses on tomato compared to nematode free plots in site Q21. Such high densities, however, are common in plastic houses of north and southern Spain after the spring crop as shown by the results of the survey conducted to detect native isolates of the fungus in nematode infested soils (Task 1.1). In site CC, the nematode increased after tomato, produced moderate root galling but did not caused yield losses. Soil temperatures at the time of fungal application were perhaps too low for adequate development of *V. chlamydosporium*. The fungus can grow between 15 and 30 ºC but temperatures below 10 ºC or above 30 ºC greatly reduce its rate of development as shown by the *in vitro* tests done to determine optimal temperature for fungal growth (Subtask 1.1.1). Apparently, the sandy soil of site Q21 was more conducive to *M. javanica* and the fungus than the loamy sand of site CC since both organisms were widely distributed and abundant in site Q21 than in site CC (Tables 6 and 7) despite similar levels of the nematode before starting the study.

Two applications of oxamyl three and 21 days after planting tomatoes consistently reduced root gall ratings in both experiments and plastic houses but had no effect on tomato yield. Final soil densities were not affected by oxamyl alone in either experiments or sites, but in site CC they were reduced in F + oxamyl treated plots in Exp.1. Eggs production was also reduced in site CC when *V. chlamydosporium* and oxamyl were used in combination in both experiments, and when used it alone in Exp. 2. In this study, oxamyl had a greater effect on *M. javanica* in the loamy soil of site CC than in the sandy loam soil of site Q21, and it was more effective in Exp. 1 than Exp. 2. The application of *V. chlamydosporium* to the autumn-winter crop and oxamyl to the subsequent spring-summer crop tended to have a greater impact on reduction of nematodes and crop damage than either the fungus or oxamyl alone. This trend, however, could not be explained by increased egg parasitism or rhizosphere colonization in F + oxamyl treated plots.

Methyl bromide was the only treatment that effectively controlled *M. javanica* for two consecutive growing seasons at the same site. The nematode remained under detectable levels for 21 months when the study was terminated. Yield increases in response to methyl bromide were obtained in tomato in site Q21, and this response persisted through the second growing season. Percent increase respect to nematode infested plots was 50 and 25% in Exp. 1, and Exp. 2, respectively. The decrease in tomato yield observed in plots with *V. chlamydosporium* cannot be directly attributed to the fungus because it did not establish in those plots. In site CC, none of the treatments affected yield of tomato in any of the experiments except for the F + oxamyl treatment that decreased tomato yield in Exp. 1. The overall increase in tomato yield observed in Exp. 2 was possibly due to improved fertilization as a result of the delivery of the fertilizer through the drip irrigation system instead of the broadcast application done in Exp. 1.

CONCLUSIONS

Chemical, cultural, and biological methods were evaluated alone, in combination or sequentially in a double cropping system of lettuce-tomato. Soil fumigation provided effective and lasting nematode control in both sites, and it also increased tomato yield in one site. Oxamyl consistently reduced nematode damage but had no effect on final populations or crop yield. Double cropping lettuce with tomato maintained low population densities, prevented nematode damage, and crop losses in site CC but it did not in site Q21. *V. chlamydosporium* alone or in combination with oxamyl did not affect nematode densities nor prevented crop damage. From these results, it can concluded that in warm winter climates it will be very difficult to achieve control of *Meloidogyne* in soils conducive to the nematode by means other than soil fumigation because population densities increase greatly in a single crop cycle in the spring-summer despite a sharp decrease after the autumn-winter crop.

Table 1. Main characteristics and history, and physical properties of soil from two plastic houses used for the evaluation of *Verticillium chlamydosporium* as a potential management agent for root-knot nematodes in Spain.

	Plastic house	
	Q21	CC
Surface (m^2)	800	530
Nematicide history	Soil fumigants	None
Previous crop	Carnation	Lettuce
Size of individual plot (m^2)	12.5	9.0
pH	8.1	8.1
Electric conductivity (dS/m)	0.40	0.58
Organic matter (%) (P/P)	0.9	2.4
Sand (%)	85.8	46.5
Silt (%)	8.1	41.0
Clay (%)	6.1	12.5
Soil texture (USDA)	sandy loam	loam

Table 2. Summary of the actions taken to evaluate the effectiveness of *Verticillium chlamydosporium* in two plastic tunnel houses infested with *Meloidogyne javanica* in Barcelona, Spain during the growing season of 1998-1999.

	Site	
Action	Q21	Can Comas
Initial nematode sampling	Spring 1988	Spring 1988
Summer crop	Tomato cv "Durinta"	Tomato cv "Kromex"
Nematode species	*M. javanica*	*M. javanica*
Transplant tomato seedlings	15 June 1998	17 July 1998
Nematode analyses	1 October 1998	1 October 1998
Tomato removed	2 October 1998	13 October 1998
Sampling individual plots	13 October 1998	14 October 1998
Methyl bromide application	30 October 1998	6 November 1998
P. initial lettuce cv "Maravilla"	16 November 1998	17 November 1998
Application of *V. chlamydosporium*	18 November 1998	17 November 1998
Transplant lettuce seedlings	18 November 1998	17 November 1998
No. chlamydospores/g soil (4 wks)	17 December 1998	18 December 1998
P. final after lettuce	16 February 1999	24 February 1999
Harvest of lettuce	22 February 1999	24 February 1999
No. chlamydospores/g soil and root	16 February 1999	24 February 1999
P. initial tomato cv "Durinta"	9 March 1999	12 March 1999
Transplant tomato seedlings	9 March 1999	12 March 1999
No. chlamydospores/g soil	10 March 1999	12 March 1999
1st application of Oxamyl	12 March 1999	15 March 1999
2sd application of Oxamyl	1 April 1999	7 April 1999
First harvest of tomatoes	8 June 1999	11 June 1999
Last harvest of tomatoes (after 6 wk)	12 July 1999	15 July 1999
P. final after tomato	12 July 1999	20 July 1999
Root gall index	12 July 1999	20 July 1999
No. chlamydospores/g soil and root	13 July 1999	20 July 1999
Egg parasitism	13 July 1999	22 July 1999
Tomato plants stalks removed	26 July 1999	4 August 1999

Table 3. Summary of the actions taken to evaluate the biomanagement strategy in field trials in two plastic tunnel houses located in Barcelona, Spain during the growing season of 1999-2000.

	Site	
Action	Q21	Can Comas
P. initial lettuce cv "Arena"	6 October 1999	7 October 1999
Fungicide treatment (Trotis)	6 October 1999	--
Transplant lettuce seedlings	18 October 1999	19 October 1999
Fungal application (1st time)	18 October 1999	19 October 1999
No. chlamydospores /g soil	15 November 1999	16 November 1999
Harvest of lettuce	1 February 2000	14 February 2000
P. final lettuce (after 17 weeks)	1 February 2000	14 February 2000
No. chlamydospores /g soil & root	1 February 2000	14 February 2000
P. initial tomato cv "Durinta"	9 March 2000	14 March 2000
Transplant tomato seedlings	9 March 2000	14 March 2000
Fungal application (2nd time)	9 March 2000	14 March 2000
No. chlamydospores /g soil	9 March 2000	14 March 2000
1st nematicide application	13 March 2000	16 March 2000
2sd nematicide application	3 April 2000	6 April 2000
First tomato harvest	15 June 2000	16 June 2000
Last tomato harvest	17 July 2000	24 July 2000
P. final tomato (after 18 weeks)	17 July 2000	24 July 2000
No. chlamydospores /g soil & root	17 July 2000	26 July 2000
Gall index	17 July 2000	24 July 2000
Egg parasitism	18 July 2000	24 July 2000
Tomato removed	7 August 2000	14 August 2000

Table 4. Initial and final population densities, gall index, and eggs per gram of root of *Meloidogyne javanica* on lettuce and tomato in a plastic house use to evaluate the effectiveness of the fungal egg parasite *Verticillium chlamydosporium* in site Q21 at Cabrils, Barcelona, Spain.

			Jueniles/ 250 cm^3 soil			Gall	
Trial	Crop	Treatments	P. initial	P. final	Pf/Pi	index[a]	Eggs/ g root[b]
Exp. 1	Lettuce	Untreated	1250 ± 650 a	330 ± 40 a	0.3± 0.1 a	0	0
		Fungus (F) [c]	980 ± 280 a	490 ± 290 a	0.5 ± 0.3 a	0	0
		F + Oxamyl [d]	1260 ± 620 a	930 ± 680 a	0.7 ± 0.4 a	0	0
		Oxamyl [e]	815 ± 390 a	360 ± 130 a	0.5 ± 0.2 a	0	0
		Methyl bromide [f]	0	0		0	0
	Tomato	Untreated	540 ± 280 a	25030 ± 12330 a	61 ± 59 a	7.3 ± 1.0 a	27580 ± 13570 b
		Fungus (F)	300 ± 150 a	25 030 ± 12150 a	98 ± 50 a	7.5 ± 0.7 a	40510 ± 17080 a
		F + Oxamyl	710 ± 270 a	20670 ± 6360 a	32 ± 12 b	6.9 ± 0.8 b	22860 ± 8600 b
		Oxamyl	420 ± 210 a	22290 ± 11040 a	65 ± 40 a	6.3 ± 0.9 c	29810 ± 13580 ab
		Methyl bromide	0	0		0	5 ± 12 c
Exp. 2	Lettuce	Untreated	2190±1030 a	240 ± 130 a	0.1 ± 0.1 a	1.42 ± 1.4 a	0
		Fungus (F)	2290 ± 985 a	280 ± 125 a	0.1 ± 0.1 a	0.38 ± 0.5 b	0
		F + Oxamyl	2960 ± 830 a	540 ± 120 a	0.2 ± 0.1 a	0.33 ± 0.6 b	0
		Oxamyl	2780 ± 750 a	310 ± 170 a	0.1 ± 0.1 a	1.38 ± 1.3 a	0
		Methyl bromide	13 ± 30 b	0		1.29 ± 1.6 a	0
	Tomato	Untreated	500 ± 280 b	10250 ± 4360 a	23 ± 11 a	7.0 ± 0.7 a	39010 ± 18040 a
		Fungus (F)	1200 ± 490 a	14240 ± 11380 a	13 ± 10 a	6.1 ± 0.5 b	53260 ± 22000 a
		F 2x[g]	1200 ± 490 a	13180 ± 12670 a	10 ± 6 a	5.8 ± 1.2 bc	47.790 ± 20300 a
		F + Oxamyl	1260 ± 460 a	11300 ± 9660 a	10 ± 9 a	5.6 ± 0.7 c	49020 ± 13470 a
		Oxamyl	870 ± 220 a	17180 ± 12960 a	22 ± 11 a	6.7 ± 0.6 a	42840 ± 11620 a
		Methyl bromide	0	0		0	0

Values are means ± standard deviation of five replicated plots per treatment.
[a] Based on a scale from 0 (none) to 10 (severe).
[b] Parasitized eggs excluded.
[c] Applied at planting of lettuce in November 1998 and 1999.
[d] *V. chlamydosporium* to lettuce and oxamyl to tomato.
[e] Split application 3 and 21 days after planting tomato.
[f] Applied at a rate of 75 g/ m^2 in November 1998.
[g] Second application of the fungus prior to tomato in 2000.

Table 5. Initial (Pi) and final population (Pf) densities, gall rating, and eggs per gram of root of *Meloidogyne javanica* on lettuce and tomato in a plastic house use to evaluate the effectiveness of the fungal egg parasite *Verticillium chlamydosporium* in site CC at El Prat, Barcelona, Spain.

Trial			Juveniles/ 250 cm^3 soil			Gall	
Exp. 1	Crop	Treatments	P. initial	P. final	Pf/Pi	rating [a]	Eggs/ g root[e]
	Lettuce	Untreated	277 ± 330a	189 ± 261 a	0.5± 0.6 a	0	0
		Fungus (F) [c]	187 ± 135 a	65 ± 47 a	0.8 ± 0.8 a	0	0
		F + Oxamyl [d]	87 ± 18 a	91 ± 63 a	1.1 ± 0.9 a	0	0
		Oxamyl [e]	185 ± 185 a	84 ± 101 a	0.8 ± 1.1 a	0	0
		Methyl bromide [f]	0	0	-	0	0
	Tomato	Untreated	278 ± 472 a	7900 ± 9130 a	54 ± 43 a	2.0 ± 1.3 a	5100 ± 4050 a
		Fungus (F)	33 ± 25 a	2998 ± 3340 ab	57 ± 59 a	1.5 ± 1.1 b	3710 ± 2960 a
		F + Oxamyl	70 ± 49 a	520 ± 670 b	7 ± 8 b	0.4 ± 0.6 d	835 ± 730 b
		Oxamyl	47 ± 57 a	4086 ± 3635 a	153 ± 189 a	0.9 ± 0.9 c	3120 ± 2850 a
		Methyl bromide	0	0	-	0	0
Exp. 2	Lettuce	Untreated	53 ± 35 a	11 ± 16 a	0.1 ± 0.2 a	0.6 ± 0.6 a	0
		Fungus (F)	96 ± 102 a	9 ± 15 a	0.6 ± 1.3 a	0.8 ± 0.6 a	0
		F + Oxamyl	28 ± 17 a	10 ± 15 a	0.4 ± 0.5 a	0.6 ± 0.5 a	0
		Oxamyl	85 ± 63 a	9 ± 16 a	0.1 ± 0.2a	0.7 ± 0.6 a	0
		Methyl bromide	0	0		0	0
	Tomato	Untreated	0	360 ± 290 ab	-	3.2 ± 1.7 a	1326 ± 836 a
		Fungus (F)	0	1480 ± 890 a	-	3.4 ± 1.3 a	867 ± 764 a
		F 2x[g]	0	560 ± 320 a	-	3.2 ±1.4 a	470 ± 850 b
		F + Oxamyl	0	290 ± 380 ab	-	0.8 ± 1.1 c	140 ± 107 b
		Oxamyl	0	220 ± 310 b	-	1.7 ± 1.7 b	530 ± 667 b
		Methyl bromide	0	0	-	0	0

Values are means ± standard deviation of five replicated plots per treatment.
[a] Based on a scale from 0 (none) to 10 (severe).
[b] Parasitized eggs excluded.
[c] Inoculated at planting of lettuce in November 1998 and 1999.
[d] *V. chlamydosporium* to lettuce and oxamyl to tomato.
[e] Split application 3 and 21 days after transplanting tomato.
[f] Applied at a rate of 75 g/ m^2 in November 1998.
[g] Second application of the fungus prior to tomato in 2000.

Table 6. Number of colony forming units (cfu) of *Verticillium chlamydosporium* (F) per gram of soil and root recovered from two plastic houses used to evaluate the effiectiveness of the fungus as a potential management agent of *Meloidogyne javanica*.

			Time after fungal inoculation			
			4 weeks	End	Before	End
Site	Experiment	Treatment		lettuce	tomato	tomato
			Number of cfu per gram of soil			
CC	1	Fungus	23000 (5)	5600 (5)	1000 (2)	0
		F + oxamyl	23000 (5)	5400 (5)	1050 (2)	550 (1)
	2	F	25 (1)	0	0	0
		F 2X	-	-	-	0
		F + oxamyl	0	0	0	250 (1)
Q21	1	F	24000 (5)	1650 (5)	1300 (2)	900 (4)
		F + oxamyl	18000 (5)	600 (5)	3550 (3)	600 (2)
	2	F	900 (4)	400 (5)	0	100 (2)
		F 2X	-	-	-	1000 (4)
		F + oxamyl	650 (3)	500 (4)	0	950 (5)
			Number of cfu per gram of root			
CC	1	F	-	2650 (5)	-	0
		F + oxamyl	-	800 (4)	-	0
	2	F	-	0	-	0
		F 2X	-	-	-	100 (2)
		F + oxamyl	-	0	-	0
Q21	1	F	-	0	-	200 (3)
		F + oxamyl	-	1100 (3)	-	200 (2)
	2	F	-	100 (2)	-	200 (3)
		F 2X	-	-	-	1000 (2)
		F + oxamyl	-	150 (1)	-	21000 (2)

In parenthesis number of plots with the fungus.

Table 7. Effect of *Verticillium chlamydosporium* (F) alone and in combination with oxamyl on percentage of parasitized eggs of *Meloidogyne javanica* on tomato.

		Percentage egg parasitism (%)	
Site	Treatment	Exp. 1	Exp. 2
Q21	Fungus (F)	1.2 (2)	3.2 (5)
	F 2X	-	4.7 (5)
	F + oxamyl	1.4 (3)	5.0 (5)
CC	Fungus (F)	0	5.2 (2)
	F 2X	-	9.3 (3)
	F + oxamyl	0	0

In parenthesis number of plots with the fungus.

Table 8. Effect of *Verticillium chlamydosporium* alone or in combination with oxamyl, and of methyl bromide on tomato yield in two plastic houses infested with *Meloidogyne javanica* in Barcelona Spain.

		Kilograms / m^2	
Site	Treatment	Experiment 1	Experiment 2
Q21	Untreated	9.8 b	9.7 b
	Fungus (F)	7.9 c	8.3 c
	F + oxamyl	9.1 b	7.8 c
	Oxamyl	10.0 b	9.8 b
	Methyl bromide	14.7 a	12.2 a
CC	Untreated	8.8 ab	12.1 a
	Fungus (F)	7.9 bc	12.1 a
	F + oxamyl	7.1 c	12.6 a
	Oxamyl	8.5 ab	13.1 a
	Methyl bromide	8.9 a	13.4 a

Mean separation within experiment and site by LSD test (P=0.05).

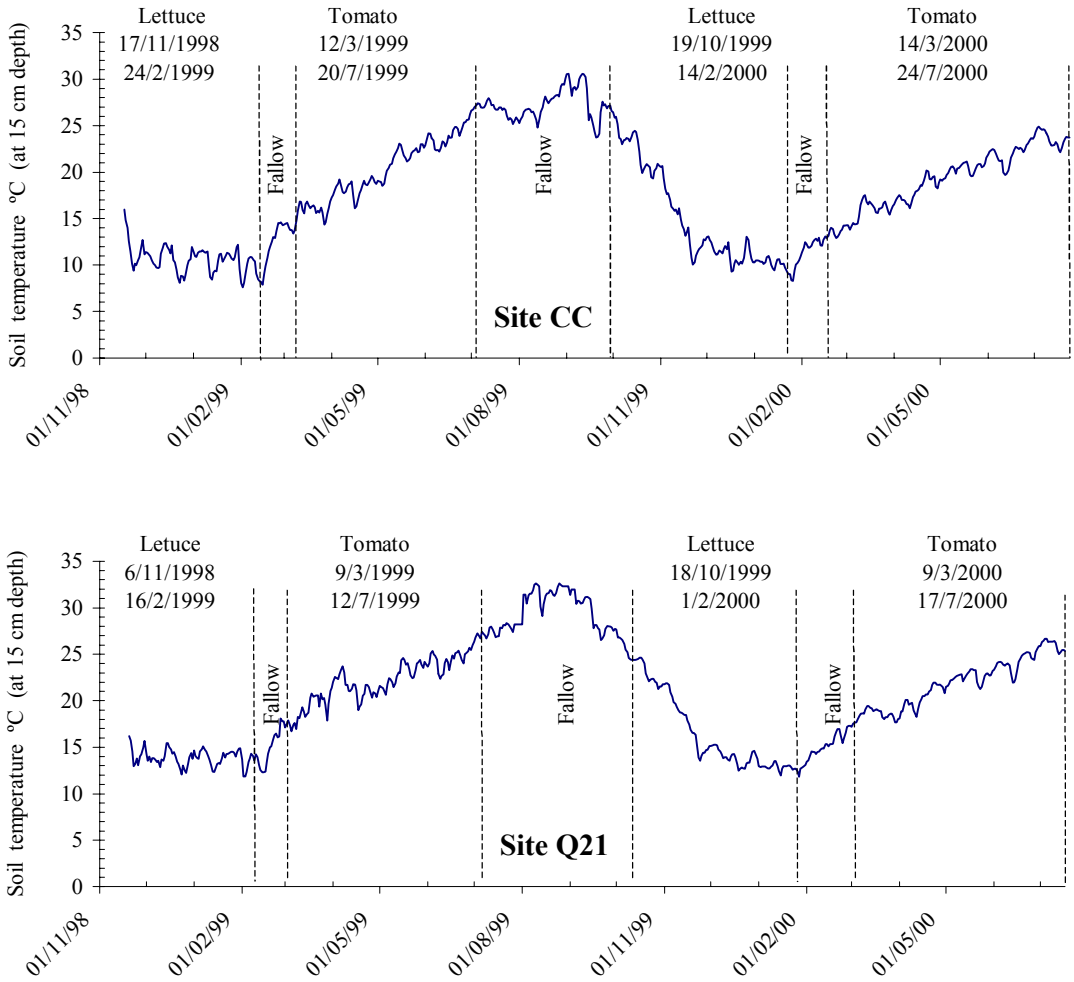

Fig. 1. Mean daily soil temperature at 15 cm depth from November 1998 to August 2000 in two plastic houses cultivated with lettuce in autumn-winter and tomato in spring-summer for two consecutive growing seasons at El Prat, (site CC), and Cabrils (site Q21) Barcelona, Spain.

DISSEMINATION OF RESULTS.

Research Agreement.

Partners: Institut de Recerca i Tecnologia Agroalimentàries and Consell Comarcal del Baix Llobregat.
Objective: Evaluation of alternative strategies to the use of methyl bromide for control of nematodes in vegetable crops in the Baix Llobregat County.
Duration: July 1998 to March 2001.

Post- doctoral research contract.

Dr. César Ornat Longarón
Duration: May 1998 – July 1999

University–Enterprise Collaboration Agreement for student training.

Student: Cristina Sànchez i Esperalba
University of Girona. School of Biology
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Student: Meritxell Juliá i Sans
University of Girona. School of Biology
Duration: July- August 2000

Student: Inma Ponsa Herrera
University of Barcelona. School of Pharmacy
Duration: July- August 2001

Final dissertation projects.

Title: Aïllament i caracterització de fong parásits d'ous per al control de nematode *Meloidogyne* spp.
Presented by: Inés Santoro Fort
Universitat de Vic. Escola d'Enginyeria Tècnica Agrícola.Vic
Date: November 1998

Title: Aïllament i caracterització de *Verticillium chlanmydosporium* per al control del nematode *Meloidogyne* spp, al litoral barceloní .
Presented by: Montserrat Sabater Ferret
Escola Universitaria d'Enginyeria Tècnica Agrícola. Barcelona
Date: January 1999.

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J. Sorribas has lectured in three courses on "Nematological problems on horticultural crops and methods of control"organized by the following Organizations:
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Institut Agrícola de Sant Isidre
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Publications

Santoro, I., Sorribas, F. J., Ornat, C., Sabater, M. Verdejo-Lucas, S. 1998. Detección de hongos parásitos de huevos de *Meloidogyne* en cultivos hortícolas. Nutri-fitos 98: 134-136.

Ornat, C., Verdejo-Lucas, S., Sorribas, F. J., Santoro, I. 1999. El nematodo *Meloidogyne* en los cultivos hortícolas de los invernaderos de Almería. Phytoma 106: 27-34.

Verdejo-Lucas, S., Ornat, C., Sorribas, F. J., Stchiegel, A. 2001. Fungal parasites of root-knot nematodes associated to vegetable crops in Spain. Journal of Nematology 33

Papers presented to Meetings.

Authors: Santoro, I., Sorribas, F. J., Ornat, C., Sabater, M.,Verdejo-Lucas, S.
Title: Detección de hongos parásitos de huevos de *Meloidogyne* en cultivos hortícolas.
Meeting: III Jornadas de Protección Vegetal de ICEA
Place and date: Barcelona, Spain, November 1998.

Authors: Ornat, C., Sorribas, F. J., Verdejo-Lucas, S., Lorenzo, M. L., Lopez-Llorca, L. V., Salinas, J.
Title: Colonización de la rizosfera de cultivos hortícolas por aislados de *Verticillium chlamydosporium*.
Meeting: X Congreso de la Sociedad Española de Fitopatología (SEF).
Place and date: Valencia, Spain, October 2000.

Authors: Ornat, C., Verdejo-Lucas, S., Sorribas, F. J.
Title: Fungal egg parasites of root-knot nematodes on vegetable crops in Spain.
Meeting: 25th International Symposium on Nematology. European Society of Nematologists (ESN).
Place and date: Herzliya, Israel. April 2000.

Authors: Verdejo-Lucas, S., Sorribas, F. J., Ornat, C.
Title: Effect of the crop on the establishement of *Verticillium chlamydosporium* in the field.
Meeting: Congress of the Organization of Nematologist of Tropical America (ONTA).
Place and date: Varadero, Cuba, June 2001.