TOMATO PRODUCTION IN SPAIN WITHOUT METHYL BROMIDE

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SUMMARY

Tomato, a basic product in Spanish horticulture, occupies 14% of the horticultural surface cultivated and contributes to 23% of the value of the sector's production. Spanish tomatoes satisfy the interior demand and have a strong export demand. About 25% of the fresh production and 50% of the canned fruit are exported. Crop management systems for tomato production in Spain require, as the principal alternatives to methyl bromide (MB), hybrid varieties with resistance to pathogens and soil fumigation with other conventional fumigants. New "long life" varieties that possess the *Mi* gene have overcome the problem of susceptibility to the nematode genus *Meloidogyne*. The "Mediterranean system" for producing tomatoes for fresh consumption is much more effective in controlling diseases and yields better quality fruit than the "Dutch system" which is based primarily on cultivation using substrates.

Keywords: fungi, nematodes, soilborne, fumigants, crop management, methyl bromide, alternatives

INTRODUCTION

In order to understand the reasons why tomato crops in Spain do not generally use methyl bromide (MB) for soil disinfestation, a preliminary analysis is necessary of cultural techniques and of the crop innovations that have been introduced in the last thirty years. This analysis should be done for crops produced for fresh consumption as well as for crops for processing (e.g., paste, sauce) and canning. Both subsectors are defined as different activities because of their production techniques as well as their geographic distribution.

Tomato production for canning is an extensive, seasonal crop confined to Mediterranean countries, whereas tomatoes destined for fresh consumption have an intensive production and extend throughout the European Community.

Both subsectors of tomato crops have followed different technological paths. Nevertheless, in both cases, technical change has sought an increase in output per surface unit and crop adaptation to less favorable climatic conditions. Developments have included out-of-season and greenhouse crop production. This change has been accompanied by new criteria in consumer conduct, which is a concern for a healthy diet. Among Europeans, disquiet exists over the degree of safety in foods, an uneasiness which carries implicit criticism of intensive production methods and their contaminating effects (Aldanondo Ochoa 1995). Regulations on the maximum residue limits for horticultural products reflects well this concern, a concern that reaches as far as to the deterioration of our surroundings, even though they may be as distant as the stratospheric ozone layer. The growing interest of the large distribution chains to establish commercial trademarks for more natural products (organic, ecological, etc.), is a phenomenon that is influencing the tomato-producing areas of Spain, starting with the advantage of scarce or nil use of MB in its crops (Bello & Tello 1997).

PRODUCTION TECHNIQUES IN THE PAST THIRTY YEARS

In the three years from 1990-1992, the EU produced a total of 13,118,000 tonnes of tomato of which 22% was produced by Spain (Aldanondo Ochoa, 1995). This analysis of the changes in production techniques will be centered on tomato for fresh consumption, since the cultivation of tomato for canning in Spain does not normally use MB.

There are two models for tomato production in the EU: Holland, Belgium, the United Kingdom, Denmark and Germany primarily use the "Dutch system" in which tomatoes are cultivated in greenhouses on substrates (principally rockwool) with a central hot water heating system, and computerized control of environmental constants and of the watering system. Generalization of soilless cultivation — partly mandated by the strict prohibition of MB — varietal improvements, innovations in the design of greenhouses and the perfection of agricultural practices, has permitted output to reach spectacular levels (400 t/ha, triple the production of Spanish greenhouses). This system is especially expensive in energy (e.g. heat, fertilizers) and in production per unit (from 20-30% above that of Spain's). Also, the system is particularly contaminating. It is calculated that substrate crops require two to three times more fertilizer than field crops. The degradation problem surrounding agricultural areas in Holland, where production is particularly intensive, has instigated government plans to minimize them.

Among other modifications to improve this system of production are savings in energy consumption, a decrease in the use of fertilizers and pesticides and, particularly, a closed circuit for recycling the water used for irrigation. In this way, these growers aim to halt one of the major sources of contamination: the filtration of pesticides and fertilizers into the waterbeds. The advantages of the system are as certain as its remarkable rise in production costs (Aldanondo Ochoa 1995).

The "Dutch system" has been considered the archetype of efficiency. Nevertheless, the growing preoccupation of consumers about the environment and the safety of foods has motivated them to reject the “Dutch tomato”, which they consider "artificial and insipid." In fact, between 1991 and 1992 the mean price fell about 28%.

Taking Spain as an example, since 30% of its production is in fresh tomatoes and 20% is exported, the "Mediterranean system" is different from the "Dutch system" in its more natural cultural practices, favored by more favourable climatic conditions. The Spanish tomato production system has a certain technological dependence on the "Dutch system" and a great heterogeneity in productive structures. The basic elements of the Spanish system are: field cultivation, or cultivation in soil under plastic or mesh-covered greenhouses without temperature or atmospheric control. Varieties exist for cultivation in sand-covered soils. Soilless cultivation is done on a small scale.

Mediterranean production in the last few years has undergone an important modernization programme. Outstanding changes include the adaptation of plastic greenhouses, the introduction of a localized irrigation system, the utilization of soluble fertilizers, varietal reconversion (Aldanondo Ochoa 1995; Diez Niclos 1995), automatization of the packaging line, and the incipient automatization of the irrigation system. A great part of these innovations were generated in the EU, especially in Holland.

One of the most important changes was created by hybrid varieties (Diez Niclos 1995). Their part in the non-utilization of MB well deserves a brief commentary. Holland maintained a monopoly on the production of the most-acceptable hybrids commercially, which were carriers of a resistance to the crop's gravest pathogens. This monopoly has gone on to be the patrimony of the "long-life" varieties, an Israeli patent which presents advantages over the Dutch varieties. “Llong-life" varieties contribute to a remarkable improvement in quality, firmness and homogeneity, although not so in flavour. It could be said that Israel has broken the seed market monopoly and has placed a new starting point in research. The qualities of these hybrids favour production areas at a distance from the market. It must be added that these "long-life" varieties at their commercial birth have been susceptible to pathogens, especially root-knot nematodes (*Meloidogyne* spp.) (Diez Niclos 1995; Tello & del Moral, 1995).

This brief analysis clearly intends what could be expressed in the following manner: the temptation to continue a technological strategy similar to that of the northern EU. Intensifying production systems to reduce unit costs may be inconvenient in the long run. The Mediterranean area enjoys relative prestige in the market place due to the naturalness of its crops (Tello 1984; Tello & Lacasa 1990; Tello & del Moral 1995).

TOMATO PRODUCTION IN SPAIN

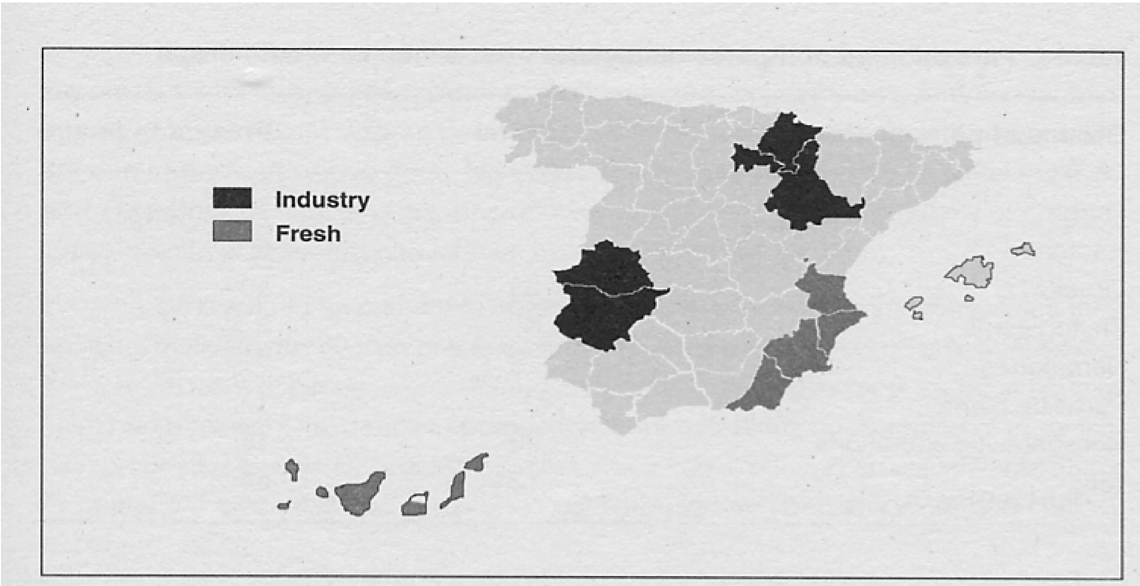
Tomato is a basic product in Spanish horticulture. It occupies 14% of the horticultural surface cultivated and contributes to 23% of the value of the sector's production. Spanish tomatoes satisfy the interior demand and have a strong export demand. About 25% of the fresh production and 50% of the canned fruit are exported. The details of production are found on Table 1, where data indicate an increase in unitary output, especially in fresh tomato, corresponding to a decrease in land cultivated (Aldanondo Ochoa 1995; Rodrfguez del Rincon 1995).

Table 1. Tomato production in Spain, 1983-1993 (Aldanondo Ochoa 1995)

TYPE	YEAR	HA (X 1000)	TONNES PER HA	TOTAL PRODUCTION
Tomato for fresh consumption	1983	43.1	39.2	1688.7
	1993	35.5	50.9	1805.9
Tomato for industrial transformation	1983	18.7	39.4	740.8
	1993	21.2	42.1	893.5

Spain's production areas are centered in the Southeast of the peninsula (Valencia, Alicante, Murcia and Almeria), the Ebro River Valley (Navarra, Rioja and Saragossa), Extremadura and the Canaries, which are areas in which 73% of the national production is concentrated (Figure 1).

Figure 1: Tomato production sectors in Spain



Nevertheless, crop systems are different. Extremadura and the Ebro River Valley are dedicated to production for canned tomatoes, sauce and paste. In this crop system, rotation every three years is habitual, a rotation that maintains tolerable disease levels, and where the use of MB has never been necessary. For this reason, these areas will not be considered in this paper (Aldanondo Ochoa 1995; Tello & del Moral,1995).

Southeastern Spain and the Canary Islands dedicate their fields to the production of fresh tomato. The crop system has been described in a previous paragraph on the "Mediterranean system." It will be commented on later from the point of view of pathosystems and soil disinfestation.

DISEASES IN SPANISH TOMATO CROPS

Diseases in tomato have undergone an important increase in the world. They have almost tripled in the last few years (Messiaen *et al.* 1991). A review of the papers published 20 years ago to prove that there were no more than thirty disease outbreaks. Today the figure is around 124 outbreaks as shown in Table 2, distributed among mycosis, bacteriosis, virosis, phytoplasmosis and non-parasitic diseases (Tello 1984; Tello & Lacasa 1990; Jorda Gutierrez 1995; Tello & del Moral 1995; Jorda & Llacer 1996; Lopez & Montesinos 1996).

Table 2: Parasitic and non-parasitic diseases described in tomato crops

GROUPS OF PATHOGENIC AGENTS	TOTAL	PRESENT IN SPAIN
Fungi	30	18
Bacteriae	7	6
Viruses	33	10

GROUPS OF PATHOGENIC AGENTS	TOTAL	PRESENT IN SPAIN
Phytoplasms	6	2
Nematodes	26	2
Parasitic plants	20	15
Total	**124**	**54**

Many of these new diseases have been described when the techniques for diagnosing them have improved, as is the case in virosis (Jorda 1995; Jorda & Llacer 1996). Others have appeared with the modification of cultural techniques (Tello & dell Moral 1995) such as the mycosis caused by *Spongospora subterrana* and *Calytella campanula* (Messiaen *et al.* 1991). Nevertheless, non-parasitic diseases reveal that new developments in crop management affect the appeareance of new pathologies. Non-parasitic diseases are confused with others caused by microbes which can result in harmful, if not counter-productive, phytosanitary provisions being put into action (Tello & del Moral 1995).

After years of observation, experience has demonstrated that MB is not effective in the soil for controlling bacteria, phytoplasms and virus, except when limiting the populations of the latter's vector agents, such as nematodes or fungi. Nor has it been recommended to control parasitic plants since they have no relevance to Spanish tomato crops (Messiaen *et al.* 1991). Therefore, the study of fungi and root-knot nematodes explains why the use of MB is insignificant in Spanish tomato crops.

FUNGI AND NEMATODES DISEASES: THEIR IMPORTANCE AND CONTROL

Table 3 outlines the inventory of fungi and nematodes described as tomato crop parasites. Of a total of 30 described, the presence of eighteen are registered in Spain whose presence does not indicate that they constitute a cause of appreciable losses (Tello & del Moral 1995).

Limiting ourselves to the pathogens that cause disease in the aerial part of the plant, the following observations must be made. *Erysiphe* spp and *Fulvia fulva* appeared superficially, without becoming more widespread with the passing of the years. On the other hand, *Alternaria dauciisp solanni*, *Botryotinia fuckeliana*, *Leveillula taurica* and *Phytophthora infestans* are responsible for important losses, regularly or sporadically, in spite of repeated and intense treatments with fungicides.

Soilborne fungi present in Spain deserve a longer commentary since they are what motivate disinfection practices with MB. As shown in Table 3, *Fusarium oxysporumisp lycopersici*, *Verticillium dahliae* and *Meloidogyne* spp are what have or have had a relevant incidence in production. Fortunately, the appearance of *Fusarium oxysporumisp radicis-lycopersici* over ten years ago produced an unjustifiable alarm "a posteriori" since this pathogen does not seem to have become more widespread (Tello & del Moral 1995).

At present, *Fusarium oxysporum fsp lycopersici* has two strains described in Spain (strain 0 and strain 1). Before hybrid cultivars with resistance to pathogens were introduced, it was one of the most important diseases, and compelled long cultural rotations (from 3-4 years) and soil disinfection. Resistant tomato hybrids permitted the escalation of fixed installations for production (irrigation, greenhouses, etc.), eliminating rotation. The combination of soil disinfection, generally based on metam sodium, and varieties with the resistant gene *I*, made the maintenance of an almost total control of mycosis possible for five years. The appearance of a new strain (strain 0) and its escalation compelled the introduction of varieties with gene *Is*, resistant to that pathogen. For 13 years since that time, the system has remained stable, in spite of continued crop intensification, with the consequent disappearance of traditional cultural tasks (Tello & Lacasa 1990; Tello & del Moral 1995).

Verticillium dahliae has shown a similar recent trend to that of *F.oxysporum fsp lycopersici* except for one important factor. The *Ve* gene introduced in the hybrid varieties, has remained stable for more than 20 years, without new fungal pathotypes being detected. It would be interesting to understand why new strains of *F.oxysporum fsp lycopersici* and *V. dahliae* have not appeared. The answer would permit a better understanding of the system and consequently, the extension of its use. In an empirical way, how some cultural techniques influence this, can be known by intuition.

Table 3: Pathogenic fungi and nematodes in tomato crops, their importance in Spain. Key (+) present; (-)not present; (1) acceptable control with phytosanitary products; (2) control with pesticides is difficult, making resistant varieties necessary.

SPECIES	PRESENT IN SPAIN	IMPORTANCE
Alternaria dauci fsp so/ani	+	1
A. tomato	-	-
A. alternata fsp lycopersici	-	-
Botryotinia fuckeliana	+	2
Calyptella campanula	-	-
Cercospora fuliginea	-	-
Colletotrichum atramentarium	+	-
C. gloeosporioides	-	-
C. acutatum	-	-
Didymella lycopersici	+	-
Erysiphe spp	+	-
Fulvia fulva	+	-
Fusarium oxysporum fsp lycopersici	+	2
F. oxysporum fsp radicis-lycopersici	+	-
F. solani	+	-
Leveillula taurica	+	2
Phoma destructiva	-	-
Phytophthora infestans	+	1
P. nicotianae var parasitica	+	-
Pyrenochaeta lycopersici	+	-
Pythium spp	+	-
Rhizoctonia solani	+	-
Sclerotium rolfsii	+	-
Sclerotinia sclerotiorum	+	-
Septoria lycopersici	-	-
Spongospora subterranea	-	-
Stemphyllium solani	-	-
St. botryosum fsp lycopersici	-	-
St. vesicarium	-	-
Verticillium dahliae	+	2
Meloidogyne spp	+	2

In root-knot nematodes of the genus *Meloidogyne*, the species most widespread in Spanish crops is *M. incognita* and *M. javanica*, where the situation has developed in a different way. The fragility of the hypersensibility Mi gene (chromosome 6) which loses its effectiveness at 32° C when in homocygosis and at 27° when in heterocygosis, provides insufficient protection during some seasons of the year (Messiaen *et al.* 1991; Tello & del Moral 1995). This circumstance makes soil disinfection treatments necessary. Nevertheless, recent experience acquired in Spain shows quite well that for such disinfections it is not necessary to revert to MB. Biofumigation and its combination with solarization, and even nematicides, have been shown to be sufficiently effective providing that the time and the technique for application are well known (Bello *et al.* 1998).

Nevertheless, recent concern about nematodes in tomato crops in some Spanish areas is not precisely over the lack of effectiveness of the /W/ gene, but rather over the fact that this gene is absent in present "long life" varieties. The most widespread varieties at the present time are shown in Table 4. A survey done during the summer of 1997 among 59 farmers from Almeria, dedicated for more than 10 years to tomato monoculture farming in greenhouses, and who use the above indicated cultivars, showed that 77% of their operations experienced difficulties with *Meloidogyne* spp.

Table 4: Resistance to soil pathogens in "long life" tomato for fresh consumption cultivated in Spain. Key: + = resistance incorporated; - = without resistance.

Cultivar Virus (ToMV)	*Meloidogyne* strain 0	*V.dahliae* strain 1	T. Mosaic	F.o. fsp	*lycopersici*
CLX-3759 F1	-	+	+		+
Daniela F1	-	+	+	+	+
Durinta F1	-	+	+	+	+
E-873 F1	-	+	+	+	+
E-875 F1	-	+	+	+	+
Gabriela	+	+	+	+	+

One interpretation of these data is the following: The escalation of the "long life" varieties of tomato lacking the *Mi* gene resistant to *Meloidogyne spp*, has caused problems in the soil that have remained at bearable levels with resistant varieties. When those cultivars incorporate the *Mi* gene, as in the case of the Gabriela variety, as indicated in Table 4, the situation will again be restored that was reached with hybrids without the "long life" gene.

CONCLUSIONS

Three conclusions made to explain the reason why MB is not used for tomato production in Spain:

1. There are a small number of pathogens that cause relevant losses in Spanish tomato crops. The reason must be looked for in the "more natural" form of tomato production when compared with other countries of the European Union. The superior quality of the Mediterranean tomato is a reputation well-earned by Spain in the European markets. This quality is a consequence of a "more natural" form of production.

2. The absence of MB in practically all of Spain's tomato crops could be explained by the stability of the genes resistant to *Fusarium oxysporum fsp lycopersici*, *Verticillium dahliae* and *Meloidogyne* spp. which has been verified in the last 20 years. The durability of the effectiveness of the resistant genes has been influenced by crop management, both because an important part of the surface area applies Almeria's type of "sand-covered soils", and because of soil disinfectants, essentially based on methyl-isothiocyanate.

3. "Long life" hybrid varieties are susceptible to *Meloidogyne* spp. due to their lack of the /W/ gene. Alternative control techniques based on biofumigation, alone or combined with solarization, and the use of nematicides developed in Spain, have demonstrated their utility when correctly applied. In any case, the introduction of the /W/ gene in "long life" hybrids has begun. Varieties with that property are offered on the seed market. This circumstance will predictably restore the situation to that generated by previous hybrid cultivars.

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ALTERNATIVES TO METHYL BROMIDE FOR TOMATO PRODUCTION IN THE MEDITERRANEAN AREA

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ABSTRACT

In the Mediterranean area, tomato soilborne pathogens used to be controlled by methyl bromide, particularly in protected cultivation. To decrease the use of this fumigant, non-chemical and chemical alternatives were developed and implemented in many Mediterranean countries. However, the success of the alternative depends on the degree of its integration in an integrated pest management programme.

Keywords: Methyl Bromide, tomato, alternatives, Mediterranean countries.

DEFINITION OF METHYL BROMIDE ALTERNATIVES

The MBTOC report (UNEP 1998) defined alternatives as: "Those non-chemical or chemical treatments and/or procedures that are technically feasible for controlling pests, thus avoiding or replacing the use of Methyl Bromide (MBr)". "Existing alternatives" are those in present or past use in some regions, "Potential alternatives" are those in the process of investigation or development".

ALTERNATIVES TO METHYL BROMIDE FOR TOMATO PRODUCTION

The existing and potential alternatives to methyl bromide (MB) in the Mediterranean countries to control tomato soilborne pathogens pest are reported in Table 1.These alternatives should be considered as components of an Integrated Pest Management (IPM) programme which includes other control methods such as sanitation, pathogen-free seeds and seedlings, weed control, improvement of plant growing conditions and other similar activities (UNEP 1998).

Non chemical alternatives

Resistant varieties

Many tomato cultivars are resistant to various soilborne pathogens. However, at the moment, no resistance is available for the control of some pathogens such as *Sclerotinia sclerotiorum*, *Didymella lycopersici* , *Verticillium dahliae* race 2, *Fusarium oxysporum* f.sp. *lycopersici* race 3, *Fusarium oxysporum radicis - lycopersici*, *Pyrenochaeta lycopersici*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. tomato and *Xanthomonas campestris* pv. *vesicatoria*. Most of the high yielding tomato hybrids used in many Mediterranean countries are susceptible to nematodes. Even for the available resistant cultivars, the rise of new races particularly of *Fusarium* and *Verticillium* is a threat to tomato production. Soil and water salinity increase the susceptibility of tomato plants to many diseases and particularly to *Fusarium* and *Verticillium* wilts. Resistant varieties become also susceptible when the irrigation water has a high salt content (Gabarra § Besri, 1999, Besri 1999, Besri 2000).

Grafting

Resistant rootstocks, provide excellent control of many tomato soilborne pathogens and particularly *Fusarium oxysporum* f. sp. *lycopersici*., *F. oxysporum* f. sp. *radicis-lycopersici*, *P. lycopersici* and *Meloidogyne* spp. This technique, which initially was considered too expensive, is now widely used at a commercial level in many Mediterranean countries. In general, without grafting, the tomato plant density per hectare is about 18,000 plants. When grafted plants are used, the same yield could be obtained with half plant population (9,000 plants/ha). In addition to controlling some soilborne pathogens, tomato grafting promotes growth, increases yield, increases plant tolerance to low temperature, extends the growth period and improves fruit quality (Besri 2000).

Organic amendments

Biofumigation with organic matter such as brassicae, compositae, swine and chicken manure, grape and olive pomace produces volatile chemicals (methyl isothiocyanate, phenethyl isothiocyanate) which have herbicidal, fungicidal, insecticidal and/or nematicidal properties (Gamliel 2000 b). Marigolds (*Tagetes spp.*) have a high suppressive effect on nematode populations. Differences, however, exist between the various species of *Tagetes* in their ability to effectively reduce nematode populations (Besri 2000, Reiss 1998).

Soil less culture

In the Mediterranean countries, many farmers have introduced soilless culture for vegetables and particularly for tomatoes as a replacement for MB fumigation. However, although soilless media are usually pathogen-free, infestation of these media by plant pathogenic micro-organisms such as *Phytophthora, Pythium, Rhizoctonia* and *Fusarium* may occur in greenhouses if proper sanitation procedures are not followed (Jenkins § Averre 1983).

Table 1: Technical Evaluation of the degree of development and the efficacy of Methyl Bromide alternatives for the control of tomato soilborne pathogens.

ALTERNATIVE	Degree of development[1]	EFFICACY			
		Spectrum of activity		Environmental dependence	
		Specific	Broad	Low	High
NON-CHEMICAL					
Resistant varieties	4	■			■
Resistant rootstocks	4	■			■
Organic amendments	4	■		■	
Biofumigation, compost, cover crops	3	■		■	
Soilless culture	4		■		■
PHYSICAL TREATMENTS					
Solarisation	3	■			■
Steam	4		■	■	
Flaming	3	■		■	
Biological control	2	■			■
CHEMICAL					
Metam Sodium	4		■		■
Dazomet	4		■		■
1,3-D	4	■			■
Chloropicrin	4	■		■	
TREATMENT COMBINATIONS					
Chemical x chemical	4		■		■
Chemical x non-chemical	4		■	■	
Non-chemical x non-chemical	4	■		■	

1: At experimental stage in laboratory, 2: At experimental stage in field, 3: At small scale, 4: At commercial level. Adapted from UNEP (1998)

Physical treatments

Solarization

Soil solarization controls many tomato pathogens (Stapleton 2000, Tjamos 2000). Though initially used only in hot regions during the summer, technological advances are extending its applicability to cooler areas such as the northern part of Italy (Stapleton 2000, Tamietti § Valentino 2000). Another application for which solarization has become common is for disinfestation of seedbeds, containerized planting media, cold-frames and tomato supports (Besri 1991, Stapleton 2000). However, despite its efficiency, soil solarization is used only on a relatively small scale as a substitute for MB.

Steam

Steaming is a well established and effective technique for soilborne pest control and is extensively used for bulk soil, soil treatments within green house. Negative pressure steam technology currently available improves energy efficiency by providing better dispersal of the steam throughout the soil and reducing treatment time. Steam is used in many Mediterranean countries in the greenhouse industry with vegetables and ornamental crops to replace MB (UNEP 1998, Runia 2000).

Flaming

In the Mediterranean area, *Orobanche ramosa* and *O. aegyptiaca* are very important parasitic higher plants on many vegetables and particularly on tomato. No variety of tomato resistant to these parasites is known. Preliminary experiments have shown that burning the *Orobanche* plants at the end of the growing season decreases the *Orobanche* seed population in the soil and consequently the parasite incidence and severity on the following crop (Besri 1999, Gabarra § Besri 1999).

Biological control

Biological control of tomato soilborne pathogens is gaining an increasing interest. *Penicillium oxalicum* reduces the incidence of *F. oxysporum* f.sp.*lycopersici* both in hydroponic and soil systems. *Trichoderma harzianum* and *T. Koningii* control *Fusarium* root and crown rot. The introduction into the soil of non-pathogenic strains of *F. oxysporum* (F.O.74) obtained from suppressive soil controls *Fusarium* wilts. However despite decades of research in the field of biological control, only a few micro-organisms are on the market and are successfully applied in practice (Vannacci and Gullino 2000).

Chemical alternatives

Chloropicrin (CP) is a very effective fungicide for the control of tomato soil-borne fungi, but not for weed and nematodes control. 1,3 Dichloropropene (1,3-D) is as efficacious as MB in controlling nematodes, but does not control fungi or insects. At high rates, 1,3-D has some efficacy against few weeds. Dazomet and metam-sodium applied to moist soil decompose to methyl isothiocyanate which is the biocidal agent. These chemicals do not provide consistent control of soilborne pathogens comparable to MB (Braun and Supkoff 1994).

Combination of alternatives

Non chemical x Chemicals

Crop rotation in combination with metam sodium applied through the drip irrigation system, is successfully used in protected cultivation to control some tomato soil-borne pathogens. The rotation adopted on a 3-year basis includes melon, pepper (hot and sweet), peas, cucumber, tomato and squash (Besri 2000). The combination of soil solarization with reduced dosage of Dazomet and of MB controls *Fusarium* and *Verticillium* wilts and *Fusarium* crown rot on tomato (Minuto *et al.* 2000, Gamliel *et al.* 2000a). Soil solarization alone or combined with low rates of 1,3-D, dazomet or MB control efficiently both nematodes and weeds and significantly increased marketable yield (Di Vito et al 2000) . The use of virtually impermeable plastic film (VIF) improved the efficacy of Dazomet (Minuto *et al.* 2000).

Non chemical x non chemicals

Biofumigation considerably shortens the time necessary to accomplish acceptable pest control through solarisation (Gamliel et al 2000b). Combination of reduced length of soil solarization and soil drenching of K. 165 (*Paenibacillus* sp.) effectively controls *F.oxysporum* f.sp. *radicis cucumerinum* in comparison with metam sodium treatment (Tjamos 2000).

Combination of chemicals

Metam-sodium, CP, 1,3-D, 1,3-D plus 17% CP (1,3-D+C-17) and 1,3-D plus 35% CP (1,3-D+C-35) control efficiently various tomato soil borne pests (Csinos *et al.* 2000).

CONCLUSION

In the Mediterranean countries, alternatives to MB for tomato production are available. These alternatives developed to control the key pests have been adopted in an IPM programme by farmers who are technically-aware and who are also on the watch for any new technology regardless of its cost. However, in the Mediterranean countries, many factors are limiting a wider application of MB alternatives. The most important are: an impression of complexity of the proposed methods, inadequate information, the low cost of MB, the activities and dynamism of the chemical companies, the weakness of the ecological demand, and the lack of specialists and extensionists in IPM.

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ALTERNATIVES TO METHYL BROMIDE FOR VEGETABLE PPRODUCTION IN GREECE

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ABSTRACT

Soil solarization with impermeable plastics singly or in combination with biocontrol agents or chemicals is efficient under Greek climatic conditions in reducing the duration of soil coverage from 4-6 down to 3 weeks and controlling equally effectively serious soilborne pathogens. This could be considered as an alternative to methyl bromide**.**

INTRODUCTION

Vegetable production in Greece has been largely dependent on the use of methyl bromide (MB) for several decades now. However, MB will not be available in the market anymore. Greek climatic and farming conditions favour theh use of soil solarization as a MB alternative for controlling soilborne pathogens in vegetable crops. Although the classical 4-6 weeks soil solarization is effective, its extensive commercial application has been prevented both in Greece and elsewhere.

Application of soil solarization during the last 25 years in Greece was effective in restricting development of disease symptoms and increasing yield with drastic reduction in the density of fungal propagules in vegetables {Antoniou *et al.* 1993 1995ab 1997; Tjamos 1983, 1991, 1992, 1998; Tjamos & Faridis 1980, 1982; Tjamos & Paplomatas 1988; Tjamos *et al.* 1987 1989, 1992). More recent data demonstrated that reduced duration of soil solarization singly or in combination with low doses of MB significantly restricted the populations of *Fusarium oxysporum* f.sp. *cucumerinum* of cucumbers even one week after mulching, while fungal populations were almost eliminated after 2-4 weeks solarization (Tjamos *et al.* 2000). A drastic reduction and even elimination of the number of sclerotia of *Pyrenochaeta lycopersici* was also demonstrated within one to two weeks after soil tarping (Tjamos *et al.* 2000b). It has been demonstrated that heat tolerant fungal or bacterial antagonists are involved in the long-term effect of soil solarization for 2-3 cropping seasons.

Soil solarization against *Verticillium dahliae* of tomatoes and globe artichokes and *Clavibacter michiganensis* subsp. *michiganensis* of tomatoes in Greece indicated that heat tolerant fungal (e.g. *Talaromyces flavus*) or bacterial antagonists (e.g. *Paenibacillus* and *Bacillus*) were involved in the longevity of the effect (Tjamos & Paplomatas 1989; Antoniou *et al.* 1993; Tjamos *et al.* 2000).

Our current work on solarization in Greece is mainly focused on the effectiveness of reduced duration treatments using impermeable plastics (polyamide plastic sheets covered with polyethylene), singly or in combination with biological or chemical agents.

COMBINATION OF REDUCED LENGTH SOIL SOLARIZATION WITH IMPERMEABLE PLASTICS AND BIOCONTROL AGENTS

Soil solarization trials were established in Peloponesse region to determine whether a short period of solarization using a triple layer plastic sheet of 40 μm thick. The plastic sheet consisted of two outer layers of common polyethylene and one of polyamide plastic sheet in the middle.

The effect of reducing the length of soil solarization combined with the use of selected K-165 antagonistic isolates against *Fusarium oxysporum* f. sp. *radicis-cucumerinum* of cucumbers, was tested in plastic houses, where cucumbers were grown in Peloponesse in South-West Greece. Experimental trials were established in very infested soils with intense symptoms during the previous cropping season. Short duration solarization (covering with impermeable plastics for 17 instead of 30-45 days and combined with a post planting root drench of K-165) was compared with disinfestation with metam-sodium at a dose of 1.2 l/m^2.

The combination of reduced length soil solarization and soil drenching of a *Paenibacillus* strain K-165 effectively controlled *F. oxysporum* f.sp. *radicis-cucumerinum*. The final percentatge of diseased plants was 0.7% compared with metam-sodium treatments of 11%. The total yield of cucumbers reached 31 tonnes/ha in solarized soils plus the antagonist, in comparison with the metam-sodium treated soils where the yield was 24 tonnes/ha (Tjamos *et al.* 2000).

COMBINATION OF REDUCED LENGTH OF SOIL SOLARIZATION WITH NEMATOCIDES

The main soilborne pathogens of tomato cultivations in Greece are *Pyrenochaeta lycopersici, Fusarium oxysporum* f.sp. *radicis lycopersici, Verticillium dahliae, Phytophthora* sp. and bacterial canker *Clavibacter michiganensis* subsp. *michiganensis*. Furthermore *Meloidogyne* sp. is becoming a limiting factor, particularly for summer plantations.

Experimental trials were carried out for the control of the main tomato soilborne pathogens for two consecutive cropping seasons. Trials started in July 2000-May 2001 for the first period and August 2001 to January 2002 for the second period.

A three-week soil solarization treatment was applied during July 2000 in tomato plastic houses by using two types of impermeable plastics singly or in combination with nematocides and/or fumigants. Five plastic house plots were covered with Orgafum® 33-33-33 containing 33% polyamide and another five with Orgafum ®40 25-50-25 with 50%. Experimental plastic house plot (40X5 m) were planted with the Jumbo tomato hybrid in January 2001, while 450 plants per plot were planted.

RESULTS

First cropping season July 2000-May 2001

Soil temperature determination in covered and uncovered soil showed that there was difference at various soil depths between the two types of plastics (50°C at 5-10 cm soil depth) while in the control uncovered sites temperature was 10-12°C lower. Plantations were frequently inspected for soilborne pathogen infections. Table 1 reports very restricted symptom development in all treatments and trials.

Table 1: Effectiveness of soil solarization singly or in combination with nematocides on the percentage of diseased tomato plants and the final total tomato fruit yield per plant in 8 experimental plastic houses

Soil treatments	**Soil borne pathogen**	**Percentage of diseased plants[5]**	**Yield[6] Kg /plant**
Orgafum ®40[1]			
SS[3] 3 Weeks + Condor 200 L/ ha	*Meloidogyne sp.*	0.22	*4.49*
SS 3 Weeks + MB[4] 250 Kg/ ha	*Clavibacter michiganensis* subsp. *michiganensis*	0.22	*4.23*
SS 3 Weeks	*Clavibacter michiganensis* subsp. *michiganensis*	0.22	*4.06*
SS 4 Weeks	*Pseudomonas* sp. Pith necrosis	0.22	*3.81*
SS 3 Weeks + Rugby 20 L/ ha	*Phytophthora* sp.	0.22	*3.82*
Orgafum ®35[2]			
SS 3 Weeks + Condor 200 L/ ha	*Clavibacter michiganensis* subsp. *michiganensis*	0.44	*4.14*
SS 3 Weeks + MB 250 Kg/ ha	*Pyrenochaeta lycopersici*	0.22	*4.01*
SS 3 Weeks	*Healthy plants*	0.0	*3.92*
SS 4 Weeks	*Pseudomonas* sp. Pith necrosis	0.22	*3.70*
SS 3 Weeks + Rugby 20 L/ ha	*Healthy plants*	0.0	*3.88*

[1] impermeable plastic sheet (polyethylene 25: polyamide50: polyethylene25); [2] impermeable plastic sheet (polyethylene33: polyamide33: polyethylene33); [3] Soil solarization [4] Methyl Bromide[5] Mean of 450 plants[6] Mean of 450 plants

Indeed all treatments were very effective against the main tomato soilborne pathogens. It is remarkable that at the end of the cropping season just 1 or 2 out of 450 plants developed symptoms of the above mentioned diseases, a figure quite negligible proving the effectiveness of the applied methods. No difference were observed in the effectiveness among the plastics. Early and late infection by *Pyrenochaeta lycopersici* in nearby control untreated plots almost reduced total tomato fruit yield by 40% and reduced mean yield to 2 -2.5 kg/ plant.

Second cropping season August 2001-January 2002

The same plots were also used for a second cropping season between August 2001-January 2002 without any soil treatment. The only difference with the previous season was the use of plants grafted on *Meloidogyne* tolerant rootstocks.

Table 2: Effectiveness of soil solarization one year after its application on the percentage of diseased tomato plants in 8 experimental plastic houses

Soil treatments	Soil borne pathogen	Percentage of Meloidogyne diseased plants[5]
Orgafum®40[1]		
SS[3] 3 Weeks + Condor 200 L/ ha	*Meloidogyne*	5
SS 3 Weeks + MB[4] 250 Kg/ ha	*Meloidogyne*	3
SS 3 Weeks	*Meloidogyne*	2.5
SS 4 Weeks	*Meloidogyne*	2.6
SS 3 Weeks + Rugby20 L/ ha	*Meloidogyne*	3.0
Orgafum®35[2]		
SS 3 Weeks + Condor 200 L/ ha	*Meloidogyne*	5.5
SS 3 Weeks + MB 250 Kg/ ha	*Meloidogyne*	2.8
SS 3 Weeks	*Meloidogyne*	2.7
SS 4 Weeks	*Meloidogyne*	3.2
SS 3 Weeks + Rugby20 L/ ha	*Meloidogyne*	3.1

[1] Impermeable plastic sheet (polyethylene 25: polyamide50: polyethylene25); [2] impermeable plastic sheet (polyethylene33: polyamide33: polyethylene33); [3] Soil solarization [4] Methyl Bromide[5] Mean of 450 plants

Data in Table 2 strongly suggest a long-term effect of solarization exceeding not only soilborne fungal or bacterial pathogens but also nematodes. Observations in nearby plantations of the same variety and same age were infected by rootknot nematodes up to 80%.

CONCLUSIONS

Soil solarization with impermeable plastics was efficient in almost nullifying the percentage of diseased plants during the first cropping season (winter-spring). Mean total tomato fruit production per plant and treatment did not differ more than 15%. No difference among 3 and 4 weeks solarization was observed, proving that 3 weeks solarization was more than enough when impermeable plastics were used. No significant differences among plastics was observed. No *Meloidogyne* infection was observed but we are dealing with twinder spring cultivation. During the second cropping season all treatments significantly inhibited root infection by *Meloidogyne* indicating a long-term effect of the method.

It seems that reduced duration of solarization using impermeable plastics could be one of valuable alternatives to MB fumigation in Greece. Research should continue to assess a combination of antagonistic organisms with soil solarization. It is possible that the duration of solarization could be further reduced if properly combined with nematocides and or other available fungicides.

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ALTERNATIVES TO METHYL BROMIDE IN SWEET PEPPER CROPS IN SPAIN

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ABSTRACT

In the Southeast of Spain over 9,000 ha of sweet pepper are grown in greenhouses. Of this land area, 1,880 ha are disinfected every year with MB (MB 98:2) to control *Phytophthora capsici* and *Meloidogyne incognita* and to mitigate against replant disease. Some alternatives to replace MB by 2005 were studied between 1997 and 2001. MB (98:2) at 30 g/m^2 applied under VIF (Virtually Impermeable Film) plastic provided the same results as MB at 60 g/m^2 applied with polyethylene (PE) plastic. However, at 16 g/m^2 with VIF plastic, pathogen control decreased and there was a yield reduction of more than 20% from the second year onwards. Dichloropropene (60.5%)+chloropicrin (33.3%) mixture applied by drip irrigation at 50 g/m^2 under PE plastic produced similar results to MB and may be a short term alternative to MB. Biofumigation with solarization, using fresh sheep manure and chicken manure or soybean flour, gave satisfactory pathogen control and production similar to MB. When the applications were repeated there was an improvement in efficacy against pathogens, yield and physico-chemical characteristics of the soil. This has proven to be a viable alternative for sustainable and organic agriculture. Grafting is another alternative which provides satisfactory soil-borne pathogen control and acceptable yield.

INTRODUCTION

In Spain, the cultivation of greenhouse sweet pepper is located in the Southeast. In 2000, 7,000 ha were grown in Almeria, 1,440 ha in Murcia and 440 ha in Alicante.

Soil growing pepper in Murcia and Alicante is disinfected every year with methyl bromide (MB 98:2), to control *Phytophtora capsici* and *Meloidogyne incognita* (Tello and Lacasa 1997; Bello *et al*. 1997; Lacasa *et al*. 1999). In these regions, replant disease was thought to be caused by production of pepper crops in the same soils for more than fifteen years (Lacasa and Guirao, 1997), and annual soil disinfection avoided production loss (Lacasa *et al*. 1999, 2000; Guirao *et al*. 2001). In the province of Almeria, MB is not used for soil disinfection because pepper is not only grown in the winter cycle but also because of the characteristics of the soil, the growing systems and frequent crop rotation.

Regulation EC2037/00 from the European Parliament and Council prohibits the use of MB for soil disinfection from December 31th 2004 which, without alternatives to MB, puts at risk the continuity of pepper cultivation in Murcia and Alicante that directly employs 3,569 growers and another 6,000 people indirectly (López and Guirao, 1998).

At the end of 1996, the Federación de Cooperativas Agrarias de Murcia (FECOAM) and the Consejería de Agricultura, Agua y Medio Ambiente de la Región de Murcia, joined in a programme to develop alternatives to MB in greenhouse sweet pepper crops. By mid-1997, the Instituto Nacional de Investigaciones Agrarias (INIA) approved the national interest research project INI SC97-130-C3 to investigate alternatives in crops using MB, including sweet pepper grown in greenhouses of the Southeast of Spain (Bolivar 1997).

From 1997 to 2001, various chemical products and non-chemical methods for soil disinfection were studied. The trials were carried out in commercial pepper greenhouses with different phytopathological problems and different levels of crop repetition. The alternatives were compared with MB (98:2) applied at 60 g/m^2 under 0.05 mm PE plastic, MB at 30 g/m^2 under 0.04 mm VIF or non-treated soil. The effects were evaluated by examination of *P. capsici* and *M. incognita* incidence, plant development, yield and weed proliferation. Each alternative was assayed in at least three greenhouses. Treatments were randomized in a complete block design with three replicates. The viable alternatives were trialled over three consecutive years in the same greenhouse.

CHEMICAL ALTERNATIVES

Methyl bromide rate reduction

Rates to achieve pathogen suppression using reduced rates of MB will be trialled until 2005. Formulations of MB 98:2 and MB 67:33 were used to make the equivalent 60 g/m^2, 30 g/m^2, 15 g/m^2 rates of MB 98:2. Fumigations were applied using VIF plastic for the 30 and 15 g/m^2 rates. Repeat trials were done in one greenhouse and the efficacy trials were carried out in greenhouses with *P. capsici* and *M. incognita* problems.

The same efficacy was observed at 30 g/m^2 under VIF plastic as at 60 g/m^2 using PE, both in greenhouses with phytopathological problems (Table 1) and when an application was repeated (Table 2). MB 98:2 at 15 g/m^2 did not provide satisfactory *P. capsici* control and the yield loss increased (higher than 18%) (Table 1) when the application was repeated (Table 2).

Table 1: Efficacy of application in MB reduced rates. Average of three assays carried out in 1997-1998.

	Percent plants with *P. capsici*	**Percent plants with *M. incognita***	**Average knot index[1]**	**Plant height (cm)**	**Marketable yield (kg/m^2)**	**Weed index (1-3)**
MB 60 PE	7.7 a	12.5 a	0.23 a	122.6 a	9.4 a	0.3 a
MB 30 VIF	12.6 a	6.3 a	0.10 a	121.7 a	8.9 a	0.2 a
MB 15 VIF	19.5 b	2.7 a	0.65 a	108.8 b	7.2 b	0.5 a
Control	53.4 c	62.5 b	3.70 b	81.5 c	3.3 c	2.6 b

Means in the same column followed by the same letter are not significantly different (P > 0.05). LSD Test. [1]According to Bridge and Page (1980).

Table 2: Effect of the reiterated use of MB reduced rates. Average of the three replicates in the same greenhouse.

	1997/98			1998/1999			1999/2000		
	[1]Average knot index	Plant height (cm)	Marketable yield (kg/m^2)	Average knot index	Plant height (cm)	Marketable yield (kg/m^2)	Average knot index	Plant height (cm)	Marketable yield (kg/m^2)
MB 60 PE	0.0 a	142.5 a	12.5 a	0.0 a	101.3 a	8.8 a	0.0 a	92.5 a	9.2 a
MB 30 VIF	0.0 a	144.2 a	12.0 a	0.0 a	103.3 a	8.9 a	0.0 a	93.8 a	9.5 a
MB 15 VIF	0.0 a	131.3 b	10.6 b	0.0 a	96.6 b	7.3 b	0.1 ab	88.8 b	8.0 b
Control	0.5 b	107.7 c	6.7 c	0.5 b	75.0 c	3.2 c	0.8 b	65.0 c	3.5 c

Means in the same column followed by the same letter are not significantly different (P > 0.05). LSD Test. [1]According to Bridge and Page (1980).

BROAD SPECTRUM ACTION FUMIGANTS

Alternatives assayed were: Chloropicrin at 50 g/m^2 applied through the drip irrigation system under PE plastic, four trials; Metam sodium (50 LS) at 150 g/m^2 applied in irrigation water under PE plastic, three trials; Dazomet (Basamid 98 GR) at 60 g/m^2 applied on soil at 60% of the field capacity, buried and covert with PE; 1,3-dichloropropene (1,3-D, 60.5%) with chloropicrin (33.3%) at 50 g/m^2 applied in irrigation water with PE plastic, 10 trials; and 1,3-D (60.5%) with chloropicrin (33.3%) at 50 g/m^2 applied on the soil over several consecutive years.

Chloropicrin and 1,3-D+chloropicrin had efficacies similar to MB 98:2 at 60 g/m^2 (Table 3). However, pathogens control was variable. When metam sodium or Dazomet were used there was a significant reduction in pathogen control and yield (Table 3). When the application of Telopic was repeated in the same soil, yield, plant development, weed control and *P. capsici* incidence were not significantly different from MB 98:2 at 60 g/m^2. The incidence of *M. incognita* with Telopic was significantly higher than MB 98:2 at 60 g/m^2, although variable through the years (Table 4).

Table 3: Application efficacy of different products as alternative to MB. Average of 1997/98, 1998/99, 1999/00 assays.

	Percent plants with *P. capsici*	Percent plants with *M. incognita*	Average knot index[1]	Plant height (cm)	Marketable yield (kg/m^2)	Weed index (1-3)
MB 60 PE	7.7 a	12.5 a	0.2 a	114.1 a	8.0 a	n.e
Cloropicrin	9.1 a	22.2 a	0.6 a	118.5 a	7.4 a	n.e
Control	53.4 b	62.5 b	3.7 b	81.5 b	3.3 b	n.e
MB 60 PE	6.9 a	8.3 a	0.1 a	154.9 a	7.6 a	0.5 a
Metam Na	30.1 b	63.0 b	2.6 b	130.3 b	6.6 b	1.5 b
MB 60 PE	8.9 a	15.3 a	0.7 a	86.0 a	6.8 a	n.e
Dazomet	18.5 b	54.2 b	2.3 b	76.0 b	5.0 b	n.e
Control	25.1 c	55.5 b	2.1 b	74.0 b	5.2 b	n.e
MB 60 PE	1.4 a	5.1 a	0.1 a	143.5 a	9.3 a	0.1 a
Telopic	6.8 a	21.1 b	0.5 a	137.3 a	9.4 a	0.1 a
Control	36.5 b	55.2 c	2.5 b	88.8 b	4.4 b	0.8 b

Means in the same column followed by the same letter are not significantly different ($P > 0.05$). LSD Test. [1]According to Bridge and Page (1980); n.e = non tested.

NON-CHEMICAL ALTERNATIVES

Biofumigation with solarization

The following were trialled:

- Timing of the organic amendments and solarization, beginning in August, September, October or November. Fresh sheep manure (EFO) (7kg/m^2) plus chicken manure (3 kg/m^2) and 3-6 weeks of solarization. Three assays.
- Type of amendment. EFO (7 kg/m^2) plus chicken manure (3 kg/m^2); EFO (7 kg/m^2) plus soybean flour (0,5 kg/m^2); EFO (7 kg/m^2) plus urea (0.25 kg/m^2) and 4-6 weeks of solarization. Two assays.
- Biofumigation with solarization (B+S) reiterated in the same soil. 1st year: 7kg/m^2 of EFO plus 2.5 kg/m^2 of chicken manure; 2nd year: 5 kg/m^2 of EFO plus 2.5 kg/m^2 of chicken manure; 3rd year: 4 kg/m^2 of EFO plus 2 kg/m^2 of chicken manure.

Table 4: Effect of the reiterated application of Telopic in the same soil through three consecutive years.

YEAR	Treatment	Percent plants with *P. capsici*	Average knot index[1]	Plant height (cm)	Marketable yield (kg/m^2)	Weed index (1-3)
1st year 1998/99	MB 60	0.4 a	0.1 a	130.1 a	8.5 a	0.05 a
	Telopic	2.4 a	0.2 a	124.5 a	8.9 a	0.08 a
	Control	25.3 b	3.1 b	89.0 b	4.3 b	1.0 b
2nd year 1999/00	MB 60	0.6 a	0.1 a	113.1 a	7.8 a	0.2 a
	Telopic	2.5 a	0.7 a	115.3 a	8.4 a	0.1 a
	Control	34.7 b	2.5 c	73.5 b	3.2 b	1.1 b
3rd year 2000/01	MB 60	1.5 a	0.6 a	143.5 a	9.1 a	0.05 a
	Telopic	3.8 a	2.8 b	149.7 a	9.2 a	0.05 a
	Control	27.4 b	6.7 c	80.1 b	3.0 b	4.6 b

Means in the same column followed by the same letter are not significantly different ($P > 0.05$). LSD Test. [1]According to Bridge and Page (1980)

Table 5: Effect of timing in biofumigation plus solarization.

	Percent plants with *P. capsici*	**Percent plants with *M. incognita***	**Average knot index[1]**	**Plant height (cm)**	**Marketable yield (kg/m^2)**	**Weed index (1-3)**
MB 60	0.2 a	8.3 a	0.1 a	132 a	9.9 a	0.3 a
Biof.+ sol. Aug	5.6 b	66.6 b	3.3 b	125 a	9.4 a	2.1 b
Control	72.1 c	97.0 c	5.5 c	91 b	4.2 b	2.8 c
MB 60	(1) -	0.0 a	0.0 a	181 a	12.2 a	0.1 a
Biof.+ sol Sept.	-	8.0 b	0.2 a	179 a	12.0 a	1.1 b
Control	-	24.0 c	2.1 b	157 b	11.1 b	1.6 b
MB 60	(1) -	0.0 a	0.0 a	180 a	12.3 a	0.2 a
Biof.+ sol Oct.	-	9.0 b	0.3 a	176 a	10.6 b	2.1 b
Control	-	26.0 c	2.2 b	156 b	11.0 b	1.7 b
MB 60	0.5 a	8.6 a	0.1 a	171 a	8.4 a	0.6 a
Biof.+ sol. Nov	13.2 b	44.4 b	1.2 b	129 b	6.9 b	1.9 b
Control	28.6 c	96.2 c	4.0 c	136 b	6.3 b	2.8 c

(1) No *P. capsici* in the greenhouses. Means in the same column followed by the same letter are not significantly different (P > 0.05). LSD Test.
[1]According to Bridge and Page (1980)

B+S initiated before mid September had results similar to MB (Table 5), both in marketable fruit and plant development. Pathogen and weed control were significantly lower with B+S than with MB. When B+S was initiated after mid-September, the results were poorer as the applications were delayed (Table 5). The best results were obtained using soybean flour as an organic amendment to keep optimal C/N levels during the B+S process (Table 6). The reiteration of B+S resulted in improved pathogen and weed control, as well as in the physico-chemical soil properties, with yield similar to that of MB (Table 7).

Table 6: Effect of biofumigation + solarization (B+S) using different nitrogen amendments.

	Percent plants with *P. capsici*	**Percent plants with *M. incognita***	**Average knot index[1]**	**Plant height (cm)**	**Marketable yield (kg/m^2)**	**Weed index (1-3)**
MB 30	0.0 a	26.1 a	0.7 a	108.0 a	9.7 a	0.4 a
B+S(EFO+chicken manure)	2.6 b	25.3 a	0.6 a	92.5 b	8.6 b	0.6 a
B+S(EFO + soybean flour)	0.2 a	26.0 a	0.8 a	89.0 b	9.6 a	0.5 a
B+S (EFO + urea)	1.5 b	21.7 a	0.5 a	87.5 b	8.1 b	0.5 a

Means in the same column followed by the same letter are not significantly different (P > 0.05). LSD Test. [1]According to Bridge and Page (1980)

Table 7: Effect of the reiterated application of biofumigación + solarization (B + S) (greenhouses without *P. capsici*).

	Percent plants with *M. incognita*	**Average knot index[1]**	**Plant height (cm)**	**Marketable yield (kg/m^2)**	**Weed index (1-3)**
MB 30	36.7 a	1.5 a	164.3 a	11.2 a	0.1 a
B + S 1st year	90.0 b	4.5 b	151.0 b	10.0 b	1.0 b
B + S 2nd year	43.3 a	1.8 a	150.2 b	11.3 a	0.4 a
B + S 3rd year	40.0 a	1.3 a	153.1 b	11.2 a	0.2 a
Control	100.0 b	6.7 c	143.0 c	9.3 b	3.0 c

Means in the same column followed by the same letter are not significantly different (P > 0.05). LSD Test. [1]According to Bridge and Page (1980)

Grafting

Capsicum annum rootstocks with different level of resistance to *M. incognita* and *P. capsici* were used. The responses of 78 rootstocks, mostly *C. annum* x *Capsicum* ssp. hybrid, were studied. Some of the rootstocks gave satisfactory pathogen control and production similar to MB (Table 8). The repetition of the same rootstock in the same soil over 3 years selected more aggressive populations of *M. incognita*. Therefore, it will be necessary to develop rootstock management strategies that counter resistance by, for example, using grafted seedlings in B+S soil, or by treating the soil with broad spectrum chemicals with specific or partial action.

Table 8: Response of different rootstocks used for grafting. Three assays.

	Percent plants with *P. capsici*	**Percent plants with *M. incognita***	**Average knot index[1]**	**Plant height (cm)**	**Marketable yield (kg/m^2)**
BM 60	0.8 a	11.1 a	0.1 a	159 a	8.2 a
Hybrid 1	2.3 c	89.2 c	2.9 b	133 c	6.3 c
2	0.5 a	70.7 b	2.2 b	147 b	7.1 b
3	1.4 b	61.2 b	2.0 b	150 b	7.0 b
BM 60	0.8 a	0.0 a	0.0 a	136.2a	6.9 a
Hybrid 2	9.4 b	60.0 d	2.2 b	120.7 b	5.9 b
25	0.0 a	10.0 b	0.3 a	127.8 b	6.2 b
26	0.7 a	40.0 c	1.6 b	109.2 c	6.0 b
27	0.0 a	36.8 c	1.0 b	114.0 bc	5.3 c
28	0.5 a	57.9 d	1.3 b	120.0 b	5.8 b
29	1.8 a	12.5 b	0.4 a	107.4 c	6.7 a
30	0.0 a	0.0 a	0.1 a	139.0 a	6.8 a
BM 60	1.3 a	20.0 a	0.6 a	143.2 a	8.9 a
Hybrid 23	0.5 a	83.3 bc	3.3 c	118.3 c	6.3 de
25	2.1 b	100.0 c	5.9 d	122.4 bc	6.7 cd
28	0.0 a	100.0 c	5.3 d	123.8 bc	6.1 de
29	0.0 a	83.3 bc	2.3 b	125.0 bc	7.5 bc
30	3.3 bc	73.3 b	4.1 c	139.8 a	8.2 ab
43	4.3 c	64.3 b	3.6 c	141.1 a	5.5 e
46	0.0 a	100.0 c	5.6 d	144.5 a	6.7 cd
56	0.0 a	60.0 b	0.6 a	128.4 b	6.0 de
57	4.0 c	40.0 a	0.4 a	124.2 bc	8.0 b
58	0.0 a	100.0 c	1.8 ab	125.1 bc	8.3 ab

Means in the same column followed by the same letter are not significantly different (P > 0.05). LSD Test. [1]According to Bridge and Page (1980)

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THE CURRENT STATUS OF ALTERNATIVES TO METHYL BROMIDE IN VEGETABLE CROPS IN FRANCE

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ABSTRACT

Vegetable production in France was valued at 2,234 million euros in 2000 and contributes significantly to the agricultural economy. Integrated Pest Managment (IPM) provides growers with a a practical solution for integrating new methods such as soilless systems or grafting to eliminate harmful fumigants without totally omitting chemical options. Methyl bromide (MB) has the highest cost per hectare of all registered agricultural compounds which has acted as a disincentives to its further use in France. The main alternatives are dazomet, dichloropropene, metam sodium, sodium tetrathiocarbonate, solarisation and steam depending on the crop and its locality. The amount of land treated with MB has decreased significantly in the past 3-4 years, particularly in strawberry production where soilless subtrates are now used. To allow better comparison between treatments, a protocol has been suggested for adoption by researchers.

Keywords: dazomet, dichloropropene, metam sodium, sodium tetrathiocarbonate, solarisation, steam, IPM, strawberry, melon, tomato, cucumber

INTRODUCTION

Vegetable crops remain an important economic part of French agriculture. Vegetable production in France was valued at 2,234 million euros (Anon 2000). All of these crops can be described as intensive, their production requiring a high level of grower technical knowledge. Controlling the pressures of soilborne pathogens is still one of the main agronomic aims and soil disinfestation is an available curative measure.

Recently, consumer demand for residue-free products of high quality, the future European registration of pesticides, and considerations of the safety of pesticides to growers are all modifying cultural practices in France. Today, integrated pest managment (IPM) provides growers with a a practical solution for integrating new methods such as soilless systems or grafting to eliminate harmful fumigants without totally omitting chemical options.

METHYL BROMIDE USES

Since 1997, the consumption of methyl bromide (MB) for soil disinfestation has decreased significantly (Table 1) due to three main reasons: International decisions taken by governments under the Montreal Protocol, inorganic residue problems for several vegetable crops which, for example resulted in the banning of the use of MB on lettuce, and because MB has the highest cost (around € 9,000 per ha) compared to all registered compounds. Despite these reasons, MB is still required in the production of tomatoes, strawberries, cucumbers, cut flowers and forest nurseries. MB is the only compound that eradicates specific pathogens like *phomopsis scleroïdes* on cucumbers.

ALTERNATIVES TO METHYL BROMIDE

It is difficult to give a precise view due to the diversity of crops and production areas in France. However, we can see two main tendencies in vegetable crops with two specific markets: the development of chemical fumigants in open fields mainly on carrots or potatoes; and the development of non-chemical alternatives in greenhouses like steam, grafting or soiless systems.

Registered Fumigants - chemical alternatives

Table 1 shows the relative change in use of five chemical compounds, the only ones registered in France as alternatives to MB (Fritsch 2001). The global use of chemical fumigants is increasing, especially 1,3-dichloropropene (1,3-D) and metam sodium. Because of its broad spectrum and easy application (liquid formulation, possibility to inject it into a drip-irrigation system), metam sodium is the potential alternative to MB in several cases, especially on strawberries. It can be used to obtain a herbicide effect, for example on leek seedlings. Dazomet has a very broad spectrum, but is too expensive compared to other chemical fumigants.

Table 1: Trends in use for soil disinfestation techniques in France in 2000.

Soil disinfestation techniques	Treated hectares	Tendency
Dazomet	1,000	stable
Dichloropropene	7,000	up
Metam sodium	4,000	up
Methyl bromide	1,203	down
Sodium tetrathiocarbonate	500	up
Solarization	200	stable
Steam	2,000	stable
Total surfaces	15,903	up

Sodium tetrathiocarbonate (commercial name: Enzone) which can be applied after planting is very effective against *Meloigogynes spp.,* especially on melons. Sometimes, resistant grafted plants can become infested by rootknot nematodes, mainly if there is a large population, and a good harvest can be maintained by using this compound.

Nevertheless, more and more growers are using new means of applying chemical fumigants: low dosages for metam sodium, irrigation pipes for 1,3-D, virtually impermeable sheets for disulfide generators (Sodium tetrathiocarbonate).

Low dosages of fumigants combined with solarization is another method used in the Roussillon region, for example, on lettuces to reduce the risk of residues.

Non-chemical alternatives

Soilless culture is increasing significantly as the replacement for MB use on tomatoes and strawberries, its main use. For both crops, we can also see an improvement in the hanging rain gutter system with organic or inorganic substrates.

The total production area of tomotoes in 2001 was 3,428 ha consisting of 1,293 ha in open fields (soil disinfestation not required) and 2,135ha in greenhouses (Anon 2001). About 950 ha are now planted with soilless systems which is particularly prevalent in the Northeast, Britanny. MB disinfestation still occurs in non-heated greenhouses in the south of France, mainly to eliminate *Pyrenochaeta lycopersici*.

Ten years ago, there were around 500 ha/year of land for strawberry production (excluding nurseries) that was disinfected with MB out of a strawberry production area 1,580 ha. Over a three or four year period, strawberry growers adopted soilless cultivation which reduced the amount fumigated with MB to only 150 ha in 2000. A mixture made of peat and organic substrates (pine bark) or inorganic substrates are used on these 150 ha.

Grafted plants ave also proven useful. The Agronomic Research National Institute's variety "Brigeor" uses hybrid vigour to give resistance to tomatoes to *Pyrenochaeta lycopersici*, *verticillium*, *fusarium oxysporum f.sp. lycopersici* and *fusarium oxysporum f.sp. radicis* and to nematodes. The "Beaufort" variety predominates because it gives resistance and "Maxi fort" seems to have better resistance against nematodes. Grafted plants are also used on eggplants to decrease susceptibility to *Verticillium dalhiae*, a pest which causes significant damage in the South of France (Lot and Garonne Valley, Vaucluse). Melons are mainly grafted with a hybrid of *Curcurbita maxima* and *C. moschata* (ex: RS 841, P360) against *Fusarium* and *Phomopsis scleroïdes* (Monnet 2001)

RESEARCH PROTOCOL FOR ALTERNATIVES

Field trials must be instigated to find potential alternatives to MB. It would be useful if scientists used a common protocol or methodology to evaluate chemical or non-chemical alternatives.

The suggested approach is to report a correlation between three important parameters in micro-plots, i.e. 1) Quantification of the main active ingredient on pathogens (for example: gas concentrations for fumigants) including a comparison with mortality obtained in the laboratory; 2) Direct biological efficacy using systematic artificial infestations; and 3) Agronomic behaviour of the crop.

A working group of scientists, technicians, contractors and soil fumigant users are currently in the process of creating an official methodology for the "Commission des Essais Biologiques", an official expert committee in France. In the near future, this protocol will be followed for each new registration for soil disinfestation compounds.

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APPENDIX 1

Table 1 : Methyl bromide consumption (tonnes) in 1997 and 2000.

Crops	Consumption 1997	Percentage share	Consumption 2000	Percentage share	Tendency
Tomatoes	410	29.2	141	19.7	-65.1
Strawberries	295	21	150	21	-49.2
Cut flowers	144	10.3	80	11.2	-44.4
Cucumber	125	8.9	100	14	-20
Horticultural nurseries	62	4.4	50	7	-19.4
Melons	56	4	10	1.4	-82.1
Arboricultural.re plant	53	3.8	55	7.7	+3.8
Aromatic plants	42	3	30	4.2	-28.6
Pepper	30	2.1	20	2.8	-33.3
Tobacco seedlings	26	1.9	5	0.7	-80.8
Carrots	25	1.8	2	0.3	-92
Courgettes	25	1.8	2	0.3	-92
Lettuces	24	1.7	2	0.3	-91.7

Substrates	24	1.7	25	3.5	+4.2
Asparagus (nurseries)	21	1.5	2	0.3	-90.5
Forester nurseries	20	1.4	26	3.6	+30
Eggplants	12	0.9	10	1.4	-16.7
Leek seedlings	5	0.4	2	0.3	-60
Vineyards replant	3	0.2	3	0.4	0
Total	1402	100	715	100	-49

BIOLOGICAL CONTROL AGENTS IN VEGETABLE CROPS AS ALTERNATIVES TO METHYL BROMIDE

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ABSTRACT

The effect of time, frequency, and multiple applications of the egg parasitic fungus *Verticillium chlamydosporium* in reducing population densities and crop damage caused by *Meloidogyne javanica* were evaluated in unheated plastic houses in Barcelona, Spain. The effect of the fungus on crop yield was also evaluated. The fungus survived in the soil throughout the growing season and parasitized nematode eggs in all the experimental conditions tested. The fungus consistently decreased plant damage in all experiments except in the post-planting fungal treatment carried out 10 weeks after planting. However, it did not affect final nematode densities or egg production. Accumulated tomato yield in nematode-free plots was always higher (P=0.05) than in nematode-infested plots whether they had been treated with the fungus or not.

Keywords: antagonist, egg parasites, fungus, Meloidogyne, plastic houses, root-knot nematodes.

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp., are a major pest in vegetable crops worldwide. Currently, nematode control is based mainly on the use of soil fumigants and nematicides. Constraints imposed on the use of nematicides by legislation and consumers are leading to the development of new management strategies including biological control.

Microbial antagonists of nematodes can be exploited as biological control agents (BCA) either alone or in combination with other management strategies. *Verticillium chlamydosporium* is a facultative parasite of nematode eggs that has been extensively investigated as a potential BCA for cyst and root-knot nematodes. The tri-trophic interaction occurring in the soil between the fungus, the plant, and the nematode is complex and several factors affect the performance of the fungus (Kerry 2000).

The objective of this research was to determine the effect of time, frequency, and multiple applications of *V. chlamydosporium* in reducing population densities and crop damage caused by *Meloidogyne javanica* in unheated plastic houses. The effect of the fungus on crop yield was also investigated.

MATERIALS AND METHODS

Procedure. The soils from the experimental sites were infested with *M. javanica*, and located at Centre de Cabrils, IRTA, Cabrils, Barcelona (site Q21), and at Can Comas Farm, Consell Comarcal del Baix Llobregat, El Prat, Barcelona, Spain (site CC). The soil was sandy loam and loam, at sites Q21 and CC, respectively. The experimental design was incomplete randomized blocks with five replicated plots per treatment.

The isolate IACR Vc 10 of *V. chlamydosporium* (provided by B. R. Kerry, IACR-Rothamsted, UK) was used for the experiments. Fungal inoculum was mass-produced on a solid medium consisting of a moist autoclaved mixture of coarse sand and corn flour that was inoculated with the fungus.

After four weeks' incubation at 25ºC, fungal biomass was recovered by washing and sieving. The concentration of chlamydospores in the fungal suspension was estimated in diluted samples using a haemocytometer (de Leij *et al.* 1993). Trials were done in a similar way unless otherwise stated. Nematode-free plots were obtained by methyl bromide fumigation (75 kg/m^2) in October 1998.

Frequency of application. To determine the effect of one or two applications of the fungus, lettuce seedlings cv Arena were planted in October 1999 and harvested after 16 weeks of growth in February 2000. Tomato seedlings cv Durinta were planted on 9 and 14 of March at sites Q21 and CC, and harvested after 17 and 19 weeks of growth respectively, in July 2000. Plots consisted of a single row 3.4 m long with six plants/plot spaced 50 cm in the row. Initial nematode density (Pi) before planting the tomato seedlings was 1,200 nematodes (J2) / 250 cm^3 soil at site Q21, and nematodes were below detectable levels at site CC. The fungus was applied at a rate of 1×10^6 chlamydospores/plant at the time of planting the lettuce seedlings in autumn, and a second application at a rate of 53×10^6 and 67×10^6 chlamydospores/plant was done at the time of planting the tomato seedlings in spring at sites Q21 and CC, respectively.

Time of fungal application. The effect of pre-planting or post-planting applications of the fungus was tested on the tomato plants. Trials were run in parallel to the previous ones. The pre-planting fungal application rate was 53×10^6 and 67×10^6 chlamydospores/plant at the time of planting tomato cv Durinta at site Q21 and CC, respectively. The post-planting application rate was 20×10^6 chlamydospores /plant. The inoculum was delivered at a volume of 800 ml/plant as a soil drench 10 weeks after planting. Plants were irrigated immediately after inoculation.

Multiple fungal applications. The effect of multiple applications of the fungus during cultivation of tomato plants was determined at site Q21. Plots consisted of two rows (3.4 m long and 1.5 m wide) with twelve plants/plot spaced 50 cm in the row. The Pi was 1190 J2/250cm^3 soil. Tomato seedlings cv Durinta were planted on 12 March, and harvested on 10 July 2001. Individual plants received a total of 122×10^6 chlamydospores, which were applied at one-week intervals for 6weeks starting 6 weeks after planting. The fungus was delivered through a drip irrigation system at a volume of 4 l/plot. Plants were irrigated immediately after inoculation.

To determine densities of *M. javanica*, composite soil samples were collected at the beginning and end (P. final) of each experiment. Individual samples consisted of five 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 extracted from 500-cm^3 soil subsamples by Baermann trays. The number of nematodes was expressed as J2/ 250 cm^3 soil. Nematode damage was evaluated after the final harvest using a gall index. Following sampling for final densities, four or eight plants/plot were dug and rated for galling on a scale of 0 to 10, where 0 = complete and healthy root system, and 10 = plant and roots are dead.

Eggs were extracted from two 10-gram root subsamples by blender maceration in 0.5% NaOCl solution for 10 minutes. Fungal densities were estimated at the end of the crop in representative 1-gram soil and root subsamples from each plot using dilution plate on a semi-selective medium (de Leij and Kerry 1991). The numbers of colonies of *V. chlamydosporium* (cfu) were counted after three weeks' incubation at 25 ºC. Parasitism of nematode eggs was assessed following the procedure described by de Leij and Kerry (1991). Eggs were considered parasitized if fungal hyphae were growing out of them.

To determine crop yield, fruits produced per four or eight tomato plants/plot were harvested as they matured. Tomatoes were collected once each week for 6 weeks. Fruits were counted and weighed, and the accumulated tomato yield was expressed as kilogram per m^2. For statistical analysis, the nematode-free treatment was excluded from nematode analysis because *M. javanica* was not detected in these plots during the trials. Data on number of nematodes in soil and eggs/g root, and on number of cfu in soil and roots were transformed to [log (x+1)], and subjected to analysis of variance using the GLM procedure of SAS version 8, and analysed by site. Tomato yield data were subjected to analysis of variance. When the overall F test was significant, means were separated by LSD procedure (P =0.05).

RESULTS

Frequency of application. The fungus was re-isolated from parasitized eggs of *M. javanica* in both plastic houses nine (Vc) and four (Vc 2X) months after its application, and was recovered from all inoculated plots at site Q21 but only from two or three plots at site CC (Table 1). Repeated fungal applications (Vc2X) tended to increase percent egg parasitism.

Fungal distribution in soil and root also tended to increase after two fungal applications at site Q21. In contrast, the fungus was not established in the soil of site CC after one application, and was only recovered from root samples after two applications.

Gall rating in fungal treated plots was lower (P=0.05) than in those left untreated at site Q21, but no effect was observed at site CC. The fungus did not affect final nematode densities or egg production.

Accumulated tomato yield was higher (P =0.05) in nematode-free than in nematode-infested plots at both sites regardless of the frequency of fungal applications. There was no difference between fungal treated or untreated plots in plots infested with *M. javanica*.

Table 1: Effect of the frequency of application of *Verticillium chlamidosporium* (Vc) on population densities of *Meloidogyne javanica* (Mj) and yield of tomato cv Durinta in two plastic houses infested with the nematode in Barcelona, Spain.

Site	Frequency application	P. final (J2/250 cc soil)	Gall[1] rating	Eggs/ g root [2]	Parasitized egg (%)	[3]cfu/g soil	cfu/g root	Yield[4] (kg/ m^2)	Fruits (m^2)	Fruit weight (g)
Q21	Vc	14240	6.1 b	53260	3 (5)	100 (2)	200 (3)	8.3 c	89 c	93 c
	Vc 2X	13180	5.8 b	47790	5 (5)	1000 (4)	1000 (2)	10.2 b	102 ab	100 bc
	Untreated	10250	7.0 a	39010	-	-	-	9.7 b	95 bc	102 b
	Mj free	0	0	0	-	-	-	12.2 a	113 a	109 a
CC	Vc	1480	3.4	867	5 (2)	0	0	12.1 b	119	104
	Vc 2X	560	3.2	2190	9 (3)	0	100 (2)	11.5 b	101	120
	Untreated	360	3.2	1330	-	-	-	12.1 b	102	127
	Mj free	0	0	0	-	-	-	13.6 a	110	128

Values are means of five replicated plots per treatment. For each site, means within a column followed by the same letter are not different according to LSD test (P=0.05). In parenthesis, number of plots with the fungus. [1] Based on a scale from 0 (none) to 10 (severe). [2] Parasitized eggs excluded. [3] Number of colonies forming units. [4] Four plants/plot, five plots/treatment.

Time of fungal application. The fungus was re-isolated from nematode eggs when applied in either pre-planting or post-planting tomato at both sites (Table 2).

Parasitism of nematode eggs occurred in all fungal-inoculated plots at site Q21, but they were only recorded from three to four plots at site CC. Tomato plants in pre-planting treated plots showed lower (P=0.05) gall rating than those in post-planting treated or untreated plots at site Q21. The fungus did not affect final nematode densities or egg production at this site. At site CC, final densities of *M. javanica* were higher (P=0.05) in post-planting treated fungal plots than in the remaining treatments.

Accumulated tomato yield was higher (P=0.05) in nematode-free than in nematode-infested plots in both sites, although yield in post-planting treated plots did not differ from that in nematode-free ones (Table 2).

Table 2: Effect of time of application of Verticillium chlamidosporium on population densities of Meloidogyne javanica (Mj) and yield of tomato cv Durinta in two plastic houses infested with the nematode in Barcelona, Spain.

Site	Frequency	P. final	Gall[1]	Eggs/ g	Parasitized	[3]cfu/g	cfu/g	Yield[4]	Fruits	Fruit

	application	(J2/250 cc soil)	rating	root [2]	egg (%)	soil	root	(kg/ m^2)	(m^2)	weight (g)
Q21	Pre-planting	13180	5.9 b	47100	5 (5)	900 (4) a	1000 (2)	10.2 b	102 ab	100 b
	Post-planting	11800	6.8 a	46420	9 (5)	0 b	50	8.4 c	83 c	100 b
	Untreated	10250	7.0 a	29010	-	-	-	9.7bc	95 b	102 b
	Mj free	0	0	0	-	-	-	12.2 a	110 a	112 a
CC	Pre-planting	560 b	3.2	2190	9 (3)	0	100 (2)	11.5 b	101	120
	Post-planting	2170 a	3.1	150	10 (4)	0	0	13.5 a	102	137
	Untreated	360 b	3.2	1330	-	-	-	12.1 b	102	127
	Mj free	0	0	0	-	-	-	13.6 a	110	128

Values are means of five replicated plots per treatment. For each site, means within a column followed by the same letter are not different according to LSD test (P=0.05). In parenthesis, number of plots with the fungus. [1] Based on a scale from 0 (none) to 10 (severe). [2] Parasitized eggs excluded. [3] Number of colonies forming units. [4] Four plants/plot, five plots/treatment.

Multiple applications. The fungus was re-isolated from eggs of *M. javanica*, and also from rhizosphere soil and tomato roots after six fungal applications at one-week intervals (Table 3). Gall rating in plants from fungal treated plots was lower (P= 0.05) than in those left untreated. The fungus did not affect final nematode densities or egg production. Accumulated tomato yield was higher (P =0.05) in nematode-free than in nematode-infested plots whether they had been treated with the fungus or not.

DISCUSSION

Verticillium chlamydsoporium was successfully introduced in unheated plastic houses in north-eastern Spain. The fungus survived in the soil throughout the growing season and parasitized eggs were recorded from nematode-infected roots at the end of all trials conducted in both plastic houses. These results indicate that the fungus maintained its virulence against *M. javanica*, and was compatible with the agronomic practices and environmental conditions of intensive agriculture in plastic houses. The fungus has a cosmopolitan distribution and has been isolated from eggs of several species of *Meloidogyne* infecting vegetable crops in Spain (Verdejo-Lucas *et al.* 2002). The fungus consistently decreased plant damage in all experiments except in the post-planting fungal treatment applied 10 weeks after planting (Table 2). However, it did not affect nematode densities in soil or root. At site Q21, levels of *M. javanica* at the beginning of each experiment were above the reported damage threshold for *Meloidogyne* (4-5 J2/ cm^3 soil), and densities increased considerably after a single crop.

Table 3: Effect of multiple applications of Verticillium chlamidosporium (Vc) on population densities of M. *javanica* (Mj), and on yield of tomato cv Durinta in a plastic house infested with the nematode in Barcelona, Spain.

Site	P. final (J2/250 cc soil)	Gall[1] rating	Eggs/ g root [2]	Parasitized egg (%)	[3]cfu/g soil	cfu/g root	Yield[4] (kg/ m^2)	Fruits (m^2)	Fruit weight (g)
Vc	10175	6 b	29810	7 (5)	3100 (4)	2150 (4)	9.9 b	90 b	103 b
Untreated	12500	7 a	32830		0	0	9.1 b	92 ab	98 b
Mj free				-	-	-	12.3 a	103 a	118 a

Values are means of five replicated plots per treatment. Means within a column followed by the same letter are not different according to LSD test (P=0.05). In parenthesis, number of plots with the fungus. [1] Based on a scale from 0 (none) to 10 (severe). [2] Parasitized eggs excluded. [3] Number of colonies forming units. [4] Four plants/plot, five plots/treatment.

Percent egg parasitism was low in all experiments, which suggests that effective establishment of the fungus in the field will most probably require several growing seasons because numerous factors affect the saprophytic phase of the fungus, including the receptivity of the soil to the antagonist. Thus, the sandy loam soil of site Q21 appeared more favourable to *M. javanica* and the fungus than the loam soil of site CC since both organisms were more widely distributed and abundant at site Q21 than at site CC.

The time and frequency of fungal applications are important factors that need to be optimized according to local agronomic and environmental conditions to improve fungal performance. Other factors to be considered are application rates and formulation of the fungus.

Because *V. chlamydosporium* is a stage-specific antagonist that only parasitizes nematode eggs, and will not prevent juveniles from entering the roots, the combination of the fungus with other control methods such as resistant plant cultivars, poor nematode host or non-fumigant nematicides would be necessary to achieve nematode control.

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ALTERNATIVES TO METHYL BROMIDE FOR TOMATOES AND VEGETABLES IN ITALY

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ABSTRACT

Italian tomato and vegetable production is economically very important. MB is largely used to control soilborne pests, and to ensure crop productivity and yield stability. The potenial for chemical and non chemical alternatives to replace MB were evaluated. This paper reports the main results obtained by a project supported by EC and Italian Government carried out by five institutions in collaboration during 1998-2001. The key in developing alternatives was soil solarization employed alone or in integrated systems (IPM) with manure (biofumigation), biological antagonists, resistant/tolerant varieties and rootstocks, or by reduced dosages of chemicals. Results showed that, for specific crops and situations, MB alternatives can be accomplished assuring good control and profitable yields. It is furthermore possible to reduce costs, increase quality and provide a safe environment.

Keywords: Soil solarization, integrated pest management, mulching films, soilborne pests, biofumigation, space structural solarization.

INTRODUCTION

The Italian production of tomatoes and vegetables is of considerable economic importance. In intensive agriculture and in the restricted area of protected crops, vegetables are continuously planted in the same field, leading to a rapid buildup of soilborne pests (SPs) attacking the plant's roots. Because of our climatic conditions, many pathogens are known to induce diseases in several major crops causing damage and economic problems. Heavy damage was caused too by nematodes belonging to genera *Meloidogyne*, *Dytilenchus* and *Pratylencus* , as well as by weeds.

To ensure crop productivity and yield stability, it is necessary to treat soil by steam or fumigants to reduce disease damage to below tolerance limit. The high cost of soil disinfestation by steam restricts its use to high-value crops, therefore soil fumigation became the most common approach for SPs control.

Chemicals allowed as preplant soil disinfestation are very few (methyl bromide, metham sodium, 1,3-dichloropropene and dazomet). Methyl bromide (MB) was the predominant choice because of its relatively short treatment period and activity on a broad range of pests.

Unfortunately, this pesticide has been identified as an ozone depleting substance requiring phase out by 2005 except for critical uses. Research was carried out in order to reduce its dosage in agriculture but retain its pathogenic activity. In soil naturally-infested by pathogens and nematodes, it was verified that MB was still active at dosages of 40 and 20 g/m^2 when the soil was covered with virtually impermeable films (VIFs) to conserve gas (Cartia & Minuto 1998).

Studies in Italy that aimed to control diseases affecting tomatoes and vegetables using alternative methods to MB started in 1980 and focused on "soil solarization" (SS) (Garibaldi & Cartia 1991). These studies over the past 20 years in Italy's climatic conditions showed its effectiveness in reducing the population density of many pathogens and nematodes (Table 1).

THE MULTIREGIONAL OPERATING PLAN

Project POM-A12 was carried out to optimize alternatives to MB and to improve soil solarization knowledge and practice. Implemented under the supervision of the Department of Agrochemistry and Agrobiology and financed by the European Community, this project was worked out in a multi-regional context, involving five research institutes and many scientists (pathologists, nemathologists, physicists, engineers) as well as the extension services from Apulia, Calabria and Sicily (Di Primo et al.1999). Nurserymen, fumigation and pest control companies, mulching film manufacturers, builders of machinary applying films on the field, disused film recycling industries and farmers too, were involved. The key in developing the project was SS employed alone or in a integrated system (IPM) with: reduced dosages of chemicals; manure (biofumigation); biological antagonists; resistant/tolerant varieties and rootstocks. The project aimed to reduce costs, increase quality and provide a safe environment.

During a three year period (1998-2001) studies were carried out to verify:

- Thermal regimes in soil subject to solarization (Gutkowski & Terranova 1991);
- The behaviour of innovative mulching films devoted to increase SS effects (Di Primo & Cartia, 1998);
- The activity of biological agents in controlling root rot diseases (Cartia & Causarano 1996);
- The efficacy of SS and IPM methods in controlling soilborne pathogens and nematodes (Cartia et al. 1997).

Biofumigation (BF), combining SS with organic amendments, offers additional option for SPs management involving chemical, physical and biological effects. Amendments were tested such as chicken manure that caused the release of volatile ammonia and an increase of soil temperature resulting in more effect on the resting structure of SPs, followed by solarization alone (Di Primo & Cartia 1998).

Mulching film is an important factor in creating favourable condition for SS treatment and in reducing soil reinfestation. Lately studies on this matter pay particular attention to coextruded plastic film having different photometric properties and color, in comparison with PE mulching film. Green coextruded film (GCF), which inhibits weeds, can be left in place as mulching soil after the solarization reducing pathogens reinfestation in the treated soil (Cascone *et al.,* 2000).

Ethylene tetrafluoroethylene (ETFE) is a greenhouse cover film that improves soil thermal regimes and SS effectiveness (Cascone *et al.* 2001).

Space structural solarization (SSS) is a promising disease management strategy which is a complementary soil disinfestation for the control of inoculum surviving on the structure. In a closed tunnel or greenhouse during July-August, air temperatures were raised to 60-65°C (Di Primo & Cartia 2001a).

Table 1: The main soilborne agents controlled by soil solarization in Italy.

Diseases and pathogens controlled	Host plant	Treatment	References
Basal necrosis	Pepper	Glasshouse	Cartia *et al.* 1987
Ditylenchus dipsaci	Onion Carrot	Field	Lombardi *et al.* 2000 Greco & Cartia 2000
Fusarium oxysporum f.sp. *melonis*	Melon	Tunnel	Di Primo & Cartia 2001
Fusarium oxysporum f.sp. *radicis – lycopersici*	Tomato	Greenhouse	Cartia *et al.* 1997
Globodera rostochiensis	Potato	Field	Greco *et al.* 2000
Meloidogyne incognita	Tomato	Greenhouse	Cartia *et al.* 1997
Meloidogyne spp.	Tomato Eggplant Pepper Lettuce/Melon	Greenhouse	Greco *et al.* 1992; Colombo *et al.* 1992 Cartia 1984; Greco & Cartia 1996 Di Vito et al. 1998

Diseases and pathogens controlled	Host plant	Treatment	References
Phytophthora capsici	Pepper	Greenhouse	Polizzi *et al.* 1994; Morra *et al.* 1995
Pyrenochaeta lycopersici	Tomato	Greenhouse	Cartia *et al.* 1988; 1989
Pythium ultimum	Cucumber	Greenhouse	Minuto *et al.* 1995
Rhizoctonia solani	Chicory	Greenhouse	Vannacci *et al.* 1997
Sclerotinia spp.	Carrot Lettuce	Field	Cartia *et al.* 1987; Triolo *et al.* 1987; Scannavini *et al.* 1993; Cartia 1996
Sclerotium cepivorum	Onion	Field	Polizzi *et al.* 1995; Di Primo & Cartia, 1998; Agosteo & Cartia 2001
Verticillium dahliae	Eggplant/Tomato Eggplant	Greenhouse Tunnel	Tamietti & Garibaldi 1982; Cartia 1984; Cartia *et al.* 1990; Tamietti & Valentino 2001

To verify the efficacy of innovative, alternative methods to MB, various experimental trials were carried out in controlling SPs affecting tomato, melon, onion and carrot. The main results are reported.

TOMATO - Fresh market greenhouse-tomato is a major vegetable crop in Italy. Among SPs affecting tomato, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), *Pyrenochaeta lycopersici* and nematodes (*Meloidogyne* spp.), are the most harmful, inducing Fusarium crown and root rot (FCRR), corky root, and root-knot respectively.

Preliminary studies carried out in the open field showed at 12 days SS reduced survival of FORL propagules significantly. The effectiveness of the pathogen's control was improved by combing SS with manure, or extending the SS treatment to 27 days. In a closed greenhouse, SS and BF with bovine manure proved effective in reducing the viability of FORL chlamidospores, disease incidence, and in increasing commercial yield. Susceptible tomato plants grafted on FCRR-tolerant hybrid rootstock (He-man), even cropped in a severe FORL infested soil, remained healthy during the growing season and gave a profitable yield (Di Primo & Cartia 2001 b).

Trials to test the effectiveness of SS, in comparison with MB, in controlling *P. lycopersici* and nematodes, were performed in greenhouse covering soil with different films. Mulching soil with EVA, GCF, and black coestrused films (BCF), soil temperature exceeded 42.5°C per 373, 265 and 68 hours respectively. Corky root and root-knot symptoms appeared very low in MB and in solarized plot mulched by EVA and GCF treatments, and statistically different from BCF and untreated control (Cascone *et al.* 2000).

The activity of five biological agents in controlling FCRR disease was evaluated on 1700 seedlings grown in nine different greenhouses. Because of the light incidence of FCRR, disease control appeared evident only by "Fus Più", and no yield increase was recorded (Polizzi & Catara 2001).

MELON - *Fusarium oxysporum* f.sp. *melonis* (FOM), the causal agent of muskmelon wilting, causes serious damage to crops grown in unheated tunnels. The efficacy of SS and BF in controlling FOM propagules, placed in the soil at depths of 15 and 30 cm, was tested in an open field and in a tunnel. In the open field, a short solarization period (12 days) induced, in propagules settled at 15 cm, a mortality of 52 and 65% respectively. Combining SS with chicken manure (1 kg/m^2), or extending treatment to 27 days, improved pathogen control. In tunnel trials, chlamidospores were settled at the same depths and soil treated by SS, BF and MB. The SS treatment carried out for 35 days killed propagules placed at 15 cm and induced a mortality of 83% at 30 cm depth. BF and MB treatments obtained 100% mortality at both soil depths. All treatments reduced disease incidence in melon plants and increased yield (Di Primo & Cartia 2001 b).

ONION -The white rot caused by the fungus *Sclerotium cepivorum* is most prevalent on onion crops. Its sclerozia that stay alive for many years is a negative factor in infected soils. In preliminary field trials carried out in Calabria region, SS induced a significant reduction of white rot (89%) and increased the marketable yield (64%) (Polizzi et al. 1995).

SS carried out for 27 days achieved the complete destruction of sclerozia buried at a depth of 15 and 30 cm. BF adding chicken manure into the soil significantly enhanced pathogen control, as early as 12 days after treatment. Sclerozia mortality at the two tested depths reached 100%. The SS of a field amended with organic manure resulted in a better control of sclerozia of *S. cepivorum* and shortened the application time, as compared to either treatment alone (Di Primo & Cartia 1998).

Good results were also obtained using mulching soil with ETFE and GCF. The viability of sclerotia, set in the soil at 20 cm depths, decreased by 26 and 21% respectively, than 10 days of SS and they were completely killed after 20 days (Agosteo & Cartia, 2001).

CARROT -Trials to control *Ditylenchus dipsaci* in carrots were carried out in Sicily to assess the effectiveness of two fenamiphos formulations in combination with SS (August 13 - Septmber 25). The carrots were sown in October. All control plots and only one solarized plot showed many dead or stunted and yellowing plants in early March. Because several generations were developed by nematodes, no significant differences were observed in numbers of nematodes in the soil and in the carrot tap roots at the end of March. However, yield parameters showed highly significant differences. At the end of March the average carrot tap root weight was 58.5g in plots which were only solarized, 62.5 – 77.2 g in plots solarized and treated with fenamiphos and only 30.7 g in control plots. At harvest (17 April), the carrot tap root yield/0.8 m^2 was 3.6 kg in the controls and as high as 8.0 - 9.7 kg in the treated plots, while the average tap root weight was 38.7 g and 92 - 97g, respectively (Greco & Cartia 2000).

CONCLUSIONS

Trials carried out in Apulia, Calabria and Sicily, during a three year period showed that there are, for specific crops and situations, various chemical and non-chemical methods that can control SPs activity and are able to replace MB. In our climatic conditions, during summertime, SS treatment can solve the problems particularly against pathogens sensitive to heat, and its linking with other control methods (IPM) can enlarge activity or extend the control to regions located in Northern Italy.

Research to evaluate and solve the problems related to finding alternatives to MB will need to evaluate what will happen when chemical alternatives to MB increase together with environmental problems.

The project “Toxicological impact of agricultural and energetic activities” is carried out in Sicily to verify which chemicals considered alternatives to MB are used in the Ragusa area in controlling SPs. Preliminary investigations pointed out that 1,3-dichloropropene is largely employed to control soilborne nematodes. It’s use appears promising - chemical residues have yet to be found in treated soil and cropped vegetables.

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ALTERNATIVES TO METHYL BROMIDE FOR TOMATO AND CUCUMBER PRODUCTION IN TURKEY

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ABSTRACT

The effectiveness of an eight weeks solarization period, alone and in combination with other treatments (1,3 dichloropropene, metam-sodium, dazomet, biofumigation, biocontrol-Mycormax , biocontrol-Promot) were compared with the effectiveness of methyl bromide (MB) and control applications. No single alternative can be recommended to replace MB. Solarization, alone or in combination with other treatments such as metam-sodium, 1,3 dichloropropene, dazomet and biofumigation can be recommended in an IPM program to manage soilborne pests in Antalya conditions. Soilless culture treatments also showed promising results in our conditions.

Keywords; Methyl bromide, solarization, biofumigation, biocontrol, dazomet, metam-sodium, dichloropropene, soilless culture, volcanic tuff, peat, tomato and cucumber

INTRODUCTION

Methyl bromide (MB) is one of the most widely used pesticides in the world. It is very effective to control a wide range of pests including pathogens, arthropods and weeds in soil and stores. In Turkey, MB is mostly used in soil fumigation. Total MB consumption in Turkey in 2000 was about 606 tonnes. More than 90% of this amount was used to control nematodes in particular, and also soilborne diseases and weeds in greenhouses in Antalya and Aegean Provinces (Doganay 2000).

Tomato is the main crop grown in greenhouses. Tomatoes account for more than 50% of the vegetable production followed by cucumber, eggplant and some other vegetables produced in Antalya (Anon 2000).

Growers prefer to use MB for better yield. If an alternative to MB is not discovered in the near future in the world, they may not be able to grow these plants because of nematodes and soil borne pests. Although MB is highly effective at controlling soil borne pests, it is destroying the ozone layer and also very toxic human and animals (Bell *et al.* 1996; Katan 1999). Therefore, it is going to be phased out in 2005 in developed countries and in 2008 in Turkey. This situation has stimulated researchers to find alternatives to MB in the world and in Turkey. To find alternatives to MB, a demonstration project was established on tomato (*Lycopersicon esculentum* Mill.) and cucumber (*Cucumis sativus* L.) plants produced under glasshouse conditions in Antalya Province, in collaboration with UNIDO.

MATERIALS AND METHODS

The demonstration project was carried out in Aksu and Kocayatak experiment stations of Citrus and Greenhouse Crops Research Institute in Antalya**.** The trail was conducted following a completely randomized block design with four replicates and approximately 6.5 da. glasshouse. In this project, the tomato variety Fantastic F144 and the cucumber variety Quamar were used as experimental plants. The treatments used in the experiments were solarization (SL), SL+1,3 dichloropropene (DD), SL+metam-sodium (MS), SL+dazomet (DZ), SL+biofumigation (BIO), SL+biocontrol-Mycormax (BCM), SL+biocontrol-Promot (BCP), MB 100% (MB100), MB 50% (MB50) and a control. Other than these treatments, soilless culture treatments [sand (S), volcanic tuff (VT), volcanic tuff+peat(VTP)] were also added to the project just for yield observation.

The surface of the soil in the solarization plots in the glasshouses was covered with 0.02 mm thick transparent polyethylene plastics for 8 weeks during the summer. DD was manually injected into soil to a depth of 15-17 cm using a hand-gun with 6 ml/m^2 dosage. MS (40 ml/ m^2) was mixed manually in tap water pooled into 10 m^2 small plots. DZ granular was spread by hand on the soil surface and incorporated into the soil with a small rotovator at 25 g/m^2 dosage. Fresh chicken manure was used at 5 kg/m^2 as the biofumigation material. Preparations containing *Trichoderma koningii* and *T.harzianum* called Promot (10 gr/L), and *Glomous intraradices* called Mycormax (35-40 gr/L), were used as biocontrol agents. MB plots were covered with polyethylene plastic and MB was applied by drip-irrigation (full dosage 70 g/m^2, half dosage 35 g/m^2).

Microorganisms were counted by diluting them in water. The counts were carried out before solarization and 8 weeks afterwards. Fungi and bacteria in 10 g of soil were counted 10^5 and 10^7 dilution, respectively.

Soil samples (5 samples from each parcel) from the glasshouses were collected to determine nematode (*Meloidogyne* spp.) density. Nematodes (J_2 larval stage) were extracted according to the modified Baermann Funnel method and counted before and after the treatment applications. Root gall indices (RGI) were determined at the end of the vegetation period reported by Zeck (1971): 0=no root system galled; 1=from 1% to 25% of root sytem galled; 2: from 26% to 50%; 3: from 51% to 75%; 4= from 76% to 100% of the root system galled.

Total yield from 21 January 2000 to 27 June 2000 was recorded from each treatment. Tomato and cucumber fruit were classified according to standard marketable sizes as Extra, Class I, Class II for tomatoes; and Extra and Class I for cucumbers.

RESULTS

The weather conditions for 8 weeks from July 26 to September 21 were favourable for all the treatments in the tomato and cucumber plots. The daily mean maximum soil temperatures were recorded for only SL, SL+BIO and the control plots and measured at 10 cm depth in the range of 43-47 °C for SL, 45-52 °C for SL+BIO and 34-46 °C for the control. Soil temperatures at 20 cm depth were in the range of 36-41°C for SL, 38-43°C for SL-BIO and 34-37°C for the control.

The fungal population in tomatoes before treatment was in the range of 29.9-39.2x10^5 in 10 g soil collected from each plot (Table 1). The fungal population for cucumber plots was very similar to that of tomato. All treatments reduced the number of fungi in 10 g of soil in the tomato and cucumber plots, but MB100 was the most effective (Table 1, 2). This was followed by MB50 and SL+MS in tomato. However MB50, SL+DD and SL+MS were effective in cucumber after MB100. At the end of the season, MB100, MB50 and SL+MS were still providing effective fungal control in the tomato and cucumber plots.

The bacterial population in tomato and cucumber plots before treatment was in the range of 50.5-56.2 x 10^7, 51.7-64.6 x 10^7 in 10 g soil, respectively (Table 1, 2). All treatments after application reduced the number of bacteria in all tomato and cucumber plots, but MB100 was the most effective. The effectiveness of MB50, SL+MS and SL+DD was very close to the effectiveness of MB100 in tomato. In cucumber MB50, SL+MS and SL+DD were also effective but not as effective as the MB100 dosage in the tomatoes. At the end of the season, MB100, MB50 and SL+MS still reduced the number of bacteria in 10 g soil in tomato and cucumber plots.

In the tomato and cucumber plots, the number of *Meloidogyne* spp. before the treatments was in the range 255.8 - 321.1 J_2 and 130.0 - 182.5 J_2 in 100 cc soil, respectively (Tables 1, 2). After the applications, all treatments reduced *Meloidogyne* spp. population, but MB100 application was the most effective (3.6, 3.3 J_2) at controlling *Meloidogyne* spp. in tomato and cucumber, respectively. This was followed by MB50, SL+DD and SL-BIO.

The highest infestation (Tables 1, 2) was observed in the control plots which had RGIs of 3.8 on tomato and 2.3 on cucumber. The level of RGI was the lowest in MB100 treatment on both tomato (0.2) and cucumber (0.02). MB50, SL+BIO, SL+DD also had low levels of RGI in tomato and cucumber.

In tomato plots, all treatments increased the marketable yield compared to the control (Table 1). The greatest yield was obtained in SL+BIO plots (20597.5 kg/da). This was followed by SL+DD, SL+DZ, MB100 and MB50 treatments. In cucumber, all treatments except SL+BCM increased the marketable yield compared to the control (Table 2). The MB50 treatment provided the greatest yield (9249.2 kg/da). The yield of MB100, SL+DD, and SL+MS treatments was also higher than that of control.

Among soilless culture treatments, sand provided the highest yield (21064.1 kg/da) in tomato. The yield using VTP (20436.2 kg/da), VT (19420.4 kg/da), MB100 (19163.1 kg/da) and MB50 (19138.9 kg/da) were very close to each other, but significantly higher than that of control (14940.9 kg/da). In cucumber plots, all the soilless culture and MB treatments provided higher yield than that of the control. The highest yield was obtained from MB50 plots (9249.2 kg/da). This was followed by VTP (8501.7 kg/da), MB100 (8066.7kg/da), VT (7829.7kg/da) and S (7763.6 kg/da).

DISCUSSION

Solarization has been reported as an alternative to MB alone or in combination with other chemicals (DD, MS, DZ), biofumigation and biocontrol agents (Di Vito *et al.* 2000; Gamliel *et al.* 2000; Minuto *et al.* 2000; Tamietti & Valentino 2000). In our experiments, solarization may also be an effective alternative method to MB either alone or in combination with other methods. SL is very suitable and applicable for use in the Antalya province due to the favourable environmental conditions. Antalya has very strong sunlight for approximately 250 days in a year and the summer (July and August) is very hot. During July and August (total 55.5 days completely sunny and clear weather) the glasshouses are empty. This situation makes solarization possible in Antalya conditions.

MB100 was the most effective treatment to reduce number of fungi and bacteria in soil in both the tomato and cucumber plots. SL+MS, SL+DD, SL+DZ and SL also significantly reduced fungal and bacterial populations and the effectiveness of these treatments was close to the MB applications in the tomato and cucumber plots. Therefore, SL+MS, SL+DD, SL+DZ and SL may be alternatives to MB, but SL+MS may be the most favorable alternative for controlling fungi and bacteria. Similar results have also been reported by Reuven *et al.* (2000) for MS, Gamliel *et al.* (2000) for SL+MS and Minuto *et al.* (2000) for SL+DZ.

The results of this experiment indicated that MB100 was the most effective treatment to reduce the number of nematodes in both the tomato and cucumber plots. SL+DD and SL+BIO were also effective for controlling *Meloidogyne* spp., in fact close to the effect of the MB treatments. Therefore, these treatments may be recommended to control *Meloidogyne* spp. in tomato in Antalya province. However, SL+BIO containing a very high level of nitrogen (especially chicken manure) may not be recommended to control *Meloidogyne* spp. in cucumber as the plants are made very susceptible to soil borne diseases. The RGI was below 1 in the SL+DD and SL+BIO plots treated in tomato and cucumber at the end of the vegetation period. Similar results for SL+DD were reported by Di Vito & Campanelli (2000).

In the treated tomato plots, all the treatments increased the marketable yield compared to the yield of the control (Table 1). The greatest yield was obtained in SL+BIO plots (20597.5 kg/da). This resulted from chicken manure which contained high level of plant nutrition materials and microbial activity.

In conclusion, no single alternative can be recommended to replace MB. Therefore, an IPM programme must be considered to manage soil borne pests in Antalya conditions. However, solarization alone or in combination with other treatments (SL+MS, SL+DD, SL+DZ and SL+BIO) may be recommended as alternatives to MB to control soilborne pests. Soilless culture treatments also showed promising results in our conditions.

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Table 1: Effects of different treatments on the incidence of fungi, bacteria and nematodes, and on the yield in **tomato**.

Treatments	**Fungi (10 gr/10^5)**			**Bacteria (10 gr/10^7)**			***Meloidogyne* spp. (J_2/100 cc soil)**			**Root-Gall Index**	**Total Yield**
	Before Treatments	After Treatments	At Harvest	Before Treatments	After Treatments	At Harvest	Before Treatments	After Treatments	At Harvest		
Control	39.2 a*	41.0 a	41.0 a	56.2 c	58.8 a	59.0 a	321.1 a	266.0 a	520.0 a	3.8 a	14940.9 d
Solarization	36.3 a	13.8 de	21.0 c	58.6 ab	16.4 d	21.3 cd	224.0 b	35.0 c	70.0 bc	1.5 b	17356.5 c
Solarization + Dichloroproene	38.6 a	13.0 de	20.6 c	62.4 a	9.5 e	16.4 d	284.0 ab	11.5 fg	40.0 de	0.8 bcde	19566.8 ab
Solarization + Metam sodium	31.2 a	11.7 e	19.5 c	55.6 c	8.5 e	16.5 d	290.3 a	30.3 cd	70.5 bc	1.14 bc	18157.4 bc
Solarization + Dazomet	29.9 a	16.4 de	21.0 c	54.6 c	20.9 cd	21.9 cd	255.8 ab	22.8 de	56.0 cd	1.06 bcd	19521.3 ab
Solarization + Bio fumigation	32.8 a	18.8 d	20.1 c	50.5 c	26.7 c	27.9 c	292.5 ab	18.5 ef	48.5 d	0.3 de	20597.5 a
Solarization + Bio control (m)	37.2 a	33.4 b	34.0 b	62.9 a	52.8 ab	53.2 ab	308.5 a	48.8 b	85.5 b	1.6 b	18006.7 bc
Solarization + Bio control (p)	35.3 a	25.3 c	25.9 c	57.8 c	49.3 b	51.5 b	260.0 ab	46.8 b	78.5 b	1.5 b	18636.7 bc
Methyl Bromid 100%	34.2 a	1.2 f	3.2 d	57.9 c	0.0 f	4.2 e	273.3 ab	3.5 g	8.5 f	0.2 e	19163.1 ab
Methyl Bromid 50%	34.0 a	2.5 f	6.4 d	56.5 c	2.0 f	8.5 e	276.0 ab	11.0 fg	30.0 e	0.4 cde	19138.9 ab

*:Treatments with the same letters are not significantly different according to Duncan Multiple Range Test, P=0.05

Table 2: Effects of different treatments on the incidence of fungi, bacteria and nematodes, and on the yield in **cucumber**.

Treatments	**Fungi (10 gr/10^5)**			**Bacteria (10 gr/10^7)**			***Meloidogyne* spp. (J_2/100 cc soil)**			**Root-Gall Index**	**Total Yield**
	Before Treatments	After Treatments	At Harvest	Before Treatments	After Treatments	At Harvest	Before Treatments	After Treatments	At Harvest		
Control	38.1 a*	38.4 a	39.1 a	61.8 a	57.4 a	60.7 a	177.0 a	130.0 a	375.0 a	2.3 a	6474.1 c
Solarization	35.7 a	14.9 c	20.9 d	53.1 a	17.9 cd	21.9 d	130.0 a	19.0 bc	58.5 bcd	1.4 b	7242.1 bc
Solarization + Dichloroproene	34.7 a	14.0 c	19.5 d	64.6 a	14.6 d	18.7 d	182.5 a	10.8 cd	20.0 gh	0.8 d	7874.3 ab
Solarization + Metam sodium	38.9 a	14.2 c	18.7 d	51.7 a	14.1 d	18.4 d	150.3 a	14.8 c	50.0 cde	1.2 bc	7844.9 ab
Solarization + Dazomet	36.4 a	15.6 c	20.1 d	55.6 a	16.2 d	20.3 d	155.0 a	13.3 cd	42.0 def	1.0 cd	6841.7 bc
Solarization + Bio fumigation	34.8 a	26.1 b	28.2 c	60.3 a	22.0 c	30.1 c	171.0 a	12.5 cd	40.0 ef	0.8 d	7646.5 abc
Solarization + Bio control (m)	33.3 a	24.5 b	33.6 b	55.4 a	50.2 b	55.7 b	166.0 a	25.3 b	60.5 bc	1.4 b	6337.0 c
Solarization + Bio control (p)	38.2 a	19.7 c	29.6 bc	57.1 a	48.3 b	52.3 b	147.0 a	26.5 b	68.5 b	1.5 b	6718.0 c
Methyl Bromid 100%	38.0 a	0.9 d	2.7 e	58.6 a	0.7 e	4.8 e	175.3 a	3.3 d	5.0 h	0.02 e	8066.7 ab
Methyl Bromid 50%	37.1 a	1.8 d	5.8 e	56.1 a	2.9 e	9.0 e	181.5 a	11.8 cd	26.0 fg	0.08 e	9249.2 a

*:Treatments with the same letters are not significantly different according to Duncan Multiple Range Test, P=0.05

ALTERNATIVES TO METHYL BROMIDE FOR SOIL TREATMENTS IN CUBA

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ABSTRACT

Methyl bromide (MB) has been used for soil treatments to control pests in Cuba since 1969. About 400 tonnes were used on a range of crops during the 1980's but in 1998 only 80 tonnes were consumed due to the development of alternatives and a national drive toward integrated pest management programmes. These programmes include control of weeds by disrupting seed development or desication of those weeds with vegetative organs, rotation crops, solarization, varieties of crops resistant to nematodes, biological control programmes for nematodes, limited chemical intervention, and the use of organic substrates. MB is no longer used in tobacco production due to the introduction of the floating tray technology. Grafted plants and bio-fumigation are under evaluation for use on protected crops such as tomato, pepper, water melon and cucumber. Steam has been used with success in seedbeds of tobacco but complicated procedures as well as high fuel costs make this alternative unattractive in Cuba.

Keywords: methyl bromide**,** alternatives, soil preparation, rotation crops, solarization, biological control, resistant varieties, IPM.

INTRODUCTION

Cuba has an agricultural economy whose development can be divided in four fundamental phases. The first prior to 1959 was characterized by a monoculture of sugar cane, the second after 1959 when there was a radical transformation starting from the laws of agrarian reform, the third was based on the recovery of fertile lands and the production was intensified, diversified and specialized, with increased yields due to chemicals and mechanization. Today, the last stage started in 1993 with qualitative and quantitative transformations that allowed conditions to be created for technological reconversion to low inputs, careful use of resources, use of biotechnological products, and with more emphasis on sustainability and preservation of the environment.

Of the cultivated land, except for the sugar cane and pasture, rice represents 25%; citrus 21%; vegetables, grains and tubers 17%; coffee and cocoa 13%; other fruit trees 9%; and tobacco 3%. In the last 10 years, an important increase has taken place in the production of vegetables, especially those grown under protected crop cultivation and in urban agriculture. The largest increases have been in cut-flower and ornamental plant production.

Methyl bromide (MB) has been used for soil treatments to control pests in Cuba since 1969. At the moment, it is used in tobacco seedbeds, protected cultivation of tomato, pepper, watermelon, cucumber, flowers and ornamental plants and substrate for coffee nursery. Large quantities of MB were used in 1980-81 when annual consumption was 400 tonnes; and during the decade of the 1980's. In 1990, 133 tonnes were used due to a programme of remarkable reduction in the use of this MB. In 1998 only 80 tonnes were consumed.

The introduction of the protected crops between 1990 and 1995 was associated with the use of MB to eliminate nematodes, soil borne disease and weeds in tomato, pepper, water melon, cucumber and other crops that have a current demand of 20 tonnes. Two tonnes were used in cut-flowers and ornamental plants. One tonne was used coffee nursery plantations. Despite increased areas dedicated to these protected crops in the last few years, the consumption of MB has not increased in the same proportion as alternatives have been sought to replace MB that would be viable under an integrated pest management programme.

The Government of Cuba endeavours to implement its commitments to the Montreal Protocol, some of them financed by the Protocol through implementing agencies like UNIDO in the project of “Phasing Out Methyl bromide in the Tobacco Sector in Cuba.” In addition, Cuba has established a system of licenses for import and export of ozone depleting substances which guarantees freezing the quantities and ensures successive elimination of these substances.

The main species of pests present in the soil that justify the use of MB in different crops include: the fungi *Phytophthora spp, Rhizoctonia solani* Kuhn, *Phythium spp*. and *Fusarium spp*; the nematodes *Meloidogyne incognita* (Kodoif and White) Chitwood and *M. arenaria*; mites of the genus *Rhizoglyphus;* and weeds *Cyperus rotundus* L., *C. esculentus* L*., Amaranthus dubius* Mart*, Parthenium hysterophorus* L., *Echinochloa colonum* L., *Eleusine indicates* L., *Rottboellia cochinchinensis* (Lour) Clayton and *Sorghum halepense* (L) Pers.

ALTERNATIVES TO METHYL BROMIDE

Soil preparation

Weeds that reproduce via seeds with a high viability can be controlled manually with great effort immediately before the flowering period and rain season. This is carried out with different kinds of implements. On the other hand, those that reproduce by vegetative organs (rhizomes, stolons, tubers, basal bulbs) require tiller and multiplow to bring to the surface the self generating organs during the dry period in order to desicate them. With 3-4 tiller passes at the end of the preparation this species decrease substantially.

The time between the manual effort to kill the weeds that reproduce by seeds is linked to the conditions existing for their germination in which rain plays a main role. Under normal conditions during the rainy period, 12-15 days between efforts are required. Those weeds that reproduce vegetatively, once the rest of the plants have risen to the surface of the soil, the time to wait depends on the susceptibility of these organs to desiccation and loss of viability. For example, *C. rotundus* requires 7-9 days, and *S. halepense* requires 8-10 days (Pérez *et al.* 1999).

Since the radical systems of several plants or their underground organs continue to live and serve as host to the nematodes, mites and fungi for weeks or months after the crop, and the fact that several species of these parasites have a high survival even without a host, it is necessary to expose these parasites to the sun to contribute to their gradual mortality. In Cuba, *Meloidogyne spp*. was largely eliminated using a disk plow to invert the soil prism in combination with tiller every 25 days for 60-90 days (Fernández 1999).

Rotation crops

Rotation crops have a big influence in the composition and population of weeds. In Cuba, the intercropping (spring crops) affect notably the incidence of annual and perennial gramineous, *C. rotundus* and *Amaranthus spp* as this proliferate in the most favorable conditions of humidity and temperature. As an exception, *P. hysterophorus L.* is distributed by the effects of the main crops. In general, variable rotations lead to the best results because they do not allow the conditions that contribute to the population growth of certain species of plants to occur repeatedly (Pérez *et al.*1990).

Some systems of rotations have been included as part of an integrated pest management programme for weeds in Cuba. To control *R. cochinchinensis* and *S. halepense,* begin with sweet potato as permanent or variable intercropping and follow this by leguminous plants such as soy and velvet bean. More than two leguminous crops reduces the incidence of these weeds, but yield is reduced. To eliminate *C. rotundus,* fallow periods with intercropping of sweet potato and corn is used.

The benefit of the potential allelopathic properties of the crops can be shown by sowing corn at 45x25 cm spacing and incorporating fresh mass to the soil so that it reaches 50-60 cm of height with a production of vegetable mass of 6.4 kg/m^2. This reduces the populations of *R. cochinchinensis*, *C. rotundus* and *S. halepense* (Labrada 1990). Equally, by sowing and incorporating *Stizolobium deeringianum Bort, the* weeds are eliminated and in the next tobacco crop the use of herbicides decrease by 20%. Intercropping of *Sorghum vulgare* Pers reduces the incidence of *C. rotundus* (Fernández *et al.* 1990). In general, the leguminous *Canabalia ensiformis (L)* and *S. deeringianum* were important species in a rotation system because of their associated production of toxins.

Succession in horticultural crops is recommended according to their susceptibility to *Meloidogyne spp*. It is known that there are very susceptible crops such as tomato, eggplant, lettuce and water melon; moderately susceptible crops such as cabbage and cauliflower; and lightly susceptible crops such as garlic and onion. Similarly, crops such as peanut, sesame, corn or the resistant variety of sweet potato CEMSA 78-354 can precede susceptible crops. Good results have been obtained in the elimination of *Meloidogyne* in tobacco with the rotation of peanut, millet, velvet bean, corn and sesame (Fernández *et al.* 1990).

The use of rotation crops for 3 consecutive years is effective for reducing the incidence of *P. nicotianae var. nicotianae* in tobacco.

Solarization

Solarization in Cuba requires about 45-60 days in periods of high temperature and sunshine that occur in July and August. As solarisation is expensive and complicated to use, it is recommended for the control of nematodes, soil born disease and weeds in seedbeds (Fernández *et al.* 1990). Good results were achieved using solarization to control *Meloidogyne*, several weeds and *P. nicotianae, R. solani* in traditional tobacco seedbed, protected crops, urban agriculture and hydroponics with located watering (Fernández 1999). To obtain the best results with solarization, it is necessary to ensure the soil is fluffy before covering, that an appropriate hermetic seal over the soil is achieved, that sufficient time is allowed, that it is carried out in the appropriate season and that infestations after solarization are avoided.

Resistant varieties

The use of resistant or tolerant varieties is economic and environmentally sure method. Today, there are some commercial varieties of tomato, tobacco and pepper that are resistant to *M. incognita* (Sasser 1989; Montes *et al.* 1998). Nevertheless, these varieties should be managed carefully, because the continuous sowing in the same area can select species or races that are in the minority initially but they can converted by resistance so that they dominate. Such a case was reported in tobacco in South Carolina where the indiscriminate sowing of varieties like “Speight G-28” controlled *M. incognita* race 1, but there emerged with force race 2 as well as *M. arenaria* (Fortum *et al.* 1994). This situation also occurred in Cuba, but the new dominant species is *M. javanica* for which a resistance source does not exist in the genus *Nicotiana* (Fernández 1999).

Recently, the varieties of tobacco “Habana-92”, “Habana-2000” and “Corojo especial” have been introduced that are less affected by ***P. nicotianae,*** compared with the traditional varieties " Criollo” and “Pelo de Oro."

BIOLOGICAL CONTROL

Trichoderma spp.

Recently in Cuba, some research has been undertaken to evaluate the use of species of the genus *Trichoderma* for biological control of *P. nicotianae; P. capsici; Pythium aphanidermatum; Sclerotium. rolfsii* and *Fusarium spp.* Their application to the soil and substrate in a preventive treatment in seedbeds or different stages crops reduces the appearance of soil borne disease caused by fungus in tobacco, pepper and tomato. *Trichoderma* could be produced by handmade methods on solid supports or in static liquid media using by-products of the sugar industry as raw materials. So much the biomass of the fungi formed for conidia, clamidospora and mycelia as the metabolites, they exercise an effective control against the sensitive pathogens. Of the *T. harzianum* strains isolated, the most promising for the control of *P. nicotianae* are "A-53" and "A-84" which give good control of the disease under field conditions (Stefanova & Sandoval 1995)

Among the benefits noted that contribute to the treatment with *Trichoderma spp* has been the stimulant effect on plant growth in different crops.

Micorriza-arbuscular

Several experiments have been carried out with the objective of knowing the interactions that settle down in the soil between the fungus formed of Micorriza-arbuscular and different pathogens of plants. The effectiveness of the micorrizas as inhibitors of the action of these noxious organisms has been demonstrated.

Experiments carried out in Cuba showed that tobacco plants treated with micorriza are less affected by *P. nicotianae.* The level of reduction of the disease compared with non treated plants depended on the strain used. *Glomus mosseae* (strain "Habana") was the most effective in the reducing the disease (80%), while other strain decreased infection between 20 and 50%, compared to 100% of plants infected without treatment. The interactions of micorriza-host-pathogen depend on the environmental conditions and the soil type. This is being taken into account in future research programmes, particularly for control of *P. nicotianae* in tobacco cultivated in sandy soil.

Paecilomyces lilacinus

P. lilacinus is a fungus which parasitises the eggs and adults of several species of a nematode phyto-parasite. It also produces toxins. Both effects are manifested in acid soil. Good results have been reported for the control of tropical nematodes *Meloidogyne spp* and *Radopholus similis* in diverse countries using products that are made locally or produced commercially. This fungus in Cuba has been produced locally and has produced good results in field conditions for control of *Meloidogyne spp* and *Radopholus similis* through preventive application to *in-vitro* banana and coffee plants that are inoculated in nurseries and then planted (Fernández 1999).

Bacillus thuringiensis

A native strain of *Bt.*, effective for controlling field populations of *R. similiis* and *P. coffeae,* is under evaluation (Fernández 1999).

CHEMICAL PRODUCTS

The use of pesticides to control most soil pests is the easiest and most effective method. However, the current tendency is toward a decrease in pesticide use. Cuba has evaluated many chemical pesticides as alternative to MB, among these currently registered for use in different cultivations are: Aldicarb, carbofuran and fenamifos for nematodes; azoxystrobina, benalaxylo + mancozeb, captan, metalaxyl, propamocarb hydrochloro against fungi of the soil, mainly *P. nicotianae;* napropamida, napropamida + metribuzin, oxadiazon, oxifluorfen, glyphosate for weeds; and dazomet as soil sterilizer.

ORGANIC SUBSTRATES

Cuba has achieved good control of *Meloidogyne* with the phlegm of sugar cane semi-composted and applied into the holes of transplanted perennial plants. Incorporation into the soil of cruciferous crops like cabbage after it has been broken into fragments and lightly fermented was reported to reduce substantially nematode populations due to the formation of toxic compounds such as ammonia and several sulphurous compounds.

INTEGRATED PEST MANAGEMENT IN CUBA

Research has been carried out with the objective of having different alternatives for the management of pests, diseases and weeds in different crops. One of the basic aspects constitutes the development of quick methods for the diagnosis and prognosis of the main species of pests and evaluation of scales established as damage thresholds. Success has been achieved in the selection of areas for IPM, the use of practical agronomic techniques, the use of chemical alternatives, and the use of biological control against insects, disease and nematodes.

An example of these results is the use of IPM and floating tray technology in traditional tobacco seedbeds where crop rotation is limited. Cuba has developed an unique solution consisting of organic substrate composed of black pit or compost of sugar cane phlegm (80%) + husk of rice (20%), mixed with *T.harzianum* to 1kg/ha in two times. In general, biological control is given priority for pest control. This technology provides healthy plants of standard size with a vigorous and protected root system. This technology requires rigorous control of quality during the production process that includes periodic monitoring of the main noxious organisms that can prosper in the humid atmosphere and under conditions of sterilized substrate, and that can prosper in the transmission of any infection when the leaves are pruned. The introduction of this technology has totally eliminated MB in tobacco cultivation in Cuba.

OTHER METHODS

Grafted plants and bio-fumigation are under evaluation for use on protected crops such as tomato, pepper, water melon and cucumber. Steam has been used with success in seedbeds of tobacco but complicated procedures as well as high fuel costs make this alternative unattractive in Cuba.

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BIOFUMIGATION AS AN ALTERNATIVE TO METHYL BROMIDE

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ABSTRACT

Biofumigation is based on the management of the bio-decomposition process of organic matter, which produces volatile substances that, by being kept in the soil, are able to regulate the population of plant parasitic organisms. Results confirm that biofumigation is as effective as conventional pesticides in the control of fungi, nematodes, insects and weeds. The practice of biofumigation increases the biodiversity of edaphic fauna, also improving physical and chemical soil properties. The main limiting factor to biofumigation is the cost of transporting biofumigants; therefore, it is important to use local resources. Biofumigation is shown to increase its efficacy over time, since harmonic systems of production are planned, based on the management of biological and environmental diversity.

Keywords: nematodes, organic matter, soil ecology, crop protection, agroecology.

INTRODUCTION

New paths of research have been opened up in crop protection, stemming from the action of organic matter, which produces gases in the process of its decomposition, that can effect the control of plant pathogenic organisms since they work as fumigants. This process is defined as biofumigation and was included as a non-chemical alternative to methyl bromide (MB) by the Montreal Protocol's Methyl Bromide Technical Committee (MBTOC 1997).

Our research has permitted biofumigation to be defined as: "the action of volatile substances produced during the bio-decomposition of organic matter for plant pathogen control." Biofumigation increases its effectiveness over time when it forms a part of an integrated production system. It has been found that generally any organic remains could act as a biofumigant, their efficacy depending on their characteristics, dose and method of application. Excellent examples of the application of biofumigation exist in vegetables, strawberry, pepper, tomato, citrus fruit, banana, grape and cut-flowers in Spain. The most utilized biofumigants are goat, sheep and cow manure, and crop remains from rice, mushroom, olive, brasica and ornamental gardens. It has been demonstrated to be just as effective as conventional pesticides in controlling nematodes, fungus, insects, bacteria, and weeds, and can regulate problems with viruses by controlling vector organisms (Bello *et al*. 2000).

This paper describes the methodology for applying biofumigation in the field in a form as accessible to technicians as farmers. The section on results evaluates first of all the efficacy of nematode control by various biofumigants from animal sources, green manure and agro-industrial remains. Lastly a synthesis is made of the principal results obtained. Three representative cases are selected that correspond to the use of biofumigation in vegetable crops at El Perelló (Sueca, Valencia), pepper crops in Murcia and tomato and melon in Uruguay.

METHODS

The methods of application of biofumigation were described by Bello *et al.* (2000), which indicated that it was an easy technique for farmers and technicians to apply, since it only differs from organic matter amendments in the choice of the biofumigant, which should be partly decomposed, and in the method of application. This method must take into account the necessity of retaining for at least two weeks the biofumigant gases produced from the bio-decomposition of organic matter, since their effect in the majority of cases is not biocide but rather biostatic. Therefore it is necessary to prolong the time of their action on the pathogens. It has been demonstrated that any agro-industrial remains, or combination thereof with a C/N relation between 8-20, can have a biofumigant effect. The use of a dose of 50 t/ha (tonnes per hectare) is recommended, although when problems with nematodes or fungus are very serious, the dose should be increased to 100 t/ha, which can be reduced through crop techniques such as application in furrows. The biofumigant should be uniformly spread so that focuses of pathogens do not appear in the future, which could create problems for the crop. Once the biofumigant is spread, it should be incorporated immediately into the soil, tilled once with a rotovator, and watered preferably by spraying until the soil is completely saturated.

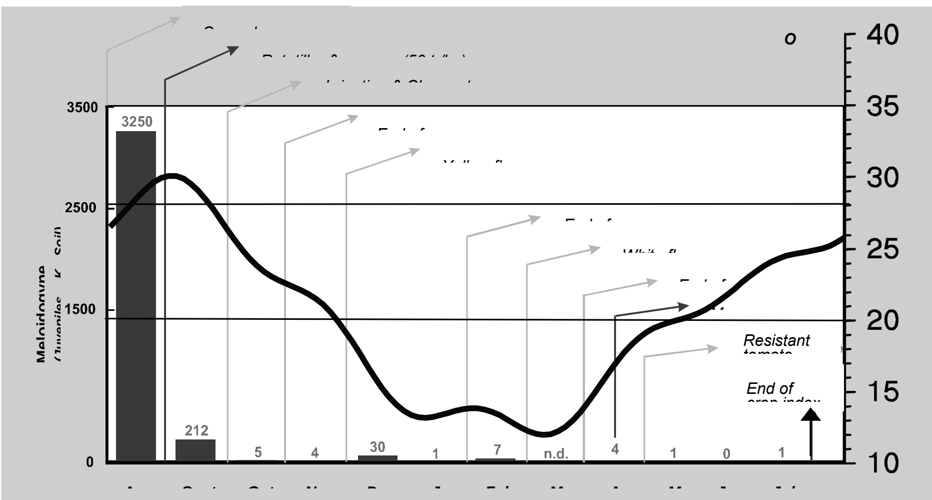
RESULTS AND DISCUSION

The great majority of the biofumigants selected were found to have an efficacy of 100% in the control of *Meloidogyne incognita*, with root knot indexes in Marmande cv. tomato plants, sensitive to this nematode, that did not exceed a value of 3.8 according to Bridge and Page's (1980) scale of 1 to 10. Values of efficacy under 100%, as well as higher indexes, are a consequence of possible errors in application or the absence of an activating substance. On the other hand, an increase in saprophagous nematodes was generally observed.

In relation to the effect of biofumigation on the height of Marmande cv. tomato plants cultivated on treated soils, values have generally been higher or, in some cases, similar to the control group. Biofumigation can also have a benevolent effect on the physical properties of the soil, particularly in respect to its compactness. In relation to the chemical properties of the soil, a general increase is observed in the majority of the parameters determined, with the exception of Ca, which presents a tendency to decrease, as well as Na, to a lesser degree. In some treatments with brasica, rice hull, sugar cane husks, wet olive pomace, and wheat straw, a drop in nitrogen content and organic matter may appear which shows up as decrease in the biomass of the Marmande cv. tomato plant grown on soils biofumigated with these materials. Therefore, it would be necessary to establish fertilization programs that would take into account the characteristics of the biofumigant and the soil where it is applied.

The cost of biofumigation is very low when local resources are used, as in the case of green manure or agro-industrial remains. Treatments with animal manure can be more expensive, especially due to transportation, although the cost can be reduced by means of the use of agricultural techniques that lower the dose of application. It must also be taken into consideration that in many crops the use of organic matter is a normal practice, differing from biofumigation only in the characteristics of the organic matter as well as the dose and method of application.

Biofumigation in vegetables at El Perelló (Valencia, Spain) (Bello *et al.* 1996) (Figure 1).

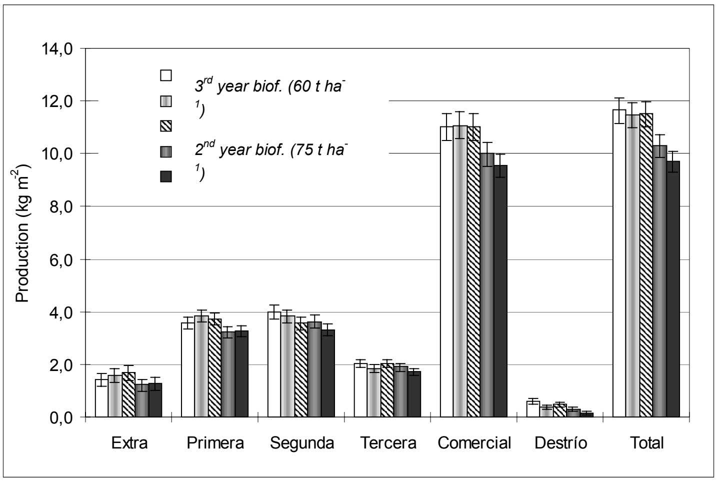


Biofumigation was carried out using a mixture of sheep manure and mushroom remains at a dose of 50 t/ha and applied in the month of August. This was followed by cultivation for about two months of trap plants consisting of short-cycle Chinese vegetables as they disrupt the biological cycle of the *M. incognita* nematode, the principal phytopathological problem in the area. The population reduction avoids the possible development of virulent populations which could appear after using tomato resistant varieties with *Mi* gen, helping in that way to reduce the total number of nematodes at the end of the tomato crop. This permitted the cultivation of sensitive plants in the second year such as cucumber or melon.

Figure 1. Biofumigation and integrated production in vegetables at El Perelló (Valencia, Spain) (Bello *et al*. 1996).

Biofumigation in pepper crops at Murcia, Spain (Bello *et al.* 2001a) (Figure 2). A mixture of sheep and chicken manure was used which was applied with solarization at a dose of 100 t/ha, during the month of August, in a greenhouse affected by *M. incognita*, strain 1, at Campo de Cartagena (Murcia). The results of continued biofumigation treatments for one, two and three years were analyzed, compared with the control group and with the MB treatments. Biofumigation provided a similar level of effectiveness as MB in the control of *M. incognita* and weeds. Some difficulty could appear in the first biofumigation treatments, but with time, the farmer may become familiar with the method and select mixtures of biofumigants and establish the most effective doses.

Figure 2. Average production by quality classes in 2000-01 campaign. LSD Intervals to 95% with Log $_{10}$(x+1) transformed data (Bello *et al.* 2001).



Biofumigation in tomato and melon crops in Uruguay. Biofumigation consisted of the application of various types of organic matter such as chicken manure plus rice hull, sheep manure, cow manure, chicken manure, crop remains, agro-industrial remains and compost (De León *et al.* 2000, 2001). These treatments were complemented with other crop practices such as crop rotation, the use of vegetable coverings and tolerant cultivars or those sensitive to *Meloidogyne*. The results obtained showed that the proposed management systems had a high efficacy in the control of *M. arenaria* and *M. incognita*. The positive effects of biofumigation on physical and chemical soil parameters were outstanding. Through the design of these alternative horticultural systems, the decrease and even elimination of the use of agrochemicals are achievable as well as a reduction in production costs (Table 1, see next page).

CONCLUSIONS

The effectiveness of biofumigation is similar to that of conventional pesticides, but at the same time, biofumigation improves soil and plant characteristics. When using biofumigation, it will be necessary to design crop production methodology for each situation. The efficacy of biofumigation is increased with time when it forms a part of an integrated production system.

The cost of biofumigation can be inexpensive, especially when animal manures, green manure or agricultural remains are used. It is actually an organic amendment and the differences are principally in the characteristics of the organic matter, the dose and the method of application. The use of local resources as biofumigants is recommended as one of the principal costs of transport can be reduced. Problems with soil fertilization and plant nutrition can arise, such as a deficit in nitrogen, but they can all be solved with adequate fertilization.

Table 1: Cost in US dollars of production using biofumigation in tomato and melon crops in Uruguay[1].

CROP / CRITERIA	PRODUCTIVE SYSTEM	
	CONVENTIONAL	**BIOFUMIGATED**
TOMATO [1]:		
Variety	Facundo (sensible)	Tommy RN (resistant)
Length of crop (Months)	6	5.5
Total (kg)	8,700	7,910
Kg per plant	4.7	4.7
kg m^{-2}	12.2	11.5
Production costs	486	376
Gross income	**2,583**	**2,707**
MELON [2]:		
Variety	Galia	Galia
Length of crop (Months)	5	5
Total (kg)	758	444.2
Kg per plant	438	484.8
kg m^{-2}	8,550	9,150
Production costs	1,618.9	1,351.9
Gross income	**3,505.5**	**3,751.5**

[1] Labour is not included; [2] Average cost on 15 farms in a conventional system

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ALTERNATIVES TO METHYL BROMIDE FOR TOMATO PRODUCTION IN BELGIUM

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ABSTRACT

Belgium has 40 years of soil disinfestation experience with the use of methyl bromide (MB) and its alternatives that have been used for growing tomatoes in soil and for intensive production of vegetables. Belgium's use of MB declined in 2001 to 102 tonnes, approximately one-third of the amount consumed in 1991, due to adoption of substrates such as rockwool, grower fear of exceeding bromide residues in MB-fumigated crops, exclusion of MB from crops grown organically and a decline in intensive agricultural land. At present, alternatives to MB are important not so much because of the restrictions on future use imposed by the Montreal Protocol but because there are several new methods under development with which Belgium already has experience including integrated soil inoculation, 'partial substrate culture', 'bio-fertilisers' and 'microbiological soil analyses'. This paper discusses these developments and other concepts that together make up the building blocks for the future success of horticulture.

Keywords: biofertiliser, biopesticide, biosoil amendment, coconut fibre substrate, ecological boomerang, integrated soil fumigation, microbiological soil analysis, partial substrate, partial laying-fallow, soil inoculation, virtually impermeable film

INTRODUCTION

Belgium is a country with a rapidly evolving horticulture. Within the intensive vegetable sector, tomatoes are financially the most important crop. Approximately 75 to 80% of the tomatoes are produced on rockwool substrate without the need for disinfestation. The remaining tomato production is, just like the production of lettuce and several other vegetables, soil-bound and still requires soil disinfestation. Statistics on soil fumigation in Belgium will therefore always relate to the intensive, soil-bound vegetable sector.

For about four decades, horticultural soils have been fumigated in Belgium with a battery of physical and chemical soil sterilization methods. Soils for intensive vegetable cultivation under glass or outdoors used to be almost exclusively chemically disinfested, and this mainly carried out by registered soil fumigators. Approximately 40% of these chemical soil sterilizations were done with MB, the remainder with chloropicrin, dichloropropene (1,3-D) and metam-sodium.

The state of affairs in 2001 looked completely different as chemical alternatives to MB have gained the upper hand. Could this be attributed to the restrictions imposed by the Montreal Protocol? Which MB alternatives or alternative combinations show great promise for the Belgian grower?

SOIL FUMIGATION IN BELGIUM

For over 40 years, soil disinfestations have been carried out in Belgium in order to eliminate accumulated microbiological soil sickness. This is the result of a insufficient crop rotation and an intensive monoculture. Approximately 20% of the intensive vegetable cultivation is sterilized by growers themselves using steam or dazomet. The remaining 80% is disinfested by registered soil fumigators who use strong chemical fumigants like MB and chloropicrin, as well as the weaker alternatives like 1,3-D and metam-sodium. The use of steam as a viable alternative remains relatively unimportant because of the hard labour required, the big energy consumption and the high price.

In Belgium MB has been continuously decreasing in significance and its consumption in 2000 was about one-third of the amount consumed in 1991. Estimated amounts in 2001 show consumption to have declined still further to about 10% of 1991 levels (Table 1).

Table 1: Reduced use of MB (tonnes) in Belgium[1]

1991[2]	1992	1993	1994	1995[2]	1996	1997	1998[3]	1999	2000	2001[3]
312.09	267.32	289.28	201.46	221.12	186.667	180.21	127.46	145.60	102.17	33.00[4]

[1] Mr. Houins - Belgian Ministry of Agriculture - [2] Reference Years (Montreal Protocol); [3] Obligatory reductions: 25% in '98 and 50% in '01 (Montreal Protocol) – [4] Estimated value.

What are the reasons for this remarkable decline in the use of MB? First of all the great attraction of the substrate culture. Indeed, 75 to 80% of the tomatoes and as much as 100% of the cucumbers and sweet peppers are now grown on substrates. Only lettuce remains 100% soil-cultivated. The precise management of crops on a thin substrate layer, the urge to implement innovations and the initially interest in 'premium prices' have made a lot of growers switch over from soil to substrate culture. This automatically resulted in a large decrease of MB for soil fumigation and implied that the introduction of a MB ceiling of 25% in 1998 and 50% in 2001 imposed by the Montreal Protocol, compared to the 1991 base level, did not pose a problem for Belgium.

So we can safely say that substrate culture is a very important MB alternative for tomatoes in Belgium, although this growing method also has to contend with typical 'substrate born pests and diseases'. These problems can be avoided by replacing the old substrate or by vacuum steaming the substrate slabs in special containers. Growing in these kinds of artificial and watery substrate slabs is not only expensive but it also entails controlling new and/or obstinate diseases and pests which often necessitates an increased use of chemicals.

A second important motive for adopting alternatives to MB is the fear of exceeding bromide residues in harvested vegetables. The enforced maximum residue levels in Belgium of 30 ppm bromide for tomatoes and 50 ppm for lettuce are being controlled more rigorously than ever and more often than in any other country. A grower that exceeds the permitted level must destroy the crop that is in non-compliance.

A third reason for the considerable increase in the use of MB alternatives is growers' uncertainty over the extent of the Belgian government's liability (dated 22 July 2000) for a crop that exceeds the maximum permitted residue level and that may not control pests and diseases adequately when a mandatory, half-dose of MB (4.5 kg/100m²) is applied under virtually impermeable film (VIF). However, grower scepticism appears to be unfounded as the soil pest and diseases are being controlled and the bromide residue norms are not being exceeded due to additional aeration and leaching measures. One could expect that increasing grower confidence in MB with VIF will increase the use of MB to more than 50 tonnes again!

Certain growers with a classic crop rotation system under glass of 'summer tomatoes - autumn lettuce - spring lettuce', have withdrawn themselves from the obligations for the use of MB under VIF by using the 'partial substrate culture' system. They still grow soil-bound lettuce, but they cover the soil up with plastic during the summer, on which they grow summer tomatoes in buckets filled with coir (coconut fibre substrate). In comparison with rock wool, coir substrate is cheaper and better naturally recyclable. Very little is known about the prevention and controlling of 'coir born pests and diseases' in this organic medium. The soil is in every way temporarily unused, therefore this technique could also be labelled as 'partial laying-fallow'.

A fourth reason for reduced MB consumption is possibly due to the exclusion of MB in the cultivation guidelines for 'Organic Food' and 'Organic Farming'.

Finally, the area of the intensive horticulture and consequently also the number of soil fumigations with MB, have been decreasing slightly over the past 10 years in Belgium.

Table 2 shows that in 5 years time the share of MB alternatives for soil fumigation, has been increasing considerably. In 1996 still 50% of the area of intensive outdoor vegetable growing and the cultivation under glass was being disinfested with strong fumigants of which 45% were with MB. The remaining 50% was being disinfected with weaker MB alternatives, of which 45% was 1,3-D. In 2001, the situation has turned over completely: 75% of weaker soil disinfectants of which 65% are 1,3-D, and 30% of strong soil fumigants of which 25% is MB.

Table 2: Use of chemical alternatives (%) to MB that became more important in Belgium[1]

		Percent in 1996	Percent in 2001
MB-ALTERNATIVES TOTAL:		55	75
Strong:	Chloropicrin	5	5
Weak:	Dichloropropene	45	65
	Metham-sodium	5	5
MB-TOTAL		45	25

[1] SEGO (Special Registered Soil Fumigators Belgium) and De Ceuster Soil Ennoblement (Belgium)

METHYL BROMIDE ALTERNATIVES

What are the experiences with these chemical MB alternatives in Belgium? First of all, they have a smaller and/or weaker action spectrum, as is shown in Table 3. They are therefore less effective for growers, especially with a very intensive monoculture e.g. all the year round lettuce growing 6-7 crops per year, and/or with less favourable growth circumstances and thus with greater disease and pest pressure.

Table 3: Action spectrum of chemical soil disinfestants[1]

	FUNGICIDE (F)	NEMATOCIDE (N)	INSECTICIDE (I)	HERBICIDE (H)
Methylbromide	FFFFF	NNNNN	IIIII	**HHHHH**
Chloropicrin	**FFFF**	**N**	**IIIII**	**HH**
Dichloropropene	**FFF**	**NNNN**	**IIII**	**HHH**
Metham-sodium	FFF	**N**	IIII	HH

[1] Actual and practical appreciation scheme for chemical soil disinfestants by SEGO (Special Registered Soil Fumigators Belgium) and De Ceuster Soil Ennoblement (Belgium)

Furthermore the weaker chemical alternatives for MB, like chloropicrin, 1,3-D and metam-sodium have several significant disadvantages. At present the concern is whether or not these alternatives have a direct, negative impact on man and environment. Because of their limited action spectrum, it is very likely that in the future growers will be applying more pesticides during cultivation. The residue cocktail of these cultural crop protection agents is unpredictable and in all respects harder to trace than the bromide residues in soil and crops. What is more, the repeated and single use of weak chemical soil disinfection alternatives can lead to uncontrollable, cumulative, soil sickness and/or biological adaptation of the soil. Such biological aberrations and/or complete ineffectiveness of the soil disinfestation agents have an ecological boomerang effect on the grower.

When selecting new disinfestation alternatives or alternative combinations, Belgian growers and soil fumigators are always guided by at least 3 parameters: the efficient action spectrum, the absence of harmful residues in harvested vegetables and the safety for the workers and the environment.

Which MB alternatives or alternative combinations show real promise for the Belgian grower in the future? Whether or not the substrate culture will become an even more important alternative for soil-bound vegetable growing and thus for MB soil disinfestations depends entirely on the consumer. Because debates on quality, taste and storage life of substrate vegetables, as well as about the increased use of pesticides in artificial substrates, will continue.

A new, beautiful, organically-based variation on the substrate culture, namely the 'partial coir substrate' or the 'partial laying-fallow system', offers promise providing sufficient microbiological diversity can be maintained in the rhizosphere.

Grafted plants and disease- and pest-resistant varieties are partial alternatives for MB. However, their protection spectrum is often very limited and in addition plant pathogens regularly break through their resistance. Besides disease and pest pressure differs from year to year.

As a matter of fact, an ideal and reliable substitute for the simple but extremely efficient molecule of MB has yet to be discovered. Many of the available alternatives still have to prove their worth in time and space. Until then MB can - as half a dose applied in a_low-emission manner under VIF - be appointed the useful role of 'correction tool'. Particularly for those cases of diagnosed soil sickness, which can not be controlled anymore by means of classic chemical or physical substitutes. A good follow-up of the bromide residue danger is still highly recommendable. It's a sad paradox that the same worldwide efforts are not made to develop workable and valid MB alternatives as the efforts that have already been made to simply ban MB. Perhaps a better application of chemical alternatives to MB can open up new opportunities for pest and disease control. Probably the action spectrum of weaker soil fumigants can be improved by applying them under VIF.

The additional inoculation of microbiological preparations (antagonists) with chemical disinfestation, so called integrated soil disinfestation, results in increased efficacy e.g., against *Pyrenochaete* or 'corky root' with tomatoes. Integrated soil disinfestation will definitely reduce pesticide use and even provide a certain after-protection. It would be a welcome prospect to know that better control of resistant forms that are hard to destroy like sclerotes of *Sclerotinia*, chlamydospores of *Fusarium* or pseudosclerotes of *Rhizoctonia* could be obtained. Provided that the soil is inoculated regularly, the frequency of the soil fumigations can be reduced. Over the last 17 years, Belgium has gained a wealth of practical experience and know-how concerning integrated soil fumigation. When using chemical MB alternatives frequently and monotonously, it is imperative to avoid biological adaptation. This is achievable with the aid of an early detection technique. In this context, Belgium has already developed an adaptation test for metam-sodium. It is also very useful to alternate chemical soil fumigants and/or to diversify microbiologically the disinfested soil with microbial preparations.

Nonetheless, horticulture in Belgium will, despite the limited available quantity for 'critical use', have to miss the comfort and the efficiency of soil fumigations with MB from 2005 onwards. Growers and soil fumigators will have to be extremely attentive to assessment and application errors. It will become of crucial importance to make the correct and ultra-early disease and pest diagnoses by means of a microbiological soil analysis, because it is only there and then that the (weak) biological inoculations stand a chance. These kinds of analyses give us an idea of the microbiological diversity in the rhizosphere and a 'forecast' of any imbalance. This technique draws a picture of the degree of infestation in the soil, of biological buffering and of the biological protection thanks to the natural presence of antagonists. The analyses will advise us on whether soil fumigation, soil inoculation or the combination of both are required. Belgium already has 7 years of experience in pest and disease monitoring.

Growers often cultivate in a biological vacuum, as is the case with recently fumigated soils or new potting soils. This is fundamentally wrong since pest and disease incidence can originate from organisms that remain viable but resistant after treatment, or they may immigrate from external sources to more easily occupy open 'niches' than the slower but useful antagonists. Consequently 'Good Horticultural Practice' obliges growers to avoid incidences of a biological vacuum in the rhizosphere by means of induction and imitation of beneficial crop rotation effects. In fact, more is needed as such potential infection sources (irrigation water, composts, substrates) must be microbiologically "dammed up" and even armed with suppressive 'bio-fertilisers'. It is better to start a new crop with a positive predominance in the rhizosphere than with a biological balance.

Microbiological preparations are often conveniently, and without all too much microbiological insight, classified as 'bio-pesticides' which results in all the unpleasant obligations of expensive and time-consuming registrations. It would be much more correct and justified to apply them – more preventively than preventive – as 'bio-fertilisers' or as 'bio-soil amendments'. In this context, the opponents of MB could support the horticultural industry by putting in place more favourable approval and legislative procedures to allow more widespread use of these natural, non-genetically modified, microbiological preparations. This may be possible under the terms of a fertiliser legislation, for example.

Finally, it should be pointed out that a healthy tomato and vegetable production in Belgium or elsewhere can only be made possible by the creation of an oxygenous rhizosphere, which stimulates the roots and increases the self-defence capacity of plants. The aerobic 'beneficials' in the soil also benefit extensively from this. Also a strong, intrinsic plant growth based on a mineral-rich, slow-release nutrition which does not stress the plants but nourishes them in a well-balanced and holistic way, can be highly recommended. The rules of good horticultural practice will have to be thought over, rewritten and complied with again, and this applies to Belgium as well.

CONCLUSIONS

Alternatives for MB in Belgium have become more important than MB itself. The switch from soil to substrate culture has made soil fumigations redundant in the cultivation of tomatoes and several other vegetables. In the soil-bound vegetable culture the grower can rely on many years of experience with chemical alternatives of which the shortcomings and possibilities are well-known and which could be applied and combined even more efficiently. Belgium has acquired a wealth of experience with innovations, like microbiological soil analyses, integrated soil fumigation, soil inoculation and suppressive substrates. Belgian growers and soil fumigators are asking to keep MB at their disposal as a 'correction tool', as long as no worthy and affordable substitute has been found. Besides there's still a great demand for recognition and a favourable validation of 'bio-fertilisers' or 'bio-soil amendments'. This would enable a symbiotic cooperation between growers and ecologists, while the truth about MB and the ozone layer still remains hazy!

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