ALTERNATIVES TO METHYL BROMIDE TO CONTROL BLACK SHANK DISEASE (*PHYTOPHTHORA NICOTIANAE* BREDA DE HAAN) IN TOBACCO IN CUBA

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ABSTRACT

Cultural, biological, and physical alternatives were investigated as methyl bromide and some other fungicides such as metalaxyl were shown to be ineffective in preventing epidemics of Black Shank Disease (*Phytophthora nicotianae* Breda de Haan) in tobacco in Cuba. Soil solarization for 30 days, the use of *Glomus spp*, and the use of the *Trichoderma harzianum* according to *P. nicotianae* soil infection levels, were all highly effective in reducing *P. nicotianae* inoculum levels and disease incidence. Disease incidence was also reduced by selecting areas for the seedbeds free of this disease and by using crop rotation in the seedbed production areas.

Keywords: Black Shank Disease, tobacco, Cuba, solarization, seedbeds, methyl bromide, alternatives

FLOATING TRAY TECHNOLOGY WITH ORGANIC SUBSTRATE AND BIOLOGICAL CONTROL AS AN ALTERNATIVE TO METHYL BROMIDE IN CUBAN TOBACCO PRODUCTION

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ABSTRACT

Farmers have played the most important role in the adoption of new technology that will lead to the phase out of methyl bromide (MB) for tobacco seedling production in Cuba. Farmers were first made aware of the requirement to phase out MB, which was followed by an increase in extension services advice and preparation of teaching materials. Using group and individual teaching techniques, almost 790 farmers were trained in workshops and conferences, and by demonstrating alternatives in the field. Almost 900 visits to individual farmers were made by extension staff. More than 250 farmers have already adopted the floating tray technology which is evidence of widespread farmer interest and the success of the programme.

Keywords: tobacco, floating tray technology, methyl bromide, alternatives, Cuba

DIMETHYL DISULFIDE AS A NEW POTENTIAL ALTERNATIVE TO METHYL BROMIDE FOR SOIL DISINFESTATION

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ABSTRACT

The use of chemical compounds and especially fumigants remains a safe curative tool to control large populations of soil-borne pathogens, nematodes and weeds. A new fumigant produced by Atofina called dimethyl disulfide (DMDS) shows promise as an alternative to methyl bromide. Preliminary laboratory experimental results showed DMDS was biologically effective against several resistant forms of soil-borne fungi. At 20°C, the DMDS calculated Concentration Time Products (CTPs, in $g.h/m^3$) required for 90% efficacy were: 1981 $g.h/m^3$ for *Sclerotium rolfsii,* 3017 for *Rhizoctonia solani,* 3203 for Ph*ytophthora cactorum* and 3249 for *Sclerotinia sclerotiorum*. The physical and chemical behaviour of DMDS was also studied using small soil columns *in vitro*. In the short term, the goal is to understand the diffusion of DMDS in soil. The results show a good diffusion of this gas to a depth of 33 cm at 20°C in sandly loamy soils. A dose of 800 kg/ha of active ingredient is expected to result in lethal CTPs in the soil.

Keywords: dimethyl disulfide, alternative, methyl bromide, nematode, weeds, soil fumigant

CYANOGEN AND CARBONYL SULFIDE AS POTENTIAL QUARANTINE FUMIGANTS FOR TIMBER

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ABSTRACT

Carbonyl sulfide is a good fumigant for insect and nematode control and a substantial amount of work has been done on stored product pests using this fumigant. An application dose of 60 g/m^3 for 48 hour will control all life stages; and 80 g/m^3 controls or inhibits most grain fungi. Carbonyl sulfide penetrates and diffuses through both hard and soft timber more quickly than does methyl bromide (MB). The sorption is much less than MB so that effective internal concentrations may be attained. Desorption is very rapid; one day of airing post fumigation results in a headspace concentration less than the Australian experimental TLV of 10 ppm. Cyanogen is a potent biocide that controls insects, nematodes, fungi and bacteria. It also kills seeds and therefore is not suitable for many grain applications. No existing timber fumigant has a comparable range of biocidal activity. Cyanogen therefore has the potential to be effective against the full range of organisms that may be associated with timber in international trade. Cyanogen penetrates and diffuses through both hard and soft timber more quickly than does MB and also diffuses across the grain. Sorption on timber is similar to MB. Desorption is more rapid than other known fumigants.

Keywords: cyanogen, timber, fumigation, insects, nematodes, fungi, bacteria, biocide

BIOFUMIGATION WITH SOLARIZATION FOR SOIL DISINFECTION AND SUSTAINABLE PRODUCTION OF GREENHOUSE-GROWN SWEET PEPPER IN THE SOUTHEAST OF SPAIN

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ABSTRACT

In the Region of Murcia in South-East Spain, 1,433 ha of sweet pepper are grown in plastic greenhouses. *Phytophthora capsici* and *Meloidogyne incognita* are the main soilborne pathogens. Every year, soils are disinfected with methyl bromide (MB, 98%) with chloropicrin (2%) at 30 g/m^2, applied under a VIF (Virtually Impermeable Film) plastic mulch. Since 1998, biofumigation with solarization has been trialled as an alternative to MB in crops grown under an Integrated Pest Management (IPM) programme and those produced under an organic programme. The following aspects were studied in commercial and experimental greenhouses, and compared to MB and non-treated soil: i) biofumigation timing ii) which organic amendments to use and iii) the effects of reiterated biofumigations. The effect of disinfection on *P. capsici*, *M. incognita*, weeds, plant development, yield and the physico-chemical characteristics of the soil were measured. The best results were obtained when biofumigation with solarization was applied at the end of August or the beginning of September. Sweet pepper yield using fresh sheep manure with soy bean flour, or fresh sheep manure with chicken manure treatments, was similar to sweet pepper yield when MB was used. The reiteration of biofumigation with solarization over two or more years led to an improvement in pathogen and weed control, higher plants, an increase in yield and improvements to the soil physical properties, with a higher level of macro- and micronutrients as well as electrical conductivity.

Keywords: biofumigation, methyl bromide alternatives, sweet pepper, greenhouses, sustainable agriculture.

TECHNICAL AND ECONOMIC FEASIBILITY OF CHEMICAL AND PHYSICAL ALTERNATIVES TO METHYL BROMIDE IN SOIL DISINFESTATION OF TOMATO IN MOROCCO

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ABSTRACT

Solarisation, optimal use of fumigants, reduced dose methyl bromide (MB), steam pasteurization of soil and soilless cultivation, all within an Integrated Pest Management programme, were evaluated as alternatives to MB for control of severe root-knot nematode soil infestations. Soil solarisation using a transparent plastic mulch (45μ) increased soil temperatures to 48°C. Soil solarisation for six weeks alone, or four weeks in combination with metam sodium or 1,3-dichloropropene and soil pasteurization, gave similar control of root-knot nematode infestations as that obtained with a full or reduced dose of MB combined with VIF. An economic feasibility assessment of these alternatives supported these technical results. The project was part of an UNIDO project PROJECT/MP/MOR 97/126 funded by the Multilateral Fund of the Montreal Protocol.

Keywords: Solarisation, methyl bromide, steam pasteurization, root-knot nematode, metam sodium, 1,3-dichloropropene, Morocco

CHEMICAL AND NON-CHEMICAL CONTROLS USED IN URAGUAY AS ALTERNATIVES TO METHYL BROMIDE FOR NEMATODE CONTROL

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ABSTRACT

This two year project focused on demonstrating the effectiveness of several alternatives to the use of methyl bromide (MB) as a soil fumigant in protected horticulture. The use of metam sodium alone or combined with soil solarization gave good results in controlling nematodes and producing, in some cases, the highest yield in summer cycles. Bio-fumigation with corn or broccoli with soil solarization performed very well but, in the case of corn, the results were outstanding and could be compared statistically to MB. Generally, organic amendments increased populations of antagonist bacteria in the soil and improved its physical and chemical characteristics. This project was performed by INIA and UNIDO was the implementing Agency of the Multilateral Fund.

Keywords: methyl bromide, metam sodium, solarization, nematodes, corn, broccoli, organic amendments, Uruguay

BIOFUMIGANTS SUPPRESS FUNGAL PATHOGENS AND WEEDS OF STRAWBERRY

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ABSTRACT

This study examined the release of isothiocyanates (ITCs) from Brassica crops into soil. It demonstrated that the quantity and diversity of ITCs released into soil varied between Brassica species. Brassica roots released greater quantities of ITCs into soil than their shoots. The release of ITCs into soil peaked 4 hours after incorporation. These factors influenced the degree that biofumigants suppessed the growth of fungal pathogens and weeds of strawberry *in vitro*. In the field, biofumigation reduced weed growth by 30% and the growth of *Phytophthora cactorum* by 10%.

Keywords: isothiocyanates, brassica, biofumigants, methyl bromide, alternative, weed suppression, fungi

MICROFLORA AND NUTRIENT CHANGES AFTER FUMIGATION

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ABSTRACT

This study compared the selectivity of four soil fumigants against saprophytic soil microflora and their effect on soil nitrogen status over time. On soil without a history of fumigation, treatment with Vorlex + CP®, Basamid®, Telone C35® or Bromafume® led to drastic changes in the population dynamics of fungi, bacteria and actinomycetes. Basamid® and Vorlex + CP® displayed the greatest selectivity against fungi. The concentration of soil nitrogen was altered significantly by soil fumigation, but was not necessarily related to an increase in crop growth. Clearly, nitrogen is not the only factor involved in the increased growth response observed in plants grown in fumigated soil.

Keywords: Vorlex, Basamid, Telone-C35, Bromafume, fungi, bacteria, actinomycetes, growth response, methyl bromide, alternative

ECONOMIC VALUATION OF METHYL BROMIDE ALTERNATIVES IN SPANISH STRAWERRY CROPS

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ABSTRACT

Experimental trials carried out in strawberry plots in southwestern Spain around Huelva on different alternatives to methyl bromide for soil disinfection were economically analysed to determine their profit per hectare, profit per kg and profit per unit cost. The trials were carried out in 1997/98 within the scope of the INIA SC97-130 project. Both physical and chemical alternatives to MB were tested in 12 different treatments. Chloropicrin (40) seemed to be a good alternative to MB (40) when considering profit per hectare. Solarisation + metam sodium (50) appeared to also be interesting for profit per kg and profit per unit cost.

Keywords: methyl bromide, alternatives, chloropicrin, solarisation, metam sodium, economic evaluation, profit

CUBAN SKILLS USED IN THE ECOLOGICAL MANAGEMENT OF PLANT PARASITIC NEMATODES

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ABSTRACT

For more than twenty years in Cuba, economic control of plant parasitic nematodes has been achieved in a range of crops using non-chemical methods. Technicians and farmers use non-chemical methods as a part of a national programme to reduce chemical pesticides. Alternatives include quarantine regulations as well as cultural, physical and biological control methods. The crops with the most non-chemical alternatives are vegetables (urban agriculture included), tobacco, coffee, banana and potato. Sometimes an alternative consists of crop rotation with a crop that is not susceptible to nematodes such as peanut, sesame, corn, sorghum, sweet potato, garlic, and onion. Other methods include trap crops, soil tillage, elimination of weed hosts, use of non-decayed organic matter, clean seedlings, selection of sites as free as possible of pests and disease, and biological control with fungi and bacteria. Integrated Nematode Management Systems have been developed for different crops such as coffee, banana, vegetables and potato.

Keywords: Cuba, non-chemical, nematode, crop rotation, trap crop, soil tillage, biological control

EFFECT OF SOIL SOLARIZATION ON THE VIABILITY OF *FUSARIUM OXYSPORUM* F. SP. *DIANTHI*

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ABSTRACT

A comparison was made of the differences between the ability of three different types of plastic sheet to solarize soil in a closed greenhouse in southwest Cádiz from late June to early August 2001. Soil temperatures were recorded over time in each of the different treatments. Sequential sampling of soil samples naturally infested with *Fusarium oxysporum* f.sp. *dianthi* buried at two depths indicated that full control of the fungal pathogen was achieved at 15 cm depth after 30 days solarization in all treatments. Pathogen viability loss was 100% at 30 cm depth after 30 days when PE CP-129 40µm was used, after 37 days using the yellow plastic 180µm treatment, and ca. 6% after 44 days of solarization with polythene 50µm.

Keywords: solarization, plastic sheet, fungal pathogens, *Fusarium oxysporum*

ALTERNATIVES IDENTIFIED FOR VEGETABLES IN UNEP'S REGIONAL DEMONSTRATION PROJECT ON METHYL BROMIDE ALTERNATIVES IN CENTRAL AND EASTERN EUROPE

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ABSTRACT

A two year evaluation of chemical (dazomet, 1,3-D+CP) and non-chemical (*Trichoderma viride,* Indian mustard or straw amendments) alternatives was conducted in field-grown cabbage, tomato and celeriac. The yield increases of cabbage and tomato following application of dazomet and 1,3-D+CP integrated with *Trichoderma* were lower than the yield when methyl bromide (MB) was used, but still the yields were commercially acceptable. Celeriac showed a stonger response to alternative treatments. Dazomet and 1,3-D+CP integrated with *Trichoderma* significantly increased the marketable yields of celeriac. The combined applications of lower rates of 1,3-D+CP or dazomet with the biocontrol agent was superior to those chemicals used alone at higher rates. The results indicate that in the production of celeriac both dazomet alone and 1,3-D+CP at a lower dosage combined with *Trichoderma* can be considered as one-to-one replacements for MB.

Keywords: dazomet, 1,3-dichloropropene, cabbage, tomato, celeriac, Poland, alternative, methyl bromide

IODOMETHANE (TM-425) - DEVELOPMENT AND REGULATORY UPDATE

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ABSTRACT

TM-425, (active ingredient: Iodomethane) is in commercial development as a replacement for current uses of methyl bromide (MB). TM-425 is an environmentally friendly, broad-spectrum fumigant applied to soil for the control of various economically important weed seeds, plant parasitic nematodes and soil borne pathogens. Target markets include strawberries (including nursery production), fresh market tomatoes, turf and ornamentals (cut flower and bulbs). Research has demonstrated there are no detectable residues in all fruits tested (less than 0.01 ppm) and its half-life in soil and air is short (less than 5 days). TM-425 has an extremely low ozone depletion potential (ODP). TM-425 is a superior quality material, which can be used as a drop-in replacement for MB. The spectrum of activity of TM-425 and its TM-442 combinations is equal to or superior to commercial standards. Additionally, there is strong evidence of increased yields resulting from treatment of fresh market strawberry and tomato fields.

Keywords: Iodomethane, TM-425, alternative, methyl bromide, tomatoes, turf, ornamentals, strawberry, tomato

INTRODUCTION

Arvesta Corporation, formerly Tomen Agro, Inc., is a multinational company having as its core business agricultural products. Arvesta focuses on premium - branded products and is active in worldwide development, registration, manufacturing and sales. Chloropicrin (TM-442), one of our proposed mix partners, is included in our manufacturing portfolio of products.

TM-425, (active ingredient: Iodomethane) is in commercial development as a replacement for current uses of methyl bromide (MB). TM-425 is an environmentally friendly, broad-spectrum fumigant applied to soil for the control of various economically important weed seeds, plant parasitic nematodes and soil borne pathogens. Target markets include strawberries (including nursery production), fresh market tomatoes, turf and ornamentals (cut flower and bulbs). Pre-plant fumigation to soil is shank applied by conventional equipment typically used for flat / broadcast, prepared raised bed or through buried drip line fumigation.

RESULTS

Field efficacy trials conducted in the United States support rates of 134 – 263 kg/Ha for TM-425. Testing has been conducted with TM-425 and in combination with chloropicrin (TM-442). Formulations in development include TM-425: TM-442 ratios of 98:2, 50:50 and 25:75. Additional formulations are under evaluation. Rates are dependent upon target species, soil conditions, texture, and cultural practices. TM-425 formulations are well suited for both field and glasshouse applications.

In 1999,Tomen Agro placed TM-425 in a Biological Development program as a soil fumigant and replacement candidate for methyl bromide. Regulatory briefings with regulatory agencies the in U.S., EU and Japan have been held. Conferences with the U.S. EPA and California Department of Pesticide Regulations have been favorable resulting in TM-425 being given an EPA top priority and the expectation of an accelerated review process. Target data package submissions and registration/sales are the following:

January 2002 United States submission; January 2003 United States registration; February 2003 Israel submission; March 2003 Israel registration; March 2005 EU and Japan Submissions; March 2006 EU Provisional Sales; December 2006 Japan Sales.

Compared to MB, there are a number of advantages when TM-425 is used. Research has demonstrated there are no detectable residues in all fruits tested (less than 0.01 ppm) and its half-life in soil and air is short (less than 5 days). TM-425 has an extremely low ozone depletion potential (ODP). The calculated ODP value is 0.0015, which US EPA confirms, makes it a non-threat to the ozone layer. Importantly, TM-425 is a liquid at ambient temperature and can be handled and stored using conventional equipment. Research confirms it is well suited for use in closed systems for pre-plant soil fumigation including buried drip line irrigation. The end result is reduced potential for worker exposure.

Data from various field trials have shown increased yields in crates/ha for strawberries. TM-425 at196 kg/ha, and TM-425 + Chloropicrin at 270 (50:50 ratio) and 331 kg/Ha (60:40 ratio) were equivalent or superior to the commercial standard, MB + chloropicrin @ 393 (263 +130) kg/ha (Table 1). Data from tomato field trials show increased yields of marketable fruit as much as 123% of yields harvested for MB (Table 2).

Efficacy results for TM-425 reflect a spectrum of activity similar to what is observed with MB-treated soil. However, TM-425 is effective at rates lower than standard MB rates. This increased effectiveness is mostly attributed to retention of a higher concentration over time in the soil. Because TM-425 is a liquid as it is injected into the soil it will penetrate further in the soil profile before it enters a gas phase. TM-425 vapor pressure and Henry's Law constant still classify this chemical as a true fumigant, which means that it will move as a gas in all directions through the soil profile.

CONCLUSIONS

TM-425 is a superior quality material, which can be used as a drop-in replacement for MB. The spectrum of activity of TM-425 and its TM-442 combinations is equal to or superior to commercial standards. Additionally, we have strong evidence of increased yields resulting from treatment of fresh market strawberry and tomato fields.

Table 1: Effect of fruit yields as a percent of yield from methyl bromide treated soil. Methyl bromide applied at 393 kg/Ha, 67:33 ratio. Average from two strawberry field trials in California.

Treatment	Rate	Ratio	Percent Yield
TM-425	196 kg/Ha	Stand alone	103%
TM-425 + TM-442	270 kg/Ha	50:50	93%
TM-425 + TM-442	331 kg/Ha	60:40	100%

Table 2: Effect of fruit yields as a percent of yield from methyl bromide treated soil. Methyl bromide applied at 393 kg/Ha, 67:33 ratio. Average from a single tomato field trial in Florida.

Treatment	Rate	Ratio	Percent Yield
TM-425	196 kg/Ha	Stand alone	94%
TM-425	263 kg/Ha	Stand alone	123%
TM-425 + TM-442	268 kg/Ha	50:50	120%
TM-425 + TM-442	331 kg/Ha	60:40	135%

AGROCELHONE, THE SOLUTION AGAINST NEMATODES AND SOIL FUNGI AS AN ALTERNATIVE TO METHYL BROMIDE

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ABSTRACT

The poster consists of number of studies performed with *AGROCELHONE N* and *AGROCELHONE NE* as alternatives to methyl bromide. These studies have been done in strawberry, tobacco and vegetable crops in Andalucia and Extremadura areas during the years 1998, 1999, 2000 and 2002. In the poster, the rates used, the application methods employed, the assessments, results and conclusions are described for each crop in turn. Trial photographs, graphs and results are included to assist in interpretation of the poster.

Keywords: Agrocelhone, dichloropropene, chloropicrin, methyl bromide, product, nematodes, fungus, efficacy trials, tobacco, strawberry, vegetables.

STRAWBERRY

Methods and application

During the seasons 98/99 and 99/00, nematicides and fungicide efficacy trials were performed on strawberry in the Huelva area with the product **AGROCELHONE N**: *Dichloropropene 59.6% p/p + Chloropicrin 34.6% p/p.* Trials were also carried out with **AGROCELHONE F** formulated with different 1,3-D and chloropicrin rates. The experimental design was randomized blocks with 3 thesis and 4 replicates.

The following test were run:

AGROCELHONE N

THESIS	PRODUCT	DOSES/Ha
1	AGROCELHONE N	180 Kg[1]
2	67% Br + 33% Cl	400 Kg
3	UTC	Plastic

[1] In some trials 500 Kg/Ha rate was applied

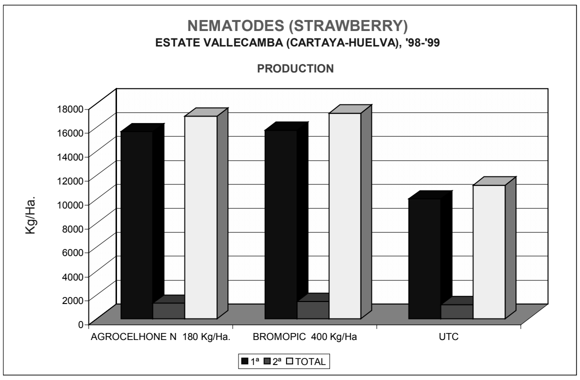
The applications were done in two passes through an injection mechanism located in the soil during the season 98/99. The plots were stamped with waterproof plastic, and it was taken away after 5 days. The treatment was performed in bands and in two passes: Alternative bands were treated in the first pass and untreated bands in the second pass. The machine used in the treatment was an specific applier for located injections to the soil. The application method used in the season '99/'00 was an injection mechanism located at soil in the plantation back. The back was stamped with waterproof plastic with the purpose of getting it ready for the transplant of the crop.

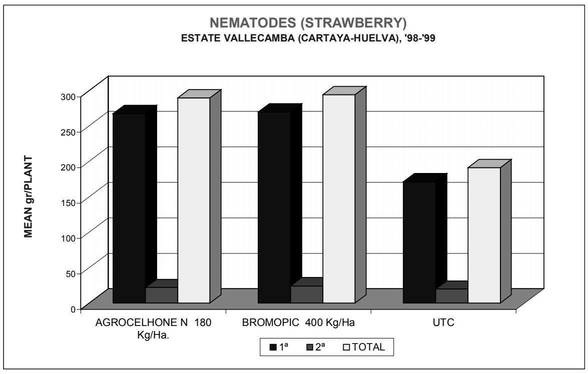
Assessments

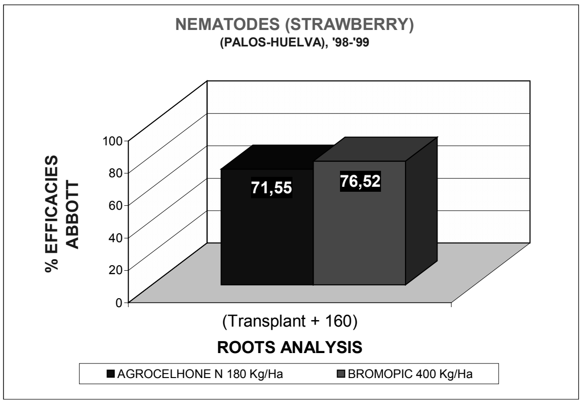
The sampling were performed on plants, seeing the air symptoms, on roots nodule index (on roots affectation index in fungus trials), on vegetative development, taking 10 randomized plants/elemental plot. The flowers/plant number and fruits/plant number were reported, taking 10 randomized plants per elemental plot. An estimation of production was done per treatment, weighing 20 fruits per repetition taken at random (all harvestable fruits of first and second quality). Moreover a sample composed of neck and roots of affected plants was taken and it was carried to the laboratory for its analysis.

CONCLUSIONS

The **AGROCELHONE N** product proves to be in most cases, good disinfectant of soil against nematodes and soil fungus. This product can be compared with the MB, reaching similar results on efficacy, vegetative development, fruit number/plant, mean weigh/fruit and production that the plots disinfected with *67% Br + 33% Cl* and better of course than untreated plots.







TOBACCO

AGROCELHONE N: *Dichloropropene 59.6% p/p + Chloropicrin 34.6% p/p*

METHODS AND APPLICATION

On 1999 three trials were performed against nematodes on tobacco crop in Talayuela (Cáceres). The experimental design of each trial was randomized blocks with 3 thesis and 4 replicates.

The application method used was through injection mechanism located at flat soil on all the land. The land was stamped with soil through an iron platform fitted to the application machine. The machine used was an specific applier for the located injections at the soil.

On 2000 two assays against nematodes in Talayuela (Cáceres), comparing **AGROCELHONE N** at 150 Kg/Ha with 1-3 D at 120 Kg/Ha. The trial had two replicates in two plots with different textures (sandy and clearly-sandy). The application method used was through injection mechanism located at flat soil on all the land. A machine was used with 3 parts.

The following test were run:

THESIS	PRODUCT	DOSES/Ha
1	1,3-D INJECTABLE	150 l
2	AGROCELHONE N	180 Kg
3	UTC	

Assessments

The assessments done in these trials in 1999 are performed through roots analysis in the laboratory in transplant and after it, with the purpose of to see the nodule index.

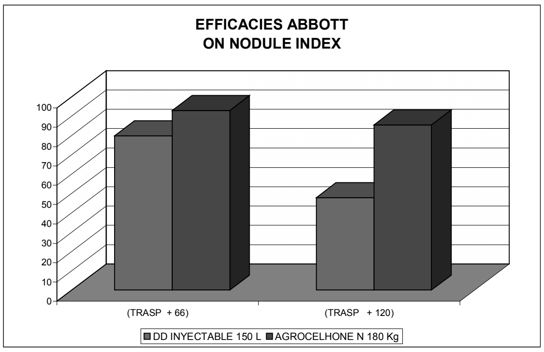
Two assessments on vegetative development are also done to see if some difference exists between treatments and control, taking 10 plants/elemental plot before flower removal, reporting the height in cm, the length and leaves wide.

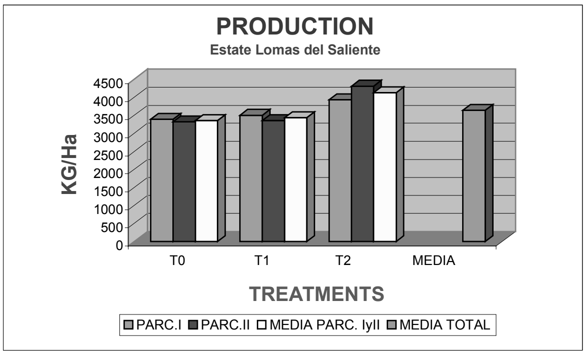
In the trials performed on 2000, the crop data were reported, such as transplant, ploughing, flower removal, phytosanitary treatments, growth rate, colouration, shoots development, pests, illness and ripeness.

- One hundred plants per elemental plot are harvested.
- Yield in Kg/Ha of tanned tobacco per foliar flat and elemental plot.
- Physical assessment of tanned tobacco per foliar flat and elemental plot.
- Chemical assessments of tanned tobacco per foliar flat and elemental plot, reporting about total alcaloides, reducer sugares, relation reducer sugar/total alcaloides and chloruros.
- The nematode attack in roots was evaluated in order to scale 0 to 10 after the last harvesting.
- A sample per foliar flat and elemental plot was taken composed by 48 samples (2 trials, 2 repetitions, 3 thesis and 4 foliar flats).

CONCLUSIONS

AGROCELHONE N proves to be a good soil disinfectant against nematodes, with high populations of *Meloidogyne* spp., in all the trials done. The product obtains better efficacies than 1,3-D INJECTABLE product, obtaining at the same time better development of the plant and higher yielding.





(*) T0 (UTC) T1 (1,3 D INJECTABLE) T2 (AGROCELHONE N)

VEGETABLES

AGROCELHONE NE: *Dichloropropene 59.6% p/p + Chloropicrin 34.6% p/p*

During last years, it has been performed trials with this product in greenhouses in several vegetable production areas (tomato and pepper crops fundamentally) in Almería and Murcia areas. It has been reached high efficacy indexs, comparable with the MB product and better than those of others disinfectants compared also (1,3-D and metam-sodium), applied through located irrigation prior to transplant of the crop on all the land and stamping the soil with a plastic. The plastic is keeping during 10-12 days after application

Nowadays, some trials with this product are being performed, in greenhouses in Los Palacios (Sevilla) area. This product is being applied through located irrigation before crop transplant, on all the land and stamped with a plastic. It must pass 28 days before transplant. The rate is 400-500 Kg/Ha. Before application it is irrigated with the purpose of get the very best humidity (60%-70%). It is applied at 2 g/l, equivalent to 2.000 ppm, and it is irrigated about 3-5 l/m^2 after application with the purpose of clean the pipelines and stamp the product in the soil.

Nowadays other assays are being performed in other vegetables with this product with the same rate and application method. The assessments are done on nodule index of nematodes and on infestation of neck and roots fungus and, in some cases, on production.

ALTERNATIVES TO THE USE OF METHYL BROMIDE IN BANANA PRODUCTION IN COLOMBIA

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ABSTRACT

In order to show the world's banana industry that there are technically and economically feasible alternatives to methyl bromide (MB) in an integrated Moko disease management programme, the fumigant Dazomet (Basamid ®) and the herbicide Glifosato (Roundup ®) were evaluated alone and in combinations, in the two main Colombian banana growing zones (Urabá and Magdalena). Results of the first replication show that the four treatments used to eradicate banana plants infected with the bacterium *Ralstonia solanacearum* (causal agent of Moko), were effective and none of the replants in post-treated plots showed symptoms of the disease from planting to flowering. The second replication showed over 90% of plants infected, 15 and 30 days after the inoculation in Urabá and Magdalena, respectively, being the disease evolution faster in the first zone. Lab analysis, on post-treatment vegetal tissue samples from all treatments, detected the presence of bacteria colonies with a similar morphology to that of the causal agent of Moko. However, no symptoms of the disease were found on greenhouse plants after the inoculation with inoculum prepared with these bacteria.

Keywords: methyl bromide, alternatives, banana, Colombia, Moko disease, dazomet

INTRODUCTION

In Colombia, there are records of methyl bromide (MB) utilization in the banana growing zone of Urabá - Atioquia up to 1993 and in the Magdalena Province there are records of its utilization up to 1995. Basically, MB was used in banana crops to control Moko disease or "maduraviche" caused by the bacterium *Ralstonia solanacearum* (Mejía 1995 & Granada 1997).

Joint efforts of producers and different institutions enabled the development of alternative methodologies and strategies for the efficient handling of diseases, without MB. In the case of Moko disease, Augura implemented the phytosanitary brigades strategy to diagnose and control the disease through the herbicidal eradication of infected plants in the Urabá and Magdalena regions, the main two areas under export-quality Cavendis*h* banana (*Musa AAA*) cultivation (Quiros 1984 & Rosero 1985). The objectives were to watch over the phytosanitary maintenance of the zones, the periodical revision of infected and neighboring farms, the prevention and control of diseases found in the area, statistical data-gathering in relation to diseases and sharing of information with other entities. These activities allowed a notable reduction in the incidence of the disease, and the proposal of new projects and research topics during the program (Mejia 1996 & Mena 1999).

This study attempts to demonstrate that there are alternatives to MB in the control of Moko disease. For this purpose, four treatments, with 2-replications, for the control of Moko disease in the two main banana growing regions (Urabá and Magdalena) in Colombia were evaluated (MB, Dazomet, Glifosato and the combination Glifosato + Dazomet). The treatments were evaluated from the economic and efficacy point of view.

MATERIALS AND METHODS

The demonstration was conducted in two 0.5-hectare Plots. One is located in Uraba, Municipality of Carepa, Antioquia Province, Colombia. The other one is located in Zona Bananera Municipality, Magdalena Province, Colombia.

To improve soil porosity, the preparation of each plot was based on the analysis of its physical properties and included weeding, sub soiling and two harrow passes. Each area was subdivided into 4 plots of 0.1 hectare each. Each experimental lot was planted with 720 plants from meristems of Great Dwarf Cavendish banana in 30 X 30 X 30 cm holes, at 2.5 m between plants and 2.5 m between rows. Each plot included 180 plants, occupying a net area of 962.5 m^2, a total net area of 3.850 m^2 for each zone.

To work with the specific bacterium, the inoculum was prepared directly from fresh diseased tissue obtained from the participating banana growing regions, to preserve the bacterium's in-situ virulence, facilitate its preparation and to avoid possible alterations to the study and the environment. For the Uraba banana growing region, the inoculum was obtained from a 1-kg Great Dwarf Cavendish banana corm infected with Moko disease, located in lot 6 of the Dioselina farm, Carepa municipality, Antioquia Province, Colombia. For the Magdalena region, the inoculum was obtained from an infected corm located in parcel 1 of the Futuro Porvenir farm, Zona Bananera municipality, Magdalena Province- Colombia.

Each corm was soaked in a 10 % sodium hypochlorite solution for 15 minutes, then placed in an isolation chamber as aseptic as possible, and washed with sterilized water. A 300 g sample was taken from the so called "medal" (characteristic internal symptom of the disease) and liquefied with 2.7 liters of sterilized water. The suspension was then filtered and the motility and amount of bacteria was microscopically determined in Colony Forming Units CFU in 100 μl present in these solutions, pouring 100 microliters of each inoculum in a TZC culture medium. Five replications were performed. A few hours after inoculum collection and preparation, 2.5 cm^3 of the bacterial suspension was injected in the central part of the pseudostem of each plant.

Four treatments for the control of Moko disease were evaluated in each region, as follows: Plot 1, with 50 g/m^2 of MB commercial product, covering the entire plot with plastic during 8 days, including the infected plants. Plot 2, with 10 cm^3/plant of Glifosato (Roundup®) 20% commercial product in distilled sterilized water, applying 25 g/m^2 of Dazomet (Basamid®), 8 days later, covering the ground with plastic for 8 days. Plot 3, with 10 cm^3/plant of Glifosato (Roundup®) 20% commercial product in distilled sterilized water and were maintained in quarantine for six month. Plot 4, with 25 g/m^2 of Dazomet (Basamid®) incorporating the product into the soil and covering the entire plot, including diseased plants, with plastic for 8 days.

From each 180-plant plot where fumigants were applied, 30 samples of Moko diseased pseudostems were selected immediately after the tents were removed and taken to the lab, in order to detect whether bacteria were present in the treated samples. To corroborate its pathogenicity, the colonies obtained *in vitro,* from tissues after treatment, were evaluated in greenhouse and the Great Dwarf banana seedlings were inoculated (2 ml/plant at $1x10^3$ UFC concentration in a PCG liquid medium). All treated plots were planted with 180 Great Dwarf banana. These plants had been under six-month constant monitoring for Moko symptoms, from planting to flowering, in order to verify product efficacy in soil and a possible relapse of the disease. When each plot showed over 80% flowering, the plants, and all vegetal material generated within the plots, were removed and buried outside the plot to prevent neighboring producers from using them as seedlings. To obtain the necessary information for an economic analysis, the number of day's wages (men-day) needed in each one of the treatments were determined.

RESULTS AND DISCUSSION

The inoculum showed an average concentration of 22 and 21 Colony Forming Units (CFU), for Urabá and Magdalena respectively. 95% of the inoculated plants in Urabá showed Moko disease symptoms, in a period of 16 days after the inoculation. This zone shows a fast evolution of the disease. 50% of the plants showed symptoms of the disease just 10 days after inoculation (Table 1). In the Magdalena region, over 90% of the plants showed symptoms of the disease 30 days after the inoculation and 100 % infestation 35 days after inoculation. This zone showed a 15-day latent stage, then, the disease developed rapidly (Table 1). As in the first year, the Urabá region showed a faster development of the disease compared with the Magdalena region. This seemed to be related to the rainfall which is heavier in the Urabá zone.

In Urabá, total day's wages needed in each treatment, in descendent order, were: first year dazomet 18.36, dazomet + glifosato 16.62, MB 7.68, glifosato 0.39; and in the second year dazomet 13.14; dazomet + glifosato 9.58 and MB 5.29; (Table 2). In the Magdalena region, in descendent order, day's wages needed in each treatment were: first year dazomet + glifosato

8.48, dazomet 8.21, MB 4.96, glifosato 0.44 and in the second year, dazomet + glifosato 8.58, dazomet 7.3, MB 4.9 and Glifosato 0.44 (Table 3). Treatments to the soil, dazomet and glifosato + dazomet, demanded more labor compared with MB which does not require incorporation into the soil. In the case of Uraba, compared with the previous semester, all treatments demanded less labor, explained by a more worked soil and lower rainfall. However, the Uraba plot still demanded more labor compared with the Magdalena region, which has a more sandy soil.

Efficacy in tissue, the number of pseudostems that resulted positive for Moko disease, per treatment, were: in the second year Urabá; MB 28, dazomet 3 and glifosato + dazomet 16. However, in inoculated greenhouse plants, no symptoms of the disease were observed. Magdalena; MB 21, dazomet 16, Glifosato + dazomet 6. None of the inoculated greenhouse plants showed symptoms of the disease. These results suggest that isolated bacteria are not pathogenic after treatment, although it could be due to an after-treatment same genus bacterial colonization during the vegetal tissue decaying process, presenting the same type of colony (see Table 4). This may occur because the TZC culture medium is not specific for Ralstonia solanacearum and other species may grow in it.

Table 1: Percentage of Great Dwarf banana plants infected with Moko disease, in Uraba Antioquia and Zona Bananera, Magdalena - Colombia.

Time (days)	Uraba		Magdalena	
	Year 1	Year 2	Year 1	Year 2
5	0	0	0	0
10	80	51	1	4
15	88	89	10	12
20	96	99	83	66
25	97		98	80
30	100		100	94

Table 2: Day's wages per 0.1 hectare required per treatment for the control of Moko disease. Uraba –Colombia.

Labor	Methyl bromide		Dazomet + glifosato		Glifosato		Dazomet	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Soil movement	-	-	6.48	4.25	-	-	6.00	6.60
Product application	1.00	0.16	0.47	0.11	0.14	0.12	0.38	0.12
Plant removal and chopping	0.20	0.10	0.21	0.10	-	-	0.20	0.09
Product incorporation	0.00	-	2.00	1.26	-	-	2.10	1.50
Plastic installation	4.75	4.00	5.63	2.83	-	-	7.80	3.80
Pathways disinfections	0.13	0.03	0.13	0.03	-	-	0.13	0.03
Plastic removal	1.60	1.00	1.70	1.00	-	-	1.75	1.00
Weed Control	-	-	-	-	0.25	0.30	-	-
Total	7.68	5.29	16.62	9.58	0.39	0.42	18.36	13.14

Table 3. Day's wages per 0.1 hectare required per treatment to control Moko disease. Zona Bananera, Magdalena - Colombia.

Labor	Methyl bromide		Dazomet + glifosato		Glifosato		Dazomet	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Soil movement	-	-	3.00	3.50	-	-	3.44	3.00

Labor	Methyl bromide		Dazomet + glifosato		Glifosato		Dazomet	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Product application	0.13	0.13	0.35	1.25	0.15	0.13	0.22	0.90
Plant removal and chopping	0.16	0.15	0.19	0.14	-	-	0.21	0.16
Product incorporation	-	-	1.10	0.61	-	-	1.15	0.50
Plastic installation	3.90	2.50	3.00	2.30	-	-	2.30	2.08
Pathways disinfections	0.14	0.04	0.14	0.07	-	-	0.14	0.06
Plastic removal	0.63	0.66	0.70	0.71	-	-	0.75	0.60
Weed Control	-	-	-	-	0.29	0.32	-	-
Total	4.96	3.48	8.48	8.58	0.44	0.45	8.21	7.30

Table 4. Isolation of Moko-causing bacteria from treated banana tissue in TZC culture medium, and re-inoculation of positive colonies in greenhouse seedlings.

TREATMENT	Uraba				Magdalena			
	Laboratory		Greenhouse		Laboratory		Greenhouse	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Methyl Bromide	1	28	-	-	1	28	-	-
Dazomet	1	3	-	-	-	3	-	-
Gifosato + Dazomet	-	16	-	-	8	16	-	-

FIELD EFFICACY

All plants planted after the treatments were monitored for Moko disease symptoms from planting to flowering. None of the plants showed symptoms of the disease. This suggests that any of these treatments is effective to control de disease in the seedling stage. To decide for any of these treatments, it is then necessary to consider the economic and environmental aspects of each one of the treatments.

Economic analysis for both zones for the first year of the project, total costs of each one of the alternative methods compared to MB. Detail cost of treatment, cost of quarantine maintenance and loss of profits or earnings (during the time between disease treatment and the starting of production). Loss of profits or earnings was calculated multiplying time in weeks without producing by the average profits obtained from a hectare in production in the same period. Comparing MB to the herbicide Glifosato, the difference in costs for each heading is as follows: treatment 95% and 96 %, quarantine maintenance –64% and –90%, loss of profits or earnings –58% and –58 %, for Urabá and Magdalena, respectively. That's to say, Glifosato costs are 95% lower in Urabá and 96% lower in the Magdalena region concerning the heading "Treatment", and they are also 64% higher in Urabá and 90% higher in Magdalena concerning the heading "Quarantine Maintenance", and so on (dates not showed).

In the MB method, the weight of each heading is as follows: Treatment 81% and 79%, quarantine maintenance 2% and 2%, loss of profits or earnings 16% and 19%, for Urabá and Magdalena, respectively. In the Glifosato method, the weight of each heading is as follows: Treatment 12% and 9.2%, Quarantine maintenance 11.5% and 10.2%, and loss of profits or earnings 76.5% and 80.6%, for Urabá and Magdalena respectively. The heading with more weight in the MB method is "Treatment", while in the Glifosato method is "Loss of profits or earnings". What is more attractive is that the Glifosato method has a cost comparative advantage of 62% in Urabá and 58% in Magdalena, in relation to MB despite Glifosato's six month quarantine. This situation invalidates the belief that lower quarantine time means more profits.

The difference in costs between the two zones, is due to less labor required in the Magdalena region to perform the treatments. Total day's wages used in the Magdalena region were lower because of its sandy soil, which requires less labor than Urabá's clayish soil.

The cost of product, plastic and labor of MB and Dazomet treatments is a disadvantage from the economic point of view compared to the herbicide Glifosato, despite the relative high loss of profits caused by Moko disease control with Glifosato. The economic analysis shows that losses caused by Moko disease treated with MB and Dazomet are three times higher than the ones caused by the disease treated with Glifosato.

It is important to note that in a commercial banana plantation, where banana plants are in different development stages (flowering, fructification, etc.), the cost of handling the disease will increase because of the harvest and stacking of vegetal and plastic material present in the treated area. Besides, in these plantations, the disease will affect all type of plants (mother, daughter, granddaughter plants), which reduces fumigants' efficacy due to the greater amount of diseased biomass that must be treated. This will not happen when the Glifosato method is used for the control of Moko disease.

ACKNOWLEDGMENTS

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FOUR YEARS RESEARCH ON IMPROVED SOIL SOLARIZATION AND OTHER ALTERNATIVES TO METHYL BROMIDE ON STRAWBERRY CROPS

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ABSTRACT

To develop alternatives to MB, it is necessary to study not only the efficacy of the treatments but also their long-term effects and the feasibility of repeating the treatment in the same field several years consecutively. A four year experiment was carried out in a strawberry field in which there were established seven treatments: non treated control; standard dosage application of MB (60g/m^2); half dosage application of MB (30g/m^2) using VIF tarp; moderate rates of manure (5kg/m^2) combined with solarization; reduced dosages of metam-sodium (MS) (35g/m^2) combined with solarization; MS at standard dosages (144g/m^2); and fresh manure at high rates (15kg/m^2). The solarization treatments were split in two sub-treatments in the last year, in such a way that a half of the solarization combined with manure treatment of the third year was replaced by solarization combined with MS and *vice versa*, so that the experiment had a total of 9 treatments. After four years of research, we can infer that improved solarization with manure offers the better possible alternative when data of the first two years are considered but there is a loss of efficacy if these treatments are uninterruptedly applied longer than two years. The replacement of MS for manure produced less marketable yield losses than the reverse replacement.

Keywords: methyl bromide, alternatives, solarization, metam-sodium, strawberry

INTRODUCTION

The strawberry industry is very important in Spain, ranking second in the world in strawberry production for fresh consumption right after the United States. Most of the land cultivated in strawberries is disinfested before planting using MB as a standard cultural technique to avoid disease incidence.

The MB consumption in Spain has been reduced by 50% since 1998, and it will continue declining up to the phase out in January 2005, according to the schedule approved by the EU. This means that there is an urgent need to find possible chemical and non-chemical alternatives to MB before that deadline.

As a consequence of the progressive importance that organic strawberry fruit production is acquiring in the European market, this cultural production system has to be included among the present and future studies; therefore, solarization by itself or even combined with fresh manure (Gamliel & Stapleton 1997) to increase the toxic effect of volatile compounds to control soil-borne pathogens, have to be taken under consideration. Furthermore, the fact that the soils of Valencia area are loam or clay ones with low drainage and they have a low organic mater content, offers an additional reason to examine the possibilities of these organic techniques as alternatives to MB. Also the possibility of reducing dosage of MB while keeping effectiveness was studied in our area by combining with solarization or using a VIF sheet (Cebolla *et al.* 1996) with a subsequent reduction of emissions to the atmosphere.

Chemical alternatives to MB, that are not harmful to the ozone layer, have been suggested by using fumigants such as MS at standard dosages or at low ones combined with solarization. Therefore, they have to be considered in this type of studies equally.

MATERIALS AND METHODS

The aim of this experiment was to compare the behaviour of treatments reducing MB dosage by using VIF tarp (3); fresh manure at large rates (15 kg/m^2) (7) and moderate rates (5 kg/m^2) combined with solarization (4); metam-sodium (MS) at standard dosages (140 g/m^2) (6) or reduced dosages (35g/m^2) combined with solarization (5). Non treated control (1) and standard dosage application of MB (60g/m^2) (2) were used as references. Tarping period for MB treatments was 5 days while the solarization treatments was 5 weeks. Manure composition was 75% fresh sheep and 25% poultry for the solarization improved treatment (4) and for the manure treatment itself (7).

The ranges of the main characteristics of fresh sheep manure for treatments 4, 5' & 7 were 51.7 –52.3 for % dry matter; 58.5-67.9 for % total Organic Matter; 1.85-2.08 for % Total Nitrogen and 17.2-18.7 for C/N ratio; and for poultry manure the ranges were 54.2-81.2 for % dry matter; 51.3-70.3 for % total Organic Matter; 2.95-7.29 for % Total Nitrogen and 6.6-13.8 for C/N ratio.

The solarization treatments were split in two sub-treatments in the fourth year, in such a way that a half of the solarization combined with manure treatment(4) was replaced by solarization with MS (4') and a half of solarization combined with MS was replaced by solarization combined with manure (5') . Manure was buried with deeply ploughing followed by irrigation once in solarization treatments and three times in the manure (7).

The experimental design consisted of four years cropping with a complete randomised block with three replicates originally (first year). The treatments were repeated on the same plots for three additional years in two locations instead of three. Single plots were established with a large size (400 to 600m^2). Due to the large differences among the marketable yield variance and that of the control in successive years, the data were converted with logarithmic transformation for statistical analysis. Percentage data were converted with arcsin transformation for statistical analysis. Duncan's multiple range tests were done for statistical comparison among treatments.

Some small pieces of roots infested by *Fusarium* were buried at 10 and 30 cm of depth before treatments application, and recovered on Komada selective media (Komada 1975) after the treatment to monitor the effect on inoculum. Missing plants were replaced with new ones to keep the plant population.

The incidence of weeds in each treatment was monitored all along the growing season as the time of removing weeds plus cleaning the plants.

Cold stored plants of cv. Pajaro were planted in two-row bed at 30cm apart in the first and fourth years while cv. Camarosa was used at 35cm apart in the second and third years of experimentation. Two variables were used for estimating plant vigour: plant diameter and plant height.

Marketable yield (expressed in g/m^2) and percentage of second quality fruit yield over marketable yield were recorded.

RESULTS

No important pathogens were detected in the soil or plants. Nevertheless, soil fatigue was observed, due possibly to the presence of a fungal complex in which *Fusarium* spp participates as a main component. Results on survival of *Fusarium* spp from small pieces of roots (Table 1) show that control and manure treatments do not destroy inocula, independently of the depth of sampling. VIF and improved solarization treatments are effective on the surface, but not as much as standard MB in depth. Only standard MB treatment eliminated completely the inoculum. However, solarization combined with manure (4) is promising in this aspect, too. The efficacy of solarization with MS has declined in the fourth year with respect to the first one. The sub-treatments (4' and 5') applied in the fourth year did not improve the fungicidal efficacy of the disinfestation.

Table 1. Survival percent of *Fusarium* after disinfestation, from biological probes.

Year	1998		1999		2000		2001	
Treatment	10cm	30cm	10cm	30cm	10cm	30cm	10cm	30cm
1 Control	100	100	100	100	100	100	100	100
2 MB60	0	0	0	0	0	0	0	90
3 MB30VIF	0	2.5	5	50	0	100	0	5
4 Sol.+Manure	0	22.5	5	10	0	60	0	20
4' SolMV							25	90

Year	1998		1999		2000		2001	
Treatment	10cm	30cm	10cm	30cm	10cm	30cm	10cm	30cm
5 Sol.+MS	12.5	50	0	55	0	50	20	45
5' SolVM							40	60
6 MS	12	40	10	75	0	50		
7 Manure 15	100	100	100	100				

Solarization combined with manure treatment (4) was similar to MB treatments in plant vigour the first two years while solarization combined with MS was similar to that one the first year only. In the following years MB treatments showed better plant vigour than the rest of them. Control and manure treatment (7), when it was applied, produced less vigorous plants. Plant failure and small plant size in manure treatment (7) could be due to phytotoxicity caused by excess manure.

The effect of weeds was significantly higher in manure and control. From the point of view of weed control, all other treatments had a similar effect as MB treatments along the experiment.

Results concerning earliness are not fully representative because of the variability in environmental conditions from one year to another (cold temperatures in winter 1998-99, severe drought in 1999-2000, and early high temperatures and drought in 2000-2001). Despite these abnormalities, there is a slight trend to increase earliness in the improved solarization treatments if we take into account the results in a global manner.

Results of marketable yield and percentage of second quality fruit yield are shown in Table 2. Duncan multiple range tests for marketable yield can offer us an approach about the efficacy of every treatment although they had to be done for each year independently because of the change of the variety and the large differences in climatic conditions from one year to other.

Table 2. Marketable yield and % of class 2 quality fruit yield over marketable along the four years.

	Marketable yield (g/m^2)				% Second quality fruit yield			
Treatment	1998	1999	2000	2001	1998	1999	2000	2001
1 Control	2325 c	2039 c	1150 c	603 d	16.2 c	23.1 b	27.7 e	20.5 c
2 MB60	3956 a	3835 a	2852 a	3076 a	8.5 a	14.6 a	12.3 a	10.9 a
3 MB30VIF	3735 a	3547ab	3123 a	3048 a	8.6 a	13.9 a	13.1 a	11.4 a
4 Sol.+Manure	4090 a	3011ab	2029 b	1553 bc	10.3 a	18.3 ab	21.0 d	19.0 bc
4' SolMV				1464 c				15.7 b
5 Sol.+MS	4028 a	2815 b	2014 b	1803 bc	9.7 a	18.9 ab	18.5 cd	16.5 b
5' SolVM				1973 b				17.0 bc
6 MS	3104 b	2131 c	1924 b		11.3 ab	23.3 b	19.5 cd	17.7 bc
7 Manure 15	2069 c	1003 d			15.1 c	34.2 c		

Regarding marketable yield, we can observe that the more stable treatments are those based on MB although all treatments seem to decline in productivity along the years.

Solarization with manure treatment does not differ significantly from MB treatments in the first two years. Nevertheless, it moves to a group of classification different from MB in the last two years. Solarization combined with MS belongs to the MB group of classification the first year only.

Results of MS treatment occupied an intermediate position while it was applied. Solarization improved with MS, even at reduced dosages works better than standard MS always. The worse results were obtained with the control (1) and manure (7) treatments. If we take into account the results of marketable yield for 4, 4' , 5 and 5' treatments in the last year, we realise that the effect of replacing manure for MS (4') is worse than that one caused by the replacement of MS for manure (5'). In fact, differences between treatments 4 and 5 are not significant in the last year while differences between treatments 4' and 5' are significant. With respect to solarization combined with MS at the dosage used in this research, results were not promising after four consecutive years of application on the same plot. Maybe this lack of effectiveness could be corrected by increasing the dosage of this fumigant.

Regarding second quality fruit yield, results indicate that MB treatments offered the lower percentage and the higher stability as well. Improved solarization treatments remain at the same level as MB in this aspect in the first two years only. The worse treatments were manure and control.

CONCLUSIONS

After four years of research, we can infer that improved solarization with manure offers the better possible alternative when data of the first two years are considered but there is a loss of efficacy if these treatments are uninterruptedly applied longer than two years. The effect of replacing MS for manure had less marketable yield losses than the reverse replacement. The only treatment that gives similar results to standard MB in marketable yield, and fruit quality is the MB 30g/m^2 with VIF sheet. This treatment allows us to follow the EU regulations of reducing consumption of MB up to its phase out.

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NON-CHEMICAL ALTERNATIVES TO METHYL BROMIDE FOR THE CONTROL OF ROOT-KNOT NEMATODES IN SOUTHERN ITALY

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ABSTRACT

Soil solarization with different plastic films and inorganic (sulphur) or organic (olive pomace and chicken manure) soil amendments were used, alone or in combination, for the control of *Meloidogyne incognita* on cantaloupe and tomato in two field trials in Southern Italy. Soil solarization positively affected cantaloupe yield and suppressed soil nematode population in the soil. No differences were found between the different plastic films. Sulphur alone was beneficial for yield and nematode suppression at all the tested rates, but no further yield and suppressivity increase was derived from its combination with soil solarization. Olive pomace and chicken manure as single treatments significantly increased tomato yield and suppressed *M. incognita* population, whereas their combination resulted in further positive effects only at the highest rates.

Keywords: cantaloupe, tomato, root-knot nematodes, soil amendments, solarization, integration.

INTRODUCTION

Use of organic and/or inorganic amendments could represent one of the possible alternatives to chemicals in the control of root-knot nematodes, *Meloidogyne* species (D'Addabbo 1995; Rodriguez-Kabana 1986). Moreover, the combination with soil solarization could enhance the nematicidal action of these amendments (Gamliel & Stapleton 1993; Greco *et al.* 1992). Two field trials were carried out in 1999-2000 to verify the suppressivity of some organic (olive pomace and chicken manure) and inorganic (sulphur) amendments largely available in Southern Italy, alone or combined with soil solarization, on *Meloidogyne incognita*.

MATERIALS AND METHODS

In a first experiment, a field infested by *M. incognita* was subdivided in 2 x 5 m plots, which were then solarized from June to August 1999 with 0.050 mm polyethylene or 0.035 mm EVA films and/or treated with three different doses (500, 750 and 1,000 kg/ha) of a 90 % sulphur formulation. Four replicates were provided for each treatment, according to a randomized block design, and untreated soil served as control. Cantaloupe (cv. Gialletto Rugoso di Cosenza) was cultivated in the plots from May to August 2000.

In the second trial, another *M. incognita* infested soil, arranged in 3 x 4 m plots, was amended with fresh olive pomace at the rates of 25, 50 and 100 T/ha and/or chicken manure at 1, 2 and 4 T/ha in January 1999. A dosage of 300 kg/ha of granular fenamiphos and untreated soil served as control. One month old tomato (cv. Tondino) seedlings were planted in the plots in the next May. In both experiments, crop yield was recorded and the final nematode population was determined in the soil.

RESULTS AND DISCUSSION

In the first experiment, the yield of cantaloupe was significantly increased by soil solarization with or without sulphur and by sulphur as single treatment. The thickness of the polyethylene did not affect the yields, which were significantly increased by EVA 0.150 mm, compared to EVA 0.035 mm. The addition of either 750 or 1,000 kg/ha sulphur previously to soil solarization was beneficial compared with the polyehylene tarping only. The sulphur application under EVA tarping did not statistically increased the yield with respect to 500 kg/ha sulphur alone (Table 1). The nematode population was significantly suppressed by either solarization or sulphur treatments: No difference was found between the two films or among the sulphur dosages and no further suppression derived by the combined use of solarization and sulphur.

In the second experiment, olive pomace and chicken manure significantly increased tomato yield and reduced *M. incognita* population in the soil, compared to untreated control, at all the tested dosages (Table 2). Combinations of 100 T/ha olive pomace with 2 and 4 T/ha chicken manure resulted in a further yield increase and were more suppressive than fenamiphos on *M. incognita*.

In conclusion, sulphur application, either alone or combined with solarization, could represent a further possible alternative for integrated nematode pest management. The suppressivity of olive pomace and chicken manure, either alone or combined, was previously reported in other experiments (D'Addabbo & Sasanelli, 1996;

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Table 1: Effect of soil solarization and sulphur on *Meloidogyne incognita* on cantaloupe.

Sulphur (kg/ha)	Soil solarization	Crop yield (T/ha)		Final nematode population (Eggs and J2/ml soil)	
-	-	12.6	ab*	11.7	a
500	-	16.4	b	3.2	c
500	Polyethylene 0.050 mm	16.2	b	3.1	c
500	EVA 0.035 mm	19.0	bc	2.9	c
750	-	17.8	bc	4.3	c
750	Polyethylene 0.050 mm	20.4	c	2.6	c
750	EVA 0.035 mm	20.2	c	4.3	c
1000	-	17.0	bc	3.7	c
1000	Polyethylene 0.050 mm	20.4	c	3.4	c
1000	EVA 0.035 mm	20.4	c	1.6	c
-	Polyethylene 0.050 mm	17.0	bc	2.1	c
-	EVA 0.035 mm	16.4	b	4.9	bc

* Means followed by the same letters in the same column are not significantly different according to Duncan's Multiple Range Test (P = 0.01).

Table 2: Effect of olive pomace (FOP) and chicken manure (CM) soil amendments on *Meloidogyne incognita* on tomato.

Treatments	**Crop yield (T/ha)**		**Final nematode population (Eggs and J2/ml soil)**	
Untreated control	44.8	a*	49.1	a
Fenamiphos 300 kg/ha	65.0	def	20.7	cdef
FOP 25 T/ha	57.1	bcd	27.6	bc
FOP 50 T/ha	53.0	ab	26.8	cd
FOP 100 T/ha	56.5	bcd	25.5	cde
CM 1 T/ha	53.5	ab	37.9	b
CM 2 T/ha	54.3	bc	26.1	cd
CM 4 T/ha	53.8	bc	20.4	cdef
FOP 25 t/ha + CM 1 T/ha	56.5	bcd	25.4	cde
FOP 25 t/ha + CM 2 T/ha	56.1	bcd	23.2	cde
FOP 25 t/ha + CM 4 T/ha	60.5	bcd	23.0	cde
FOP 50 t/ha + CM 1 T/ha	60.8	bcd	20.4	cdef
FOP 50 t/ha + CM 2 T/ha	63.3	cde	21.0	cdef
FOP 50 t/ha + CM 4 T/ha	61.0	bcd	20.6	cdef
FOP 100 t/ha + CM 1 T/ha	64.1	def	16.0	def
FOP 100 t/ha + CM 2 T/ha	73.8	f	14.5	ef
FOP 100 t/ha + CM 4 T/ha	71.8	ef	11.3	f

* Means followed by the same letters in the same column are not significantly different according to Duncan's Multiple Range Test (P = 0.01).

NON-CHEMICAL ALTERNATIVES TO METHYL BROMIDE IN URUGUAY

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ABSTRACT

Methyl bromide (MB) is used in Uruguay on vegetable crops, tobacco, cut flowers and seedbeds. MB consumption increased from 4 t in 1993 to some 59 t in 2000, equivalent to a 15-fold increase. Non-chemical alternatives to MB are reported, based on the use of biofumigation, solarization, resistant varieties and crop management that farmers are already applying. Results confirm that MB is not necessary in vegetable and strawberry crops in Uruguay. MB phase out is urgent because of the risk that this chemical represents to workers and farmers.

Keywords: nematodes, biofumigation, solarization, resistant varieties, agro-ecology

INTRODUCTION

Methyl bromide (MB) is used in Uruguay by horticulture under greenhouse, cut flowers and substrate disinfections in seedbed preparation. According to the General Direction of Agricultural Services from the Agricultural-Grazing-Fishery Ministry (GDAS), the volumes imported increased significantly from 40,189 kg imported in 1999 to 58,506 kg in 2000 following a crescent trend for this year. It is remarkable that by 1997 the amount of MB imported was less than 10 tonnes and in 1993 it was around 4 tonnes (MBOTC 1995).

In most of Uruguayan crops there is no use of MB. It is used in tomato, pepper, cucumber and melon which are cultivated under greenhouse mainly in Salto, as well as in tobacco nurseries in Artigas. MB use in Uruguay is unusual and it is restricted to areas of intensive horticulture. Notably, Uraguay is one of the very few countries in which strawberries are produced without MB. For all these reasons, we consider that Uruguayan experiences should be better known and could be used as examples for other regions (De León & Peyrou 2000).

Among sanitary problems caused by soil pathogens that affect intensive horticulture that leads to the use of MB as a control measure, we find damage caused by nematodes belonging to *Meloidogyne* genus *(M. arenaria* and *M. incognita*). Monoculture, temperature and moisture increases in crops grown under greenhouses, the intensive use of pesticides and fumigation products, the incorrect use of resistant varieties have together made phytosanitary problems worse.

Among non-chemical alternatives there are solarization and biofumigation, besides a variety of agricultural practices, resistant varieties, biological control agents (Casanello *et al*. 2001; De León *et al*. 2000abc; 2001ab); and among chemical ones dazomet and metam sodium are used (INIA-ONUDI 2001).

NON CHEMICAL ALTERNATIVES

Biofumigation was evaluated in Uruguay using different organic crop remnants, green manures, resistant varieties and other agricultural techniques under an integrated production system.

Biofumigation

One of this alternatives which is being developed with success in many countries and in Uruguay is biofumigation, defined as: “The action of volatile substances, produced by organic matter bio-decomposition, in plant’s pathogens” (Bello *et al.* 2000 a, b). The use of this materials helps either to solve the environmental problems generated by organic crop remnants. This technique is cheap and easy to put into action, and combined with other practices such as solarization it may become more efficient by means of enabling it’s application in different seasons and areas with optimum temperatures, despite temperature is not a limitation variable. Biofumigation success is enhanced when included in an integrated crop management system. A wide variety of materials have been tested as soil amendments that are useful for control of nematodes, fungi and weeds.

Since 1996 there have been carried out evaluations in several crops and productive regions from Uruguay about the effects of biofumigation in controlling nematodes belonging to *Meloidogyne* genus. The success of this practice have been confirmed in Tacuarembó for salt-wart and tomato crops, and in Montevideo and Canelones for tomato crops. Besides we consider that biofumigation effects are the key factor in the low incidence of nematodes and other soil pathogens in horticultural crops located in Bella Union, Canelones, Montevideo and other areas (De León *et al.* 2000abc; 2001ab).

In the trials, different biofumigants were tested which reached similar efficiency and in some cases better than conventional chemical phytosanitary products. Tested were: chicken dung with rice husk, hen dung with rice husk, cattle and sheep dung, rice husk, broccoli, corn and other agro-industrial residues (Bello *et al.* 1999; 2000ab). The difference with the pesticides available in the market is that they do not have negative effects neither over the environment nor over people's health (consumers and farmers). Furthermore, biofumigants do not have restrictions applied in integrated production systems or ecological agriculture.

Additionally we want to point out that using agricultural residues, which are an excellent organic matter source, is to make them work both as soil improvers and as soil pathogen controllers. In that way the amount of soil nutrients increases improving soil productive capacity by means of a higher offer of available nutrients for plants. Soil structure is also ameliorated, promoting permeability and aeration resulting in increased effectiveness of organic fertilization, it increases microorganism activity and biological soil process associated, both by population growth and enrichment of biodiversity.

Uruguay is carrying out a project to develop alternatives to MB as well as to avoid its use. It began in 1999 and its results had been exposed recently confirming the existence of alternatives as biofumigation which achieves similar or better performance than MB in pathogen control, using several organic materials: rice husk, chicken dung with rice husk, broccoli, sweet corn and cauliflower residues (INIA-ONUDI 2001).

The search for alternatives for MB is not only for environmental and human health reasons, but also for economic ones. The application of MB in 1000 m^2 of greenhouse, at its recommended doses of 100 g/m^2, costs US$710. On the contrary, biofumigation for the same area costs US$75 including labour.

Solarization

Solarization is used in horticultural regions from Salto and Bella Unión, and is a way of soil pasteurizing using summer sun radiation to increase soil temperatures. It is implemented by covering previously humidified soil with transparent nylon during the warm season. This technique had obtained a variable efficiency to control nodule producer nematodes (*Meloidogyne)* (De León *et al.* 2000 c; Casanello *et al.* 2001).

Resistant varieties

The use of resistant varieties is being applied in tomatoes, but it is necessary to take into account that resistance may not be effective when the soil temperature is high, when the plant roots are attacked by fungi, or more frequently, when resistant plants are exposed to virulent populations.

It has been reported that tomato varieties with *Mi* gene, which awards resistance against nodule producer nematodes (*Meloidogyne arenaria, Meloidogyne incognita and Meloidogyne javanica),* are very affected by those pathogens and, in some cases, even making the crop unfeasible. This occurs recurrently in northern Uruguayan departments, specially Salto and Artigas, where soil temperatures often exceed 27 ºC, one of chief ways resistance is broken.

This situation is serious since resistant varieties have been considered as a major alternative to MB. Several populations of *Meloidogyne incognita* coming from resistant tomato plants roots have been studied and it was find that they maintained their virulence in chambers with temperatures under 25ºC. This leads us to think that conventional cropping practices are contributing to the selection of virulent populations which could make resistant varieties non-viable in the future (De León *et al*. 2001 a).

Other cropping practices

The most frequently used are organic residue mulching, in order to regulate soil temperature, reducing the length and number of *Meloidogyne* genus nematodes cycles while dropping the number of adventitious plants. It is of major importance to design integrated production systems, with crop rotation, introducing brief cycle crops, such as lettuce, that function as trap plants. It is also important to accurately plan the planting time and sanitary procedures in order to lessen pathogens risks.

CONCLUSIONS

Biofumigation has a similar efficiency as conventional pesticides for controlling vegetable soil pathogens, with the additional benefit of improving soil and plant qualities. The methodology required to put biofumigation into practice under commercial field conditions is approachable for both technicians and farmers. Its success is enhanced when included as part of an integrally managed production system.

We consider that the obtained results show that there are effective alternatives to MB tested in different productive regions of Uruguay. Biofumigation, due to its efficiency and low cost, is becoming the principal alternative practice in different productive areas in Uruguay. It is necessary to bring forward the date of MB elimination in order to reduce its deleterious impact on farmer and worker health and on the environment.

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INFLUENCE OF PREPLANT SOIL FUMIGATIONS ON *TRICHODERMA* POPULATIONS IN STRAWBERRY PRODUCTION FIELDS

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ABSTRACT

This paper is part of the national project INIA SC 97-130 on methyl bromide (MB) alternatives for preplant soil fumigation on several crops, among them the strawberry crop. Several treatments were tested over three years at two places in Huelva province. The influence of these treatments on *Trichoderma* populations was evaluated by soil isolated onto selective media. These populations have been increased year after year and significant differences were detected between the treatments and the control. The major populations were observed after MB treatments.

Keywords: methyl bromide, strawberry, *Trichoderma*.

INTRODUCTION

The field trials and laboratory assays reported are part of the national project INIA SC 97-130 on methyl bromide (MB) alternatives for preplant soil fumigation on strawberry crop. Several treatments were tested for over three years at two locations in Huelva (southern Spain), where more than half the strawberry acreage in Spain is situated (Hancock 1999). The majority of the hectarage is under small tunnels for early production of fresh market fruit. Plants are grown in intense annual systems on raised beds with preplant fumigation (López - Aranda & Bartual 1999), basically using broad-spectrum fumigants as MB and chloropicrin. Because of the use of MB for years, Huelva soils show low levels of soil pathogens, but crop yields are better in fumigated soil than in not fumigated ones.

Species of *Trichoderma* are primarily studied for their ability to control plant disease through mycoparasitism and/or the production of antimicrobial compounds (Bailey & Lumsden 1998). These species can affect plant growth (Lindsey & Barker 1967; Wright 1956). Determination of these effects depend on many interactions that take place in the soil between *Trichoderma* spp., other microorganism, changes in the soil environment, and the plant root (Bailey & Lumsden 1998). Soil fumigations affected the environment and induced variations on *Trichoderma* populations, and after MB treatments these fungi can reproduce rapidly (Munnecke *et al.* 1981). The objective of this report was study the native *Trichoderma* after different fumigations.

MATERIALS AND METHODS

Two field trials were carried out at two locations in Huelva: Moguer (eastern coast) and Cartaya (western coast). The design of each experiment was in randomized block with three replicates. Fumigations (Table 1) were applied before planting with cv. "Camarosa".

Soil samples were taken after fumigations by a vertical graduated drill (0-20 cm). Samples were air dried. 10 g of soil was suspended in 99 ml of agar (0.3%) distilled water medium, shaken for 15 minutes and 0.1 ml aliquots were spread on Petri dishes with *Trichoderma* Selective Medium (TSM) with a glass rod (Elad *et al.* 1981). Plates were incubated for 7 days at 25°C.

Mean separation of data was analysed according to the least significant difference, calculated on square root transformed data using Newman and Keuls test, at $P < 0.05$.

TABLE 1: Fumigants treatments.

Treatments	Description
Control	Control without disinfestation
MB (20) Pref. Beds VIF	MB-Pic. (50/50) 20 g/m^2 preformed beds black VIF
MB (40) Pref. Beds	MB-Pic. (50/50) 40 g/m^2 preformed beds
Telone C-35 (40)	Telone C-35 telopic 40 cc/m^2 preformed beds
Metam S 175	Metam Sodium 175 cc/m^2 preformed beds
Dazomet (50)	Dazomet 50 g/m^2 preformed beds
Chloropicrin (40)	Chloropicrin 40 g/m^2 preformed beds

RESULTS

Tables 2 and 3 show the results obtained after the treatments. In the first year, the number of *Trichoderma* spp. propagules was the lowest, without significant differences between the control and the treatments. After one crop and two treatments, soils fumigated with MB or Telone showed important populations of *Trichoderma* spp., with significant difference in all cases between MB and the control, but there were no significant differences between MB doses. The same effect was observed incremented in the last year.

When soil was treated with Metam Sodium or Dazomet the number of propagules was very low or zero. The exception was observed at Tariquejo in the last year as there were problems of humidity on pre-plant dip irrigation at the moment of Dazomet incorporation.

Figure 1 shows the evolution of *Trichoderma* population. After repeated treatments with MB, Telone and chloropicrin the number of popagules increased year by year.

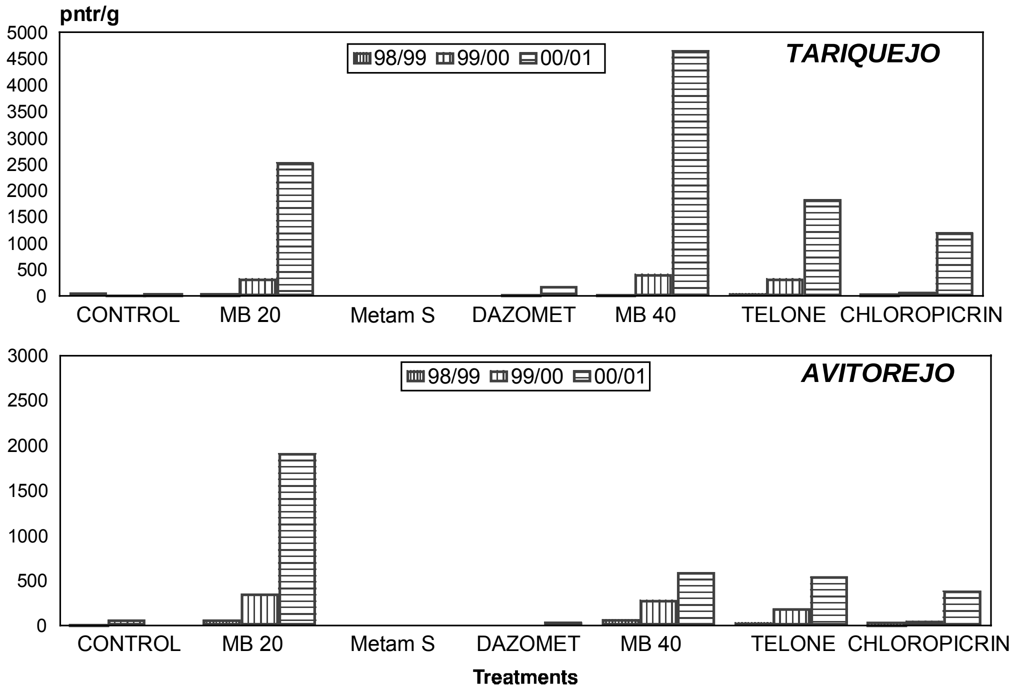
TABLE 2: Propagules number of *Trichoderma* spp. per gram of soil (pntr/g) (Tariquejo)

	pntr/g		
Treatments	**1998/99**	**1999/2000**	**2000/01**
Control	66.0 a	6.6 b	39.6 c
MB (20) VIF	33.0 a	336.6 a	2534.7 ab
MB (40)	13.2 a	415.8 a	4657.0 a
Telone C-35 (40)	52.8 a	336.6 a	1831.7 ab
Metam S 175	0 a	0 b	0 c
Dazomet (50)	0 a	19.8 b	188.12 bc
Chloropicrin (40)	26.4 a	79.2 b	1207.9 abc

TABLE 3: Propagules number of *Trichoderma* spp. per gram of soil (pntr/g) (Avitorejo)

	pntr/g		
Treatments	**1998/99**	**1999/2000**	**2000/01**
Control	6.6 a	66.0 bc	0 b
MB (20) VIF	66.0 a	356.43 a	2801.9 a
MB (40)	72.6 a	287.13 a	594.0 a
Telone C-35 (40)	33.0 a	191.4ab	544.5 ab
Metam S 175	0 a	0 c	0 b
Dazomet (50)	0 a	0 c	39.6 b
Chloropicrin (40)	26.4 a	52.8 bc	386.1ab

Figure 1.- Evolution of *Trichoderma* populations for three years.



CONCLUSIONS

Some fumigants such as MB, Telone or chloropicrin induced an increase of *Trichoderma* spp. in soils. These organisms could affect the plant growth and it could be an explanation for the beneficial effect of MB over the strawberry crop.

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QUARANTINE TREATMENT OF STORAGE INSECT PESTS UNDER VACUUM OR CO_2 IN TRANSPORTABLE SYSTEMS

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ABSTRACT

The objective of our investigation was to identify the combinations that enhance the effectiveness of insect control, based on vacuum or CO_2 in combination with increased temperatures, as a quarantine treatment to control storage pests. In laboratory studies with *Lasioderma serricorne* exposed to low pressures at 30ºC, LT_{99} value for adults was 15 h when exposed to 25 mm Hg. *Trogoderma granarium* diapausing larvae were the most resistant species, requiring 172 h exposure under the same conditions. The effect of CO_2 at 45°C for reducing the exposure time for diapausing larvae of *T. granarium* showed that, by increasing the CO_2 concentration to 90%, the LT_{99} decreased to about 10 h, whereas at 35ºC the LT_{99} value was 29 h. *Ephestia cautella* larvae were shown to be the most resistant stage to 90% CO_2 at 40°C, with an LT_{99} value of only 6 h. For *Oryzaephilus surinamensis* under the same conditions, the LT_{99} value was 9 h for the most resistant egg stage. These encouraging results led to the idea of developing a transportable flexible storage system to render the technology a practical tool for the control of insect pests. Experiments were carried out using a 15m^3 plastic container termed the "Volcani Cube™" or "GrainPro Cocoon™". Bioassay field trials demonstrated that complete mortality of test insects composed of all four developmental stages of *E. cautella* and *Tribolium castaneum* was observed when exposed to a vacuum for 3-days.

Keywords: quarantine treatment, methyl bromide, alternatives, vacuum, CO_2, storage insects, transportable systems, *Trogoderma granarium*

INTRODUCTION

Although there are a number of suggested potential chemical and non-chemical alternatives to methyl bromide (MB), each has limitations that prevent it from being a direct replacement for MB in all its current uses (Bell *et al*. 1996). Controlled atmosphere (CAs) can fulfill a specific niche where use of other fumigants is unacceptable such as treating organic foods. CAs are limited by the long exposure times required to produce complete mortality (Navarro and Jay 1987), and are similar to those required for phosphine (PH_3) fumigations (Navarro and Donahaye 1990). In cases where rapid disinfestation of commodities is required, the possibility of using CO_2 at temperatures raised to levels that will not adversely affect the commodity should be considered.

Investigations on effects of low pressures on storage insects were carried out by Back and Cotton (1925), Bare (1948), and later on by Calderon *et al*. (1966), and Navarro and Calderon (1969; 1972a; 1972b). Recently Mbata and Phillips (2001) investigated the effects of temperature and exposure time on mortality of three stored product insects exposed to low pressure. Insect mortality under low pressure is predominantly a result of oxygen deficit and not due to physical pressure effects (Navarro and Calderon 1979).

In a first attempt to use low pressures to preserve cacao beans quality, Challot and Vincent (1977) used polyethylene bags applying a low pressure of 600 mm Hg. Although 600 mm Hg may be effective in maintaining the product quality and prevent ingress of insects, storage insects can tolerate this pressure. For mortality of storage insects, low pressures below 100 mm Hg are required.

Gas tight flexible structures using the hermetic storage method have been developed and are in use on an industrial scale (Navarro *et al.* 1988; 1994; Navarro *et al.* 1990). These structures enable the treatment of modified atmosphere or fumigation (Navarro *et al.* 1995), and they are termed “Volcani Cubes™” or “GrainPro Cocoons™” (Navarro *et al* 1999). The use of these flexible storage facilities to maintain low pressures of 25-30 mm Hg was reported recently (Phillips *et al*. 2000; Navarro *et al*. 2001).

This paper reports on the effects of exposure time and treatment temperature on the mortality of different life stages of stored product insect pests exposed to increased temperatures and a constant low pressure or under a CO_2 enriched atmosphere, and it reports on the application of transportable systems that use these combinations for quarantine treatments.

MATERIALS AND METHODS

Temperature, low pressure and CO_2 combinations

For low-pressure treatments, absolute pressures of 25, 50 and 100 mm Hg at temperatures varying from 18° to 35ºC were tested. For CO_2 treatments, concentrations varying from 60% to 90% of CO_2 in air at temperatures varying from 30° to 45°C were tested.

Test insects

Diapausing larvae of Khapra beetle *(Trogoderma granarium)* were obtained by holding active larvae without food for one month at 28ºC (Lindgren and Vincent 1960). Adults of *Oryzaephilus surinamensis, E. cautella* and *Lasioderma serricorne* were taken from laboratory cultures reared on standard artificial diet. Eggs (0-2 days old), pupae and adults (1-2 days old) and larvae (4-15 days old) were taken from the same batch. Two Perspex slides each with 50-drilled "wells" were used to individually place 100 eggs from each of the studied species (Navarro and Gonen 1970). Following treatment, larvae, pupae and adults were transferred to small jars (50 ml) and maintained at 28±1°C and 65±5% r.h. Adults and larvae were provided with food.

Statistical analysis

To determine the lethal time to obtain 99% mortality (LT_{99}) data were subjected to probit analysis (Daum 1979). Results in this paper are presented without detailed statistical analysis to show the ranges of exposure times needed to control the test insects.

Application of the technology

The tested transportable system was made of flexible PVC which had been in use commercially for hermetic storage of commodities to control insect disinfestation by modified atmospheres (Navarro *et al.* 1999). Experiments were carried out using a 15-m^3 capacity plastic container called the “Volcani Cube™” or “GrainPro Cocoon™”. The pressure was maintained between 25 to 29 mm Hg and bioassay consisted of all four developmental stages of *E. cautella* and *T. castaneum*.

RESULTS AND DISCUSSION

Effects of low pressures and increased temperatures

Table 1 shows partial results obtained on three developmental stages of *L. serricorne*. Although the LT_{99} value for *L. serricorne* adults exposed to 25 mm Hg at 30ºC was 15 h, there is an apparent resistance of this species to low pressures. Eggs exposed to 25 mm Hg even at 30^0C needed 75 h exposure to attain LT_{99} value. Bare (1948) also observed greater tolerance of *L. serricorne* eggs compared with other stages exposed to low pressure. Mortality values for diapausing larvae of *Trogoderma granarium* are shown in Table 1. When the pressure was decreased to 25 mm Hg and the temperature raised to 35°C, the LT_{99} value was 146 h; at 30°C under the same pressure, it was 172 h. These lengthy exposures are comparable with 6 and 7-day exposures required for phosphine fumigation (Navarro and Donahaye 1990). These findings may also be compared to those of Calderon and Navarro (1968), on non-diapausing larvae at 25ºC and 65% rh, where complete mortality was obtained within 120 h exposure to 20 mm Hg.

Effects of CO_2 and increased temperatures

Table 2 shows the effectiveness of the combination of CO_2 at temperatures in the range of 35ºC to 45ºC on *Ephestia cautella.* The pupa was the most resistant stage when exposed to 90% CO_2 with an LT_{99} value of 17 h at 35ºC, and only 3 h when exposed at 45ºC. The adult was the most sensitive stage of *E. cautella* requiring only 4 h of exposure to 90% CO_2 at

35ºC. Results on *O surinamensis* developmental stages show that increasing the CO_2 concentration resulted in decreasing the LT_{99} value. Generally, the eggs were the most resistant stage; at 40ºC and 90% CO_2 a six h exposure was required for an LT_{99} value. Mortality values for diapausing larvae of *T. granarium* at 45°C show that increasing the CO_2 concentration to 90%, the LT_{99} value decreased to 10 h, whereas at 35ºC the LT_{99} value was 29 h.

T. granarium is one of the most serious pests of stored cereal grains and oil seeds, and is subject to strict quarantine regulations in the US, Australia and several other countries. It is a voracious feeder of grain products. The larvae can hide in cracks of the storage structure in a state of facultative diapause and can remain in this condition for years. It is particularly difficult to control with insecticides. Consequently, many quarantine treatments are mandatory when products such as rugs, spices and cereal products are imported from infested countries. In such situations, MB is still the only effective fumigant against this pest. Present distribution of *T. granarium* includes Western Africa through the Northern Indian subcontinent. Results shown in Table 2 may serve as guidelines to the possibility of applying increased temperatures for the quarantine treatment of the most resistant diapausing larvae of *T. granarium.*

TABLE 1: Effects of temperature and low-pressures on LT_{99} (hours to obtain 99% mortality) values for *Lasioderma serricorne* at various development stages and for *Trogoderma granarium* diapausing larvae.

Pressure (mmHg)	Temperature (ºC)	*Lasioderma serricorne*			*T. granarium*
		Eggs	Larvae	Adults	Diapausing larvae
25	18	-[1]	-	47	-
	25	-	-	26	>360
	30	75	-	15	172
	35	-	-	-	146
50	18	-		157	-
	25	-	191	43	>360
	30	-	49	15	260
	35			-	>360
100	18	136	-	-	-
	25	75	-	75	>360
	30	40	-	-	>360
	35	-	-	-	>360

[1] Data not available

Rigid metal chambers have been in use for the implementation of vacuum fumigation in agricultural commodities (Bond 1984). These structures are expensive and are not easily transportable. In order to render the technology practical, the possibility was recently investigated of using CO_2 or low pressures to control storage insects in a transportable system (Phillips *et al*. 2000). Bioassay in field trials at 22° to 25°C demonstrated that complete mortality of test insects composed of mixed ages of *E. cautella*, and *T. castaneum* was possible following 3-days exposure to vacuum. For quarantine treatments of durable commodities, these flexible storage containers could be considered useful for applying vacuum or CO_2 as alternative methods to MB or other toxic fumigants.

TABLE 2: Effects of temperature and CO_2 concentrations in air expressed in LT_{99} (hours to obtain 99% mortality) values for *Ephestia cautella* and *Oryzaephilus surinamensis* at various development stages and for *Trogoderma granarium* diapausing larvae.

Insect species	Temp. (°C)	30				35				40				45			
	CO_2 (%)	60	70	80	90	60	70	80	90	60	70	80	90	60	70	80	90
Ephestia cautella	Eggs	-[1]	-	-	-	23	23	17	9	16	12	8	5	9	5	3	2
	Larvae	-	-	-	-	60	27	20	12	17	9	6	6	5	4	2	2
	Pupae	-	-	-	-	56	37	17	17	36	10	8	4	7	4	4	3
	Adults	-	-	-	-	20	14	6	4	6	5	3	2	3	2	2	2
Oryzaephilus surinamensis	Eggs	-	-	38	22	29	25	22	9	15	7	6	6	-	-	-	-
	Larvae	-	-	-	-	-	-	-	-	8		2	2	-	-	-	-
	Pupae	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-
	Adults	21		22	9	26	11	8	4	12	11	6	3	-	-	-	-
T.granarium	Diapausing larvae	-	-	-	-	38	29	-	-	24	28	20	-	15	17	15	10

[1] Data not available

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METHYL BROMIDE ALTERNATIVES FOR CARROTS AND OTHER VEGETABLE CROPS IN NORTHWEST COAST OF CADIZ (SPAIN)

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ABSTRACT

Carrot are produced on about 2,900 ha in the Cadiz area. Soil-borne problems are root-knot nematodes, *Pythium* sp., "cavity spot" and nutsedges. Field experimental design was randomized blocks with three replicates. Chemical (MB, Telone-C17, PIC, Telone II), non-chemical (solarization + biofumigation) and mixed (solarization, + metam-sodium + Telone II) treatments started in July 1997. Four carrot cultivations were carried out between October 1997 and May 1999. The final experiment was carried out on potato (August-December 1999). Good sanitary conditions were evident. The results suggest alternatives to MB exist for carrot and other vegetable crops produced in the Cadiz area.

Keywords: carrot, potato, chemical, non-chemical, alternatives, solarization, biofumigation, metam-sodium, nematodes

INTRODUCTION

Cut-flowers are mainly produced on about 900 ha of coastal area near Cadiz in Spain. These cut-flower cultivations under plastic greenhouses are carried out in very small farms and they are associated with field (open-air) production of several vegetable crops. Field production usually begins in the autumn with cultivation of carrot for the European fresh market. The area of Cadiz has about 2,900 ha of carrot cultivation with typical yields of 5 kg/m^2 of commercial roots. "Mokum" is the most important carrot cultivar in the area. This carrot cultivation is followed by other field vegetable crops like potato, tomato, etc.

Traditionally, the Northwest coastal area of Cadiz has used cold or heat diffusion of MB (98-2) for decades. Soils have a sandy texture and the environment (soil and water) is alkaline. The main pathological soil-borne problems are root-knot nematodes (*Meloidogyne incognita*), *Pythium* sp. and several kind of weeds with very special invasion of yellow nutsedges (*Cyperus* spp.).

The field trials reported are part of the National project INIA SC 97-130 on "Short-term Methyl Bromide (MB) Alternatives for Pre-Plant Soil Fumigation in Several Crops". These real scale experiments have been carried out in several plots located in the Experimental Farm of CIFA-Chipiona (Cadiz). The plots have been cultivated with carrots, potatoes and other vegetable crops for decades where records show the presence of *Meloidogyne incognita, Pythium* sp., carrot "cavity spot", and several kinds of weeds (nutsedges and *Malva* sp.). Experimental design was in randomized complete blocks, with three big replicates of 250 m^2/treatment. Chemical and non-chemical fumigation treatments started in July 1997 (Table 1).

TABLE 1: Soil fumigant treatments in carrots.

Treatments	Description
Control	Control without soil fumigation
MB standard	Standardized cold diffusion MB(98-2), 50 g/m^2
MB VIF	Cold diffusion MB(98-2), 25 g/m^2 under VIF transparent film
Telone C17	Injection 1,3 dichloropropene + chloropicrin, 60 cc/m^2
Chloropicrin alone	Injection chloropicrin, 40 cc/m^2
Telone II VIF	Injection 1,3 dichloropropene, 20 cc/m^2 under VIF transparent film
Soil solarization alone	For 6 weeks

Treatments	Description
Soil solarizat. + MB	Soil solariz. and simultaneous cold diffusion MB(98-2), 10 g/m^2
Soil solarizat. + MS	Soil solariz. and simultaneous emulsion Metam Sodium, 60 cc/ m^2
Soil solarizat. + Telone II	Soil solariz. and simultaneous injection 1,3 dichloropropene, 25 cc/ m^2
Soil solarizat. + Biofumigation	Soil solariz. and simultaneous incorporation 5 kg/m^2 mushroom compost
Soil solarizat.+ cuasi-biofumigat.	Soil solariz. and simultaneous incorporation 3 kg/m^2 chicken manure

After soil fumigation, four carrot cultivations cv. "Mokum" were planted in the same experimental plots, as it is usual in the area. The sowing calendar was as follows. First cultivation: sowing date October, 28th, 1997 and harvest date March, 12th 1998. Second cultivation: sowing date May, 18th, 1998 and harvest date August, 11th, 1998. Third cultivation: sowing date September, 26th, 1998 and harvest date January, 19th, 1999. Fourth cultivation: sowing date February, 10th, 1999 and harvest date May, 18th, 1999. Soil samples were taken before and after fumigation treatments as well as before and after each sowing. In addition, plant carrot samples were taken during the four growing seasons. In all cases, including controls without soil disinfestation, a good sanitary status was evident. Neither root-knot nematodes nor fungi attacks appeared. Agronomic results are presented in Table 2.

TABLE 2: Commercial yield of roots (kg/m^2). Carrot "Mokum". Four cultivations (1997-1999).

Treatments	1º cultiv	2º cultiv	3º cultiv	4º cultiv	Av. four cultivations	Average relative yield
MB VIF	3.44 abc	6.06 a	5.10 ab	3.24 a	4.46 a	108
Solarizat. alone	4.47 ab	4.79 abc	5.02 abc	3.38 a	4.41 a	107
Solar.+ chicken man.	4.73 a	4.91 abc	5.34 a	2.54 a	4.38 ab	106
Telone C-17	3.52 abc	5.62 ab	4.47 abcd	3.40 a	4.25 ab	103
Chloropicrin	3.55 abc	5.27 abc	4.53 abc	3.50 a	4.21 ab	102
Solar.+MB	3.81 abc	5.19 abc	4.91 abc	2.88 a	4.20 ab	102
MB standard	2.56 c	5.93 ab	4.44 abcd	3.56 a	4.12 ab	100
Solar.+ MS	4.48 abc	4.17 bc	4.69 abcd	2.99 a	4.08 abc	99
Telone II VIF	2.96 bc	5.34 abc	4.30 bcd	2.99 a	3.90 abc	95
Solar.+Telone II	3.26 bc	4.43 abc	4.81 abcd	3.01 a	3.87 abc	94
Solar.+ mushroom comp.	3.84 abc	3.71 c	4.24 bcd	2.56 a	3.59 bc	87
Control (no disinf.)	3.70 abc	1.74 d	4.68 abcd	2.86 a	3.25 c	79 $P < 0.05$

In order to confirm these trends, a final experiment with autumn potato, cv. "Jaerla", was carried out in the same soils and experimental design of the previously described carrot trials. The following MB alternative treatments were selected and they were located in the replications that had contained them in the former carrot experiments, with some dosage modifications: Controls without soil fumigation; cold diffusion MB (98-2), 40 g/m^2 under VIF transparent film; soil solarization alone for four weeks (August); soil solarization and simultaneous emulsion with Metam Sodium, 80 cc/ m^2; soil solarization and simultaneous injection of Telopic (Telone C35) 40 cc/m^2; soil solarization and simultaneous biofumigation (5 kg/m^2 of fresh chicken manure). Fumigant treatments began in July, 8th and finished in August, 9th, 1999. Autumn potato dates of planting and harvesting were: August, 13th and December 14th, respectively. Once again, in all the cases a good sanitary status in plants and potato tubers was evident. At the moment of harvesting, tubers were classified in several commercial categories. A summarized report of yield results is presented in Table 3.

TABLE 3: Commercial yield of tubers (kg/m^2). Potato "Jaerla". 1999 results.

Treatments	**kg/m^2 harvested**	**Relative yield**
Solarization+ MS	2.80 a	125
Solar.+Telopic	2.53 ab	113
Solar.+ fresh chicken manure	2.45 ab	109
Solarization alone	2.40 ab	107
MB (VIF	2.24 bc	100
Control (no disinf.)	1.81 c	81 $P < 0.05$

These results suggest that several chemical and non-chemical alternatives to standardized cold or hot diffusion MB (98-2), 50 g/m^2 are able to maintain adequate sanitary and productivity levels on carrot cultivation and other field-grown vegetable crops in the Northwest coastal area of Cadiz.

METHYL BROMIDE ALTERNATIVES EVALUATED IN STRAWBERRY PRODUCTION IN UNEP'S REGIONAL DEMONSTRATION PROJECT IN CENTRAL AND EASTERN EUROPE

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ABSTRACT

A two year evaluation of the chemical (dazomet, 1,3-D+CP, metam sodium) and the biological (*T. viride* B35, *P. fluorescens* PSR21) alternatives for methyl bromide (MB) was conducted in three commercial strawberry farms in Poland. The tested alternatives were applied separately or in combinations. All the chemical agents were applied for preplant soil fumigation according to the recommended procedures. The tested biocontrol agents were used for dipping the root system of the plantlets just before the planting. In the experiments, the performance of all the evaluated alternatives was inferior to MB. However, taking into account the improvements in the plant vigour and in the productivity related to the use of some alternatives, 1,3-D+CP at a higher rate (500 l/ha) as well as combination of *T. viride* B35 with reduced dosage of 1,3-D+CP (375 l/ha) can be considered as a possible replacements for MB. Nevertheless, supplementary application of herbicides will be necessary. Metam sodium at the highest doses (1,500 l/ha) alone or combined with the biocontrol agents controlled the Verticillium wilt, but its effectiveness was not acceptable for commercial reasons. Dazomet did not significantly improve the productivity of strawberry fruits and runners. However, in the absence of the major soil-borne pathogen (*V. daliae*) the application of dazomet as well as metam sodium combined with *T. viride* gave a similar improvement of the fruit production as MB. Moreover, the efficacy of biocontrol agents applied separately was inconsistent.

Keywords: strawberry, soil treatment, transplant treatment, biocontrol agents, integration

INTRODUCTION

A prompt selection and implementation of viable alternatives to methyl bromide (MB) in Central and Eastern European countries is very important for meeting its 2005 phase out requirement. To assist the countries in the region in identifying MB alternatives, UNEP has launched a regional demonstration project in Poland to evaluate the range of chemical and non-chemical alternatives on horticultural crops. Poland has the highest level of MB consumption among the Central and Eastern European countries. In these countries, MB is predominantly used on horticultural crops such as peppers, tomatoes, cucumbers, strawberries and some ornamental plants.

Effective control of the soil-borne strawberry disease complex is a precondition to obtain pest-free propagation material. The direct control of Verticillium wilt is a one of special importance. Until now, sufficiently resistant cultivars have not been available commercially (Shaw & Larson 2001). Germplasm was not found containing obvious genetic diversity that would be useful for developing cultivars specifically adapted to the sublethal effects of organisms in nonfumigated soils (Fort *et al.* 1996; Shaw & Larson 1996). Strawberry productivity can be substantially reduced by growing plants in soil that has not been fumigated prior to planting, even in the absence of lethal pathogens (Chandler *et al.* 2001; Hancock *et al.* 2001).

MATERIALS AND METHODS

Three demonstration experiments were conducted on commercial strawberry farms during 2000 and 2001.

The first experimental site was located at Kielczewo (central western Poland) with the cultivar Camarosa. Each chemical treatment was applied in strips of 2.5 m wide and 250 m long. The transplants were planted on April 20 each year. The soil fumigation was done in the beginning of November 1999 and in mid-October 2000. MB and 1,3-D+CP were applied by an injection method using a tractor injector together with a simultaneous covering of the soil with a double layer of PE film. Dazomet and metam sodium were sprayed on soil, mixed with a rototiller and the soil was compacted with a roller. The following chemicals were applied in 2000: A. MB (Metabrom 98) - 600 kg/ha; B. Dazomet (Basamid GR®) - 500 kg/ha; C. 1,3-D+CP (Telopic®) - 380 l/ha; D. 1,3-D+CP - 530 l/ha; E. Metam sodium (Nemasol®) - 800 l/ha; F. Metam sodium - 1,200 l/ha; G. Control. In year 2001 the following soil treatments were included: A. MB - 600 kg/ha; B. 1,3-D+CP - 375 l/ha; C. Control. In the treatments B, C, E and G in 2000 and in all the treatments in 2001, 1/3 of the transplants used for each band were also dipped in a suspension of *T. viride* B35 or *P. fluorescens* PSR21. The suspension of *T. viride* (10^9 c.f.u./ml) was made in water with starch (40 g/l). The suspension of *P. fluorescens* (10^9 c.f.u./ml) was prepared in a solution of 0.1 M $MgSO_4$ and 0.1% of CMC.

The second experimental site was located at Przysieka (central Poland) with cultivar Tudnew. Each chemical treatment, integrated or not with biocontrol agents, was set up on plots 4.6 m wide (4 rows) and 45 m long. The transplants were planted on 20April 2001. The applications of fungicides and biocontrol agents were done similarly as it was described in experiments at Kielczewo. The soil fumigations were done in October 2000 with the following treatments: A. MB - 600 kg/ha; B. Metam sodium - 1,000 l/ha; C. Metam sodium - 1,200 l/ha; D. Metam sodium - 1,500 l/ha. In the treatments with metam sodium, the transplants were also dipped in the suspension of biocontrol agents.

The third experiment was set up in the southern part of Poland in 2000 with the cultivar Senga Sengana. Soil and transplant treatments were done similarly as at Kielczewo. The transplants were planted on 18May 2000. The fumigation was done in November 1999 except for metam sodium which was applied at the end of March 2000. Only the 1,3-D+CP was applied at different doses (500 and 700 l/ha).

The laboratory evaluation of the effectiveness of the tested alternatives was carried out. For these purposes, the presence of the phytopathogenic fungi on roots was evaluated by a plate count. The surface was disinfected with acidified chloramin. T 100 root fragments (~0,5 cm long) were placed on a selective agar media for isolation of *Fusarium* (Burgess et al. 1988), *Phytophthora* (Jeffers & Martin 1986) and *Verticillium* (Jordan 1971).

RESULTS AND DISSCUSSION

The first assessment of weed control by the chemical treatment was performed at planting time at all locations. In both years the bands treated with MB in all locations as well as the band with dazomet in 2000 were apparently free from primary weed infestation. At Kielczewo in the bands treated with 1,3-D+CP in the both years and with a lower dose of metam sodium in 2000, the percentage of ground covered by weeds was only slightly lower than that in the control bands. At this location, the higher dose of metam sodium (1,200 l/ha) reduced the weed infestation by about 50% in 2000. The dominant weed species were *Arthemidae, Echinochloa crus-gall, Erodium cicutarium* and *Viola tricolor*. The weed infestation of untreated bands and the ranking order of the chemicals in controlling weeds at Brzezna was similar as at Kielczewo in 2000.

In the second year after the fumigation at Brzezna, practically no effect of the soil fumigants on weeds was observed. The dominant weed species were *Cirsium* spp., *E. crus-gall, Galinsoga parviflora* and *Agropyron repens.* In the experiment at Przysieka the herbicidal effectiveness of Nemasol at all three doses evaluated one week prior to planting was very low and the reduction of the weed infestation did not exceed 30%. The dominant weed species were *Arthemidae, E. crus-gall, E. cicutarium* and *V. tricolor*. The second assessment of the weed infestation done at the end of July in both years revealed that commercially acceptable weed control was achieved only in bands treated with MB in all locations.

In the experiment at Brzezna in 2000, the vigour of the plants in untreated bands at the early growing period was visibly poorer than in the MB, 1,3-D+CP and metam sodium (1,200 l/ha) treated bands (Table 1). The stimulating effect of MB and 1,3-D+CP soil treatments was even more pronounced at Kielczewo in 2001. However, the effect of 1,3-D+CP was significantly lower than the effect of MB (Table 1). In most cases, both biocontrol agents applied as a transplant drench treatment stimulated noticeably the development of the mother plants in comparison with the chemicals applied alone at Brzezna (Table 1).

TABLE 1: The effect of MeBr and alternatives on plant vigour in field conditions expressed by the area of plant (cm^2).

Soil treatment	Transplant treatment		
	Control	**T. viride**	***P. fluorescens***
Experimental site, Kielczewo 4 July 2001, cv. Camarosa			
Control	805 c*	822 c	699 c
1,3-D+CP 375 l/ha	1,149 b	1,148 b	1,149
Methyl bromide 600 kg/ha	1,360 a	1,356 a	1,388
Experimental site, Brzezna 28 September 2000, cv. Senga Sengana			
Control	4,822 b	4,768 b	5,366
Dazomet 500 kg/ha	4,755 b	4,754 b	5,005
1,3-D+CP 500 l/ha	5,634 a	5,400 ab	5,480
1,3-D+CP 700 l/ha	5,285 ab	4,836 b	4,836
Metham sodium 800 l/ha	4,882 b	4,913 b	4,955
Metham sodium 1,200 l/ha	5,061 ab	5,269 ab	5,310
Methyl bromide 600 kg/ha	5,434 ab	5,356 ab	5,699

** Values followed by the same letters within the frame of the same experimental site are not significantly different within the columns according to Newman-Kuels test ($\alpha < 0,05$),*

At Przysieka, the vigour of the plants in the bands treated with Nemasol during the early growing period was substantially poorer than that of the MB-treated soil (data not shown).

The phytopathological studies of strawberry plant root infestation revealed a lack of root infestation with *Phytophthora* and *Fusarium* at Brzezna in 2000. We have isolated sporadically fungal colonies (< 4%) on selective media for *Verticillium* from untreated bands. Sampled roots from Kielczewo in 2000 were infested with *Phytophthora* in the range from 44% to 11% in the control and in the MB treated bands, respectively. Several isolates were identified as *P. cactorum*. The plants affected by this pathogen were randomly scattered all over the field. The chemical treatments alone and in the combination with the biocontrol agents significantly reduced (about 50%) the spread of this pathogen in comparison with untreated bands.

In 2001 on selective *Fusarium* medium, single colonies (< 7%) were detected from roots sampled at Kielczewo. No symptoms of *Phytophthora* were visible during the vegetation period and no isolates on the root segments were found. The enumeration of *Verticillium* showed that the significant number of root segments (41–50%) sampled from the untreated bands was colonised by this pathogen in 2001. Most of the selected isolates were identified as *V. dahliae* but the characteristic visible disease symptoms were not observed in the field. The great number of root segments (45-47%) sampled from the Nemasol-treated bands at Przysieka were infected by *Verticillim* and this observation could be connected with observed severe symptoms of Verticillium wilt (Table 2). Most of the selected isolates were identified as *V. dahliae*. Moreover, a few cultures isolated on selective media revealed also the presence of *F. culmorum* and *F. oxysporum*.

TABLE 2: The Verticillium wilt incidence at Przysieka expressed as percentage of wilted plants.

Soil treatment	Transplant treatment		
	Control	**T. viride**	***P. fluorescens***
Metham sodium 1,000 l/ha	16.3	5.6	14.4
Metham sodium 1,200 l/ha	12.8	8.8	4.1
Metham sodium 1,500 l/ha	1.6	8.8	3.1
Methyl bromide 600 kg/ha	0.7	N.D.	N.D.

TABLE 3: The effect of MB and alternatives on the productivity of runners.

Soil treatment	**Transplant treatment**		
	Control	**T. viride**	***P. fluorescens***
Experimental site, Kielczewo 2000, cv. Camarosa			
Control	26	25	26
Dazomet 500 kg/ha	30	35	33
1,3-D+CP 380 l/ha	26	34	31
1,3-D+CP 530 l/ha	33	ND	ND
Metham sodium 800 l/ha	22	28	27
Metham sodium 1,200 l/ha	33	ND	ND
Methyl bromide 600 kg/ha	49	ND	ND
Experimental site, Kielczewo 2001, cv. Camarosa			
Control	48	64	40
1,3-D+CP 375 l/ha	61	64	65
Methyl bromide 600 kg/ha	53	84	74
Experimental site, Przysieka 2001, cv. Tudnew			
Metham sodium 1,000 l/ha	54	71	62
Metham sodium 1,200 l/ha	71	58	59
Metham sodium 1,500 l/ha	64	66	69
Methyl bromide 600 kg/ha	72	ND	ND

Table 3 shows that MB and 1,3-D+CD had a positive influence on the mother strawberry plants productivity. In addition, the treatment of transplants with the biocontrol agents planted in the bands fumigated with MB resulted in a well-pronounced improvement of the productivity. Moreover, the treatment of transplants with *T. viride* alone increased the productivity of transplants in the control plots. Sivan & Chet (1993) described similar synergistic effect in a experiments with controlling the crown and root rot of tomato.

The data obtained indicate that, in the production of strawberry transplants, 1,3-D+CP alone or at a lower dosage integrated with a biocontrol agent *T. viride* B35 could be considered as possible replacements for MB with regard to the control of soil-borne pathogens, but not as a weed control agents.

The effectiveness of 1,3-D+CP in controlling soil-borne pathogens in our trails was similar to that described by Larson & Shaw (2000) as well as Rieger *et al.* (2001). The results of runners' production and the mycological studies indicate that Nemasol controlled the Verticillium wilt at higher doses but the effectiveness was not acceptable for commercial reasons (Table 3). The additional transplant treatments with biological agents did not significantly improve the effectiveness of Nemasol. In strawberry production, Nemasol could not be considered as an alternative equivalent to MB. Others reported similar results in the production of tomato (Locascio *et al.* 1997) and in the first year production of strawberry (Riegier *et al.* 2001).

The yield of fruit collected during 2001 showed that 1,3-D+CP in lower dose gave a similar effect as the MB treatments (Table 4). All the other chemical treatments did not influence the yield of strawberries. In addition, a significant stimulating effect was observed on virtually all plots treated with *Trichoderma* (except for the higher dose of 1,3-D+CP) in comparison with the corresponding plots treated with fumigants alone. The assessments of the yield confirmed the results from the previous year when plant vigour had been estimated which was the performance of 1,3-D+CP applied alone or in combination with both tested biocontrol agents was very good and very similar to MB. Moreover, the results of combining *T. viride* and dazomet showed a similar effect to MB combined with this biocontrol agent in terms of fruit production. The results of the plant performance and the mycological analyses suggest that some minor pathogens may also have influenced the strawberry growth in Brzezna.

In these experiments, the performance of all the evaluated alternatives was inferior to the consistent, broad-spectrum control provide by MB, as reported previously (Locascio *et al.* 1997; Larson & Shaw 2000). However, taking into account the improvements of plant vigour and their higher productivity, the use of 1,3-D+CP at a higher rates and the combination of *T. viride* B35 with reduced dosage of 1,3-D+CP (375 l/ha) suggests a possible replacement for MB in the production of strawberry.

Table 4: The effects of MB and alternatives on the fruit production of strawberry cv. Senga Sengana (t/ha).

Soil treatment	Transplant treatment		
	Control	T. viride	*P. fluorescens*
Control	16.4	19.4	17.9
Dazomet 500 kg/ha	13.3	20.9	18.5
1,3-D+CP 500 l/ha	19.3	19.2	18.1
1,3-D+CP 700 l/ha	17.0	14.0	20.4
Metam sodium 800 l/ha	14.4	20.5	11.7
Metam sodium 1,200 l/ha	14.7	19.3	17.3
Methyl bromide 600 kg/ha	19.4	21.1	19.8

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CARBONYL SULFIDE AND CYANOGEN AS POTENTIAL NEW SOIL FUMIGANTS

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ABSTRACT

Penetration of carbonyl sulfide (COS) and cyanogen (C_2N_2) through soil, and sorption or uptake of the fumigants by soil, was tested on a variety of soil types from New South Wales (NSW) and Western Australia (WA). Both COS and C_2N_2 diffused and penetrated through the soils faster and deeper than methyl bromide (MB) and carbon disulfide. Soils of different moisture content were fumigated in sealed gas jars. Sorption of the fumigants was measured by monitoring the loss of the fumigants in the headspace. Cyanogen was rapidly and strongly sorbed by all soils, followed by COS and MB. That is, cyanogen and COS were partitioned with higher ratio into soils than MB, which means that there is less emission to air. Both fumigants were stable in soil for 3-5 hr, after which they were broken down to naturally occurring soil components, such as H_2S and CO_2. Both COS and C_2N_2 were shown to control 1st-instar whitefringed weevil, *Graphognathus leucoloma* (Boheman), nematodes (*Steinernema carpocapsae*) and soil pathogens (*Fusarium graminearum, Bipolaris soroikiniana, Pythium irregulare and Rhizoctonia solani*).

Keywords: methyl bromide, alternatives, carbonyl sulfide, and cyanogen, whitefringed weevil, soil pathogens, nematodes

INTRODUCTION

More than 80% total fumigant of methyl bromide (MB) is used for soil fumigation. Given the limited existing alternatives for soil fumigation, it is important to develop new materials for this application that meet the requirements of being highly toxic to soil insects, mites, nematodes, and pathogens, easy and safe to apply and minimally deleterious to the environment.

CSIRO Entomology, Stored Grain Research Laboratory (SGRL) has developed and patented two new fumigants (Banks *et al.* 1992; Desmarchelier and Ren 1996), carbonyl sulfide (COS) and cyanogen (C_2N_2) to replace methyl bromide (MB) in a variety of applications. Carbonyl sulfide is currently being commercialised for use on grain. Cyanogen appears to have particularly good potential for timber. However, soil fumigation is one of the largest uses of MB that will be phased out under the terms of the Montreal Protocol. This preliminary work is aimed at assessing the potential of COS and C_2N_2 for use as soil fumigants.

MATERIALS AND METHODS

Penetration of COS and C_2N_2 through soil

The procedure for studying sorption was first to condition the soil to 27% (gingin sand) and 52% (pemberton loam), as determined by oven drying method. Second, the soil sample, loosely packed, was weighted and then transferred (with 95% full) to a 700 mL PVC column (7 cm $\phi \times$ 18 cm h) equipped with sampling ports on the wall of column. Fumigant (60 mg/L) was injected at bottom of column. The fumigant concentrations at different levels were measured by gas chromatography (GC).

Laboratory bioassays on insects, nematodes and fungi

Tests were conducted in 200 ml glass bottles equipped with an airtight cap that allowed gas injection through a septum. The insect cage (containing about 50-60 whitefringed beetle larvae) was placed into the bottle and then covered with soil (30% full). Fumigants were injected into separate bottles with airtight syringe. Controls were sets of 50 larvae in sealed bottles containing the soil sample.

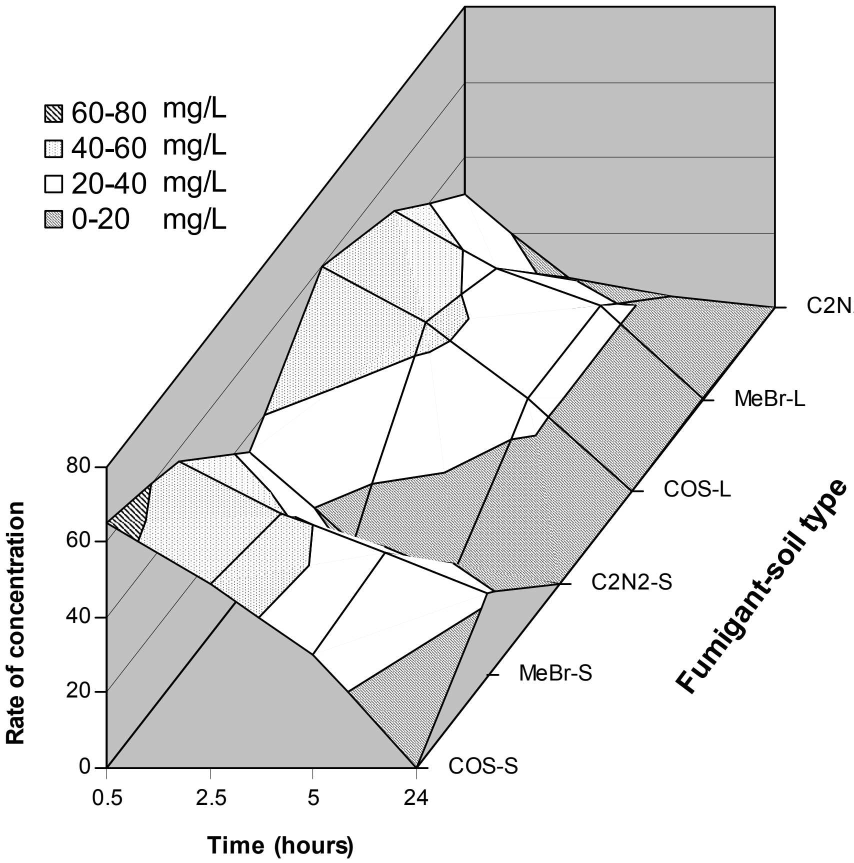
The flasks (275 ml) were sterilised and the bottom centimetre filled with a growth medium (potato dextrose Agar). The centre of the flask was inoculated with a pathogen. The flasks were then sealed. Each of the above operations was performed in a laminar-flow cabinet. The sealed flasks were then placed in a room at 25°C. The pathogens were allowed to grow until they had attained a colony diameter of approximately 30 mm. Fumigant was then injected through the septum. The colony diameter was measured, in both control and treated pathogens, after a holding period of 24 hours. After 24 hours, the control pathogen had not grown to the edge of the flask (<800 mm). The growth of the pathogen in treatments could then be calculated as a proportion of that in the control.

RESULTS AND DISSCUSSION

Penetration of COS and C_2N_2 through soil

Both COS and C_2N_2 diffused and penetrated through the soils faster and deeper than MB and CS_2 (Figure 1).

FIGURE 1: Penetration of COS and C_2N_2 through soil and sorption or uptake of the fumigants by a variety of soil types (S-gingin sand and L-pemberton loam).



Cyanogen was rapidly and strongly sorbed by all soils, followed by COS and MB. That is, C_2N_2 and COS were partitioned with higher ratio into soils than MB, which means that there is less emission to air. Both fumigants were stable in soil for 3-5 hr, after which they were broken down to naturally occurring soil components, such as H_2S and CO_2.

Laboratory bioassays on insects, nematodes and fungi

TABLE 1: Toxic to 1st-instar whitefringed weevil (Larvae) *Graphognathus leucoloma* (Boheman), at 25±2C°, with soil (30% fill) and 5 hours exposure.

L(CXt) mg h/L	C_2N_2	COS	MB
L(CXt)50	30	300	100
L(CXt)95	50	370	135

TABLE 2: Toxic to nematodes (*Steinernema carpocapsae*) at 25±2C°, with soil (30% fill) and 5 hours exposure.

L(CXt) mg h/L	**C_2N_2**	**COS**	**MB**
L(CXt)50	25	110	75
L(CXt)95	40	210	100

TABLE 3: Toxic to soil pathogens at 25±2C° and 24 hours exposure.

Pathogen	**Fumigant (mg/L)**		
	C_2N_2	**COS**	**MB**
F. graminearum	4	200	80
B. soroikiniana	6	250	65
P. irregulare	4	170	45
R. solani	8	150	55

Assessment of total environmental impact

Neither COS nor C_2N_2 are listed as greenhouse gases. The threshold limit values (TLV) for COS and C_2N_2 of 10 ppm (v/v) compares favourably with both MB (5 ppm) and phosphine (0.3 ppm). They are naturally-occurring gases present at low concentrations in the atmosphere, soil, ocean, vegetation and grain.

The average total worldwide release of COS was at about 3 million tonnes/year of which less than one third was related to human activity (HSDB 1994). Recent investigations have shown that COS plays an important role in the chemistry of the global atmosphere and in the biogeochemical sulfur cycle (Sze and Ko 1980; Gregory *et al.* 1993; Ren 1999). One of the major sinks for COS is direct removal from the atmosphere by plants and soil microorganisms (Bremner and Banwart 1976; Brown *et al.* 1986). The atmospheric half-life for carbonyl sulfide is at about 2 years (HSDB 1994). The most important fate process for carbonyl sulfide in water is volatilisation where the half-life is about 2.3 hours (HSDB 1994).

The chemistry of C_2N_2 is also well understood. It is a colourless gas with a boiling point of -21.2°C. It has an almond-like odour, which becomes acrid and pungent at high concentrations, making it detectable to the user should a leak occur. Unlike most fumigants, C_2N_2 is readily soluble in water, with 1 volume of water dissolving 4 volumes of C_2N_2. In aqueous solutions it is slowly hydrolysed to form oxalic acid and ammonia. At low pH, C_2N_2 reacts to form derivatives of formic acid and hydrogen cyanide (HCN). Hydrogen cyanide is found in nature in some vegetable substances, e.g., bitter almond, peach stones, cherry and sorghum; it is usually combined in glycoside molecules and is released when they are broken down by enzymes during metabolism.

Guideline Maximum soil concentrations for HCN residential soil is 250 mg/kg free HCN, 500 mg/kg complexed. For commercial or industrial soil 1250 mg/kg free HCN, 2500 mg/kg complexed (NEPM, 1999). However, the data from laboratory studies showed 150 mg/kg of C_2N_2 can control all target organisms (include insect and nematodes <80 mg/kg; soil borne pathogens and soil fungi <120 mg/kg). In the case of 100% applied C_2N_2 (150 mg/kg) converts to HCN. The levels of HCN residue are about 78 mg/kg, which are much lower than that maximum soil concentration. Cyanogen can be directly/indirectly utilised by plants or organisms.

CONCLUSIONS

Carbonyl sulfide and C_2N_2 appear to have potential as soil fumigants to replace MB. Formulations and application methods are being investigated to develop good agricultural practice for these fumigants.

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FURFURAL-BASED BIOFUMIGANT MIXTURES FOR CONTROL OF PHYTOPATHOGENIC NEMATODES AND WEEDS

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ABSTRACT

The nematicidal and herbicidal activities of furfural (2-furfuraldehyde), and mixtures of furfural and mustard oils and naturally occurring isothiocyanates, were studied in greenhouse and microplot experiments with soils naturally-infested with a variety of plant parasitic nematodes and weeds. Nematode species present included root-knot nematodes (*Meloidogyne arenaria* and *M. incognita*), the reniform nematode (*Rotylenchulus reniformis*) and the lesion nematode (*Pratylenchus brachyurus*). Weed species were: nutgrasses (*Cyperus esculentum* and *C. rotundus*), crab grass (*Digitaria sanguinalis*), Jimson weed (*Datura stramonium*) and a variety of other weed species. Data from these experiments indicate that a variety of effective broad-spectrum formulations of furfural can be developed for the control of economically important soil-borne pests. Furfural-based biofumigants were very effective against phytopathogenic nematodes but had no deleterious effects against beneficial microbivorous nematodes. The biofumigants have herbicidal activities at commercially feasible rates against most common weeds but must be applied at high rates (> 500 kg ai/ha) to control nutgrass-type weeds.

Keywords: biofumigants, 2-furfuraldehyde, herbicide, horticultural crops, isothiocyanates, microbivorous nematicides, nematodes, mustard oils, nematicide, plant parasitic nematodes, root-knot nematodes, soil fumigation, weed control.

INTRODUCTION

There are a number of plant species that have relatively few problems caused by soil-borne pests. Some of these plants, e.g. brassicas, mustards, radishes, have been used as rotational crops to suppress nematodes and soil-borne fungal pathogens. Cruciferous plants produce a variety of mustard oils some of which are very active against plant pests. Other plant species, e.g. oregano, thyme, lemon grass, produce simple volatile monoterpenes, terpenoids and aromatic compounds with considerable activity against phytopathogenic fungi, nematodes and other soil-borne plant pests (Harborne & Baxter 1993; Grainge & Ahmed 1988).

Research on the nematicidal and microbiocidal properties of these volatile, naturally occurring compounds has shown promise for development of new management strategies for nematodes and other soilborne plant pathogens (Soler-Serratosa 1993;Soler-Serratosa *et al.* 1996). Furfuraldehyde, a product obtained from sugarcane bagasse and similar biomass materials (Merck Index 1989), alone, and in combination with mustard oils, for example, was shown to be effective for the management of weeds, insects, nematodes and other soilborne pathogens (Rodríguez-Kábana 2000; Rodríguez-Kábana *et al.* 1993; Rodríguez-Kábana & Walters 1992).

In contrast to MB and other similar “broad-spectrum” soil fumigants (biocides), natural fumigants are directly active against target pests, while favoring or stimulating select groups of beneficial microbial species in soil (Canullo *et al.*1992; Chavarria-Carvajal & Rodriguez-Kabana 1992; Soler Serratosa *et al.,* 1994). Many of the selected microorganisms are antagonistic to phytopathogenic nematodes and fungi, e.g., species of root-knot (*Meloidogyne*) and cyst (*Heterodera*) nematodes, the reniform nematode (*Rotylenchulus reniformis*), fungi such as: *Rhizoctonia solani*, *Fusarium* spp., *Verticillium* spp, and bacteria. The mode of action of these natural fumigants (selector compounds) involves short-term direct action against target pests and long-term activity through selection of beneficial microorganisms antagonistic to the pests.

Prolonged use of mixtures of selector compounds can enhance suppressiveness of soils against pests through enrichment of the soil microflora with species antagonistic to the pests. In essence, applications of these mixtures can result in benefits typical of those obtained with pest-suppressive crop rotations without the need to dedicate the land and time necessary to implement these rotations. These possibilities led the Nematology team at Auburn University to explore the use of furfural-based biofumigants as alternatives to methyl bromide (MB) for the control of weeds, nematodes and other soil-borne pests.

MATERIALS AND METHODS

Greenhouse Experiments. The nematicidal activity of an emulsifiable formulation of furfural (2-furfuraldehyde; Illovo Sugar Ltd., Durban, South Africa) was studied in a greenhouse experiment with a soil naturally infested with the reniform nematode (*Rotylenchulus reniformis*). A 5% (v/v) aqueous emulsion of the chemical was added to one kg amounts of soil to deliver furfural rates of: 50, 100, 200, 300, 400 and 500 uL/kg soil. The treated soil was mixed well and transferred to IL capacity 10-cm-diam cylindrical plastic pot. Untreated soil (control) and each rate of the chemical were represented in the experiment by 14 pots (replications), one-half of which were covered with a plastic bag held down with a rubber band around the pots while the other 7 were left uncovered. The pots were placed on a greenhouse bench and soil samples for nematological analysis (salad bowl incubation technique) were collected two weeks after application of the material.

In another greenhouse experiment of identical design, an aqueous emulsion containing 17% furfural was added at rates of 170 - 1700 uL ai/kg soil to a soil infested with crab grass (*Digitaria sanguinalis*), purple nutsedge (*Cyperus rotundus*), Jimson weed (*Datura stramonium*) and a variety of other weed species. In other experiments of similar design as those described, mixtures of furfural with small amounts (<20%) of mustard oil or various naturally occurring isothiocyanates were applied to nematode and weed infested soil at rates of 50 - 1700 uL ai/kg soil. Variables studied in the experiments were weed and nematode numbers.

Microplot Experiment. A microplot trial was conducted on the Auburn University's campus microplot facilities to determine the efficacy of a Multiguard FFA™ formulation (Harborchem, Cranford, New Jersey) as a MB alternative. The emulsifiable formulation consisted of a mixture of furfural containing 25% allyl isothiocyanate. The chemical material was applied to soil by drenching-in an aqueous emulsion in sufficient quantity to penetrate 40 cms into the soil profile. A microplot consisted of a one-ft^2 (929 cm^2) area delimited by a *terra cotta* chimney liner (2.54 cm- thick wall) embedded 41 cms deep into the soil and protruding 2 cms above the soil. Soil in the microplots was a loamy sand with pH= 6.2, organic matter content <1.0%, and cation exchange capacity < 10 meq/100 g soil. The soil was typical for Alabama and was infested with a variety of plant parasitic nematodes including root-knot nematodes (*M. arenaria, M. incognita,* and species of *Helicotylenchus, Hoplolaimus, Paratrichodorus, and Pratylenchus*), southern blight (*S. rolfsii*), and typical damping off (*Rhizoctonia, Pythium*) and wilt (*Fusarium, Neocosmospora*) pathogens. The microplots were infested with nutsedge (*Cyperus esculentum* & *C. rotundus*) and other weeds which, in combination with the other pests present in the soil, represented closely the problems faced by producers in fields requiring fumigation with MB.

Multiguard FFA was applied at rates in the range of 0, 2.5, 5.0, 10, 15, 20, 25 and 37/5 g ai/plot (1.0 g ai/plot is approximately equivalent to 100 kg ai/ha.). The treated soil was covered with standard polyethylene plastic for 3 weeks, after which the the cover was removed, the weeds were counted and soil samples were collected for nematological analyses. Treatments in the trial were arranged in a randomized complete block design and each treatment was represented by 8 replications (plots).

Statistical Analyses. Data from the experiments were analyzed by standard procedures for analyses of variance. When F values were significant ($p \leq 0.01$) differences among means were evaluated for significance according to Duncan's multiple range test. Unless otherwise indicated differences referred to in the text were significant at $p \leq 0.01$.

RESULTS

Greenhouse Experiments. Numbers of the reniform nematode in soil declined sharply in direct response to increasing rates of furfural with the sharpest reductions in numbers occurring at rates ≤ 200 uL/kg soil. There were no nematodes in samples from covered pots that received furfural at rates ≥ 200 uL/kg soil. The reniform nematode was not eliminated in any of the samples from uncovered pots.

In the experiment with weeds, application of furfural to covered pots at rates ≥680 uL/kg soil eliminated all weeds 14 days after treatment but in the uncovered pots only the two highest rates resulted in consistent and adequate control (>70%) of most weeds. Data from experiments using mixtures of furfural mustard oil or naturally occurring isothiocyanates indicated that application of these mixtures to soil resulted in superior nematicidal and herbicidal activities than were obtained from furfural alone or the other active ingredients in the mixtures.

Microplot Experiment. Application of Multiguard FFA at 2.5 g ai/plot eliminated or drastically reduced populations of plant parasitic nematodes in soil (Figure 1A). This was in direct contrast to the pattern of response obtained for microbivorous (benefitial) nematode populations; numbers of these nematodes were either unaffected or greatly stimulated by applications of Multiguard FFA (Figure 1B).

Most weed species were controlled by Multiguard FFA at rates 5 – 10 g ai/plot. However, nutgrass control required applications ≥ 15 g ai/plot to obtain satisfactory control of these weed species.

CONCLUSIONS

Data from these experiments indicate that a variety of effective broad-spectrum formulations of furfural can be developed for control of economically important soil-borne pests. Results also indicate that furfural-based biofumigants, while very active against phytopathogenic nematodes, have no deleterious effects against beneficial microbivorous nematodes. These biofumigants have herbicidal activities against most common weeds but must be applied at high rates (> 500 kg ai/ha) to kill nutgrass-type weeds.

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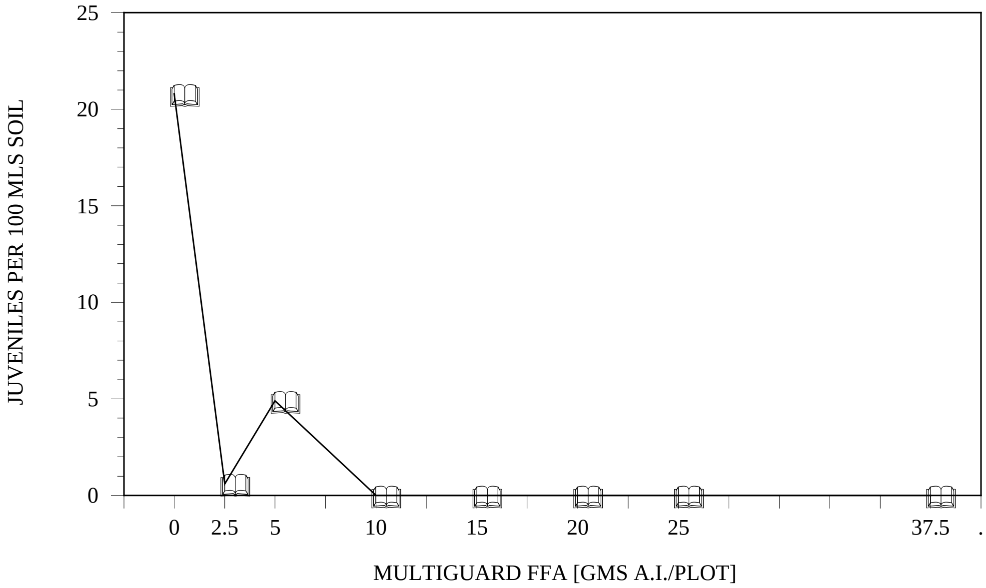

Figure 1A. Effect of applications of Multiguard FFA (Furfural + Allyl Isothiocyanate) on populations of root-knot nematode (*Meloidogyne* spp.) in a microplot experiment at Auburn University (1.0 g/plot is approximately equivalent to 100 kg/ha)

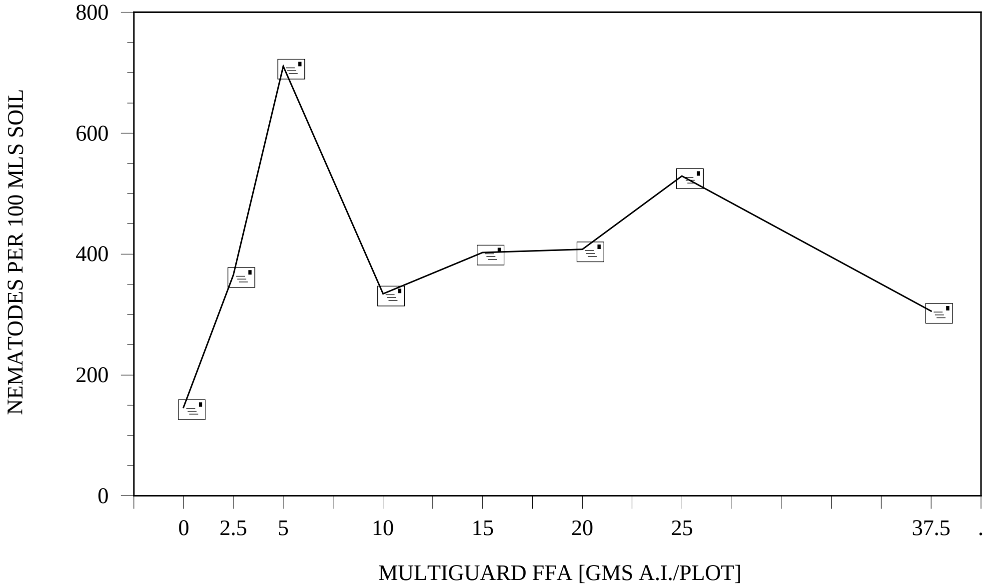

Figure 1B: Effect of applications of Multiguard FFA (Furfural + Allyl Isothiocyanate) on populations of beneficial microbivorous nematodes in a microplot experiment at Auburn University.

POTENTIAL OF PROPYLENE OXIDE AS A SUBSTITUTE FOR FUMIGATION OF SOIL WITH METHYL BROMIDE

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ABSTRACT

The nematotoxic properties of propylene oxide ($CH_2O\ CH\ CH_3$) were studied in greenhouse and microplot experiments. In greenhouse experiments with soil from a cotton field infested with the phytopathogenic reniform nematode (*Rotylenchulus reniformis*), numbers of the reniform and beneficial microbivorous nematodes in pre-plant soil samples, taken two weeks after treatement of the soil, declined sharply in response to increasing propylene oxide (PO) rates up to 750 mg/kg soil; dosages $\geq$750 mg/kg soil resulted in 100% kill. The same pattern of response to PO applications was observed for soil and root populations of *R. reniformis* at termination of the experiment 8 weeks after planting of 'Young' soybean (*Glycine max*). Significant populations of microbivorous nematodes were observed in control and all PO-treated soils at the end of the experiment; soil and root populations of these nematodes increased directly in response to PO dosages between 125-1000 mg/kg soil but declined in soils treated with rates $\geq$1125 mg. Weights of fresh roots and shoots were increased by all PO rates but the highest increase for the two variables was observed in response to the lowest PO dosage (125 mg/kg soil). In other greenhouse trials, application of a 5% aqueous PO solution to a soil infested with crabgrass (*Digitaria sanguinalis*), yellow nutsedge (*Cyperus esculentum*), Jimson weed (*Datura stramonium*) and a variety of other weed species resulted in 100% control of all weeds at rates >600-800 mg ai/kg soil. Results from a microplot experiment demonstrated that PO injected directly into soil to a depth of 36 cms was effective in controlling phytopathogenic nematodes, yellow nutsedge and other weeds at rates of $\geq$ 12 ml/m row (approx. 231 - 278 L/ha, depending on bed or row width). Drenching soil with a 20% aqueous solution of PO was 40-50% less effective than direct injection of the chemical for control of weeds in the microplots.. Data from these experiments, considered with current price, commercial availability, and relatively low mammalian toxicity, indicate that PO has great potential for development as an alternative to methyl bromide for fumigation of soils.

Keywords: herbicide, horticultural crops, propylene oxide, methyl bromide, microbivorous nematicides, nematodes, nematicide, plant parasitic nematodes, soil fumigation, weed control.

INTRODUCTION

Propylene oxide (PO) is a flammable, colorless ethereal liquid, soluble in water (40.5% by weight, 20^0C), miscible with alcohol, ether and other like solvents, with LD_{50} = 1.14 g/kg administered orally to rats (Merck 1989; Smyth *et al.* 1941). It is used as an intermediate in the preparation of polyethers to form polyurethanes, in the synthesis of propylene glycols, lubricants, surfactants and demulsifiers. PO, like gaseous ethylene oxide ($CH_2O\ CH_3$), has been used for laboratory and small scale fumigation of soils and other materials, such as food stuffs, microbiological nutrient media (Dhingra & Sinclair 1985; Klarman & Craig 1960; Hansen & Snyder 1947; Smyth *et al.* 1941; Tuite 1969; Thompson & Gerdemann 1962; Warren 2001; Watson *et al.* 1966).

In contrast with ethylene oxide, the liquid nature of PO at ordinary atmospheric pressure and temperatures, simplifies its use as a fumigant. These and other practical considerations led Nematology Research at Auburn University to study the feasibility of using PO as a substitute for methyl bromide (MB) as a soil fumigant. A preliminary report on results from this effort has been presented (Rodriguez-Kabana 2001).

MATERIALS AND METHODS

Greenhouse Experiments. The nematotoxic properties of an aqueous solution (2.5%, v/v) of PO (Aldrich, Milwaukee, WI 53201, U.S.A.) were studied in a greenhouse pot experiment with soil from a cotton field infested with the reniform nematode (*Rotylenchulus reniformis*). The soil was sandy loam with pH 6.2, organic matter content $\leq$1.0% and cation exchange capacity

≤ 10 meq/100 g soil. PO was added pre-plant to the soil at rates of 125 - 1500 mg/kg soil in increments of 125 mg. The treated soil was mixed well and transferred to one liter capacity 10-cm-diam cylindrical plastic pots. The pots were then covered with standard transparent polyethylene (1ml) tarp. Each experiment contained a control and 12 treatments each with 7 replications (pots) arranged in a randomized complete block design. After two weeks, the covers were removed, soil samples for nematological analyses (salad bowl incubation technique) were collected and 'Young' soybean planted (5 seed/pot). In other greenhouse trials, a 5% aqueous PO solution was applied to soil infested with crabgrass (*Digitaria sanguinalis*), yellow nutsedge (*Cyperus esculentum*), Jimson weed (*Datura stramonium*) and a variety of other weed species. Experimental design and procedure for these experiments were as described for the experiment on nematicidal activity.

Microplot Experiment. A microplot trial was conducted at the Auburn University's campus microplot facilities to compare the relative efficacy of PO applied as a drench in an aqueous solution of the chemical and application of the undiluted PO by direct injection into the soil. A microplot consisted of a one-ft^2 (929 cm^2) area delimited by a *terra cotta* chimney liner (2.54 cm- thick wall) embedded 41 cms deep into the soil and protruding 2 cms above the soil. Soil in the microplots was a loamy sand with identical properties to the soil used for the greenhouse experiments. The soil was typical for Alabama and was infested with a variety of plant parasitic nematodes including root-knot nematodes (*M. arenaria, M. incognita,* and species of *Helicotylenchus, Hoplolaimus, Paratrichodorus, and Pratylenchus*), southern blight (*S. rolfsii*), and typical damping off (*Rhizoctonia, Pythium*) and wilt (*Fusarium, Neocosmospora*) pathogens. The microplots were infested with nutsedge (*Cyperus esculentum* & *C. rotundus*) and other weeds which in combination with the other pests present in the soil represented closely the problems faced by producers in fields requiring fumigation with MB. In the drenching application, PO was added to the microplot as a 20% (v/v) PO aqueous solution by mixing the required amount of the solution in sufficient water (final volume = 2L/microplot) to penetrate 40 cms into the soil profile. Direct injection of PO was to a depth of 36 cms in a 5-cm-wide trench dug along a diagonal line in the square microplot; after application the soil on both sides of the trench was pushed in to cover the trench. The untreated (control) and treated microplots were covered with standard polyethylene plastic for 3 weeks, after which the the cover was removed, the weeds were counted and soil samples were collected for nematological analyses (salad bowl incubation technique). Treatments in the trial were arranged in a randomized complete block design and each treatment was represented by 8 replications (plots).

Statistical Analyses. Variables studied in the experiments were weed and nematode numbers. Data from the experiments were analyzed by standard procedures for analyses of variance. When F values were significant ($p \leq 0.01$) differences among means were evaluated for significance according to Duncan's multiple range test. Unless otherwise indicated differences referred to in the text were significant at $p \leq 0.01$.

RESULTS

Greenhouse Experiments. Numbers of the reniform and microbivorous nematodes declined sharply in response to increasing PO rates up to 750 mg/kg soil; dosages ≥750 mg/kg soil resulted in 100% kill. The same pattern of response to PO applications was observed for soil and root populations of *R. reniformis* at termination of the experiment 8 weeks after planting (Figure 1). Significant populations of microbivorous nematodes were observed in control and all PO-treated soils at the end of the experiment; soil and root populations of these nematodes increased directly in response to PO dosages between 125-1000 mg/kg soil but declined in soils treated with rates ≥1125 mg. Weights of fresh roots and shoots were increased by all PO rates but the highest increase for the two variables was observed in response to the lowest PO dosage. In other greenhouse trials, application of a 5% aqueous PO solution to a soil infested with crabgrass (*Digitaria sanguinalis*), yellow nutsedge (*Cyperus esculentum*), Jimson weed (*Datura stramonium*) and a variety of other weed species resulted in 100% control of all weeds at rates >600-800 mg ai/kg soil.

Microplot Experiment. Results from the microplot experiment demonstrated that PO injected directly into soil to a depth of 36 cms (14 inches) and covered with polyethylene was effective in controlling nematodes and yellow nutsedge and other weeds at rates of ≥ 12 ml/meter row (approx. 25-30 gal/A, depending on bed or row width. Drenching soil with a 20% aqueous solution of PO was 40-50% less effective than direct injection of the chemical for control of weeds in the microplots (Figures 2A and 2B).

CONCLUSIONS

PO is a wide spectrum soil fumigant. Results from experiments at Auburn University indicate that direct injection of the undiluted chemical into soil is more efficacious than applications based on delivery of aqueous solutions by drenching soil. Data from these experiments considered with current price, commercial availability, and relatively low mammalian toxicity indicate that PO has great potential for development as an alternative to MB for fumigation of soils.

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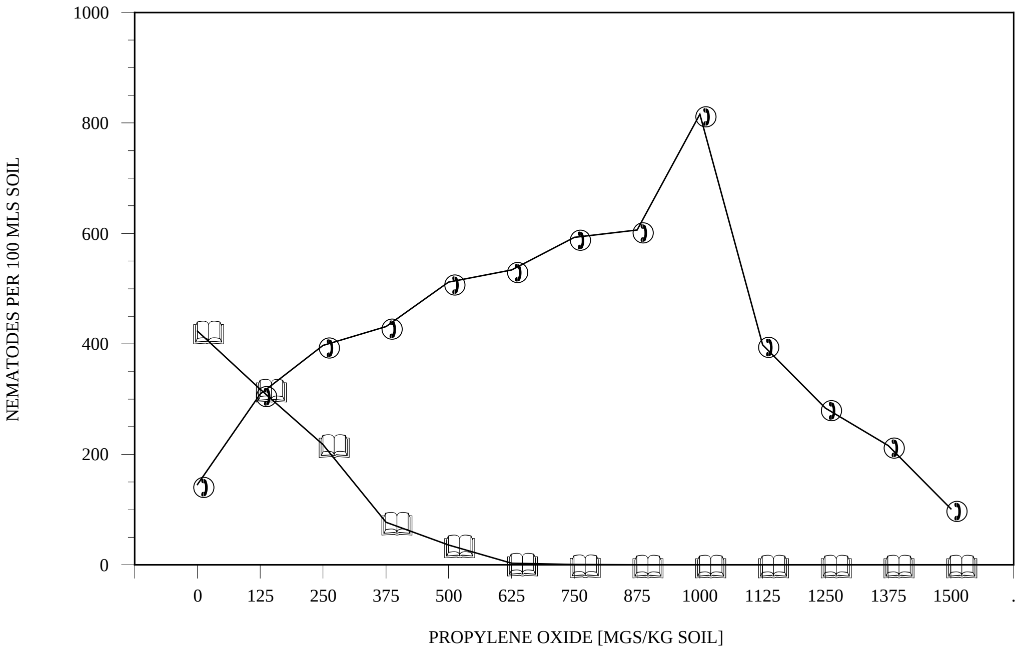

Figure 1: Effect of applications of propylene oxide on final populations of reniform nematode (*Rotylenchulus reniformis*.; diamonds) and microbivorous nematodes (circles) in a greenhouse experiment (1.0 mg/Kg soil is approximately equivalent to 2 kgs/ha)

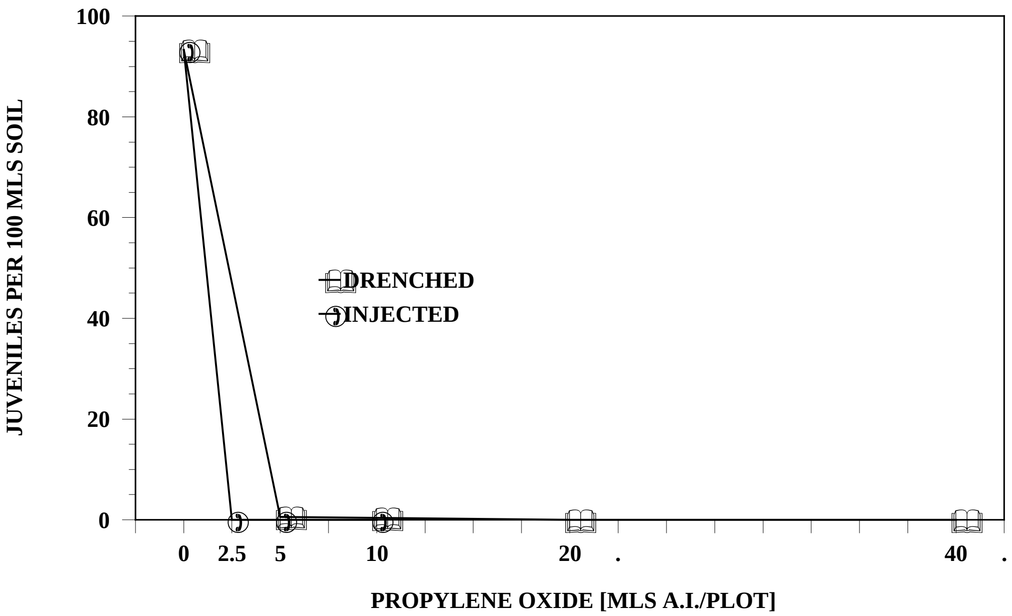

Figure 2A: Effect of drenched (diamonds) and direct injection (circles) applications of propylene oxide on populations of root-knot nematodes (*Meloidogyne* spp.) in a microplot experiment at Auburn University's microplot facilities (1 ml/plot is approximately equivalent to 32 L/Ha on a 1 meter-wide bed basis).

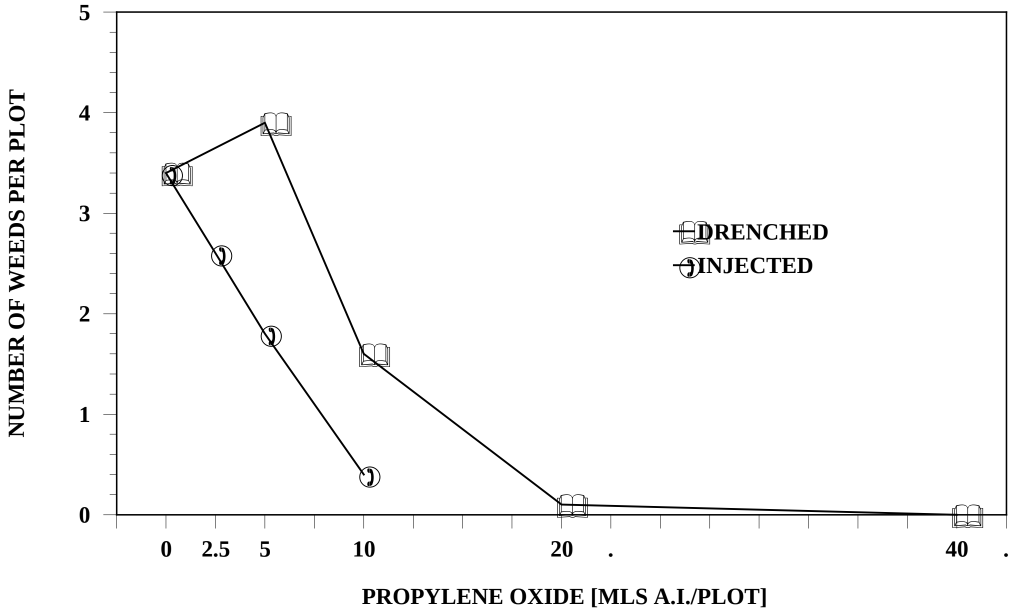

Figure 2B: Effect of drenched (diamonds) and direct injection (circles) applications of propylene oxide on populations of yellow nutgrass (*Cyperus esculentum*) in a microplot experiment at Auburn University's microplot facilities (1 ml/plot is approximately equivalent to 32 L/Ha on a 1 meter-wide bed basis).

METHYL BROMIDE PROJECTS IN DEVELOPING COUNTRIES

- ROLE OF THE MONTREAL PROTOCOL AND GTZ

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ABSTRACT

The GTZ poster provides information about major uses of methyl bromide (MB) in developing countries, and changing patterns of MB consumption, including trends in Morocco, Turkey and other major consumers. It gives an overview of the Montreal Protocol MB projects in developing countries, information about the crops/uses covered, and a summary of the results available to date. It also describes GTZ's approach to MB phase out and results of the GTZ projects, highlighting information relevant to MB users in Europe.

Keywords: developing countries, GTZ, methyl bromide, alternatives

METHYL BROMIDE USE PATTERNS

Developing countries use MB for the same purpose as European users: to control a wide range of pests/diseases in high-value horticultural crops and commodities. Approx. 70% of MB used in developing countries is for soil fumigation - for crops such as strawberry, tomato, pepper, cucurbits, cut flowers and tobacco seedbeds. The remainder is about 20% for durable commodities (eg. grains), 8% for perishable commodities (eg. quarantine treatments of fresh fruit) and about 3% for structures (MBTOC 1998). These estimates are due to be up-dated by MBTOC in 2002.

In 1998, developing countries used about 17,760 tonnes MB for soil and stored products, plus an additional amount (which is very difficult to quantify) for quarantine and pre-shipment (QPS). Developing countries accounted for approx. 25% of global MB consumption in the late 1990s.

Table 1 shows that MB use (excluding QPS) in developing countries increased by about 40% between 1994 and 1998. However, preliminary data for 1999 and 2000 indicate that MB consumption has stabilised or fallen in many countries. This change is largely a result of the Montreal Protocol activities and MB projects which are being carried out in most regions.

TABLE 1: MB consumption trends in developing countries 1994 - 1999, excluding QPS

Year	MB consumption excl. QPS (tonnes)
1994	12,790
1995	14,405
1996	14,424
1997	15,467
1998	17,760
1999	15,500 preliminary estimate

In 1998 MB consumption in developing regions was greatest in Latin America (41%), followed by Asia/Middle East (29%) and Africa (29%) (Table 2). The use patterns for MB vary greatly from region to region. In Latin America MB is used primarily for soil fumigation. In Asia it is used mainly for grains and other durable commodities, with the exception of China which uses MB mainly for soil fumigation. In Africa it is used for both soil and commodities.

TABLE 2: Reported methyl bromide consumption by region in 1998, excluding QPS

Developing regions	MB consumption excl. QPS (tonnes)	
Latin America & Caribbean	7,206	41%
Africa	5,143	29%
Asia, Middle East & Pacific	5,156	29%
CEIT	257	1%
Total developing countries	17,760	100%

Use patterns also differ greatly from one country to the next, even among those who grow similar crops. Analysis of 84 developing countries found that 70% used less than 50 tonnes MB per year, while 12% consumed more than 500 tonnes:

- 12 (14%) countries consumed zero MB
- 47 (56%) consumed less than 50 tonnes MB
- 16 (19%) consumed 50 - 500 tonnes MB
- 10 (12%) consumed more than 500 tonnes MB

In 1999, the countries consuming more than 500 tonnes MB were: Argentina, China, Costa Rica, Egypt, Guatemala, Mexico, Morocco, South Africa, Turkey and Zimbabwe.

MONTREAL PROTOCOL CONTROLS

Traditionally, the Montreal Protocol schedules have allowed ten extra years for developing countries to phase out ozone-depleting substances. MB consumption will be frozen in developing countries in 2002 (at 1995-98 average level), followed by a 20% reduction in 2005 and phase-out by 2015, excluding QPS. In 2003, the Montreal Protocol will review this schedule and may decide to introduce additional reductions.

However, the Protocol encourages all countries to phase out ozone-depleting substances faster than the international schedule, wherever possible. The Protocol's Multilateral Fund (MF) provides assistance for developing countries to do this, in partnership with UNEP, UNDP, UNIDO, the World Bank, and development agencies of industrialized countries such as Australia, Canada and Germany (GTZ). By the end of 2001, the MF had approved MB projects in more than 50 countries, comprising 44 demonstration projects to transfer and adapt alternatives, and more than 50 projects which provide information, technical assistance, training and/or policy development. Recently, the MF has approved 26 projects which plan to reduce and phase out major uses of MB in key developing countries, by encouraging the supply of alternative products and services, training growers how to use alternatives, introducing controls on MB imports, and making national action plans; in most cases the countries have made commitments to phase out major MB uses by 2006/7 at the latest.

For example, Turkey will introduce alternatives for tomato, cucumber, cut flowers and dried fruit, and has made a commitment to reduce MB imports/consumption from 840 tonnes in 1997 to 34 tonnes in 2006. Morocco has several projects that will introduce alternatives for major crops, and plans to reduce MB imports/consumption from 1,600 tonnes in 1998 to 275 tonnes in 2006. A number of countries who export to the EU aim to phase out MB early, because they expect that, as 2005 approaches, European retailers, food manufacturers and agricultural organisations may press for labelling or other measures to discourage developing countries from using MB fumigation.

GTZ APPROACH

Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) implements technical co-operation with developing countries on behalf of the German Ministry for Economic Cooperation and Development (BMZ). Early phase-out of MB has been given high priority in its environmental and rural development activities.

GTZ is working with partner countries to adapt and implement alternative production systems that are environmentally sound and economically viable, meeting the long-term needs of farmers, rural communities, purchasing companies and consumers. MB projects include components such as the development of action plans, MB import controls, awareness raising, policy dialogue with growers, trials to adapt and improve alternatives for local conditions, training programs for farmers and extension personnel, investment assistance, and development of new local industries to supply alternative products and services. GTZ focuses on farmers and farm-based activities, favouring a participatory process that fully involves farmers, agricultural organizations and other stakeholders. Projects are designed to build up local expertise and capabilities, so that changes and improvements will have a lasting impact.

When selecting suitable alternatives for technology transfer, projects typically review the available information from MBTOC and other technical sources about alternatives used commercially by farms in relevant climates/conditions. Examples of alternative techniques in commercial use are listed in Tables 3 to 7. Frequently, several techniques have to be combined in order to control the wide range of pests that MB controls. So an IPM approach has proved very useful in the process of selecting alternatives: identifying the target pests, identifying the effective controls for each pest in turn, and then selecting combinations of treatments or practices to control the full spectrum of pests in the location.

TABLE 3: Examples of alternative techniques used for tomatoes and peppers

Alternative techniques	Examples of commercial use
Solarisation + IPM	Jordan, Morocco, Japan, Israel, USA
Steam	Belgium, Netherlands, UK
Substrates	Canary Islands, Morocco, Spain, Belgium, Canada, Denmark, Netherlands, UK
Resistant varieties, grafting	Developing countries, Japan, Morocco, Spain, USA
Fumigants	Egypt, Jordan, Lebanon, Morocco, Tunisia, USA, Europe

TABLE 4: Examples of alternative techniques used for strawberries

Alternative techniques	Examples of commercial use
Solarisation	Jordan, various industrialised countries
Substrates	Malaysia, Indonesia, Netherlands, UK
Organic amendments, composts	Many countries
Crop rotation	Many countries
Resistant varieties	Japan, Denmark
Fumigants	Egypt, Jordan, Lebanon, Morocco, Tunisia, Netherlands, Spain, UK

TABLE 5: Examples of alternative techniques used for cut flowers

Alternative techniques	Examples of commercial use
Solarisation	Lebanon, industrialised countries
Steam + IPM	Colombia, Europe
Substrates	Brazil, Canada, Europe
Organic amendments, composts	Many countries
Crop rotation	Many countries
Resistant varieties	Many countries
Fumigants	Brazil, Colombia, Costa Rica, Morocco, non-A5 countries

Compiled from: MBTOC 1998

TABLE 6: Examples of alternative techniques used for tobacco seedbeds

Alternative techniques	Examples of commercial use
Substrates, float system	Brazil, South Africa, USA
Biofumigation	South Africa, Zimbabwe, USA
Fumigants	Brazil, Japan, USA

Compiled from: MBTOC 1998

TABLE 7: Examples of alternatives used for grains and other stored products

Alternative treatments	Examples of countries where alternatives have been used
Phosphine	Germany, Philippines, Thailand, UK, Zimbabwe and many other industrialised and developing countries
Carbon dioxide	Australia, Indonesia, Philippines, Vietnam
In-transit carbon dioxide	Australia
In-transit phosphine	Europe, USA
Phosphine mixed with carbon dioxide or nitrogen	Australia; Cyprus and Germany
Nitrogen	Australia, Germany
Gas-flushed retail packs	Thailand (commercial trial)
Hermetic storage	Israel, Philippines
Vacuum chamber	Indonesia
Heat treatment	Australia (prototype)
Cold treatments	Mediterranean, USA
Freezing	Europe (for premium grains)
Inert dusts (where conditions appropriate)	Australia, Canada, Germany

Compiled from: MBTOC 1998, Prospect 1997, GTZ 1998, USDA-APHIS 1998

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EFFECTS OF CHEMICAL AND NON-CHEMICAL ALTERNATIVES TO METHYL BROMIDE ON STRAWBERRY NEMATODES IN SOUTHERN SPAIN

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ABSTRACT

Two field trials were made each year between 1998 to 2001 in two localities of Huelva, the strawberry area of southern Spain. An assay included chemical alternatives to methyl bromide (MB) soil fumigation, and another one composed of non-chemical alternatives including solarization and biofumigation. Plot situation was repeated each year but plot treatments differed by slight changes on the kind or amount of the fumigant employed. Plants were harvested at the end of each season and the incidence and severity of knotted roots were recorded, number of *Meloidogyne hapla* females were also measure. Some chemical alternatives, like Telopic (DD+chloropicrin) or chloropicrin, showed a very effective nematode control, however the non-chemical alternatives were not successful. The lesion nematode, *Pratylenchus penetrans,* had reduced populations but, to a certain extent, showed a similar behaviour. Linear coefficients of correlation between disease measures and yield or agronomic traits were estimated in these fields, and it is possible to conclude that at least a part of crop losses could be explained by the presence of the root-knot nematode on strawberry roots.

Keywords: strawberry, methyl bromide, *Meloidogyne hapla*, alternatives, nematodes.

MATERIALS AND METHODS

During 1997-98 to 00-01 seasons, as part of INIA SC 97-130 National Project, assays on MB alternatives were made in the localities of Cartaya and Moguer, representative of the strawberry production area of Huelva, southern Spain. A chemical alternatives and a non-chemical alternatives experiment, disposed as a randomised complete block design with three replications, was performed each year for each locality.

The experiment was considered dynamic in a sense that treatment of a specific plot could be changed along years using new technologies, such as Preformed beds (Pb) and/or mulched with VIF plastic that allowed a reduction in the amount of fumigant applied. Fumigation methods and cultural practices were described previously (López-Aranda *et al*. 2001). A four year summary of the treatments is presented in Table 1.

Table 1: Summary of preplant soil fumigation treatments sequence through the years.

Plot treatments	Year 98	Year 99	Year 00	Year 01
Chemical alternatives:				
Control	Control	Control	Control	Control
Metam Na	MB (67%) Pb 40[1]	Metam Na Pb 125	Met Na Pb 175	Metam Na Pb 175
Dazomet	MB (67%)[2] 20	Dazomet 50	Dazomet 45	Dazomet Pb 50
MB Pref. beds	MB (67%)[2] Pb 40	MB (50%)[2] Pb 20	MB (50%)[2] Pb 20	MB (50%)[2] Pb 20
Telopic	Telone C17 60	Telone C35 40	Telone C35 Pb 40	TeloneC 35 Pb 40
Chloropicrin	Chlorop 40	Chlorop 40	Chlorop Pb 40	Chlorop Pb 40
MB	MB (67%) 40	MB Pb (50%) 40	MB (50%) Pb 40	MB (50%) Pb 40
Non-chemical alternatives:				
Control	Control	Control	Electromag.wav	Electromag.wav

Plot treatments	Year 98	Year 99	Year 00	Year 01
Solarization	Solarization	Solarization	Solarization	Solarization
Biofumigation	Biofumigation	Biofumigation	Biofumigation	Biofumigation
Sol+Metam Na	Sol+Metam Na 50	Sol+Met Na 100	Sol+Metam Na 75	Sol+Metam Na 75
Sol+MB/Telopic	Sol+MB (67%) 10	Sol+MB (50%) 10	Sol+MB (50%) 10	Telone C35 Pb 20
MB	MB (67%) 40	MB (67%) 40	MB (67%) 40	MB (67%) 40

1: g/m^2 or cc/m^2 . 2: with VIF plastic.

Before planting, a sample of plants was analysed (nematodes were never found). At the end of the season 10 plants/plot were collected with their complete root system carefully washed, observed under binocular microscope and nematodes were extracted from 25 g of roots using the sugar centrifugation method. *Meloidogyne hapla* and *Pratylenchus penetrans* were the species present. Diseased roots were also evaluated with a 0-4 scale (0=No symptoms; 4=more than 95% of root system galled). Data obtained or transformed as log, sqr or arcsen were analysed as serial experiments (MSTATC 2.1).

RESULTS AND DISCUSSION

Table 2: Measurement of disease caused in Strawberry plants by *M. hapla* after different soil fumigations with chemical alternatives.

	Location: Cartaya[1]			Location: Moguer[1]		
	Incidence[2]	Severity[3]	♀/g [4]	Incidence[2]	Severity[3]	♀/g [4]
Control	73.5 a[5]	2.23 a	13.6 a	2.5 a	0.42 a	8.37 a
Metam Na	55.5 b	1.65 b	12.4 a	0.0 b	0.00 a	0.00 a
Dazomet	16.2 c	0.84 c	5.2 b	0.0 b	0.00 a	0.00 a
MB Pref. beds	15.0 c	0.64 c	1.3 b	0.0 b	0.00 a	0.00 a
Telopic	7.7 c	0.47 c	0.4 b	0.0 b	0.00 a	0.00 a
Chloropicrin	7.6 c	0.50 c	0.5 b	0.0 b	0.00 a	0.00 a
MB	7.5 c	0.39 c	0.5 b	0.0 b	0.00 a	0.00 a

1: Mean of 10 plants/plot, 3 blocks/year and 4 years; 2: Percentage of disease plants; 3: Severity (0=no symptoms; 4=more than 90% of roots affected); 4: Number of females/g of roots; 5: Means followed by the same letter are not different under a MDS test, ($P<0.05$).

Results on Table 2 showed that at Cartaya the best nematode control was obtained with Telopic (DD+Chloropicrin), Chloropicrin and MB. Abbot´s efficacies respective to nonfumigated tests were over 75% and reached 97% in Telopic control of female number.

Table 3: Measures of disease caused in Strawberry plants by *M. hapla* after different soil fumigation with Non-chemical alternatives.

	Location: Cartaya[1]			Location: Moguer[1]		
	Incidence[2]	Severity[3]	♀/g [4]	Incidence[2]	Severity[3]	♀/g [4]
Control	54.2 a[5]	2.01 a	13.5 a	15.0 a	0.99 a	13.1 a
Solarization	36.7 a	1.48 ab	5.8 ab	8.3 ab	1.20 a	10.3 a
Biofumigation	32.5 ab	1.10 bc	5.0 ab	0.8 b	0.25 b	0.2 a
Sol+ Metam Na	26.7 b	0.63 bcd	3.6 bc	0.0 b	0.00 b	0.0 a
Sol+MB/Telopic	12.5 b	0.72 cd	1.7 bc	0.0 b	0.00 b	0.0 a
MB	0.8 c	0.08 d	0.0 c	0.0 b	0.00 b	0.0 a

1-5: As indicated in Table 2.

Solarization and biofumigation(Table 3) were not effective in controlling disease compared to Solarization+MB/Telopic or MB, used as chemical checks between the non-chemical alternatives. A similar result was obtained for *P. penetrans* at Cartaya location where the largest population was found.

Table 4: Lineal Correlation coefficients between Disease measures and Agronomic traits[1]

	Severity	**♀/root g**	**Plant diameter**	**Number of leaves**	**Fruit size**	**Yield**
Incidence	0.791 ***	0.579 ***	**-0.212 *****	**-0.192 *****	**-0.426 *****	**-0.361 *****
Severity	-------	0.678 ***	**-0.237 *****	**-0.197 *****	**-0.459 *****	**-0.347 *****
♀/root g		-------	**-0.006** ^{NS}	**-0.085** ^{NS}	**-0.291 *****	**-0.141 ****
Plant diameter			-------	0.409 ***	0.328 ***	0.652 ***
Leaf number				-------	0.350 ***	0.192 ***
Fruit size					-------	0.465 ***

1: Obtained from 294 or 312 couples of observations; ***: P≤0.001; **: P≤0.010; *: P≤0.050; ^{NS}: P>0.050.

Table 4 shows the correlation coefficients between subjective (severity) and objective (incidence and female number) disease measures in respect to yield or other agronomic traits (plant diameter, number of leaves and fruit size). Because all coefficients were negative we can infer that in these fields part of crop losses could be accounted for by the presence of root knot nematode. Fruit size as a component was especially affected.

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SOLARIZATION AND ITS COMBINATIONS: THE FIRST YEAR RESULTS OF A DEMONSTRATION PROJECT

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ABSTRACT

Methyl bromide (MB) consumption in Turkey increased from 643 tonnes in 1990 to 1319 tonnes in 1998. Turkey plans to phase out MB by 2008 even though the Montreal Protocol does not require phase out until 2015. The Eastern Mediterranean Region of Turkey is one of the foremost agricultural areas and strawberry and vegetable crops have been extensively cultivated. In the region, a World-Bank-supported Project was initiated in 2000 to introduce farmers to MB alternatives for use on strawberry, pepper and eggplant crops. Solarization, and its combinations with dazomet (400 kg/ha), chicken manure (10 tonnes/ha) or straw (500 kg/ha), were demonstrated at various sites. The effect of the treatments on soil borne diseases, weeds, nematodes and crop yield were measured. All alternatives gave comparable results with MB. Combinations of solarization with dazomet or chicken manure seemed effective alternatives. Solarization alone or with *Trichoderma* could be an inexpensive choice. These results and other activities under this project influenced farming agricultural practices in Turkey.

Keywords: strawberry, pepper, eggplant, solarization, dazomet, chicken manure, *Trichoderma*

INTRODUCTION

Methyl bromide (MB) consumption in Turkey increased from 643 tonnes in 1990 to 1319 tonnes in 1998. MB is used for soil fumigation, especially in cut-flowers, strawberry and vegetable crops grown under protected conditions in Turkey. Vegetable cultivation under protected conditions reached 44,000 ha and strawberries 8600 ha in 1999. Strawberry and vegetable crops have been extensively cultivated in the Eastern Mediterranean Region of Turkey. MB is also extensively used (150 tonnes in pepper, eggplant, and strawberry production in the East Mediterranean Region), and the farmers consider no other alternatives. However, solarization and its combinations with chemicals or organic materials are considered as applicable alternatives in Mediterranean conditions.

In the region, a World-Bank-Supported-Project was initiated in 2000 to introduce MB alternatives to strawberry, pepper and eggplant producers in order to meet Turkey's plan to phase out MB by 2008 even though the Montreal Protocol allows a MB phase out by 2015. In this paper, the first year's results of the demonstration projects with solarization and its combinations will be presented.

MATERIALS AND METHODS

Treatments were carried out successfully at 9 sites consisting of 6 pepper plastic houses, a strawberry plastic house, an open field strawberry crop and an open field eggplant crop. Treatments, number of replicates and plot sizes differed due to field size and crop. Combinations of solarization with dazomet (400 kg/ha), chicken manure (10 tonnes/ha) or *Trichoderma* spp. were applied to all fields. Combination of solarization and straw at 500 kg/ha was applied in a pepper plastic house. Following application of dazomet, chicken manure or straw, plots were covered with plastic tarp. In strawberry planting ridges were prepared before covering. *Trichoderma* spp. was applied firstly at seed beds, then periodically once a month with drip irrigation. Non-treated and/or MB-applied checks were set in suitable fields. Demonstrations lasted 4-6 weeks except for strawberries that continued for 3 and 7 weeks during July and/or August. The usual cultivation processes were applied during the growing season. Demonstrations were assessed periodically for soilborne diseases, nematodes and weeds. Yield was also recorded.

RESULTS AND DISCUSSION

In the strawberry demonstration sites, no plant parasitic nematodes were detected but a 20% incidence of *Fusarium oxysporum* and *Rhizoctonia solani* were observed in the check plots. All treatments gave 100% disease control (Table 3). The main problem was weeds but different species were abundant in two demonstration sides. Before transplanting *Cyperus rotundus* was detected at one site (Silifke) and summer annuals such as *Amaranthus* spp. were common in the other site (Yaltir). At the end of the season, weed intensity showed similar trends. Also, in the screen house, the effect of treatments was determined in pots, which were filled with soil taken after the tarp was removed from the field. Table 1 shows the average weed intensity in a square meter at early and late field counts and total germinated weed number in soil samples. A total herbicide was applied before transplanting in all treatments except MB treated plots in Silifke, and MB applied plots in Yaltir. Plants were transplanted earlier at MB applied plots in Yaltir. In Silifke, even solarization alone gave acceptable yields but *C. rotundus* control was not satisfactory. On the contrary, in Yaltir there was very good weed control but MB gave the best yield. However, solarization with dazomet seemed to be an alternative to MB.

TABLE 1. Effect of treatments on weeds and total yield in strawberry demonstration sites.

Treatment	SILIFKE Weed intensity Early	Late	Pot	Yield (t/ha)	YALTIR Weed intensity Early	Late	Pot	Yield (t/ha)
Solarization	50	105	67	23	18	6	3	30
Solarization+Dazomet 40	35	46	40	24	4	9	4	44
Solarization+Manure	50	57	42	25	6	9	9	32
Methyl Bromide	1	0	8	22	NA	NA	NA	50
Untreated check	NA	NA	NA	NA	NA	33	69	NA

[1]NA: Not applicable

TABLE 2. Effects of treatments on the nematode population as percentage of nontreated check during the growing season.

DATE	TREATMENTS and CONTROL RATES (%)[1] Solarization+Basamid	Solarization+Trichoderma	Solarization+ Manure	Solarization+ Straw	MB
03.10.2000	100.0	100.0	100.0	100.0	100.0
19.10.2000	98.8	100.0	100.0	100.0	100.0
07.11.2000	100.0	100.0	100.0	100.0	100.0
28.11.2000	89.9	100.0	80.0	100.0	97.8
19.12.2000	100.0	100.0	100.0	100.0	100.0
09.01.2001	96.3	99.2	79.9	99.3	100.0
30.01.2001	99.7	100.0	100.0	100.0	99.3
20.02.2001	100.0	100.0	100.0	100.0	97.0
13.03.2001	100.0	99.3	99.9	98.1	97.9
03.04.2001	79.7	98.7	96.2	94.3	91.6
25.04.2001	99.5	95.5	99.8	70.9	90.4
16.05.2001	72.9	88.8	94.4	93.2	87.4
06.06.2001	74.6	91.4	95.8	99.6	59.4
23.07.2001	40.8	59.3	84.9	0.0	0.0

[1]100 is complete control and 0 shows no control

Fusarium spp. were detected in pepper demonstration sites. MB and solarization with dazomet gave better disease control compared to other treatments (Table 3). Weed flora varied among fields but *Portulaca oleracea, Seteria* spp., *Amaranthus* spp., *Solanum* spp., *Echinochloa colona, Seteria viridis, Eluicina indica* and *C. rotundus* were common. Solarization+dazomet was the best control among MB alternatives. In general, solarization alone and solarization+manure gave satisfactory weed control. Pepper fields were heavily infested with *Meloidogyne* spp. Galling index was assessed using Zeck Scale and overall averages of six pepper plastichouses were 0.10, 0.89, 0.98, 1.92, 2.04, and 5.95 for solarization+ *Trichoderma*, solarization+manure, solarization+dazomet, MB, solarization+straw, and check, respectively.

Table 2 shows the effects of treatments on the second term infective larva of the nematode. Combinations of solarization with basamid, manure or *Trichoderma* were the most effective applications. MB and solarization+straw was partially effective. However, it can be said that all treatments effectively controlled nematodes.

Plant parasitic nematode was not detected at the eggplant demonstration site. The treatments controlled weeds but not very well. However, broomrape control was almost 100% in all treated plots. Also, broomrape infestation was seen in mid-April in non-treated checks, which was two weeks earlier than solarized areas. *Fusarium oxysporum* and *Sclerotinia sclerotiorum* were observed in the field. Solarization+dazomet controlled diseases better than solarization+*Trichoderma* or solarization+manure (Table 3).

TABLE 3: Average disease incidence for pepper, eggplant and strawberry in the demonstration sites.

CROP	**TREATMENTS AND DISEASE RATES (%)**					
	Solarization + Basamid	**Solarization + Manure**	**Solarization + *Trichoderma***	**Solarization + Straw**	**MB**	**CHECK**
Pepper	2.5	7.5	7.5	7.5	0.0	32.5
Eggplant	20.0	37.5	31.2	NA	NA	57.5
Strawberry	0.0	0.0	NA	NA	0.0	20.0

TABLE 4: Yield results in pepper and eggplant demonstration fields

Crop/Field	**TREATMENTS AND YIELD (TONNES/HA)**				
	Solarization + Basamid	**Solarization + Manure**	**Solarization + *Trichoderma***	**MB**	**Check**
Pepper 1	89	75	70	NA	61
Pepper 2	95	96	85	108	48
Pepper 3	81	94	81	88	60
Eggplant	105	112	91	NA	NA

Solarization and its combinations gave comparable results to MB in controlling nematodes, diseases and weeds, and crop yield (Tables 1 and 4). Combinations of solarization with dazomet or chicken manure seemed effective alternatives. Solarization alone or with *Trichoderma* can be an inexpensive choice. This results and other activities under this project influenced farmers as in this growing season (2001-2002) more farmers applied solarization. Solarization with manure was the most popular combination. It is apparent that there is need for further research related to solarization such as duration, humidity, timing, and other alternative combinations as well as demonstration of the treatments.

DICHLOROPROPENE WITH CHLOROPICRIN APPLIED BY DRIP IRRIGATION AS A VIABLE ALTERNATIVE TO METHYL BROMIDE IN SWEET PEPPER GREENHOUSES

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ABSTRACT

Methyl bromide (MB 98:2) soil fumigation is commonly used to control the soil borne pathogens *Phytophthora capsici* and *Meloidogyne incognita* and to maintain acceptable yield levels in the 1,800 ha of sweet pepper grown in the Southeast of Spain (Murcia and Alicante). The restriction in MB use led to testing a blend of dichloropropene and chloropicrin (TELOPIC: Telone C-35, 60.5% of 1,3-dichloropropene and 33.3% of chloropicrin) applied through a drip irrigation system under plastic mulch as an alternative to MB. In the last three growing seasons, trials were undertake to determine: i) the adequate rate of applications, ii) the method of application and plastic setting, iii) the effect of reiterated application, compared to MB applied at the normal dose (60 g/m^2 of MB 98:2). In commercial greehouses with different phytopathological problems, the effect of treatments on *P. capsici* and *M. incognita*, weeds, plant development and yield were measured.

The best results were obtained at doses from 400 l/ha to 500 l/ha applied under polyethylene mulch (PE). The use of VIF plastic (Virtually Impermeable Film) improved the results and allowed a reduction in the application rate. The orientation of the plastic, in relation to the direction of the drip irrigation tape, did not have any influence on the efficacy of disinfection. Applications of Telopic at 400 l/ha repeated over three years in the same soil gave the same level of control of *P. capsici* and weeds, and produced the same yield and plant development as MB at 60 g/m^2 with PE. The control of *M. incognita* was slightly less than with MB.

Key words: dichloropropene, chloropicrin, sweet pepper, greenhouses, methyl bromide alternatives.

INTRODUCTION

Meloidogyne incognita and *Phytophthora capsici* are the main soil borne pathogens in the 1,800 ha of greenhouse peppers grown in the Murcia region and the south of Alicante. In this area, peppers have been cultivated over the last eighteen years. This monoculture is grown in more that 95% of the area. Soil is disinfected every year with methyl bromide (MB 98:2) in order to control soil borne pathogens and to compensate for yield losses caused by crops repeatedly grown on the same land. Since 1998, due to a restriction on MB use, the mixture dichloropropene and dichloropicrine (Telopic EC: Telone C-35; 1,3-dichloropropene (60.5%) + chloropicrin (33.3%) from Dow Agrosciences) was trailled as an alternative to MB with its broad spectrum of activity. Applications were done by drip irrigation under a plastic mulch.

MATERIALS AND METHODS

The trials were carried out in commercial greenhouses known to have problems with *P. capsici* and *M. incognita* and where peppers have been grown for more than twelve years. The aims of the trials were:

Trial a) To determine the application rate the following treatments were assayed: T1 – Telopic at 300 l/ha with 0.05 mm PE plastic; T2 – Telopic at 400 l/ha with 0.05 mm PE plastic; T3 – Telopic at 500 l/ha with 0.05 mm PE plastic; T4 – Telopic at 200 l/ha with 0.04 mm VIF plastic; T5 – Telopic at 300 l/ha with 0.04 mm VIF plastic; T6 – MB 98:2 at 60 g/m^2 with 0.05 mm VIF plastic; T7 – Control. Non treated soil.

Trial b) Plastic set up was assayed for the following applications: T1 – MB 98:2 at 60 g/m^2 with 0.05 mm VIF plastic; T2 – Telopic at 500 l/ha with 0.05 mm PE plastic in the same direction as irrigation tape; T3 – Telopic at 500 l/ha with 0.05 mm PE plastic perpendicular to the irrigation tape; T4 – Telopic at 400 l/ha with 0.05 mm PE plastic perpendicular to the irrigation tape; T5 – Telopic at 400 l/ha with 0.04 mm PE plastic perpendicular to the irrigation tape; T7 – Control. Non treated soil.

Trial c) Reiteration of Telopic application in the same soil. Two assays were carried out in two greenhouses every year. Trial 1999/2000: T1 – Telopic 2 years at 50 gl/m^2 with 0.05 mm PE plastic; T2 – MB 98:2 at 60 gl/m^2 with 0.05 mm PE plastic; T3 – Control. Non treated soil. Trial 2000/2001: T1 – Telopic 3 years at 50 gl/m^2 with 0.05 mm PE plastic; T2 – MB 98:2 at 60 gl/m^2 with 0.05 mm PE plastic; T3 – Control. Non treated soil.

In all the experiments, the pepper variety Orlando was grown at 2.5 plants/m^2. Growing seasons were from December to the end of August. Treatments were randomized in a complete block design with four replicates per treatment. The following parameters were measured to determine application efficacy: percentage of plants killed by *P. capsici*, percentage of plants infested by *M. incognita*, average *M. incognita* root-knot index (0-10, according to Bridge and Page 1980), plant height, marketable yield and weed soil colonization index (0-3). In all cases, the plastic was kept on the soil for 21 days. Applications were carried out in November. Telopic was applied by Venturi and MB by fumigation.

RESULTS

Rate of application

When the application was done under PE plastic, the best results were obtained using the 500 and 400 l/ha rates. There were no significant differences between any of the parameters tested. When the application was done under VIF plastic, there was an improvement in efficacy that makes it possible to reduce the rates of application. In all the cases, *P.capsici* control was significantly better with MB (98:2) than with Telopic (Table 1).

Table 1. Results of disinfection at different Telopic rates applied by drip irrigation.

	% plants *P. capsici*	% plantas *M. incognita*	Average knot index (1)	Plant height (cm)	Marketable yield (kg/m^2)	Weed index (1-3)
Telopic 300PE	21.7 c	16.6 ab	0.6 abc	105.7 b	7.8b	0.2 a
Telopic 400PE	8.6 b	0.0 a	0.0 a	112.0 ab	9.0 ab	0.1 a
Telopic 500PE	4.9 b	4.1 ab	0.1 a	117.0 a	8.9 ab	0.1 a
Telopic 200VIF	10.7 b	8.3 ab	0.3 ab	116.2 a	9.1 a	0.2 a
Telopic 300VIF	6.9 b	20.8 b	1.1 bc	111.8 ab	8.8 b	0.1 a
MB 60	1.3 a	0.0 a	0.0 a	119.1 a	10.1 a	0.1 a
Control	50.1 d	25.0 b	1.1 c	83.0 c	3.8 c	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).

Plastic set up

Non significant differences were found between longitudinal and perpendicular settings for any of the parameters tested. Therefore, commercial application is viable as cost is reduced. VIF plastic enhanced Telopic disinfection efficacy. The results yielded with Telopic were similar to MB 60 (Table 2).

Table 2. Influence of plastic setting on Telopic application by drip irrigation

	% plants *P. capsici*	**% plantas** *M. incognita*	**Average knot index (1)**	**Plant height (cm)**	**Marketable yield (kg/m^2)**	**Weed index (1-3)**
MB 60	5.0a	15.8 a	0.4a	158.8a	10.4a	0.08a
Telopic 500PE Long.	15.3abc	10.0a	0.4a	150.2b	9.9a	0.04a
Telopic 500PE Perp.	20.6bc	15.0a	0.5a	153.7ab	9.7a	0.00a
Telopic 400PE Perp.	28.6c	30.0a	0.7a	149.6b	9.6a	0.26a
Telopic 400VIF Perp	11.9ab	20.0a	0.6a	156.0ab	10.7a	0.00a
Control	79.0d	85.0b	5.1b	78.0c	3.3b	2.13b

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).

Reiteration of Telopic application in the same soil

Disinfection efficacy using Telopic at 50 g/m^2 repeatedly was not different from MB 60. Only in the third year was there a significant reduction in *M. incognita* control, although without any impact in plant growth or marketable yield (Table 3). The results indicate that Telopic EC applied by drip irrigation is an alternative to MB.

Table 3. Results of reiterated use of Telopic applied by drip irrigation.

	% plants *P. capsici*	**% plantas** *M. incognita*	**Average knot index (1)**	**Plant height (cm)**	**Marketable yield (kg/m^2)**	**Weed index (1-3)**
MB 60	0.7 a	3.6a	0.1a	114.3a	8.0a	0.7a
Telopic 2 years	2.6 a	5.4a	0.7a	115.4a	8.5a	0.3a
Control	46.1 b	57.8b	3.2b	74.0b	3.2b	1.5b
MB 60	1.7a	20.0a	0.6a	149.8a	9.2a	0.00a
Telopic 3 years	4.1a	47.5b	2.7b	145.3a	9.3a	0.04a
Control	28.2b	100.0c	6.7c	80.1b	3.8b	4.62b

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).

REFERENCES

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