

ALTERNATIVES TO METHYL BROMIDE IN STRAWBERRIES IN SPAIN

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ABSTRACT

Strawberry cultivation consists of mother plant production and plant production for fruit. Since most of the mother plants used in Spain are imported from the United States, it is absolutely essential to understand the applicability of US trends and potential solutions on alternatives to MB for strawberry cultivation in Spain. As result of a four-year research programme in Huelva, several short-term MB alternatives have been developed that include chemical, non-chemical and mixed treatments to meet the requirements of several types of cultivation systems: conventional, integrated pest management and organic production. Adoption of these alternatives by strawberry growers for prolonged and extensive use will depend on EC legislation, consumer responses, scientific explanations for current results, changes to machinery and cultivation practices, and the ability of growers adopt procedures for using new technology.

Keywords: methyl bromide, short /medium-term alternatives, policies, trends, implementation, new compounds.

INTRODUCTION

The ban on the use of methyl bromide (MB) for strawberry cultivation in Spain has very important international and domestic consequences for an agricultural sector divided into two economic, technically and geographically different specialties: plant and fruit production. Plant production is carried out in high-elevation nurseries located in Central-Northern part of Spain (Castile-Leon) mostly dependent on mother-plants obtained from big Californian nurseries. Fruit production is carried out in coastal areas of Huelva, Cadiz, Barcelona, Valencia and other regions using daughter-plants multiplied in the Spanish high-elevation nurseries.

It follows that the alternatives to MB adopted by low and high-elevation Californian nurseries will have a direct influence on the phytosanitary status of plant material multiplied in Spain. In addition, alternatives to MB to be adopted by high-elevation Spanish nurseries will have a direct influence on the status and efficiency of plant material cultivated for fresh fruit production in Spain. Therefore, it is absolutely essential for the development of short-term alternatives to MB for strawberry cultivation in Spain to understand US trends and potential solutions on alternatives to MB, particularly those under development in California and Florida.

Fruit production

Strawberry fruit production is annual (one season) from October until end of May in Huelva. Farm size and grower specialization make it very difficult to alternate strawberries with other crops such as vegetables and/or with fallow periods using cereals.

The main phytosanitary soil-borne problems are *Phytophthora cactorum*, *Verticillium* spp. root-knot nematodes (*M. incognita*) and weeds (in particular yellow nutsedges). However, the most important problems have been grey mould (*Botrytis cinerea*), powdery mildew (*Sphaeroteca macularis*), anthracnose (*Colletotrichum* sp.), two-spotted spider mites (*Tetranychus urticae*) as well as abiotic disorders of fruit malformation during the first part of cropping season. These phytosanitary problems are not related to MB.

Plant production

The cultivation system for plant production is annual (one season) from April until October-December in the area of Castile-Leon. Alternate crops with other cereal and industrial crops (e.g. sugar beet) are possible. It is customary to change location from time to time by means of farm-leasing.

About 95% of the mother plants are imported from big Californian nurseries annually. So far, the general sanitary status of the Spanish nurseries has been satisfactory due to MB fumigation. Main

phytosanitary problems are *Phytophthora cactorum*, *Verticillium* spp., *Colletotrichum* sp., phytoplasmas and weeds.

Due to the certification and control system carried out in high-elevation nurseries and the soil fumigation practices and telluric conditions in the area for fruit production, it is difficult to conceive of massive and intense attacks of soil-borne pathogens in Huelva.

QUESTIONS AND REMAINING CHALLENGES

Alternatives under development

As result of a four-year research programme in Huelva (López-Aranda *et al.* 2002), several short-term MB alternatives have been developed for strawberry which have been developed to field demonstration stage. These MB alternatives are chemical, non-chemical and mixed, to fulfill the several types of cultivation systems: conventional, integrated pest management and organic production that require different demands on pesticides utilization. The following possibilities have been established: a) annual shank-application of 1,3 dichloropropene (1,3-D)+chloropicrin (61:35) under pre-formed raised beds (40 cc/m² of treated area); (also, shank-application with half-dosage (20 cc/m² of treated area) under black VIF sheets; b) annual incorporation of Dazomet located under pre-formed raised beds (50 g/m² of treated area); c) soil solarization (4 weeks, August) with simultaneous shank application of metam sodium (75 cc/ m² broadcast area); soil solarization (4 weeks, August) with simultaneous biofumigation (fresh chicken manure incorporation, 4-5 kg/ m²).

These alternatives could be appropriate short and medium-term alternatives to MB where inoculum levels of lethal soil-borne strawberry pathogens are low, as in Huelva. However, there are question marks and remaining challenges for short and mainly medium-term alternatives. How will these alternatives address national and EU policy on pesticides utilization, customer and consumer expectations? What are the scientific explanations for our results? What can we expect from new short-term alternatives yet to be trialled? How will the research results from the National Project INIA be implemented?

Results to date

Shank-application of 1,3-D+chloropicrin (61:35) under pre-formed raised beds has been the best agronomic result as a MB alternative (Telone C-35, Telopic and similar soil fumigants like Agrocelhone). This MB alternative has better potential for future development on strawberry cultivation in Spain than other solutions, particularly for conventional system. However, what will be the future EU policy on (cis or trans) 1,3-D and/or chloropicrin utilization (e.g. Regulation 414). So far, these kind of fumigants have provisional registration and authorization in Spain for strawberries.

On the other hand, soil solarization in combination with biofumigation (simultaneous incorporation of large amounts of organic matter, e.g. chicken manure), is an appropriate alternative solution for integrated crop management and organic production systems. The EU bans the agricultural use of big quantities of livestock manure – will there be a change to this ban? What will be the customers/consumers response to the use of massive amounts of organic biofumigant for fruit production?

Scientific explanations for our results

Our results and trends, carried out in “steady conditions” of lethal soil-borne pathogens under low inoculum pressure, have shown important morphological and agronomical differences (40-50%) among treatments only two-three years after trials initiation. A single answer that explains the effect of soil-borne pathogen activity, caused by *P. cactorum* and/or *Verticillium* wilt, is not enough. Our results could also be related to breaking of soil stress phenomena due to physical-chemical soil status (mainly Nitrogen liberation) and/or allelopathic processes and/or with the action of sub-lethal microorganisms like black root rot complex (*Pythium* spp., *Rhizoctonia* spp., *Cylindrocarpon* spp., etc.), and/or the interaction of all these factors. These would be diverse areas to study, but worthy of answers.

Implementation of research results

We have selected shank application as general model system for chemical alternatives (like Telopic, chloropicrin alone, metam sodium, metam potassium and others) in order to take advantage of the

whole technology, security protocols, machinery and practical experience obtained from decades of MB use. However, other practical solutions for fumigant application are possible, e.g., pre-planting fumigation with drip irrigation. In the case of strawberry cultivation in California, the best MB alternative carried out has been drip application with InLine (1,3 dichloropropene-chloropicrin 61:35) (Caulkins, pers. com.). In the case of Dazomet as MB alternative, the key factor is to undertake trials to improve localization and incorporation practices, utilization of VIF film technology, and/or combinations with other compounds (e.g. Telopic).

Special equipment (machinery) and procedures have to be improved if solarization is to be used in Huelva. Better still, it may be necessary to introduce new technologies like sprayable plastic polymers for this purpose (Gamliel *et al.* 2001).

Finally, other short and medium-term MB alternatives could be tested under our domestic field conditions: e.g., iodomethane alone or with chloropicrin, sodium azide, propargyl bromide and other products.

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SOIL SOLARIZATION AND BIOFUMIGATION IN STRAWBERRIES IN SPAIN

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ABSTRACT

The use of soil solarization combined with biofumigation resulted in increased soil organic matter content, elimination of an high number of weed species, increased vegetative and productive plant growth in strawberries in Huelva compared with soil solarization alone. The combination treatment requires growers to pay for the plastic sheeting and the costs of its application. Incorporation of chicken manure at 25 tonnes per ha was included at the same time that the soil was tilled when that sheets were removed. The application cost per hectare was about € 850 which was 24% cheaper than shank-applied MB under pre-formed beds.

Keywords: Strawberry, case-study, non-chemical, mixed technique, chicken manure, weed control.

INTRODUCTION

Methyl bromide (MB) has been used as soil fumigant for soil-borne pathogens and weeds since World War II. Its applications in strawberry cultivation started in California during the 1950's. MB soil fumigation practices in Spain were imported from California by highly specialized pioneers (farmers and agronomists) located in Andalusia, during the 1960's, along with the new technology for strawberry production using new plant varieties. MB is due to be phased out by 2005 following the requirements of the Montreal Protocol (see Bolivar 2002) and the European legislation (see Batchelor 2002). About 33% of the MB in 1995 in Spain was used for strawberry cultivation.

Soil solarization and biofumigation are some of the short-term, non-chemical alternatives suggested for strawberry cultivation which are described more fully below. The fumigants 1,3- dichloropropene-chloropicrin, dazomet and metam sodium are considered short-term chemical alternatives to MB. Some of them e.g. metam sodium, can be used in combination with soil solarization or shank-applied under VIF plastic sheets. All these options have been examined in the Spanish National Project INIA SC 97-130 "Alternatives to the conventional use of MB environmentally safe and in a cost effective manner". The results support a 75% reduction in MB consumption in 2003 on strawberry cultivation, and that short-term alternatives for Huelva's strawberry production exist (López-Aranda et al. 2000).

SOIL SOLARIZATION

Soil solarization is an easy disinfection technique based on the use of soil heating, due to the solar radiation during summer, that elevates soil temperature (at field capacity of water content) in a field previously covered with transparent polyethylene (PE) sheeting. Elevated soil temperatures are reported to be lethal or sub-lethal for soil-borne pathogens and weeds (Katan 1981). This method of soil disinfection is less effective on mobile organisms like root-knot nematode that are able to move away to cooler soil temperatures during periods of solarization. Soil solarization is also reported to be more effective in soils that have a high organic matter content. Combining solarization with reduced dose of chemical fumigants such as metam sodium (75-100 cc/m²) achieved similar results to MB (López-Aranda *et al.* 2000).

BIOFUMIGATION

Biofumigation uses gases and other products from organic amendments and residue biodegradation as fumigants against soil-borne pathogens (Bello *et al.* 1999). Biofumigant materials include livestock manure, refuse from the waste paperbin, fishing factory waste, numerous agricultural and food industrial waste, as well as plant residuals with allelopathic compounds (Hoitink 1988). Nitrogen compounds (like ammonium and nitrates), organic acids and a great number of volatile substances are responsible for this biocidal activity (Mian *et al.* 1982).

SOIL SOLARIZATION AND BIOFUMIGATION

Katan (1981) suggested that organic residuals incorporation into the soil could increase the effectiveness of solarization. Lacasa *et al.* (1999) showed that biofumigation applied in combination with solarization between July to October is effective for controlling soil-borne pathogens. Solarization is recommended for 30-45 days during the months when the soil temperatures exceed 50°C, although when combined with biofumigation, the soil temperature could be as low as 40°C. As a negative effect, it is necessary to point out that soil biodiversity losses have been observed.

SOIL SOLARIZATION AND BIOFUMIGATION IN STRAWBERRIES IN HUELVA

As consequence of the first results found out by the Spanish National Project INIA SC 97-130, soil solarization in combination with biofumigation was applied in plots dedicated to strawberry cultivation at the Experimental Farm "El Cebollar" (Moguer, Huelva). These plots were never fumigated and began to be cultivated with strawberry from 1992 without any soil disinfestation practice.

Six to seven weeks of solarization, from the first half of July until the beginning September, were carried out annually each summer from 1995 to 1998. From July 1999, chicken manure was applied before application of the solarization film. Chicken manure dose were about 25 tonnes/ha, less than those utilized by Huelva's farmers. Before the organic amendment incorporation, the soil was sprinkler irrigated until the field capacity of the water content was attained. After the chicken manure was incorporated, the soil was covered with transparent sheets by means of a prototype machine designed using funds from the National Project INIA SC 97-130. The polyethylene (PE) sheets were 50 microns thick and 3.30 m wide. This kind of plastic was the same as the one utilized for traditional broadcast of MB shank-application in the area. Soil strips of 40-50 cm wide remained without disinfestation among the PE sheets. Plastic sheets were removed after 6-7 weeks (beginning of September) and then harrow passes were given to improve soil homogeneity. The same solarization/biofumigation practices have been repeated in the summers of 2000 and 2001.

The use of soil solarization combined with biofumigation has resulted in the following improvements at our Experimental Farm: a) Increased soil organic matter content; b) Elimination of an high number of weed species (in particular purslane, *Portulaca oleracea*, very frequent in the summer time); c) Increased vegetative and productive plant growth compared with soil solarization alone (13% of yield increasing last 2001 season in cv. "Camarosa"). The combination treatment requires growers to pay for the plastic sheeting and the costs of its application. Incorporation of chicken manure at a similar dosage was included at the same time that the soil was tilled when that sheets were removed. The application cost per hectare is about € 850 which is a 24% cost reduction in comparison with shank-applied MB under pre-formed beds (€ 1,120).

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CASE STUDY: *TRICHODERMA* AS AN ALTERNATIVE TO METHYL BROMIDE IN STRAWBERRIES

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ABSTRACT

Trichoderma is known for being the most used biocontrol agent in Agriculture. Most of the isolates of the genus *Trichoderma* that have been found to act as mycoparasites of many economically important aerial and soil-borne plant pathogens. Phasing out methyl bromide (MB) will have a disproportionate effect on southern European emerging economies, and will increase dependence on chemical control measures. An EU-funded project aims to develop integrated biological, physical and low-chemical approaches to strawberry crop protection based on naturally occurring strains of *Trichoderma* active against the principal fungal pathogens *Colletotrichum*, *Phytophthora* and *Botrytis*, and to find strains of *Trichoderma* active with solarization. For postharvest treatments, novel biocides will be extracted from *Trichoderma* proteins to determine their potential for protecting the strawberry fruit from pre- and post-harvest diseases. Synergistic effects of the various control measures developed will be explored in an effort to recommend integrated strategies for strawberry production with minimal chemical input.

Keywords: Biocontrol, *Trichoderma*, cell wall degrading enzymes, solarization, IPM.

INTRODUCTION

Trichoderma is known for being the most used biocontrol agent in Agriculture. Most of the isolates of the genus *Trichoderma* that have been found to act as mycoparasites of many economically important aerial and soil-borne plant pathogens (Chet 1987), have been classified as *T. harzianum* Rifai. This leads to the fact that the species *harzianum* is generally considered as a mycoparasite and synonymized as a "biocontrol agent". However, the taxonomic status of *T. harzianum* has been unclear for a long time, as Rifai (1969) and Bissett (1991) used different concepts for its definition.

We have recently described (Hermosa *et al.* 2000) the presence of at least four different species – *T. harzianum* s.str., *T. atroviride*, *T. longibrachiatum* and *T. asperellum* within biocontrol isolates identified as "*T. harzianum*". The combination of these species in the same formulation facilitates their effectiveness to colonize soils and protect plants against their pathogens.

Trichoderma spp. evolve numerous mechanisms for both attacking other fungi and enhancing plant and root growth. Several new general methods for both biocontrol and plant growth promotion have recently been demonstrated and it is now clear that there must be hundreds of separate genes and gene products involved in these processes, including mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogen's enzymes (Monte 2001).

A few of these genes have been patented and used for plant transformation (Lorito *et al.* 1998), and they are in the process of reaching the market, but new sets of novel genes are being developed for their biotechnological application. In addition, *Trichoderma* strains have beneficial effects in agricultural systems other than pathogen control, principally through increasing soil fertility by active breakdown of organic matter. All these features facilitate the use of *Trichoderma*, at different levels, as an alternative to soil fumigants against plant pathogenic fungi.

Phasing out methyl bromide (MB) will have a disproportionate effect on southern European emerging economies, and will increase dependence on chemical control measures. Strawberry crops in California are predicted to decline by 25% without MB and similar reductions may be expected in Mediterranean countries where strawberries are farmed intensively on US-style models. MB is used as a soil sterilant before planting in polythene tube systems and is effective for controlling nematodes,

soil insects, weeds and fungi. However, the non-specific action of MB destroys natural, microbiologically-mediated disease suppression in the soil and can facilitate rapid recolonization by pathogens.

PROJECT DESCRIPTION

We are working in a EU-funded project (FAIR6-CT98-4140) to develop integrated biological, physical and low-chemical approaches to strawberry crop protection. Our aims are a) To promote natural, biological, physical and low-chemical control strategies for fungal pathogens as alternatives to MB and chemical fungicides in European strawberry cropping systems by developing consumer- and environment-low risk alternatives; b) To select naturally occurring strains of *Trichoderma* active against the principal fungal pathogens *Colletotrichum*, *Phytophthora* and *Botrytis*, and to explore their use alone and in combination with other targeted methods in laboratory and field conditions; c) To assess the efficacy and survival of selected *Trichoderma* strains in conjunction with solarization techniques, and to investigate the effects of their application to beneficial soil organisms; d) To develop novel biocides from *Trichoderma* proteins to protect the strawberry fruit from pre- and post-harvest diseases (Figure 1); and e) To explore the synergistic effects of the various control measures developed, and to recommend integrated strategies for strawberry production with minimal chemical input.

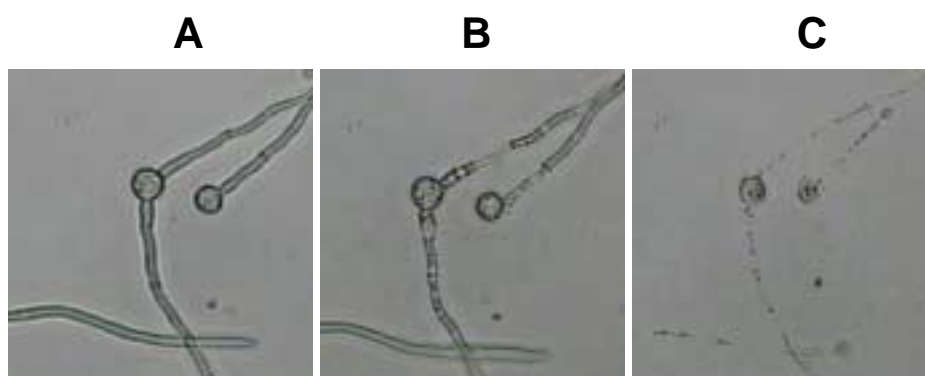


Figure 1: Detailed microscope image of *T. harzianum* 9.3 action on *B. cinerea* hyphae at 0 (A), 30 min (B) and 7 hours (C) after culture filtrate addition.

There are three major novel strands in this project, involving synergies between different control measures. Firstly, combinations of selected (but not genetically modified) *Trichoderma* strains are being explored as soil and foliar additives, in order to increase the effectiveness and range of pathogen control. Secondly, biocontrol strains are integrated with solarization techniques for partial soil sterilization and further colonization with highly competitive and beneficial fungi. Selected *Trichoderma* strains are compatible with solarization preferentially, allowing the maintenance of permanent pathogen-antagonistic soil systems. Thirdly, the combination of traditional *Trichoderma* biocontrol systems with novel antifungal agents derived from *Trichoderma* enzymes which are being tested as environmentally friendly foliar sprays or post-harvest treatments. As a result, one of the *Trichoderma* cell wall degrading enzyme genes is being used to transform strawberry plants.

We have also investigated the synergies of biocontrol organisms and their products with minimal doses of chemical control agents to develop protocols which maximize control but minimize inputs. The consortium has identified and promoted optimal control systems using components from our IPM spectrum, which should result in effective control of strawberry diseases on a long-term sustainable basis. The experimental processes will act as a model for developing environmentally friendly control measures for pests and pathogens of other MB-dependent crops.

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SODIUM AZIDE FOR SOIL PEST CONTROL IN CROPS WITH FEW OR NO ALTERNATIVES TO FUMIGATION WITH METHYL BROMIDE

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ABSTRACT

Stabilized liquid formulations of sodium azide (NaN_3) can be a "one-to-one" substitute for methyl bromide (MB) in horticultural crops. NaN_3 can be delivered through irrigation water and does not need specialized equipment for application to the soil. Greenhouse, microplot and field studies at Auburn University demonstrated that the chemical is directly toxic to nematodes, weeds, and soilborne fungal pathogens that cause vascular wilts, root rots and damping off. NaN_3 is particularly effective against purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentum*), Carolina geranium (*Geranium carolinianum*), bur clover (*Medicago* spp.), and other weeds that are very difficult to control. Previous long-term studies at Auburn University showed that soils treated with NaN_3 are enriched with beneficial fungi (*Gliricium*, *Trichoderma*) species of which are major antagonists of the southern blight pathogen (*Sclerotium rolfsii*) and damping off fungi (*Rhizoctonia*, *Pythium*, *Fusarium*).

keywords: azides, inorganic azides, herbicide, horticultural crops, hydrazoic acid, nematicide, root-knot nematodes, sodium azide, soil fumigation, strawberry, weed control.

INTRODUCTION

Na and K azides are salts of hydrazoic acid (HN_3) that have been explored in a limited manner for their pest controlling properties in the past. These materials are solids, readily soluble in water, and can be formulated as granules or liquids. Azides are potent metabolic inhibitors affecting the activities of a variety of oxidative enzymes, notably those involved in the electron transport system of respiration. There is ample information on the toxicological properties of sodium and potassium azides on humans (TOXLINE 2001).

These compounds are hypotensors (Merck Index 1989) and were used in the 1950's for treatment of certain types of cancers in humans and more recently in formulations to fight AIDS. Extensive studies have demonstrated that azides are not carcinogenic. Currently, sodium azide (NaN_3) is used principally by the auto industry in air bags. While azides of heavy metals such as Cu, Pb, Hg, are unstable and explosive, those of sodium and potassium are considered safe and stable under ordinary conditions (Moeller 1952).

Sodium and potassium azides when added to soils ($\text{pH} < 7.0$) release HN_3 which is converted chemically by reaction with water to NH_4^+ and to nitrate through the action of nitrifying bacteria (Parochetti & Warren 1970).

Field research at Auburn University in the 1970's in a commercial pine nursery and with several row crops showed that granular formulations of sodium and potassium azides applied to soil demonstrated broad spectrum activity against weeds, nematodes, and soilborne phytopathogenic fungi (Kelley & Rodríguez-Kábana 1979b; Rodríguez-Kábana & Robertson, 2000; Rodríguez-Kábana *et al.* 1975; Rodríguez-Kábana *et al.* 1972). Similar results were obtained in other areas of the U.S. and in Belgium with high-value horticultural crops (van Wambeke *et al.* 1984, 1985; van Wambeke & van den Abeele 1983). Microbiological studies of soils treated with NaN_3 for several years indicated that, in contrast to MB-fumigated soils, those treated with azide showed increased population levels of a group of fungi (principally species of *Trichoderma* and *Gliricium*) antagonistic to a broad spectrum of soilborne phytopathogenic fungi (Kelley & Rodríguez-Kábana, 1975, 1979a, 1981). The mode of action of sodium and potassium on soil-borne pathogens is based on short-term direct toxicity, but may also involve as yet undetermined long-term effects through enrichment of the soil with microbial species antagonistic to the pathogens.

Sodium and potassium azides can be formulated as granules (attapulgite clay, diatomaceous earth) or in a variety of liquid formulations. Key to the stability of these formulations is that the pH remains greater than 9.00. This can be accomplished for granular formulations by including sufficient sodium and potassium carbonate to buffer the granules at pH 9.5 - 10.0. Granular formulations were used to control weeds and soil-borne pests typically located in the top 7 - 10 cm of the soil profile. However, for other pests (nematodes, *Armillaria*, *Verticillium*) and deep-rooted crops (grapes, fruit, and nut trees), liquid formulations are more suitable. Delivery of azide to the desired fumigation zone may be difficult if reactivity of HN_3 in the soil-air space and atmosphere is too rapid and results in a concentration of the active compound too low for effective pest control. One way to achieve a more uniform distribution of the chemical might be to apply the chemical through a drip irrigation line. Recent work at Auburn University demonstrated the feasibility of preparing liquid formulations of sodium and potassium azide suitable for delivery through drip irrigation systems.

Preliminary fieldwork indicates that sodium and potassium azides possess considerable flexibility for preparation of formulations suitable for specific crops and situations. The activity of this material is influenced by soil pH. The optimal application method in the alkaline soils is different from that best suited for acid soils. The generally favourable properties of NaN_3 as a potential substitute for MB prompted the Nematology team at Auburn University to conduct research to develop new formulations for field use of the compound and to evaluate NaN_3 as an alternative to MB for control of nematodes, weeds, and pathogens in high-value cropping systems.

MATERIALS AND METHODS

Microplot Experiments. Microplot trials were conducted on the Auburn University's campus microplot facilities to determine the efficacy of new experimental formulations for NaN_3 as a MB alternative. A microplot consists of a one-ft² (929 cm²) 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 were loamy sands with pH = 6.2, organic matter content <1.0%, and cation exchange capacity < 10 meq/100 g soil. The soils are typical for Alabama and are 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. rolfii*), 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 a typical experiment, NaN_3 was applied at rates in the range of 25 - 300 kg ai/acre using various formulations. The treated soil was covered with standard polyethylene plastic for two weeks, after which the plastic was removed and crops (eggplant, tomato, or okra (*Abelmoschus esculentum*)) were planted. Each crop was grown in accordance with standard commercial practices. Treatments in each trial were arranged in a randomized complete block design with eight replications per treatment. Variables studied in the experiments were pest population densities (weeds, nematode numbers, disease incidence), crop growth parameters, phytotoxicity, yield, and product quality. Data were analyzed using standard statistical methods, primarily Analysis of Variance (ANOVA).

Field Experiments. A strawberry trial was conducted in the 2000 season near Plant City, Florida, in a sandy field infested with the sting nematode, yellow nutsedge and other weeds. Plots were 200 meters long and NaN_3 (preplant at 100 Kgs/Ha) was compared with MB at 400 kg/ha. The azide was applied through the drip irrigation system (2 tapes/bed) in enough water to penetrate the soil profile to a 45-cm depth. In 2001, a strawberry trial was established in a field at the Auburn University's Experiment Substation, near Brewton, Alabama. NaN_3 was applied preplant at 150 kg/ha as described for the Florida experiment. In both experiments, data were collected on nematode and weed populations and on yield.

RESULTS

Data obtained from microplot experiments indicate that Na azide is a potent nematicide effective for controlling root-knot nematodes (*Meloidogyne* spp.) and many other nematodes important in eggplant, tomato, green pepper, strawberry, tomato and other high value crops. The compound must be used pre-plant with a 7-10 day wait period before planting. Although Na azide does not require

plastic cover after application to soil its performance is considerably enhanced by covering the soil (Figures 1a, 1b and 1c).

Field experiments with strawberry conducted in Florida and Alabama in 2000 and 2001 demonstrated that pre-plant applications of Na azide at rates of 100-150 Kgs/Ha eliminated weed and nematode problems and was equal or superior to MB in yield response.

CONCLUSIONS

Data from microplot and field experiments indicate that NaN_3 can be applied easily by drenching or through drip irrigation systems without need of special equipment or exposure to workers. The pre-plant wait period required is similar to that for MB and well within the logistical requirements for crop production. Sodium azide applications in the range of 80 - 150 kg/ha are optimal for control of nematodes, weeds and other soil-borne pests. These rates are economical and compare favorably with MB.

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FIGURES

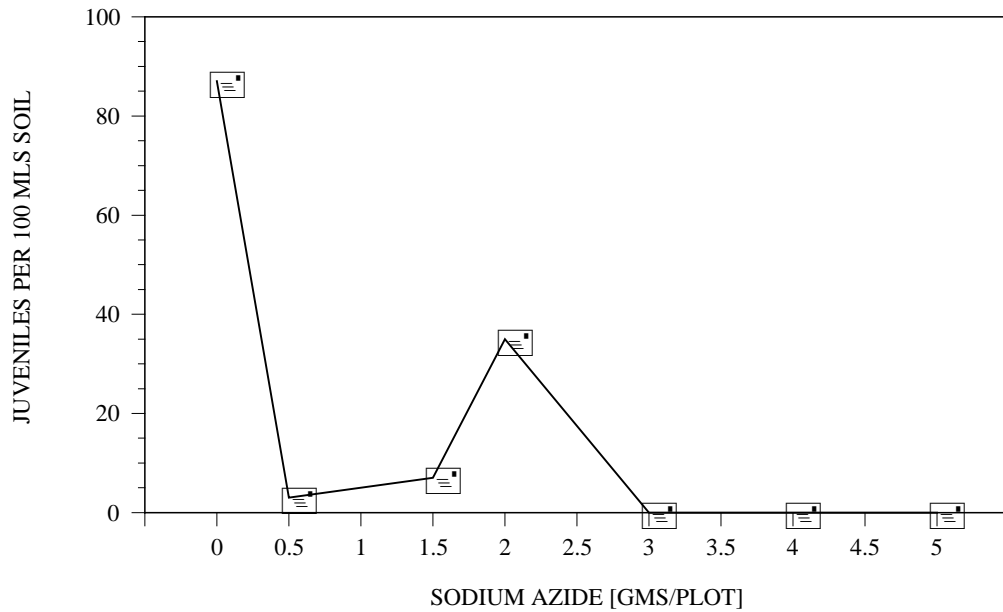


Fig. 1A. Effect of preplant applications of sodium azide on final populations of the root-knot nematode *Meloidogyne incognita* in a microplot experiment with 'Black Beauty' eggplant (1 g/plot is equivalent to 100 kg/ha).

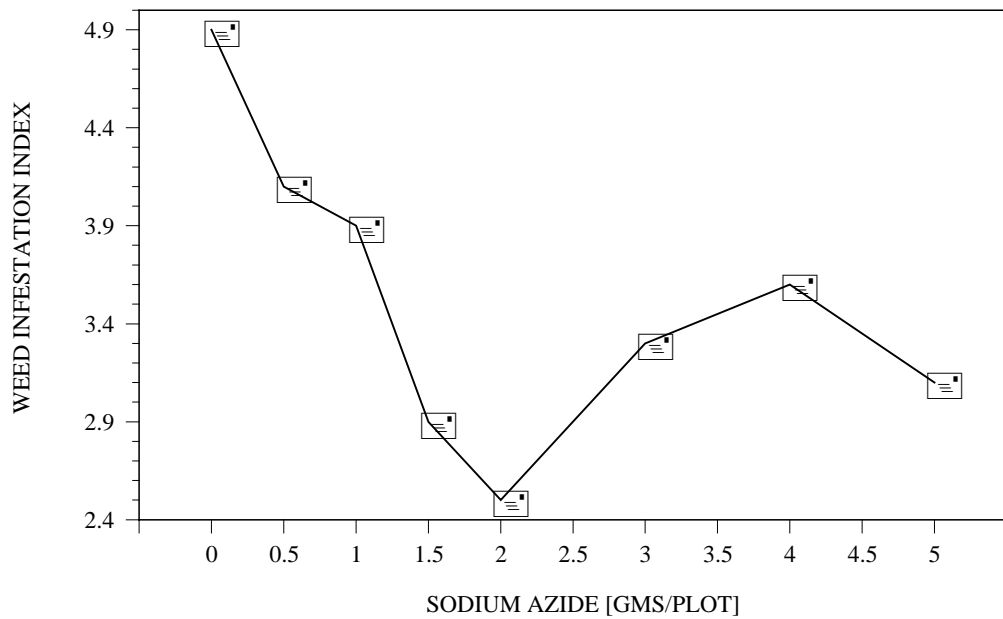


Fig. 1B. Effect of preplant applications of sodium azide on weed populations in a microplot experiment with 'Black Beauty' eggplant. Index scale: 1= no weeds to 5= plot completely covered with weeds. The principal weed species were yellow nutsedge *C. esculentum* and crabgrass *Digitaria sanguinalis*.

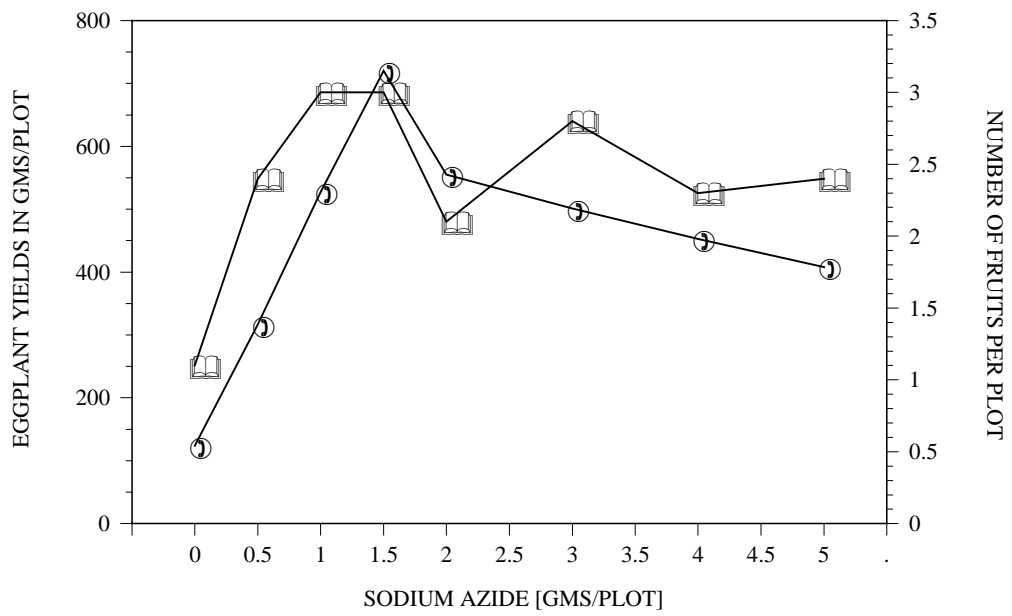


Fig. 1C. Effect of preplant applications of sodium azide on number of fruits [diamonds] and yield (circles) in a microplot experiment with 'Black Beauty' eggplant.

SPANISH STRAWBERRY PLANT NURSERIES AND THE USE OF METHYL BROMIDE AS A SOIL DISINFECTANT

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ABSTRACT

Methyl bromide (MB) with chloropicrin has been used in strawberry production in Spain since the 1980s and is effective for controlling fungal pathogens, nematodes and weeds. Increased production in recent times has been accompanied by the need to increase phytosanitary quality. In the 1990s, the development of high-elevation nurseries located in region with suitable soils and climate resulted in increased production that was linked to disinfection using MB. This combination led to Spain being confirmed as the main strawberry plant producer in terms of quality and quantity after the United States of America. Over the last five years, the nursery sector has cut MB use from 446 tonnes in 1997 to 190 tonnes in 2001 which reflects a real 42.6% reduction in the use of MB. At the present time, MB is the most important working tool available enabling strawberry plant nursery operators to produce strawberry plants with sufficient phytosanitary quality and guarantees. The continued use of MB is requested after 2005 at least for nurseries as MB is critical for the whole strawberry-growing sector, not only because of its importance at the initial stage in the nurseries but also because of the repercussions that a prohibition on its use in nurseries would have on the fruit-producing areas.

Keywords: methyl bromide, alternatives, nurseries, phytosanitary, weeds, pathogens

INTRODUCTION

A number of strawberry plant varieties have been grown in Spain for more than thirty years. The sector grew rapidly from the mid-1980s, which resulted in strawberry plant cultivation in nurseries on irrigated land increasing up to 1 000 hectares.

Methyl Bromide (MB) has been used as a soil disinfectant since the 1980s. As it was also effective in combating weeds, within a few years of being introduced it had taken the place of other products such as metam sodium and metam potassium which were much less effective in controlling fungal pathogens, nematodes and weeds. The accepted application was 600 kg of a 98:2 mixture of MB and chloropicrin per hectare.

The introduction of the use of MB led to increased production per hectare: 250 000 – 300 000 for the Tioga variety, 400 000 for Douglas and 450 000 – 500 000 plants per hectare for Chandler, the variety whose use of MB became an established and widespread practice. Increased production was accompanied by increased phytosanitary quality, cutting to a minimum the incidence of diseases such as *Phytophthora* sp., *Verticillium* sp., *Rhizoctoria* sp., and nematodes such as *Meloidogyne* sp. and *Aphelenchoides* sp., which were formerly real scourges, not only causing serious damage in nurseries but also causing growers considerable economic problems in the fruit-growing areas where plants carrying the inoculum were sent.

Finally, there was less time spent on weed control which further increased the economic return from the crop.

In the 1990s, the development of high-elevation nursery techniques, the increased professionalism of commercial nurseries, the suitability of the soil and climate, and of course soil disinfection using MB, led to Spain's position being confirmed as the main strawberry plant producer in terms of quality and quantity after the United States of America.

CURRENT SITUATION

In 1997, 800 hectares of strawberry plants were grown in Spain, 95% of which were disinfected using a 98:2 mixture of MB and chloropicrin at a rate of 600 kg per hectare, making a total of approximately 446 tonnes of MB used overall in nurseries.

In 1998 and 1999, the mixture was diluted for the first time to 67:33 with a reduction in the application from 600 to 400 kg per hectare. The same period saw the land area dedicated to the crop increase to 950 hectares. Approximately 242 tonnes of MB were used in nurseries.

In 2000 and 2001, the mixture was diluted further, with a 50:50 mix being widely used, and the number of hectares cultivated rose to 1000, 950 hectares of which were disinfected with an application of MB at 400 kg per hectare. Approximately 190 tonnes of MB were therefore used in nurseries annually.

To sum up, over the last five years, the nursery sector overall has cut MB use from 446 tonnes in 1997 to 190 tonnes in 2001. This reflects a real 42.6% reduction in the use of MB, indicating the sector's commitment to cutting down on the product while optimising its efficiency.

FUTURE PROSPECTS

Faced with no imported or produced MB being permitted to be and placed on the European Community market for soil fumigation from 1 January 2005, the Spanish association of strawberry plant nurseries (*Asociación Española de Viveristas de Planta de Fresa - AEVVPF*), has taken part in the Spanish project to find alternatives to MB since 1998. The following results of that project should be highlighted:

1. When land which has not been disinfected previously is used, only chloropicrin-based alternatives have any real disinfectant effect and even these do not control weeds sufficiently.
2. In the crop cycle for a nursery disinfected with 400 kg of a 50:50 MB/chloropicrin mixture per hectare the average time spent on weed control is 20 days per hectare. The cost increases to 26 days if chloropicrin with Telone is used and up to 38 days if solutions such as Metam sodium or Metam potassium are used. This entails a 90% increase in the costs of manpower which is already in itself scarce and expensive.
3. The volume of production in terms of the number of saleable plants per hectare in some cases has fallen to 30%.
4. The phytosanitary quality of the plant is considerably reduced, causing sites of vascular disease to emerge requiring increased manual treatment and increased application of fungicides and pesticides. This brings about a greater incidence of resistant pathogen strains which are ever more aggressive and difficult to control in nurseries and also entails increased production costs and a reduced volume of production.
5. Since this is a crop where phytosanitary quality must be officially certified, prohibition of the use of MB as a soil disinfectant would mean that every year a large number of plots of ground could not be certified and to a certain extent it would cease to be viable to export such plants since their health could not be guaranteed.

CONCLUSIONS

There is no escaping the conclusion that, without effective disinfestation, nurseries would be responsible for transferring increasingly aggressive pathogen strains to growing areas along with the plants. These areas would then carry the inoculum but would have increasing limited means of defence, given that pest-control treatments in fruit-growing areas are increasingly hazardous in view of the residual - and occasionally lethal - nature of the active substances in the fruit, the trend towards integrated production crops (IPM) where the use of such active substances is prevented and limited, the increased sensitivity of consumers and the relevant legislation in force in the countries of Europe which is increasingly exacting and restrictive.

At the present time, MB is the most important working tool available enabling strawberry plant nursery operators to produce strawberry plants with sufficient phytosanitary quality and guarantees.

None of the alternatives currently being investigated is a reliable substitute for MB in nurseries as they:

- Increase the costs of soil disinfection per hectare;
- Increase the manpower costs for weed control;

- Increase the costs of cleaning and spraying with active substances;
- They do not guarantee plant health or the consequent successful certification of plants

The greatest risk is undoubtedly the spread of pathogens to fruit-producing areas, multiplying the economic impact we have already noted for nursery areas, since a single hectare of nursery produces enough plants for approximately 10 hectares of fruit crops.

In the light of the above, we consider MB to be key to the stability of strawberry growing and therefore request that its continued use be authorised at least for nurseries, since it is critical for the whole strawberry-growing sector, not only because of its importance at the initial stage in the nurseries but also because of the repercussions that prohibiting its use in nurseries would have on fruit-producing areas. MB use should therefore be maintained in nurseries in order to safeguard the health of strawberry crops from the very first stages.

STRAWBERRY PRODUCTION AND THE USE OF ALTERNATIVES TO METHYL BROMIDE IN HUELVA (SPAIN)

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ABSTRACT

The cultivation of strawberries in Huelva has had to overcome a gradual decrease in initial profitability by increasing yield. This has necessitated the use of soil fumigants, especially MB. The reduction requirement in the use of MB imposed by Regulation EC 2037/00 and the Protocol of Montreal have been rigorously followed, while maintaining profitable cultivation, using novel techniques (such as shank-applications under preformed beds only, VIF film utilization, and others) and also by reducing the MB percentage in MB-PIC commercial formulations. The Spanish National Project on short term MB alternatives allows growers to be, according to initial data, optimistic that short term chemical alternatives will be ready. Nevertheless, these solutions will have to assure cost effective production. There remains some doubt on the availability of alternatives for the medium and long term. According to the reports so far, the availability of healthy plant material from our high-elevation nurseries with intact agronomic potential and at a reasonable cost is not at all guaranteed without the use of MB. For this reason, the strawberry sector thinks it is of utmost importance to use MB and consequently requests Critical Use authorisation for the continued use of MB in Spanish high-elevation nurseries after 2005.

Keywords: strawberry, Huelva area, fruit production, nurseries, Methyl Bromide, productivity, alternatives, future perspectives, critical uses.

INTRODUCTION

An analysis of the potential impact of the unavailability of methyl bromide (MB) for strawberry production in the area of Huelva should be carried out taking into account the evolution of costs in the cultivation of strawberries and expectations of the crop's continued economic viability.

Strawberry cultivation in the area of Huelva has maintained profitability from the beginning of the 1980's when today's production practices were put in place. Nevertheless, the strawberry sector has been confronted, season after season, with a constantly growing curve of production costs. The costs have been due particularly to labour costs. This situation combined with static or even decreasing market values for fresh strawberries in the European and National markets has been so far solved by growers increasing overall yield. Based on the need to continue this level of economic activity that generates more than 60,000 direct jobs and more than 4.5 million day's work per year in the area of Huelva, the strawberry industry must have all the keys to allow it produce to its full potential.

The results of the Project of High National Interest (INIA) "Alternatives to the Conventional Use of MB Environmentally Safe and in a Cost Effective Manner", in which our strawberry Professional Organization (Freshuelva) has been collaborating from their beginning, clearly demonstrated the dramatic drop in yield when no treatments are used against soilborne problems. Productivity in non-fumigated controls could decline by as much as 40%, even in absence of lethal strawberry soilborne pathogens. This phenomenon could be possibly attributed to soil stress and/or sub-lethal microorganism activity. For this reason, the strawberry cultivation system in Huelva has been traditionally developed with the use of soil fumigants, generally MB, in order to ensure levels of productivity (unitary yields) and consequently adequate profitability for this crop (López-Aranda *et al.*, 2000, 2001a, 2001b).

The new situation due to the Montreal Protocol guidelines has created special problems that our strawberry growers, acting as pioneers in the world, have solved with strong discipline by

means of technical novelties such as shank applications under pre-formed beds only, combinations of MB-Pic with decreasing amounts of MB formulation, VIF film technology utilization, and other treatments. These new technologies have allowed them to comply with the gradual MB reductions imposed by the European Commission Regulation EC 2037/00, in agreement with the Montreal Protocol. In the case of strawberry in Huelva, MB doses have been reduced by about 80% compared to the 1980's which is without precedent in intensive agriculture in Mediterranean conditions.

Meanwhile, an appropriate answer from our Public Administrations to the anxious strawberry sector wishing to comply with the regulations, acting with a much wider perspective to preserve the environment but at the same time very worried by the potential elimination of a decisive element for the viability of the cultivation, the mentioned INIA Project started to find short-term alternatives that could merge environmental safety with the survival of a large agrarian economic and social activity found in strawberry cultivation in Huelva. For that, we would like to choose this particular occasion to transfer to sincerely thank the whole scientific team of this Project and very especially Dr. López-Aranda on behalf of the strawberry growers of Huelva for their invaluable services.

With the normal caution in these kind of situations and still in a provisional context, the well-known Project data so far (López-Aranda *et al.* 2000, 2001a, 2001b) allowed a certain trust to be maintained in the chemical short term alternatives to MB. Technically viable and cost-effective alternatives will be received with interest by growers and without a doubt they could be practiced once they will acquire the knowledge to use them.

We think that the aim of this case-study is not to carry out a rigorous technical analysis of the new MB short-term alternatives that are beginning to be defined as these must be formally presented to the strawberry sector by the scientific team of this National Project. Nevertheless, we repeat, these alternatives to be considered as technically viable, and would have to have the same statistical significance of effectiveness as MB. Otherwise, growers could be expected to accept a decrease in strawberry production in the area. This possibility would have to be evaluated carefully by the strawberry growers and to decide if they are able to accept such a production decrease.

Strawberry high-elevation nurseries is a specific topic of other papers submitted in this International Conference. For this reason, we are not going to consider it with detail. Nevertheless, it is an important factor for the viability of our southern strawberry cultivations. Due to the necessity of plant material with a good phytosanitary status, with all full agronomic potential and at a reasonable price.

It is possible to define as very difficult the situation in the Spanish high-elevation nurseries taking account the results so far of the activities of National Project INIA developed in Castile-Leon and its consequences for fruit production in our area. According to those trends (Melgarejo *et al.* 2001), the availability of healthy plant material with full agronomic potential at a reasonable price is not at all guaranteed in absence of fumigation with MB in these high-elevation nurseries. For this reason, unfortunately, and on the contrary that we have expressed regarding the fruit production situation, in this Conference and on behalf of the survival of the strawberry cultivation in the area of Huelva, we claim as indispensable the concession of the Critical Uses for MB utilization in the case of Spanish strawberry high-elevation nurseries.

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