ENDURE

European Network for Durable Exploitation of crop protection strategies

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Thematic Priority 5
FOOD and Quality and Safety

Deliverable DR1.20

Guidelines for (i) soil steaming, soil solarisation and biofumigation as alternatives to soil chemical disinfectants (ii) landscape management to improve conservative biological control, in field vegetables cropping systems

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Dissemination Level	
PU Public	**PU**
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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Glossary

ENDURE European Network for Durable Exploitation of crop protection strategies

GSL: Glucosinolate
ITC: Isothiocyanate

Definitions

Summary

Write a short summary of your Deliverable. This summary must be 2 pages maximum but very informative and must include the following elements:

Objectives

The objective of the deliverable is to make a point on past and/or ongoing researches, within the groups involved, on what has been done or is going on the alternatives to the use of pesticides in field vegetable cropping systems. DR1.17 showed the importance of minor uses in field vegetable cropping systems and how diverse the situation was within the EC members regarding the number of available options for control of a given pest in a given crop.

Here special focus has been made on the control of soil-borne pests, diseases and weeds with the phasing-out of most of the soil disinfectants and on control of insects, considering the concerns about the toxicity of insecticides, especially neurotoxics.

Rationale: describe the approach/methodology you chose to reach the objectives

We organized two workshops, one held in Le Rheu from 30 September to 2 October 2008, the second one during the Endure Annual Meeting on 22 October 2009 in Wageningen. Between these two events we worked through exchange of written materials.

The analysis has been done from communications given during the two workshops, publication from the involved teams and results from current experimentations conducted by some partners

Soil borne pests and pathogens remain difficult to control when chemical soil disinfectants are not available. Soil steaming can be an efficient alternative in terms of control but may be slow to perform and costly in energy. Applying the technique only on the row with soil cultivation between rows during the crop can be a good alternative as far as weeds are concerned and possibly for most soil borne diseases, especially for transplanted crops. Machinery technology is still improving and should lead to better efficacy and efficiency in the near future. Biofumigation, although showing incomplete and sometimes inconsistent control, has still to be regarded as a promising option to control soil borne pests and diseases and probably also weeds. The variability observed in the results is probably partly due to the Brassicacae (and other plants) that were used and the specific glucosinolates they contain (and ITCs they can release) regarding the sensitivity of the pests and diseases targeted, as well as the way those biofumigant crops were managed. Most of the work done so far on biofumigation was applied research with big expectations on ready to use technology. The indirect control through changes in the soil microflora has also to be more documented on an epidemiological perspective.

As far as insects are concerned, numerous options are given here as alternative to the use of pesticides in commercial crops, from selected use of pesticides on margins only where pests are attracted, inundative biological control, and/or conservative biological control. One important point which comes out of this study is that there is not a unique recipe and that efficient solutions will come from local analysis of the problem to control as well as the potential offered by the production situation. This means that local adaptation will be needed to determine which plants to grow as insectary plants, which insect enemies to promote, for example. Results are shown from different regions of Europe and from Reunion Island on field margins approaches, studies have been conducted at more broader scale or are underway. Nevertheless there is a need for research on landscape and functional ecology to propose a framework in which such local studies will be thought and developed. There is also a need for more research on chemical ecology, as it offers the opportunity of

developing chemistry with new modes of action allowing regulation of plants – pests – pests enemies interactions.

Considering the information available on these different topics it appeared difficult to formally write down guidelines to be used by growers or advisers, as initially thought when proposing the framework for this case study. Thus, this report has to be considered as giving some insights on new techniques which are promising but which will have to be adapted to local situations, before being successfully implemented.

Teams involved:

AU, CIRAD, INRA, PPO, SSSUP, UDL/IRTA

Geographical areas covered:

Denmark, France, Italy, Martinique, Reunion Island, Spain, The Netherlands

1. Sharing of experience and main achievements on soil steaming, solarisation and biofumigation as alternatives to soil chemical disinfectants

1.1. Introduction

Soil chemical disinfection has been extensively used for many years in field vegetable cropping systems. It provided an efficient method for controlling soil-borne pests and diseases as well as weeds allowing growers to organize crop succession according to market demand even though they often increased soil-borne pests, diseases and weeds risks. The major effective soil fumigant, methyl bromide, has been identified as one of the worst depleters of the ozone layer. Consequently, the Montreal Protocol (1987) signed by 187 countries, outlawed the use of methyl bromide in most countries as to 2005 leading to research on alternative methods throughout the world. The 1,3-dichloropropene and metam-sodium have been some of these alternative to methyl bromide and are either phased out with derogatory measures in some countries, or under question.

Field vegetable growers are looking for alternatives to these fumigants as soil-borne pests diseases and weeds are important treats that must be controlled in current cropping systems. Some of them, from soil steaming to biofumigation, will be reviewed in this chapter, illustrating expertise from Endure member.

See also the site of the AlterBromide project financed by the EU (FP6): http://www.alterbromide.org/albro/rw/pages/home.en.do

1.2. Soil disinfection by means of activated steaming – The “Bioflash® System” (SSSUP)

by Andrea Peruzzi[1], Marco Fontanelli[1], Michele Raffaelli[1], Marco Ginanni[2] & Paolo Bàrberi[3]

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Introduction

A really promising soil disinfection system alternative to chemical fumigation is soil steaming. In this regard, a new system for soil disinfection (called Bioflash®) performed by means of steam and activating compounds (CaO and KOH) was developed by the Celli company (Forlì, Italy) in co-operation with MAMA - DAGA and CIRAA “E. Avanzi” of the University of Pisa (Peruzzi *et al*., 2002 and 2008).

This innovative technique was tested from 1999 onwards and several multidisciplinary experiments were carried out within two consecutive Italian research projects, taking into account the mechanical, operative, economic, agronomic and phytopathological aspects, yielding very interesting results and numerous publications (Bàrberi *et al*., 2009; D’Errico *et al.*, 2002 and 2007; Lenzi *et al.*, 2004; Luvisi *et al.*, 2006; Moonen *et al.*, 2002; Peruzzi *et al.*, 2002 and 2008; Triolo *et al.*, 2004 and 2007, Tesi *et al*., 2007). Moreover, the Bioflash® system is non toxic for crops and does not produce negative effects in the soil such as a biological void (Gelsomino *et al*., 2002 and 2007).

The Bioflash® system

The Bioflash® steaming system (Fig. 1) includes the distribution and incorporation into the soil of low environmental impact activating compounds (KOH or CaO) that are compatible

with subsequent cultivation, and are able to react exothermically with steam by releasing an additional quantity of thermal energy for soil heating.

Activating compounds allow to reach higher temperatures for a longer time in the soil and are also able to directly control parasites and germinating weed seeds. These substances have been chosen according to their low environmental impact and their positive side-effects on soil (e.g. pH correction and soil nutrient addition). Furthermore, the use of the Bioflash® system allows to plant crops immediately after the steaming treatment. The Bioflash® system is realized by means of machines that distribute and incorporate different rates of activating compounds in the soil (using a rotary tiller powered by a hydraulic engine) and steam by means of different injection dispensers realized on purpose.

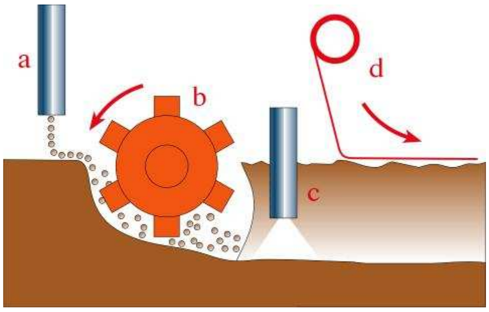

Fig.1. Scheme of the disinfection treatment with the Bioflash® system: a) activating compounds distribution; b) compound incorporation in the soil by means of the rotary tiller; c) steam injection, d) soil compaction and mulching with plastic film.

Fig. 2. The "Celli ECOSTAR SC600" self propelled machine during a soil disinfection treatment.

The most recent and innovative steaming machine of the series is the truck self-propelled Celli Ecostar SC600 (other drawn, mounted and 4WD self-propelled operative machines were also developed) (Fig. 2). The Celli Ecostar SC600 machine has a small size and hence it is well suited to use in greenhouses or tunnels. This was the machine utilized in all our trials.

Moreover four different steam injection systems were developed and tested (Fig. 3):

- single bar, for deep steam distribution (down to 15-20 cm depth),
- double bar , for very deep steam distribution (between 15 and 35 cm depth),
- carter bar, for very shallow steam distribution (5-10 cm of depth)
- mixed system, characterized by the concurrent use of single and carter bars. The tested steam distribution ratio between the upper and the lower bar was 1:1, 2:1 e 1:2 (Peruzzi, 2002, 2008).

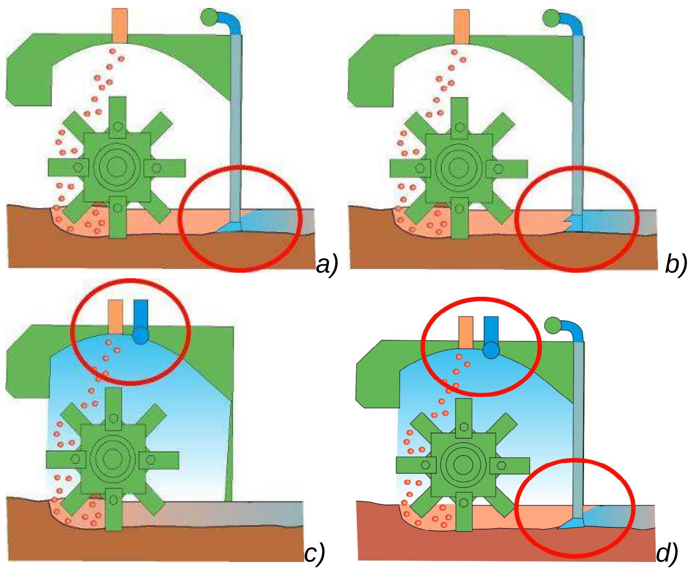

Fig. 3. Scheme of the different injection systems and different steam distribution: a) single bar; b) double bar; c) carter bar inside the rotary tiller; d) mixed steam distribution.

Biological results achieved with the Bioflash® system

In this report, the most recent results obtained in the experiments carried out in the period 2002-2008 on the Bioflash® system, are shortly summarized.

Effect on fungi and viruses

Specific tests were performed on the following combinations: *Sclerotinia minor* and *S. sclerotiorum* / lettuce and radish, *Pythium* spp., *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *lycopersici* / tomato, *S. minor and F.oxisporum* f.sp. *basilici* / basil (Fig. 4), *Rhizoctonia solani* /radish and rocket.

Experiments carried out from 2002 to 2008, allowed to obtain homogeneous and satisfying results concerning the reduction of artificially induced infections (on average -85/95% with respect to the untreated control). Phytoiatric effects appeared appreciable on the whole range of pathogens.

Furthermore, specific trials were carried out to test the effect of the Bioflash® system on viruses. TMV (Tobacco Mosaic Virus), which inoculum source is mainly infected plant debris in soil, showed a significant reduction in terms of virus density and infectivity on a following crop, after treatment.

Further experiments were run on sclerotia of *Sclerotinia sclerotiorum*, *S. minor* and *S. rolfsii* and good results were achieved again on all species but *S. sclerotiorum*. In that case, thermal treatments stimulated sclerotia germination.

In all cases, soil temperature seemed to be the most important parameter to evaluate the phytoiatric effectiveness of the system, especially when calculated as temperature sum, thus taking into account both temperature values and exposure time (Luvisi, 2006; Triolo, 2004 and 2007).

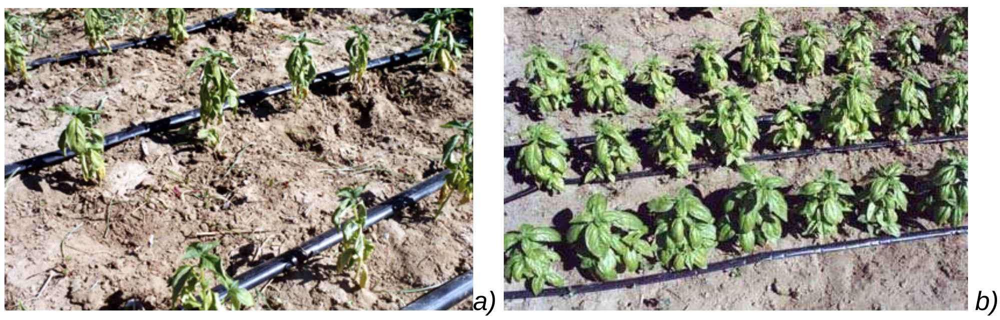

Fig. 4. Excellent control of Fusarium oxysporum f.sp. basilici on basil: (a) untreated control; (b) plot treated with the Bioflash® system.

<u>Effect on nematodes</u>

To test the effect of the Bioflash® system on soil borne nematodes, different trials were recently run for the following combinations: *Meloidogyne incognita*/courgette and lettuce, *Globodera rostochiensis*/potato.

A significant reduction of nematode infection (number of larvae, galls and cysts) was obtained, with values ranging from 65 to 95 % compared with the untreated control.

Results obtained in more recent trials carried out on rocket and radish showed a significant increase in bacteriophagous nematodes. Additionally, the Bioflash® system seems to be able to increase the benefits of mycorrhization (D'Errico, 2002 and 2007).

<u>Effect on weeds</u>

The Bioflash® system also allowed to reach a relevant suppression effect on the weed seed bank. Specific experiments were carried out with the deep injection system (20 cm depth) to determine the weed suppression potential of soil steaming plus activating compounds (KOH or CaO) to boost soil temperature (T). Different combinations between compound types and rates (from 0 to 4000 kg ha^{-1}) were tested in experiments carried out in field and controlled environment. Treatment effects were assessed on field weed vegetation, seedbank and seedling emergence of three winter (*Alopecurus myosuroides*, *Matricaria chamomilla* and *Raphanus raphanistrum*) and four spring annuals (*Amaranthus retroflexus*, *Echinochloa crus-galli*, *Fallopia convolvulus* and *Setaria viridis*).

The weed seedbank was clearly suppressed by activated steaming: total seedling emergence was inversely related to increasing KOH rates both in the 0-10 cm and 10-20 cm soil layers, while for CaO the relationship was significant only in the 0-10 cm layer. Winter annuals were more sensitive to KOH than CaO and spring annuals had a more pronounced species-specific response to treatments. There was a strong negative relationship between compound rate and seedling emergence for all species. *A. myosuroides* was the most sensitive species to the steam alone treatment (77% reduction), whilst *M. chamomilla* and *E. crus-galli* were the least sensitive.

Further experiments were carried out to test the effect of the new typologies of injection systems (the standard one plus shallow injection and mixed system) on an artificial infestation of *Brassica juncea* (Indian mustard) and on the natural weed seedbank. As expected, shallow steaming was the most effective treatment (up to 100% weed control) in the 0-7 cm soil layer. Deep steaming (performed by means of the single bar) depleted the weed seedbank up to 95% only in the deeper soil layer. The most homogeneous weed control effect along the soil profile was obtained by means of the mixed systems, with differences depending on the distribution ratio used (Fig. 5) (Bàrberi *et al*., 2009; Moonen *et al*., 2002; Peruzzi *et al*., 2007).

a)

b)


Fig. 5. Weeding effect of carter bar steaming treatments on Brassica juncea: (a) untreated control; (b) steamed plot.

Effect on soil characteristics

Specific experiments were carried out to evaluate the effect of steaming treatments on chemical, microbiological, and molecular soil properties. Soil was monitored during two consecutive radish cropping cycles, for a total time of about 3 months.

Soil pH showed little variations during the period of trial. Organic carbon did not vary sensibly during the experiment and, at the end, treated plots showed on average higher values with respect to untreated control at the beginning. NO_3-N content sensibly decreased during the period (from 40-60 to 10 mg kg^{-1}). Exchangeable potassium trend showed quite considerable variations and peaked in superficially-treated plots.

Bacterial counts showed that treatments did not affect considerably soil microbial molecular structures, being a proof that steaming treatments do not modify the bacterial community. Moreover, a significant increase in *Streptomices* species was monitored. This fact is very important, taking into account the key role they have for biological control of soil borne pathogens (Gelsomino, 2002 and 2007).

Effect on crop yield and quality

Results of the experiments carried out in the period 2002-2008 on several vegetable crops (lettuce, radish, rocket, basil, tomato, courgette, potato, strawberry, etc.) point out that the use of the Bioflash® system allowed to obtain yield increases (ranging from 20 to 300%) with respect to the untreated control (Peruzzi, 2009).

Specific tests were recently carried out again on rocket and radish (Fig. 6). Soil steaming treatments allowed to considerably increase the quality of vegetables, in terms of nitrates content reduction and total fresh and dry matter yield increase. In this respect, the most effective steam injection systems seemed the shallow and mixed ones. On average, the yield increase was ca. 15% with respect to the untreated control, while decrease in NO_3-N uptake was 13% (Lenzi, 2004; Tesi, 2007).

Fig. 6. Rocket development in the greenhouse after steaming treatments.

Future perspectives

The future development of the Bioflash® system, considering its highly promising depleting effect on weed seedbank, consists in the realization of a new prototype for band-steaming.

Band steaming is a very effective technique, able to control surface weed seeds just within the crop row, thus optimising treatments and considerably reducing energy use and costs.

This innovative method would be the first one utilizing activated steam and could solve one of the major problems in organic and integrated vegetable production, consisting in the large amount of labour required for hand weeding.

In this regard, the University of Pisa is actually developing a new drawn machine able to treat 9 to 12 “strips” on three raised beds with a total working width of ca. 5 m, in order to increase the working speed and productivity of activated steam treatments (Fig. 7).

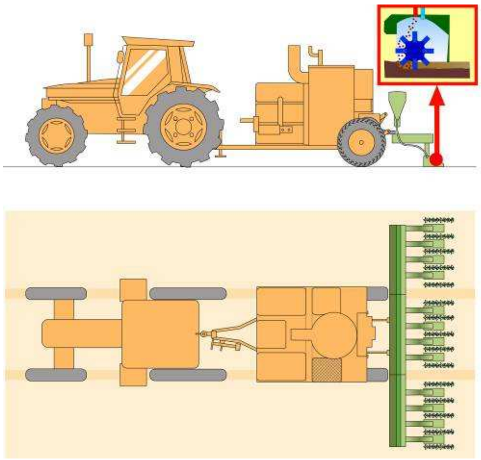

Fig. 7. Scheme of the drawn machine that will be realized to perform band steaming, one of the future perspectives for the Bioflash® system.

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1.3. Band steaming and biological activation of the soil for weed and diseases control in carrot (AU)

by Bo Melander & Sabine Ravnskov

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Introduction

Mobile soil steaming has the potential for reducing laborious intra-row hand-weeding in row crop systems where herbicides are not used. Mobile soil steaming is commercially used on raised beds, especially in short-term field salad crops with a strong need to control soil-borne pathogens (Pinel *et al.*, 1999). Steam is applied to the whole bed area and down 50 to 100 mm in the soil depending on the steaming time. Steaming causes high mortality of weed seeds and soil-borne pathogens, this could lead to effective and long-term weed and disease control.

It should be noted that the current soil steaming technology has three major disadvantages in Danish vegetable cropping. The consumption of fossil energy is extremely high, with diesel fuel use ranging from 3500 to 5000 l/ha; secondly, it is time consuming, requiring 70 to 100 hours to treat one hectare; and thirdly it is not allowed for organic vegetable cropping as many beneficial organisms are also affected. This has lead to the idea of band-steaming where only a limited soil volume is steamed, enough to control weed seedlings that would otherwise emerge in the rows (Melander & Jørgensen, 2005). The width of the treated intra-row band depends on how close to the crop plants inter-row cultivation is carried out. Inter-row hoeing in single-line sown onion, leek and carrot can leave only a 50 mm wide untilled strip in the row with no negative impact on crop growth and yield (Melander & Rasmussen, 2001). Steaming down to a moderate soil depth of 50 to 60 mm appears to be sufficient considering that most weed seeds in the seed bank are small and will predominantly emerge from the top 20 mm of the soil profile.

Band steaming has now been approved for weed and disease control in organic vegetables cropping. The energy consumption has been substantially reduced now requiring 500-600 litre oil ha^{-1}. However, several aspects still need to be studied and improved to further develop the technology. More studies are needed to explore whether the energy

consumption can be further reduced. Soil temperatures attained in practise appear to be well above those needed according to laboratory studies. Many soil organisms are either killed or significantly reduced by steaming. There is a need to clarify whether the addition of biological substrates can stimulate the re-colonisation of the steamed soil. Especially, whether beneficial organisms are more quickly in doing so than those with pathogenic and antagonistic features.

This presentation shows the first experiments made in carrot with the aim to study whether reduced maximum soil temperatures of steaming can lead to weed and disease control effects similar to higher temperature more commonly used in practise; and secondly whether biological substrates can stimulate crop growth, suppress pathogenic organisms and help beneficial organisms to re-colonise the steamed soil.

Materials and methods

The experiments are conducted at two organic locations in 2009 and 2010

a. Sandy soil, Jutland (56°3' N, 8°58' E)

b. Reclaimed bottom of the sea with a high clay content, Zealand (55°48' N, 11°29' E)

Crop

Carrots at both sites.

Experimental factors

Band-steaming intensity, 2 levels:

1. 75-80°C maximum soil temperature measured in the top soil of the band using an infra-red thermocouple
2. 60-65°C maximum soil temperature measured in the top soil of the band using an infra-red thermocouple

Application of preparations for biological activation of the soil, 8 levels:

1. No application
2. Substratum 1), *Bacillus* sp. product (biocontrol agent).
3. Substratum 2), *Pea meal* (organic substrate affects the microbial community)
4. Substratum 3), *Arbuscular mycorrhizal fungi* (Stimulates growth, nutrient uptake, disease control)
5. Substratum 6), *Chitosan* (organic substrate affects the microbial community), only at site a.
6. Substratum 7), *Pea meal* + *Israeli AM*, only at site a.

Experimental design

Two-factorial randomized block experiments arranged as a split-block design with steaming intensity as the main-plot and biological activation as the sub-plots. Five blocks included at both sites.

Experimental treatments

Band steaming was applied with band-steamers owned by the local farmer. The steamers were pre-adjusted to target the experimental temperatures before entering the experimental plots. Sowing of the carrots was done a few days later by retrieving the steamed bands. The activating substrates were applied just prior to sowing where all substrates were placed in or close to the sowing depth of carrots.

Inter-row hoeing, and pre-emergence flaming at site b., is the only weed control method applied in addition to band-steaming within the experimental area.

Apart from the experimental treatments, the carrots are grown according to the organic cropping standards normally used by the farmer.

Assessments

- Marketable carrot yield
- Disease attack on carrot roots
- Weed counts at the 3-4 true leave stage of carrot
- Aboveground crop growth at harvest
- Disease infestations and colonization of mychorrhiza recorded 3 times during the growing seasons

Results and discussion

Weeds

The intra-row effects of band steaming were calculated relative to the weed occurrence in the unsteamed inter-row area of the carrot crop. The high steaming intensity resulting in the highest maximum soil temperature reduced total weed numbers significantly more than the lower maximum temperature attained with a lower steaming intensity as seen in Figure 1. The effect was most profound at site a. (sandy soil) while pre-emergence flaming to some extent masked the effect of steaming at site b. (humus soil). However, the highest soil temperature had still more than halved the weed density at site b. Moreover, steaming has a longer lasting effect than flaming where only emerged weed plants are affected allowing re-emergence to take place. Principal weed species at site a. were: Black-bindweed (*Polygonum convolvulus*), Knotgrass (*Polygonum aviculare*) and Fat-hen (*Chenopodium album*). At site b., the principal species were: Common Chickweed (*Stellaria media*), Shepherd's Purse (*Capsella bursa-pastoris*) and Hedge mustard (*Sisymbrium officinale*).

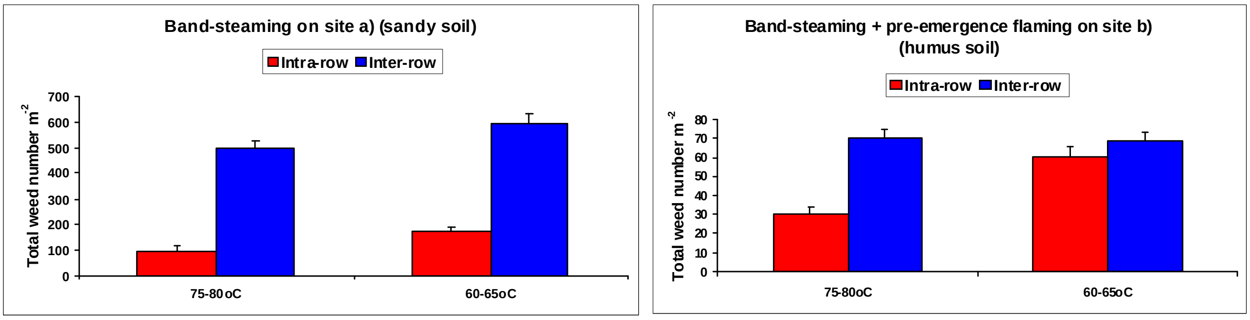

Figure 1. Effects of band steaming on the total weed number emerging intra-row and inter-row in organic carrot at two Danish sites, a) and b). Two steaming intensities were used resulting in the maximum temperatures shown under each set of columns. Standard errors of the means are shown on top of each column.

The weed reductions achieved with the highest steaming intensity at both sites are in line with the results seen with band steaming at other locations and in other growing seasons.

Hand-weeding

Reducing weed number will affect the need for hand-weeding proportionally in a linear way. Based on other experiments, measuring the relationship between weed number and hand weeding in direct-sown vegetables (Melander & Rasmussen, 2001; Van der Weide et al. (2008)), the weed density obtained with high steaming intensity at site a. would result in a time consumption of 120 hours ha^{-1} for hand weeding as opposed to 520 hours ha^{-1} for

unsteamed soil. For site b) the figures are 50 hours ha^{-1} for the high steaming intensity as opposed to 90 hours ha^{-1} for unsteamed soil.

Energy consumption

The oil consumption can be reduced by 15-20% when going from the high steaming intensity to the low one. The energy consumption used in practice to attain a max. soil temperature of approx. 80°C is approx. 600 liters oil ha^{-1} but may vary according to the equipment.

Diseases

Unfortunately, no data are available yet regarding the incidence of diseases and how they might have been affected by the experimental treatments.

Carrot yield

Preliminary analyses on marketable yield did not show any negative yield effects associated with the two steaming intensities (yield results from site a. in Figure 2). This is in line with previous studies and from practical band steaming experiences made in crops such as sugar beet, carrot and parsnip (and lettuce with mobile soil steaming). Actually steaming tended to increase marketable yield in previous studies.

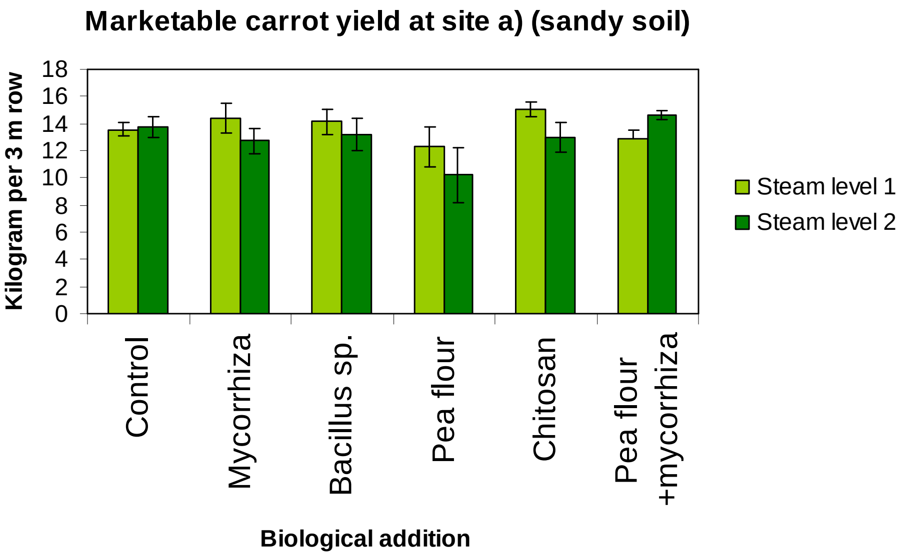

Figure 2. Effects of two band steaming intensities (high level 1 and low level 2) plus subsequent addition of various biological substrates on the marketable yield of carrot at site a) (a sandy soil). Standard errors of the means are shown on top of each column.

Adding a biological substrate has not shown any unambiguous effects on marketable yield apart from a minor yield decrease at site a., which was not present at site b. The reason for this yield decline has not been clarified yet. However, there was a tendency for an improved canopy growth due to adding *Pea flour*, *Chitosan* and *Pea flour + mychorrhiza* at site a. The effects of biological substrates on the disease infestation level have not been analyzed yet.

Conclusion

Reducing soil steaming intensity and thereby lowering maximum soil temperature seems to lower the effects on weed emergence. This will affect the time for hand weeding, which may double following a lower steaming intensity. Thus, lowering steaming intensity to save energy should be counterbalanced against an increased need for further weed control.

If hand-weeding is the only option, a reduction in steaming intensity may not be feasible when taking the current wage level in Denmark into account.

Adding biological substrates to steamed soil have not shown any significant benefits yet based on very preliminary analyses. Possible effects on the disease level still need to be analyzed. In general further investigations are needed to understand the use of these substrates in terms of application techniques and impact on crop, soil, pathogens and other microorganisms in the soil.

Band steaming receives a lot of attention in organic vegetables cropping owing to its high potential for enlightening the burden of manual weeding. However, its undesired effects on non-pests organisms are still a controversial issue among growers. A better understanding of the use of biological substrates could result in a stronger acceptance of band steaming provided that the biological substrates can demonstrate beneficial effects.

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1.4. Biofumigation for controlling root diseases on carrot (INRA)

by Françoise Montfort and Danielle Breton, INRA, UMR BiO3P, BP 35627, F-35653 Le Rheu

Introduction

Methyl bromide, long used as the principal fumigant in agriculture, was highly successful worldwide because of its consistent performance without the need to modify the cropping systems in place. However, the deleterious effects of this compound on the ozone layer resulted in the prohibition of its use in 2005 in developed countries and by 2015 in developing countries (Montreal Protocol, UNEP, 1987). Metham sodium has long been used as a substitute for methyl bromide for managing many soilborne plant pests in vegetable and potato production systems. In moist conditions, it gives rise to methyl isothiocyanate (methyl ITC), which has broad-spectrum biocidal activity against nematodes, fungi, insects and weeds (Richardson et al., 1969). This compound decomposes rapidly to generate a compound with no known toxic effects on human health, and is therefore considered less dangerous than other synthetic pesticides. Methyl ITC, like many other ITCs, is produced naturally by the plants of several genera from the *Brassicaceae*, *Capparaceae* and *Caricaceae* families (Fahey *et al*., 2001). Members of the *Brassicaceae* produce the broadest range of ITCs, these compounds having a common chemical structure but differing in terms of the functional group R. The potential use of these natural compounds as an alternative to

metham sodium has been the object of many studies and continues to be the focus of considerable interest.

The term “biofumigation” was coined by Kirkegaard *et al*. (1993) as “a crystallising term” to describe the suppressive effects of *Brassica* species on noxious soilborne organisms due specifically to the release of isothiocyanates through the hydrolysis of glucosinolates (GSLs), catalysed by myrosinase isoenzymes (Matthiessen et al., 2006). Allelopathy, defined by Rice (1984) as the direct or indirect, positive or negative effect of a plant on another, through the release of biochemical compounds into the environment, has long been observed in *Brassica,* giving these plants their reputation as “poor companion plants” (Matthiessen et al., 2006). The term “biofumigation” was then used to distinguish between the general phenomenon of allelopathy and the use in agriculture of isothiocyanates from biological sources for the suppression of soilborne pests and diseases (Brown and Morra, 1997).

As the technique of biofumigation is based on the use of isothiocyanate-generating brassicas as biologically active green manures, it generally involves growing a biofumigant crop during the intercrop phase. This crop contains precursors of toxic compounds (ITC in the case of *Brassicaceae*), which are released principally after the crushing of the crop residues. The toxic potential of biofumigant crops is greatest during flowering (i.e, when the rate of GSL in vegetative tissues is maximum, Bellostas et al., 2007).

Variability in production of ITC amongst brassicaceas

GSLs currently identified are diverse, depending on the plants considered, as well as the biological activity of their hydrolysis products (ITCs). So far 120 have been described (Mithen 2001).

Different Brassicacae have been tested for their toxicity against 2 fungi, *Pythium violae* and *Rhizoctonia solani* responsible for diseases on carrots. The tests consisted in measuring the growth inhibition of the mycelium of these fungi, in an atmosphere containing volatile compounds of a given amount of crushed tissues of the plant obtained at flowering stage (considered as the stage where content of glucosinolates in leaves is at its maximum). The results are given in the Figure 1.

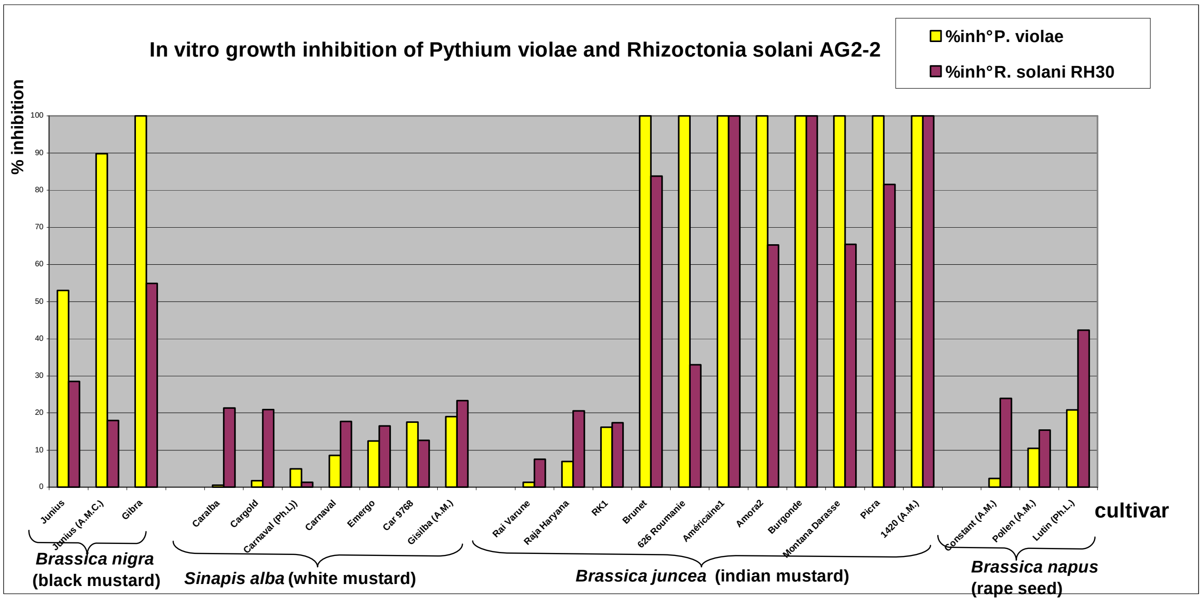

Figure 1: Growth inhibition of *Pythium violae* and *Rhizoctonia solani* in presence of crushed tissues of different Brassicacae (F. Montfort, unpublished)

Although cultivars showed different activities within a given species, European lines of *Brassica juncea* appeared to offer the largest potential activity against these two fungi.

Benefits of biofumigation go beyond a direct toxic effect of ITCs

Previous tests in microcosms showed that if ITCs have a strong effect on the soil inoculum quickly after the crushing and incorporation of mustard tissues, the protection effect lasted longer after the emission and disappearance of the volatile compounds that were produced. Furthermore, artificial infestation of the soil with new and alive pathogenic inoculum after the volatile compounds have been released and lost in the atmosphere produced low level of infection revealing that beyond the direct and toxic effect of ITCs on the resident inoculum, incorporating crushed brassicas residues gave some soil disease suppression probably due to changes in the microbial communities (Motisi et al., 2009a)

Field studies on control of *Rhizoctonia solani* on beet and *Pythium sulcatum* on carrots through biofumigation with Indian mustard

The first field trials conducted to test the efficacy of biofumigation with *B. juncea* on *Rhizoctonia solani* were conducted on sugar beet, the results being given in Figure 2.

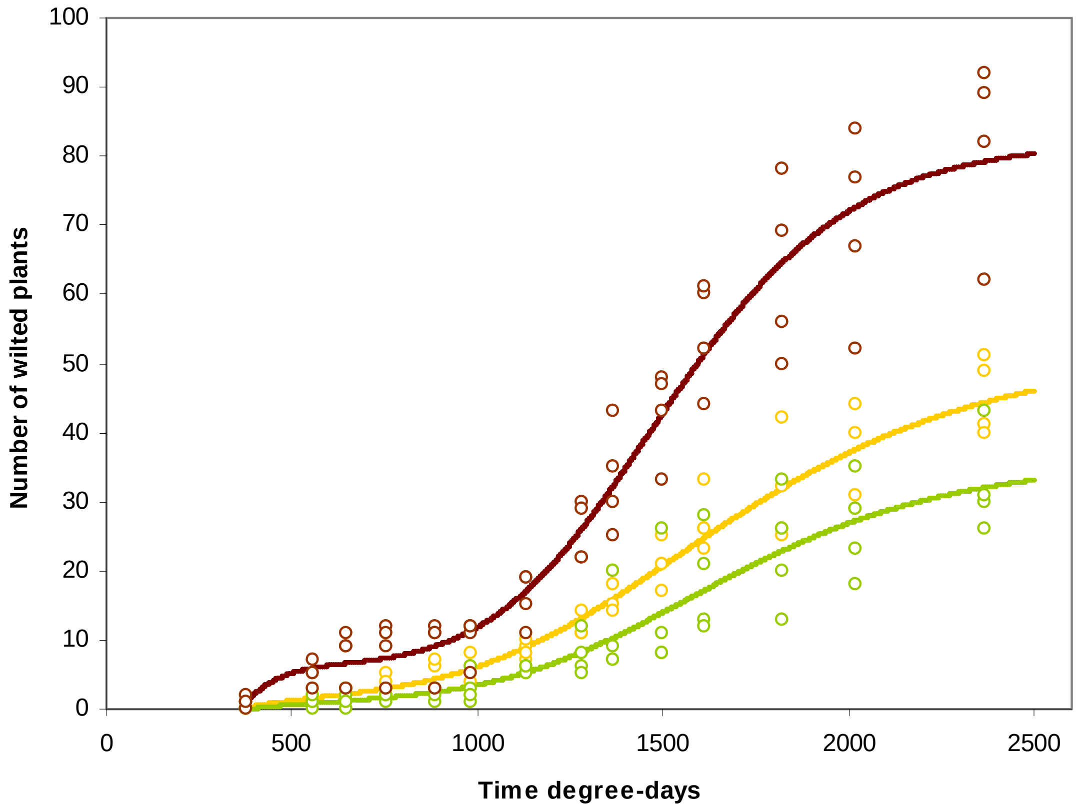

Figure: 2 Control given on Rhizoctonia brown rot by cropping mustard between wheat and beet in a wheat sugar beet rotation (brown: bare soil, yellow: mustard grown and pulled out, green: mustard grown, crushed and incorporated into soil, from Motisi el al., 2009)

Beet was grown after wheat followed or not by a crop of Indian mustard in summer, before beet sowing at the beginning of the following spring. In the classic wheat – beet rotation (without any mustard crop between wheat harvest and beet sowing), *R. solani* developed throughout the season of beet crop with a final level of around 80% of plants showing wilting (brown curve). Growing mustard during 2 months following wheat harvest

and without crushing and incorporating mustard residues (plants pulled out and exported from the field, yellow curve) gave some control, but the best results were achieved when residues were also incorporated into soil –green curve) (Motisi et al., 2009b)

A similar experimentation has been conducted on carrots but focusing on cavity spot caused by *Pythium sulcatum*. Growing *Brassicae juncea*, crushing and incorporating plant residues were compared to bare soil between two crops of carrots, the first one being inoculated with *P. sulcatum*.

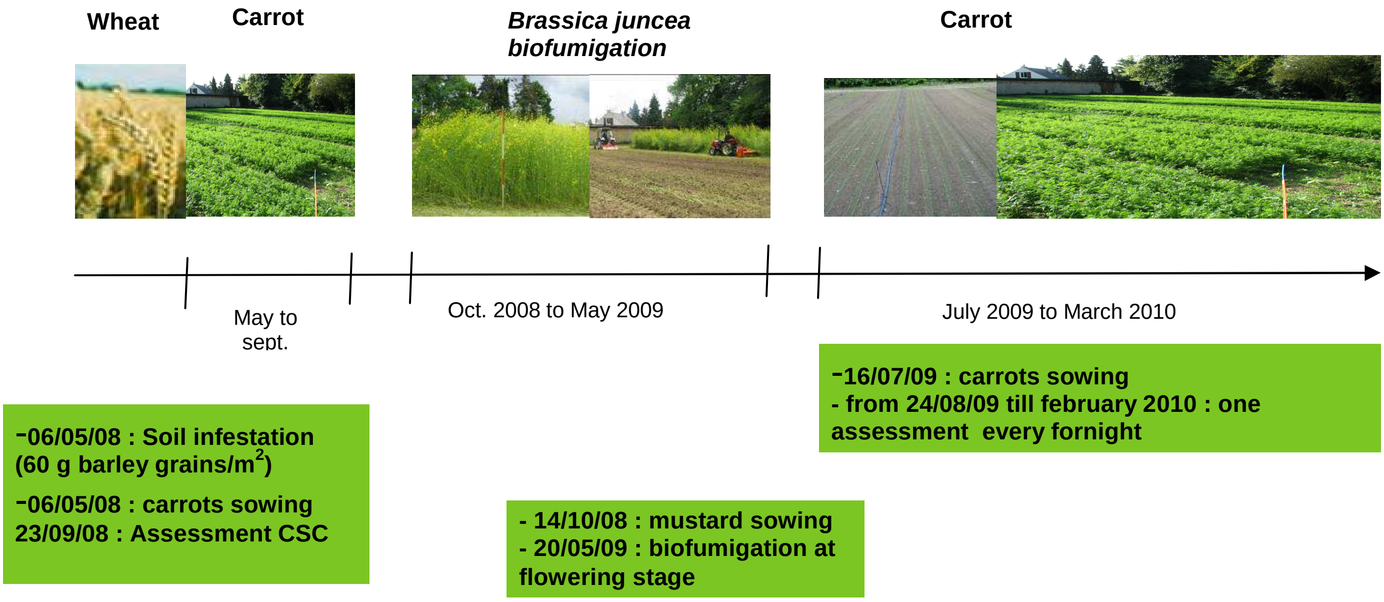

Figure: 3 Experimentation set-up in Le Rheu for testing the efficacy of biofumigation on cavity spot on carrot (INRA)

Disease incidence for cavity spot was measured from 59 days after sowing to 129 days after sowing and showed an increase up to almost 90% of infected plants with no difference whatever the treatment (bare soil *vs* mustard and biofumigation) between the two crops of carrot (Figure 4).

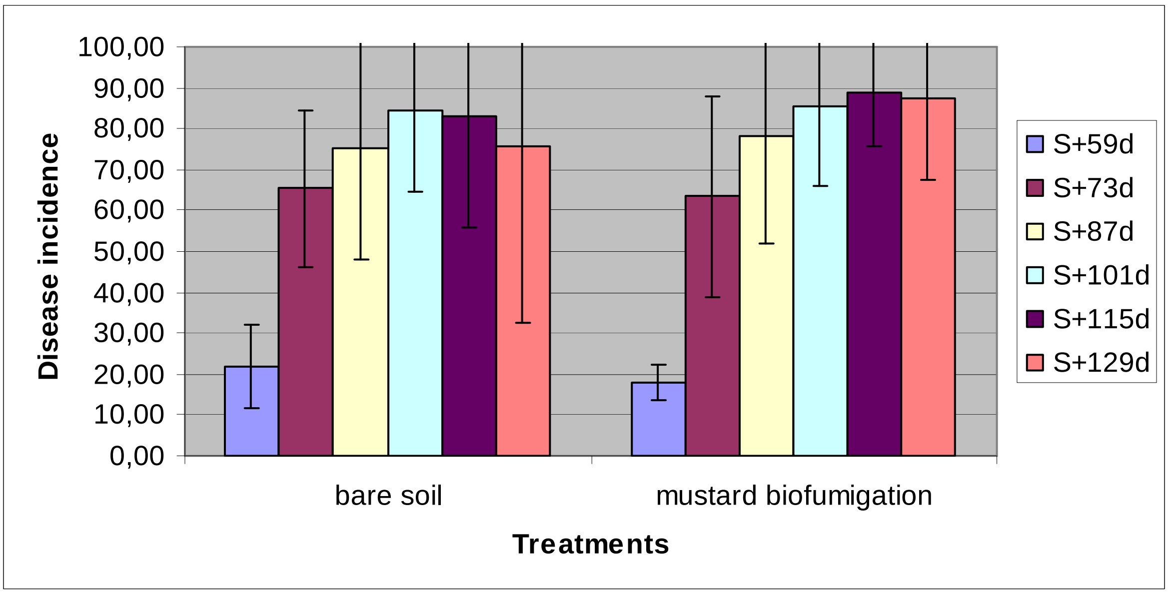

Figure: 4 Disease incidence (cavity spot) on carrots after biofumigation between two consecutive crops, compared to bare soil (percentage of plants showing symptoms within the crop)

A slight difference can be observed on diseases severity (Figure 5), showing some control of biofumigation. When mustard was grown and crushed residues incorporated into soil, disease severity was maintained at 10% when it increased regularly to 15% in the case of bare soil between the two crops of carrot.

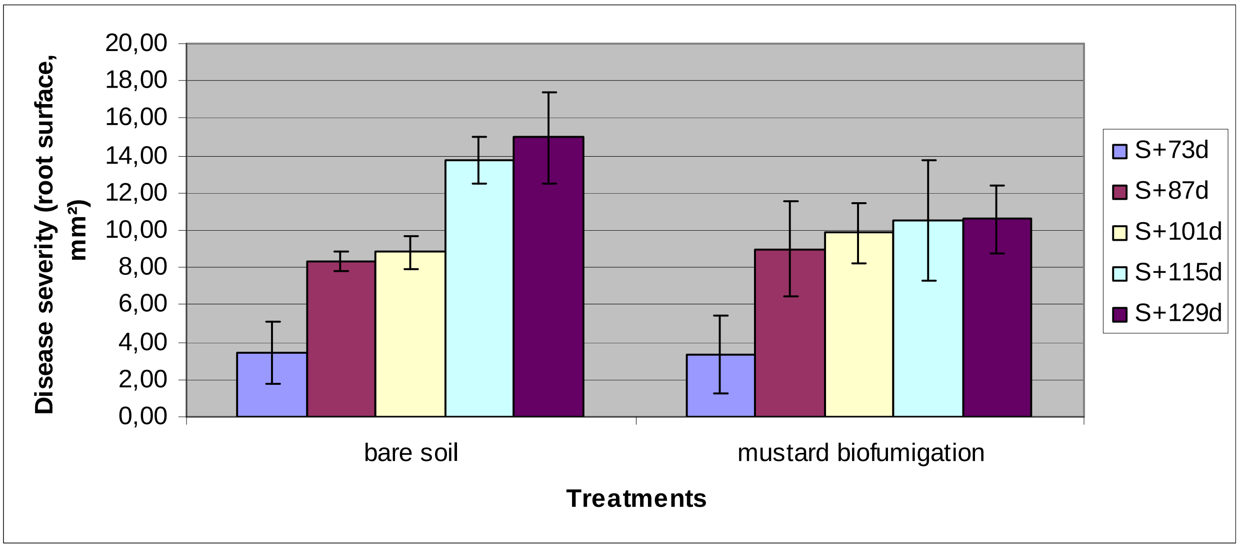

Figure: 5 Disease severity (cavity spot) on carrots after biofumigation between two consecutive crops, compared to bare soil (disease severity is the surface of the root showing necrosis, expressed in mm^2)

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1.5. Research on the potential of biofumigation as a means of control of bacterial wilt on solanaceaous crops (CIRAD)

by Peninna Deberdt, Pôle de Rech. Agron. de la Martinique, BP 214, 97285 Le Lamentin Cedex 2 - Martinique

Biofumigation refers to the suppression of soil pathogens by the release of antibiotic compounds, principally isothiocyanates, resulting from the hydrolysis of glucosinolates from the tissues of Brassicas (cruciferous plants) grown in rotation or as green manure crops (Kirkegaard and Matthiessen, 1999).

Bacterial wilt (*Ralstonia solanacearum*) is one of the world most important bacterial diseases, particularly in tropical areas (Hayward, 1991). In Martinique, this soilborne bacterium is particularly damaging on solanaceous crops such as tomato, eggplant, potato pepper and tobacco, and more recently on curcubits (Wicker *et al*., 2007).

Cruciferous plants were screened for their biofumigating potential vs *R. solanacearum* for the first time in Australia (Akiew, Trevorrow and Kirkegaard, 1996). Studies demonstrated that biofumigation using Indian mustard (*Brassica juncea*) green manure was effective in reducing the level of bacterial wilt in the soil and reducing the severity of the disease in a tobacco crop grown in succession (Akiew and Trevorrow, 1999). Akiew *et al*. (2005) designed a screening procedure to specifically test the isothiocyanate-related toxicity of a range of brassicas. Information from laboratory experiments supported the findings of Matthiessen and Shackleton (2005) suggesting that mustards with high content in propenyl glucosinolate would be the most suppressive biofumigant if conditions conducive to isothiocyanate release could be satisfied in the field. The effectiveness of a range of biofumigants for suppression of bacterial wilt was then evaluated in a highly bacterium-infected field. Results showed that all brassicas tested delayed the onset and reduced the incidence and severity of bacterial wilt. At harvest, mustard was clearly superior to other species in reducing wilt incidence and increasing tomato yield (Matthiessen and Kirkegaard, 2006). These latter results demonstrate that careful selection of Brassicas green manure can result in significant levels of biofumigation–based control in the field.

Altough the term of biofumigation was first used, and always referred to the antibiotic effects of isothiocyanates released from brassicas plants it can, and should be extended to the other volatile bio-substances with the same qualities exuded from other plant species. Thiophene, a heterocyclic, sulphurous compound with strong antibiotic activity, is released by *Tagetes* spp. and can thus also be used for biofumigation properties by its roots system (Terblanche and De Villiers, 1998). A study showed clearly that *Tagetes patula* reduces pathogen inoculum and bacterial wilt severity on tobacco in field conditions, in South Africa (Terblanche, 2002). Plants belonging to the Alliacae family also contain molecules with biofumigating effects potentially applicable to crop protection. Degradation of *Allium* spp tissues releases sulphur volatiles compounds such as thiosulphinates and zwiebelanes which are converted into disulfides having antibiotic activities against pests and diseases (Arnault *et al*, 2004). Yu (1999) showed that a rotational cropping system using *Allium tuberosum* reduced *R. solanacearum* population in the soil and the severity of bacterial wilt on tomato in greenhouse conditions, in China.

Screening of different crops for resistance to bacterial wilt and for adaptability to tropical regions is underway. We are evaluating cruciferous plants (Fig.1) and others as Asteraceae (*Tagetes* spp.) and Alliacae plants (Fig.2) as rotational crops in combination with biofumigation to control bacterial wilt on solanaceous crops.

Fig.1: Crops of *Raphanus sativus* in Martinique field conditions.

Fig. 2: Crop of *Allium fistulosum* in Martinique field conditions.

For a rotational/biofumigant plant to be effective in disease control it is generally desirable that it is not host to the targeted pathogen so that a decline in population or inoculum occurs during its growth. Studies underway at PRAM (CIRAD, Martinique) include the screening of potential rotational/biofumigant plants species for non hosting effect and rhizospheric suppression of *R. solanacearum* (Fig.3). That being the starting point to crop selection in view of a specific crop rotation system in combination with biofumigation.

Fig. 3: Screening of potential plant species for non hosting effect in greenhouse conditions

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1.6. Evaluation of biofumigation in the Netherlands (PPO)

From different contributions by researchers of Applied Plant Research gathered by Huub Schepers, Applied Plant Research, Lelystad, NL

Numerous experimentations have been conducted in The Netherlands, using different plants with the purpose of biofumigation again different soil-borne pests and diseases. An experimentation conducted between 2006 and 2008 compared 12 different crops: *Brassica juncea, Erica sativa, B. napus, B. campestris/B.napus, B. carinata, B. oleracea, Raphanus sativus, Sinapis alba, Sorghum bicolor, Tagetes patula, Lolium perenne, Crambe abyssinica*, to treatment with metam-sodium, seed meal (*B. carinata*), biological soil disinfestation (= incorporation of organic material: *Avena strigosa*) and bare soil (=black fallow) serving as a

control. The effects of these different treatments were tested on *Pratylenchus penetrans* and *Verticilium dahliae*, by measuring the increase or decrease of populations during the treatment and the consequences on a following potato crop (Korthals et al., 2008).

The results showed that most of the crops increased Pratylenchidae populations compared to black fallow at the exception of *Tagetes patula* for which almost no nematods larvae were detected at the end of the crop. The worst crops (= the ones that increased the most the Pratylenchidae populations were Sorghum, *B. carinata* and the mixture of *B. napus* and *B. campestris*. When evaluated just before seeding of the potato crop (9 months later) treatments which gave the best population decline over all, were Tagetes, Metamsodium and biological soil disinfestation.,

As far as *V. dahliae* is concerned only *Raphanus sativus* seemed to favour the fungus during the crop, seed meal giving a good control while other treatments did not differ from black fallow.

Following these treatments, potato yield was increased after *T. patula*, the association of *B. napus* and *B. campestris, B. carinata, R. sativus, and Sorghum* although these last ones had a positive effect either on *V. dahliae* or *Pratylenchidae* (Korthals et al., 2008).

Thus biofumigation crops may increase yield although the direct effect on pathogenic populations might be nil or even favourable. Mechanisms are thus more complex than initially thought and effects on other microbial populations than might exert and indirect control of pathogens has to be looked at.

Others studies conducted by Huub Schepers to evaluate biofumigation with TerraProtect (a blend of *Brassica juncea*, *Raphanus sativa* and *Sinapis alba*) against black rot on carrots (caused by different soilborne fungi such as Alternaria, Chalaropsis, Thielaviopsis, Mycocentrospora and Acrotecium) gave inconsistent results from reduction of the disease in one experiment, increase in another and no effect in three of them.

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1.7. Conclusions

Soil borne pests and pathogens remain difficult to control when chemical soil disinfectants are not available.

Soil steaming can be an efficient alternative in terms of control but may be slow to perform and costly in energy. Applying the technique only on the row with soil cultivation between rows during the crop can be a good alternative as far as weeds are concerned and possibly for most soil borne diseases, especially for transplanted crops.

Biofumigation, although showing incomplete and sometimes inconsistent control, has still to be regarded as a promising option to control soil borne pests and diseases and probably also weeds. The variability observed on the results is probably partly due to the brassicacaes that were used and the specific glucosinolates they contain (and ITCs they can release) regarding the sensitivity of the pests and diseases targeted. Most of the work done so far on biofumigation was applied research with big expectations on ready to use technology, whilst there has been a tremendous theoretical work done on the genetics of glucosinolates production thanks to Arabidopsis. So much is known today on the diversity of glucosinolates produced by Brassicacae, their kinetics, the tissues where they are produced, the stages at which they accumulate, and the genes that are responsible for production and regulation of production. This should diffuse towards applied research in order to optimize

intrinsic production of glucosinolates and release of ITCs during the crop growing and after crushing and incorporation. The indirect control through changes in the soil microflora has also to be more documented on an epidemiological perspective.

2. Sharing of experience and main achievements on landscape management to improve conservative biological control

2.1. Introduction

Vegetable crops with only 2% of the EU-27 arable land (eurostat, 2005) compared to 56% dedicated to cereals, are considered as minor crops although they represent 8.9% of the overall output value (*vs* 9.6% for cereals). This means that there are fewer and fewer pesticide options for controlling pests, diseases, weeds in these systems as there is a to low economic interest for the pesticide industry applying for the approval of new pesticides. Vegetable growers have thus to adapt and are looking forward to methods, other than pesticides, which can efficiently control (or prevent from) pests, weeds and diseases. Biological control is often considered as a possible alternative to pesticide use is rarely used as a major and deliberate component of pest management.

Ehler (1998)[1] discuss two forms of biological control, (i) importation of exotic enemies against either exotic or native pests (i.e. classical biological control), (ii) conservation and augmentation of natural enemies that are already in place or are readily available. Conservation covers actions that preserve or protect natural enemies (compatible treatments like use of selective or non-persistent pesticides), augmentation actions that increase the population of natural enemies (e.g. inoculative or inundative releases). For some authors like Barbosa (1998)[2], conservation biological control involves the use of tactics and approaches based on the manipulation of the environment (i.e. the habitat) of natural enemies so as to enhance their survival, and/or physiological and behavioural performance, and resulting in enhanced effectiveness. In general, habitat manipulations may entail the elimination or mitigation of detrimental conditions, or the enhancement or induction of favourable factors that are lacking in the habitat, or present at inadequate levels.

As more “classical” methods of biological control are studied elsewhere in ENDURE (RA4.3, the exploitation of natural biological processes), the objective of Task d is to report past experiences and current research activities on landscape and field margin management to improve conservative biological control

2.2. Field margins management to improve conservative biological control of fruit flies on vegetable crops (Reunion Island, CIRAD)

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Context

In Reunion Island, many pests and diseases threaten the sustainability of the horticultural cropping systems, forcing farmers to resort to synthetic pesticides, with all the risks that this entails for human health and the environment. Fruit flies (Diptera: Tephritidae)

[1] Ehler, L.E., 1998. Conservation biological control. In Conservation Biological Control (Barbosa Ed). Academic Press, pp. 39-54. San Diego, CA.
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figure among the main pests for solanaceous crops (in particular field-grown tomato, *Lycopersicum esculentum* Mill.) and cucurbits: cucumber (*Cucumis sativus* L.), zucchini (*Cucurbita pepo* L.), melon (*Cucumis melo* L.), etc. Losses of as much as 80 % of tomato and 100 % of cucurbit crop harvests have been frequently observed. According to farmers, fruit flies damage to vegetable crops have become significant from the 1990s. Four fruit fly species belonging to the Tephritidae family cause major damage to vegetable crops in Reunion: *Bactrocera cucurbitae* (Coquillet), *Dacus ciliatus* Loew and *D. demmerezi* (Bezzi) on Cucurbitaceae, and *Neoceratitis cyanescens* (Bezzi) on Solanaceae (primarily the tomato).

The use of chemical insecticides, which is the base of most control programmes for vegetable crops, does not provide any sustainable solutions, as it poses environmental and human health risks, whether for users or consumers. Work relating to the biology and behaviour of these species has been conducted in the past few years by Cirad in order to develop new agroecological control strategies suited to local conditions.

The approach initiated in Reunion is consistent with the agro-ecological approach, the conceptual bases of which are recapped by Deguine *et al.* (2009). It is also completely consistent with the guidelines adopted in certain countries for vegetable fruit fly control based on a large-scale management approach (Area-Wide Pest Management). Fly population control need to be implemented at production area level, with collective and coordinated resources, to promote a new agro-ecological balance centred on the role of functional plants, habitat management and development of natural beneficials, on a landscape scale.

Monitoring and basic knowledge on life history traits

Among the research actions to be developed, those relating to acquisition of knowledge of fly bio-ecology are the first to intensify. While *B. cucurbitae* is a fly whose biology has been well studied, the same does not apply to the three other species found in Reunion (*D. ciliatus*, *D. demmerezi* and *N. cyanescens*). The aim of the studies is to establish the main bio-ecological characteristics of these flies, and they cover the circadian rhythm, particularly the flies' movements between cultivated plants (Cucurbitaceae, Solanaceae) or uncultivated plants (primarily borders).

The first results are the following. In chayot systems, populations of flies roost in the crop although they roost on border plants in other cucurbit systems (zucchini, cucumber, …). Circadian rhythms have been observed in these agroecosystems. Movements between borders and crop are also observed during the day: roosting is the main activity of the adults. Leks and mating occur in the border plants and females go and lay eggs on the fruits of the crop at certain hours of the day according to the species.

Techniques that can be applied at the field scale, including margins

These agroecological techniques are based on three pillars: sanitation, habitat manipulation, biological control.

1. Sanitation

Unsellable fruits, especially those housing larvae, left on the ground or on plants, are a major "inoculum". Elimination of these fruits must be systematic so as to reduce subsequent infestations. It is also advisable to quickly destroy crop residues after the last harvest.

In Reunion, an original technique of sanitation to help controlling tephritid fruit flies using a tent-like structure called augmentorium has been implemented (figure 1). It aims to sequester the adult flies emerging from infested fruit while allowing the escape of parasitoids.

In addition, it is possible to produce compost in the augmentorium in a sustainable reasoning of agroecosystem management. This technique has already been successfully tested in Hawaii (Klungness *et al*., 2005).

To be effective, this technique has to be used at a large scale both in terms of time (several months or years) and space (exploitation, landscape) with concerted practices by the farmers, who should use this sanitation technique together. Even if the augmentorium is one of the components of the agroecological protection against fruit flies, it may play a central role in terms of Conservative Biological Control of these pests.

Figure 1. Augmentorium implemented in Reunion Island

2. Assisted push-pull

This technique mixes two methods:

- "push-pull", with trap crops, such as corn, where the populations roost during the day (figure 2);
- "attract & kill", using an attractive and adulticide product, applied on some small plots and which attracts (with protein bait) and kills (with a biopesticide) fruit flies.

It is a technique based on the role of margins or hedgerows of the fields. Furthermore, the populations of beneficial insects are enhanced by the choice of the trap crop which can be attractive for them.

Trials are currently conducted on this technique, the modalities of planting trap plants in the agroecosystem (border, patch, band), the modalities of using the product against the fly populations.

Figure 2. Border plants (corn) around a zucchini crop

3. Male Anihilation Technique

A few chemical substances, known as parapheromones, have the property of strongly attracting the males of certain fruit fly species in the same way as sexual pheromones. Consequently, these products can be used in trapping systems. Several types of trap, based on “Tephritrap®” used in fruit orchards, have been tested locally (figure 3). Only two vegetable fruit fly species present in Reunion, *B. cucurbitae* and *D. demmerezi*, are attracted by a known parapheromone, the Cuelure.

Figure 3. Parapheromonal trap for M.A.T.

Conclusion

The chemical control against vegetable fruit flies in reunion has proven to be greatly insufficient in limiting damage to the crops concerned. The absence of parapheromones for all species, genuinely effective food attractants which are approved and have sufficiently long-lasting action, the existence of short crop cycles repeated all year round without no-host plant zones, the ability of the flies to live for several months and move around easily, the presence of high population levels probably resulting from an imbalance, the presence of scattered plots and the difficulty in implementing preventive measures may explain the failures observed up till now.

In the future, fly population control should be implemented at production area level, with collective and coordinated resources, to promote a new agro-ecological balance centred on the role of functional plants, habitat management and development of natural beneficials, on a landscape scale. Other alternative methods (MAT, BAT, etc.) may be used as complements. These environmentally friendly techniques can therefore be included under Organic Agriculture and IPM approaches.

Research studies will focus on bioecological aspects of the fly species and also on natural enemies, both on predators (ants, ground beetles, rove beetles) and on parasitoids (including *Psyttalia fletcheri*). Tri-trophic interactions (pests, natural enemies, plants) will be a key object for study in this approach. A work programme is being initiated to analyse via micro-satellite markers the genetic variability of vegetable fruit fly populations, by the following parameters: host plants (wild *vs.* cultivated), altitude gradient (0 to 1200 m) and seasonal temperatures (winter-summer). This work will enable to specify the importance of

reservoir plants and shelter plants in the reproduction of fruit flies, and gain a better knowledge of dispersal, seasonal migration and practical details of the winter season transition. Finally, the implementation of different agroecological techniques and their aggregation for farmer uses will be carried out.

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2.3. The use of insectary plants in lettuce crops to enhance key natural enemies of aphids and thrips in Catalonia, north-east of Spain (UdL/IRTA)

By Judit Arnó, Rosa Gabarra, Oscar Alomar, IRTA

Mediterranean vegetable growing areas are landscapes characterised by the coexistence of several annual crops, grown on rather small farms (2-3 ha), and with a variety of species grown simultaneously all year round (e.g. lettuce, tomato, potato, brassica crops, cucurbits, etc). Greenhouses tend to be only partially sealed, and the boundaries between greenhouses and field crops often become blurred. Because of asynchronous transplantation times, there may also be overlapping fields of the same crop. As many vegetables share the same pests (e.g. whitefly and thrips) problems are exacerbated as there is a continuous carry-over of pests throughout the year that is hardly interrupted, even in winter. But the heterogeneity in the landscape may also provide abundant refuges and sources for entomophagous thus favouring natural biological control in crops (Albajes and Alomar, 1999; Alomar et al., 2002; Gabarra et al., 2004; Castañe et al, 2004).

Vegetable production is highly intensive, with up to three crops being produced on the same piece of land each year. There is also periodic destruction of non-crop vegetation along field margins (e.g. as part of cultural practices to reduce pest infestations). The discontinuous nature of such ephemeral habitats makes the establishment of natural enemies more difficult than in more permanent habitats and natural enemies must re-colonize the fields each time (Gabarra et al., 2004). The creation of ecological infrastructures to provide required resources for natural enemies may be a viable strategy to enhance biological control in these agroecosystems.

Here we present a summary of our work related to the use of insectary plants in lettuce crops to enhance key natural enemies of aphids (*Nasonovia ribisnigri*) and thrips (*Frankliniella occidentalis*), two of its major pests. Three steps were undertaken to cope the objective of our work: (1) determine the key natural enemies that spontaneously colonize non sprayed lettuce crops; (2) identify plant species that may contribute to act as a refugee for these key natural enemies providing them with food and/or ovipositing sites; and (3) propose and evaluate the utility of a margin or flower strips in the biological control of *N. ribisnigri* and *F. occidentalis.* Part of this work is described in Alomar et al. 2006 and Alomar et al. 2008.

1. Identification of key natural enemies was conducted during by sampling experimental lettuce plots and commercial lettuce fields along Catalonia (NE of Spain).

Number of natural enemies were counted and identified. Predatory larvae were reared into adults for further identification.

Hoverflies and *Orius* were identified as the most abundant natural enemies present in lettuce crops. Hoverflies are especially abundant in winter and early spring when the highest aphid populations are recorded. *Orius* are the predominant group during summer.

2. Identification of candidate plants was done in two separate fields at our research institute in Cabrils (Catalonia, NE of Spain). Native or naturalized plant species were selected from those mentioned in the bibliography as of interest for *Orius* and/or hoverflies and transplanted in 2.25 m^2 plots in a complete randomized-block design with 3 replications. Natural enemies and pests were sampled periodically and data on their abundance were used to select plants for an ecological infrastructure.

Many of the 25 plant species tested had abundant *Orius* populations, and many of them also had nymphs, indicating that adult *Orius* did not only profit from plant and prey resources, but also reproduced. However, *Orius* were quite late in appearing on some of the plants (e.g. *Ocimum basilicum, Thymbra capitata*), possibly because of their late flowering. When the plants selected were restricted to those bearing *Orius* between February and mid-May, only 5 species were selected, and when not only *Orius* abundance but also the risk of exacerbating pest and disease problems in the crop were considered, *Vicia sativa* and *Lupinus hispanicus* were selected as candidate plants to enhance *Orius abundance*.

Adults of predatory hoverflies were observed on most of the 32 plant species in the field set. However, on less plant species adults were observed resting or feeding on flowers. For hoverflies, the plants with most visits (e.g. *Diplotaxis arvensis*) also had very abundant thrips populations, or were arbustive and slow growing (e.g. *Cistus* spp.), and therefore, *Centaurea cyanus* and *Lobularia maritima* were selected.

3. Evaluation of the utility of a margin or flower strips in the biological control of lettuce pests. Based on the results presented above, we defined a preliminary mixture of 4 plant species (*Lobularia maritima*, *Centaurea cyanus*, *V. sativa*, and *L. hispanicus)*, that we compared to a single-species margin of *Lobularia*. Two controls were included in the experiment: (1) a pesticide control according to conventional practices; and (2) a natural control, without pesticides. The experiment was conducted in a 3600 m^2 field. Insectary plots were set in the middle of lettuce plots in a complete randomized-block design with 4 replications of each of 4 treatments. The field was planted twice, in spring and summer. For the summer crop, we kept the same lay-out of the treatments to keep the insectaries *in situ*, but replaced both legume plants with *O. basilicum* and *Achillea millefolium* two weeks before transplanting the new crop. Each week, predator populations were sampled in the insectary patches and, lettuces from each plot were destructively sampled in the laboratory to count the number of aphids, thrips, other major pests (e.g. Lepidoptera), and predators. Lettuce sentinel plants infested with *N. ribisnigri* were used to monitor hoverflies movement/dispersion in the field.

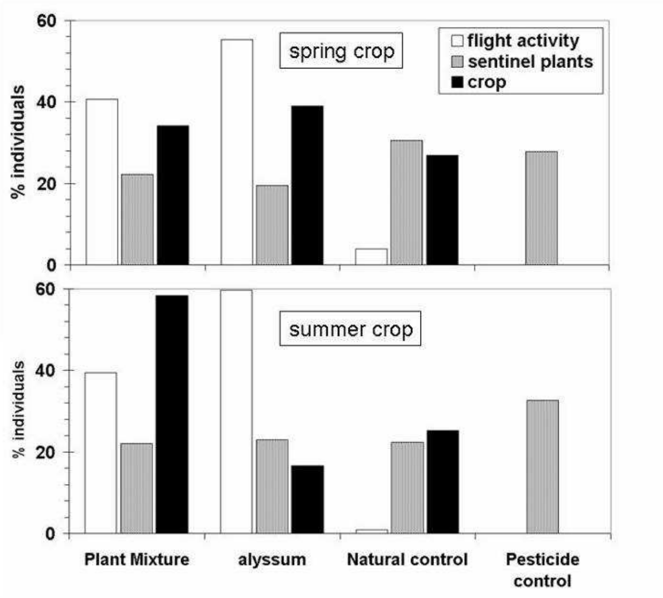

Figure 1. Relative percentage distribution of all recorded hoverflies in each of four treatments during the spring and summer lettuce crops: (a) flight activity in the centre of the plots (insectary plant patches or equivalent lettuce plants); (b) oviposition on sentinel plants; (c) eggs and larvae established in the crop. Figure from Alomar et al. 2008.

The visual observation on insectary patches or equivalent central lettuce plants confirms that adult hoverflies were active in the field, being attracted by the flowers (figure 1). No adults were seen in the no-insectary plots. However, hoverflies did locate and oviposit on sentinel plants across all four treatments, thus indicating that adults did disperse all over the field. In the spring crop, hoverfly larvae and eggs were recovered from lettuces in both insectary treatments and natural control plots but, no from the pesticide control plots. As a result of hoverfly establishment in the no-pesticide plots, aphids were controlled from average peak levels of aprox. 200 down to 5 aphids/plant in 2 weeks and to the same level as in the pesticide plots. No remains of hoverfly larvae were present in marketable lettuce heads. In the summer crop, very few hoverflies established in the no-pesticide plots, probably due to low aphid levels.

Orius were recovered from insectary plants from mid-may onwards. *Lobularia* harboured higher *Orius* populations in the plant mixture, and did maintain *Orius* during the crop-free period. During the summer lettuce crop, *Orius* did build-up slowly in the insectary plants but did specially increase in the newly transplanted basil that harboured almost three times as many *Orius* as *Lobularia*. In the spring crop, very few *Orius* established in lettuce plants, but in the summer crop, *Orius* nymphs were already established on lettuces by the 2nd week after transplant, without significant differences among the 3 no-pesticide treatments. Thrips maintained at similar levels in both crops (up to approx. 6 thrips/plant), and the same level of thrips control was obtained in the pesticide than in the non-pesticide treatments. *Orius* also preys *N. ribisnigri*, and its establishment in the lettuce may also have contributed to the control of aphids.

Since no a clear advantage was observed in using a mixture of plants vs. a single species marginflower margins of *Lobularia* were established on one side of 5 commercial fields selected along Catalonia (NE of Spain), and 2 treatments plots were established in each field: (1) the BIO-plot close to the flower-margin with no insecticides applied, and (2) the CHEM-plot where insecticides where applied according to pest advisors/farmers criteria. To evaluate pest and natural enemy abundance in lettuce, lettuce heads were destructively sampled in the laboratory. Adult hoverflies activity was recorded by visual observations in the flower-margin and the crop. To assess the population abundance of key pests and predators

in the *Lobularia* margins we shook parts of the plant parts over a white pan and counted the number of major predator groups (*Orius*, other Heteroptera, etc), and thrips. Insects were returned to the flower patch. Voucher specimens were kept to confirm identification.

Aphids did establish in all fields, although with variable degree of infestation and were controlled by the chemical treatments. In the non sprayed BIO-plots, closer to the flower-margin, the number of aphids was initially higher than in the chemical treatment, but aphids were also controlled, and to the same level as in the chemical treatment. The use of pesticides (CHEM-plot) did not allow the establishment of predators in the lettuce crop. On the other hand, avoidance of chemicals allowed the establishment of hoverflies, and other predatory groups (e.g. *Orius* spp.) that ensured the biological control of the lettuce aphid. The flight activity of adult hoverflies was clearly concentrated close to the field margin, but adults did also fly to the centre of the field. This result indicates that adult hoverflies would be attracted to the companion plants, but also disperse to the field, thus demonstrating the importance of environmental diversification in order to ensure the presence of predators inside the crop.

Conclusions

The results confirm that biological control shows great potential for aphid and thrips control in spring and summer lettuce, and providing plant resources in the field ensured the presence of key predators: adult hoverflies were attracted and aggregated on flower patches, and predatory bugs did establish in the insectary plants. Being highly mobile, both predators did disperse to plots without flower patches and established on lettuce plants unless limited by pesticide treatments. As a result of predator establishment, prey populations were reduced below the economic threshold. Moreover, no other pests were recorded from the lettuce crops as a result of adding the insectary plants.

As mentioned above, Mediterranean vegetable growing areas are complex landscapes characterised by the coexistence of several annual crops in rather small fields. Such ephemeral habitats make the permanent establishment of natural enemies more difficult than in more stable crops, and beneficials must re-colonize the fields each time. Under such circumstances, the establishment of predators in a crop should not only be seen as a net benefit for a given field, but also as a net contribution to the enhancement of predator populations that will benefit other neighbouring crops (e.g. cucurbits) within the agricultural mosaic. Moreover, many of those predators are polyphagous, and will contribute to control other pests (e.g. Arnó et al., 2008).

However, the abundance and diversity of natural enemies can vary according to the composition of the surrounding landscape, and it is necessary to confirm that the presence of insectary plant margins in simple landscapes is also sufficient to ensure biological control of major lettuce pests.

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2.4. Landscape and functional agro-biodiversity a region of the Netherlands (PPO)

From a work done by Paul van Rijn[1], Eefje den Belder[2], Janneke Elderson[2], Marian Vlaswinkel[3], Frans van Alebeek[3]

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See references of the original papers at the end of this chapter for more complete details, summarized by P. Lucas

In the Netherlands, a study was conducted at the regional scale on initiative of the Dutch farmers organization (LTO) and two ministries responsible for agriculture and environment. The aim was to increase biological diversity, to enhance natural enemies of pests, and ultimately to reduce the use of pesticides. The so-called Functional Agro-Biodiversity (FAB) pilot started in 2004 on four arable farms. To enhance functional biodiversity, the waterboard and other local institutions were asked to improve the quality and management of semi-natural vegetations on dykes, ditches, road sides and hedgerows. At the field level, project farmers created field margin strips with perennial grasses and/or an annual flower mixture. Moreover farmers were advised to restrain the use of insecticides and to preferentially use selective insecticides that have little effect on the main natural enemies. The impact of these measures on natural enemies and pests were studied during 2005, 2006 and 2007 in wheat, potato and Brussels sprouts (van Rijn et al., 2008a). On Brussels sprouts the main pests species in this area were mainly aphids and whiteflies (*Brevicoryne brassicae, Myzus persicae, Aleyrodes proletella*), moths (*Plutella xylostella, Mamestra brassicae*), and (root) flies (*Delia radicum*). In some years, thrips and slugs may also cause significant crop losses. To control these pests (as far as possible) the farmers used to treat the plants with Imidacloprid before planting, and spray the fields 8 or 9 times a year with other insecticides (e.g. Lambda-cyhalothrin and Dimethoate). Within the framework of FAB trap cropping and enhancing biological control of pests have been addressed, considering their potential impact on pesticide reduction.

Trap crops were used on margins of the field in order to prevent pests from entering the field. For diamondback moth several plant species were tested: yellow rocket and Indian mustard. Previous studies have shown that these two plants are preferred as oviposition

substrate over cultivated cabbage. Yellow rocket has the additional advantage of being unsuitable as host plant for the larvae. Unfortunately, Indian mustard appeared to be unsuitable due to its short life cycle compared to the growing season of Brussels sprouts and yellow rocket remains low during the first year and did not flower before the second year. This indicates that biological characteristics with regards to insects behaviour are not the only factors to take into account but also agronomic factors.

For cabbage whitefly (*A. proletella*) among the 10 plant species tested by cropping two 9 m^2 plots adjacent to the an experimental sprout field, Chinese cabbage, *B. campestris* var. *chinensis,* attracted some whiteflies, but only young kale plants, *B. oleracea* var. *acephala*, attracted many whiteflies. The latter plant may therefore be an effective trap crop, especially since killing off the pest on this host plant appeared to be feasible: Treating the plants with an experimental insecticide against cabbage whitefly killed 95% of the eggs.

Conservation biological control consisted in a 3 meter wide annual flower strip sown adjacent to each target field surveyed. Parasitoids are important natural enemies of cabbage moths and butterflies, whereas (cabbage) aphids are also attacked by the larvae of hoverflies, lacewings and gall midges. As these natural enemies solely feed on sugar sources or pollen during their adult stage, the flower species were selected for their suitability in providing (floral) food for the natural enemies and the low risk of supporting pests. The mixture included Buckwheat, Borage, Common Vetch, Coriander, Fennel, Cornflower, and Corn Marigold. To match the flowering period with the long growing period of Brussels sprouts the mixture was sown in May and supplemented with short Sunflowers. The fields were sampled every 3 weeks in a fixed grid at various distances from the edge for recording the pests and their natural enemies (if possible) per species and life stage. The main results were quickly communicated with the farmers, to help them with pest management decisions. In addition to commercial fields, small experimental sprouts field with field margins have been created where no chemical insecticides were applied after planting, in order to evaluate the impact of natural enemies.

In the experimental fields the numbers of natural enemies (especially hoverflies, gall midges and parasitoids) were much higher than in the commercial fields as growers used insecticides in those fields. By the end of August, however, their numbers declined rapidly each year. In the experimental fields cabbage aphid (*B. brassicae*) levels remained low or at least stable during June, July and August, but increased exponentially by early September, causing economic damage to the sprouts by November. The resurgence of the cabbage aphids in September when natural enemies, such as hoverflies, virtually disappear, suggests that natural enemies can play an important role in keeping the aphids under control during summer, although not always at a sufficiently low level.

Of all caterpillars, those from diamondback moth were the most numerous, especially in 2006 (when no experimental field was available) and 2007. In all years and months about 50% of the pupae appeared to be parasitized (mainly by *Diadegma semiclausum*). The experimental field was treated an few times with a *Bt* product, when the infection tended to surpass the action threshold. Ultimately no economic damage from this species to the sprouts has been observed.

Discussion

When using Functional Agro Biodiversity as a means for pest control one can consider not only methods to augment natural enemies, but also methods to diminish pests directly. The reduction of pest refuges that can act as sources of reinfestation is one example at the landscape scale. Trap cropping is another example at the field level.

Implementation of the first method may be difficult as it requires concerted action of various growers in the region. The second method may be applied only after some technical issues have been solved. This require serious studies on *e.g.* (1) the attractiveness of trap plants relative to the crop at different stages of development, (2) the level of pest reduction within the crop that can be obtained and (3) the type of pest management needed to prevent secondary spread of the pest.

The conservation of natural enemies can only be effective when pesticides that are harmful for natural enemies are not or only incidentally applied. In a crop such as Brussels sprouts, where many pests have to be controlled at the same time, this is a challenging task.

When measures at the landscape and farm level to support natural enemies and diminish pest pressure, are effective for some pests only, we may consider the efficient production and release of natural enemies against other pests.

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2.5. Chemical ecology, conservation biological control and field margins management in France (INRA)

From a presentation by Anne Le Ralec and work from Anne-Marie Cortsero's group (UMR BiO3P, INRA-Agrocampus Ouest-Université de Rennes 1), summarized by P. Lucas

Landscape ecology

Researches on insects that affect vegetable crops from the Brassica family and on their natural enemies, with a special focus on parasitoid insects, are conducted at UMR BiO3P in Brittany (France). Several programs are developed in the group in areas such as genomics, chemical ecology, behavioural ecology or plant - insect relationships …

So far studies have been conducted mainly at the individual insect-parasitoïd-plant interaction or at the crop scale but new projects are dealing with landscape ecology and applications to pest control in field vegetables is underway.

One of these program concerns vegetables of the Brassica family, such as cauliflower, which is a crop of great economic importance in Brittany, but also carrots.

These crops are exposed to several phytophagous insects but it was decided to focus our study on flies, such as *Delia radicum* and *Psylla rosae* and the cabbage (*Brevicoryne brassicae*) aphid for several reasons.

These species were chosen because of their situation towards pesticides. There will be no more available insecticides against the vegetable flies at the end of the present year. These insects are important pests and it is quite impossible to produce cabbages, turnips or carrots during the flies periods of activity, without chemical treatments. The available alternative methods, such as protecting nets, are too expensive and not always effective.

On the other hand, many treatments directed against aphids are probably not necessary, as these insects are prone to be controlled by numerous natural enemies. But there is a need for demonstrating this natural regulation process to convince growers not to do these treatments.

Another reason for focusing on these two groups of phytophagous insects is that they appear to have some interesting differences in their population dynamics.

For example, *D. radicum* has 3 annual generations and 3 flying periods. This species is attacked by several natural enemies. However, they generally do not maintain the fly populations under the economic threshold. Finally, some preliminary results indicate that this pest could be favoured by hedges around fields. These structures could create favourable climatic conditions leading to precocious infestation and development of the fly.

Conversely, *B. brassicae* has many generations per year, depending on temperature. In Brittany, it reproduces all year long by parthenogenesis. But it is attacked by a great number of natural enemies, like parasitoids, predators (ladybirds), Syrphids, … and also by entomopathogenous fungi. In this case, hedges could have a negative impact on aphid populations by acting as a source for these natural enemies.

The results obtained on these two insect pests should then be generalized to other species sharing the same population dynamics traits and could lead to the description of insect pest types based on these traits and common management schemes.

The main objective of the program is to search for relationships between landscape structure and composition and the damages caused by pest insects in vegetable crops. The first step is to describe the landscape structure in areas showing various degrees of structural complexity. Then, will be investigated whether correlations between these features, insect populations and/or damages in the fields exist. Finally, the mechanisms implied in the observed variations to propose methods of landscape management to limit the crop damages will be analyzed.

The program began year 2008 and so far, only description of the surveyed zones has been done. Two sub-units of 7 and 12 km^2 respectively have been selected in an area of cauliflower production in north Brittany. The landscape in this region is characterized by the presence of banks around the crop plots.

Figure 1: Banks and vegetation around plots in the surveyed areas in Northern Brittany.

These banks are covered by diverse herbaceous species or often carry hedges as shown on Figure 1.

Using official digital maps (Institut Géographique National) and aerial photographs, the landscape structure has been described in the 2 sub-units. These two sites are very close one to the other, in the same climatic area, with similar agricultural practices and equivalent size of plots (around 1 ha or less), but differing in the density of hedgerows. The density of banks and hedges is much higher in the Saint-Adrien area than in the Ploubazlanec area.

Following this description, impact of these structures on the colonization of the plots by the cabbage fly and the cabbage aphids and on their population dynamics inside the plots is underway. The same landscape characterization is under progress for other areas of field vegetable production in western France in order to have broader references.

The monitoring of colonization by the phytophagous insects and insects enemies of several plots in each site is currently being done as well as the evaluation of the level of

damages in each plot. Correlations will then be sought between these data and various landscape features such as length and floristic composition of the banks and the hedges, the forest density, density of other crops and grassland, etc. This will be done at different scales, using concentric buffers, because it has been shown that landscape features could act differently on insect populations at a small or a large scale.

It is expected that mechanisms which explain differences in insect colonisation or density will be identified, in order to figure the landscape traits which can be manipulated to limit insect damages. One main point is to assess the positive impact of some types of hedges on plot colonization by the adult flies. Another is to identify the sources of the insects which colonize the field. As a matter of fact, almost nothing is known about the distance of migration of the emerging flies of the 3 successive generations. Such knowledge could lead to targeted destructive methods of the overwintering forms of the flies.

Chemical ecology

Interactions among organisms are under the strong influence of chemical compounds. In insect-plant interactions, volatile chemical plays a key role in foraging activities. These volatiles, originating either from conspecifics, preys, or their habitat are often used by foragers to make useful decisions for finding resources or avoiding danger (Bell and Cardé, 1984). The importance of these infochemicals in insect-insect and plant-insect interactions led to the emergence of new pests management practices.

Delia radicum is a major pest of cabbage crops in northern Europe. Due to more constraining laws relating to insecticide use, new strategies to control this pest are urgently needed. Manipulating insect behavior through infochemicals is a promising approach. The recent identification of dimethyl disulfide (DMDS) as a compound that both attracts the main predators of *D. radicum* and inhibits oviposition by the fly gives a challenging opportunity to develop such strategy. A study was performed to confirm such potential of DMDS, in the field (Ferry et al., 2009). Through the 8 weeks of the first egg laying peak of the fly, the potential of artificially increasing the levels of this molecule in the close vicinity of broccoli plants was assessed on 1/attraction of predators, 2/stimulatation of predatory activity and 3/reduction of damage done by the fly. Despite a lower number of *D. radicum* eggs as food resource, DMDS effectively increased predator catches in treated plots (119 *Aleochara bilineata* caught in treated plot, while only 21 in control plots). However, damages done by the fly were of the same magnitude order in treated plots than in control ones. Number of *D. radicum* larvae and pupae recovered in plant roots were similar, despite the important decrease in eggs laid. This result, together with the observation that the numbers of eggs predated in artificial patches were lowered in the presence of the molecule, seems to indicate that increasing DMDS amounts disturbed the foraging activity of the fly predators. It is possible that the large amount of the molecule present in treated plots might have hidden other volatiles cues to the predators, thereby diminishing their efficiency in finding eggs. Another possibility would be that DMDS presence acted as a diversionary cue. DMDS abundance in treated plots could indicate the presence of abundant resources that were actually not present. Predators might have been confused by this signal, spending time searching for high quality patches that did not exist, rather than predating existing ones (Ferry et al., 2009).

Plant breeding for biological control ?

For more details see Cortesero et al., 2000 (complete reference below)

Plant breeding and biological control have mostly been parallel but independent pest management practices in the past. While plant breeders have almost exclusively focused on selecting varieties with enhanced direct defenses against pests, biological control workers have mainly concentrated on improving natural enemy traits, such as reproduction and hostfinding efficacy. The authors of this review feel that there is an urgent need for bridging these two pest management practices and to emphasize the importance of managing plant attributes from a tritrophic perspective. There are numerous attributes through which a plant

can affect insect pests and insects enemies. Plants provide shelters, they mediate host/prey accessibility (morphological traits such as waxy surface, foliar pubescence, …), they provide host/prey finding cues (visual, chemical), they influence host/prey suitability (toxic allelochemicals, nutritious quality) and provide supplemental food resources (e.g. nectar).

There is thus potential in manipulating morphological traits as well as physiological traits of plants, in order not only to escape from pests but to favour natural enemies. This is certainly a big challenge in crop protection against insects for the coming years

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2.6. Conclusion

Numerous options are given here as alternative to the use of pesticides in commercial crops, from selected use of pesticides on margins only where pests are attracted, inundative biological control, and/or conservative biological control. One important point which comes out of this study is that there is not a unique recipe ad that efficient solutions will come from local analysis of the problem to control as well as the potential offered by the production situation. This means that local adaptation will be needed to determine which plants to grow as insectary plants, which insect enemies to promote, for example. Nevertheless there is a need for research on landscape and functional ecology to propose a framework in which such local studies will be thought and developed. There is also a need in more research on chemical ecology, as it offers the opportunity of developing chemistry with new modes of action allowing regulation of plants – pests – pests enemies interactions.

Conclusion

As described in a previous work from the Field Vegetable Case Study[3], vegetables are subject as other crops to phasing out of pesticides, but without a renewal with new active ingredients as they do not offer a significant market for the pesticide industry to seek registration. Thus, vegetables are really concerned with minor uses and with the challenge of proposing alternative methods of control for weeds, pests and diseases.

Among the most urgent problems are soil-borne pests and diseases and weeds with the interdiction of most of the soil disinfectants and insects with the concern about some insecticides, especially neurotoxics.

For soil-borne pests, diseases and weeds, soil disinfection through steaming might be an alternative, especially band steaming which reduce costs (and consumption of fossil energy) and time compared to broad soil steaming. This should be sufficient to protect plants from diseases and pests and can be completed by inter-row hoeing. New progress in machinery technology could even reduce costs and improve efficiency further on. Biological activation after soil steaming must be an objective for research as this offers the opportunity for re-colonization of the disinfected soil with useful microorganisms like biocontrol agents for example.

Biofumigation gains a renewed interest with researches intending to understand the mechanisms of action. They are complex and acts through different processes: during the biofumigant crop (equivalent of a break crop), during the crushing and incorporation of residues (the *sensu stricto* biofumigant effect, with the flush of ITCs), and during the decomposition of the residues (green manure effect and changes in the soil microflora). Looking at these different modes of action in an epidemiological perspective should help defining criteria which make a crop a good fumigant one according to a set of soil-borne pests and/or diseases. This might even lead to plant breeding programs for plant used specifically as biofumigant crops (*sensu lato* as defined above).

For insects, a set of methods is proposed here, from field margins to landscape management. All rely on biological control or/and chemical ecology. Behind the general concepts, they have to be adapted to local situations. For example insectary plants must have adequate life cycles and provide shelter and resource for key natural enemies at the right periods to cover the crop length and ensure protection against the target pests. Push-pull strategies and insectarium, also need local adaptation, showing the importance of local experimental stations. Landscape management is more complex to establish but is a key point to create a background reducing pest development through enhancing of regulation mechanisms.

The concentration of vegetables crops in specific production basins should help interactions between growers for strategies at the needed regional landscape scale. Chemical ecology should gain more interest from scientists as semio-chemicals are a key component of recognition between insects and plants, insects and insects and probably will be at the center of new methods of biological and integrated control of insects.

[3] http://www.endure-network.eu//about_endure/all_the_news/vegetable_study_reveals_ppp_situation