LITERATURE REVIEW OF THE BIOCIDAL CROPS MUSTARD (*Brassica juncea*) AND WILD ROCKET (*Eruca sativa*)

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Growers' Summary

- plant-derived chemicals from biofumigant crops offer an additional, sustainable control tool to reduce pathogens and weeds
- a range of biocidal crops are being marketed in the UK, but none offer blanket control of all pathogens, including those of particular interest, *Brassica juncea ISCI 99* and *Eruca sativa NEMAT*
- The effectiveness of many biocidal crops is difficult to predict
- There has been no scientific assessment of the use of biofumigants in horticultural crops in the UK
- Field assessments are required to refine a biofumigation strategy
- Seeding rates are 10kg/ha for *ISCI 99* and 8kg/ha for *NEMAT.* Both will cost approximately £100 per ha. Establishment costs are estimated to be about £75 per ha.
- In the light of developing technology for formulations of biocidal plant products, growers need to consider the advantages of growing such crops solely as a green manure for soil structure or for crop cover for weed control
- The cost:benefit of using biocidal plants as pellets or as a meal will need to be assessed
- After many years research, biofumigants are now used routinely in the USA and parts of Europe

Introduction

In order to assess the potential of *Brassica juncea* (available in the UK as ISCI 99) and *Eruca sativa* (available in the UK as NEMAT), growers need to be aware of current research into the use of Brassicaceae crops generally as biofumigants. Many growers are familiar with manufactured products for soil sterilisation, used to reduce the incidence of pests (particularly plant-parasitic nematodes), diseases and weeds. The active ingredients in these commercial products include dazomet and metam-sodium, which both release methyl isothiocyanates. The isothiocyanates are a group of volatile compounds that are also produced when plant tissue is damaged and most researchers believe their role in nature is to provide protection against pests and pathogens. They are produced when plant cells are damaged and the glucosinolates they contain react with water and the enzyme myrosinase at neutral pH (also found in plant tissue). However, there are different types of glucosinolates which vary in their reactivity and the quantity of isothiocyanates released. *Brassicaceae* species contain high levels of these glucosinolates and so offer potential as biofumigants for a natural release of isothiocyanates when the plants are chopped and used as a green manure.

Whilst biofumigation is the term generally used to describe the exploitation of the myrosinase-glucosinolates system, it is also used to describe the use of other plant-derived chemicals for the control of pathogens. Nematode suppression has occurred following soil incorporation of cyanogenic sudangrass hybrids into soil before cropping with carrots (Widmer & Abawi, 1998), and has been shown to be correlated with the amount of free cyanide released into soil (Widmer & Abawi, 2002). Poultry manures also have potential to suppress nematodes whether through stimulation of antagonistic microbes (Kaplan *et al.,* 1992) or by production of ammonia (Rodriguez-Kabana, 1986). Lucerne soil amendments have also been reported to suppress nematodes (Mankau, 1968; Mankau & Minteer, 1962; Johnson *et al*., 1967) and have shown potential to reduce plant disease caused by soil-borne fungi (Asirifi *et al.,* 1994; Nam *et al.,* 1988; Okumura, 2000). Sulphur volatiles produced by *Allium* species also show good potential for pest and disease pathogens (Auger *et al.,* 2004). Amendments with high N contents are generally recognised as being more effective against nematodes than those with lower N contents (Mian & Rodriguez-Kabana, 1982).

Whilst biofumigation seems to be practical and involve little expenditure, efficacy is, in several cases, still far from that which can be obtained with the synthetic compound treatments (Lazzeri *et al.,* 2004). There is no 'blanket' activity against all pests, diseases and weeds by any biofumigant. For example, research in France into the effectiveness of a range of green manures against *Aphanomyces* root rot of pea has not, so far, found a successful candidate (Moussart *et al.,* 2004). Increased research and usage should highlight those crops which have consistently been effective against certain pests and diseases. However, whilst there has been some work on the identity of glucosinolates and their respective efficacy in Europe (Quinsac *et al.,* 2004; Sørensen *et al.,* 2004) the limited amount of research into the complicated reactions that occur in the soil during and after release of the isothiocyanates means that the effectiveness of many biocidal crops will remain difficult to predict.

The quality and quantity of glucosinolates present in cruciferous plants varies according to the genera, species, cultivar and their location in the plant, and thus gives different biofumigants different properties. This has given rise to blends being developed to provide maximum control for particular pest or disease situations. Trials have also been done to investigate the most glucosinolate-productive parts of these plants. For example, in-vitro work in Australia (Bianco *et al.,* 2001) illustrated how root material from a mixture of *Brassica napus* and *B. campestris* was more effective against *Rhizoctonia fragariae* than the shoots from this mixture, suggesting that it might be worthwhile macerating the whole plant, not just the foliage, when incorporating biofumigants into the soil. The mixture used in this in-vitro study also produced 8 times more and a greater variety of isothiocyanates than the use of *B. juncea,* a popular biofumigant crop, alone. However, this literature study has illustrated that results from many in-vitro tests are contradicted when assessed in field situations. It has taken many years' research in Washington State, U.S.A., to develop a blend of *Sinapis alba* and *Brassica juncea* to control major pathogens of potato, namely *Meloidogyne chitwoodi* (Columbia root-knot nematode) and *Verticillium dahliae* (potato early dying disease) and weeds (McGuire, 2004a).

Whilst there is increasing knowledge about the characteristics of individual biofumigants there are common traits to be evaluated when considering their use. For example, a key characteristic of the release of the isothiocyanates (either from the enzyme hydrolysis in biofumigants or manufactured chemicals) is that it occurs within a few hours. Indeed, research on biofumigant crops has estimated that degradation may be faster (20 minutes) or slightly slower (7 hours) but the rate of degradation seems to be related to the level and particular types of glucosinolates involved (Aires *et al.,* 2004). Persistence, or the length of time over which isothiocyanates are produced, might also be related to soil pH and moisture levels (Bianco *et al.,* 2001) as well as temperature.

Other factors to be considered, which also apply when using manufactured sterilants, include the production of a fine tilth of soil, a minimal presence of clods, prompt and efficient incorporation and a quick, efficient surface seal. In The Netherlands, where the use of biocidal plants in strawberry, asparagus and woody ornamentals is under investigation, the soil surface is lightly compacted and irrigated after the biofumigant or green manure crop has been incorporated and then covered with a plastic film for 6-10 weeks under warm conditions in the summer. The anaerobic conditions that develop form additional toxic fermentation products but details of the success in controlling pathogens and weeds are not available. In the case of biocidal plants, other factors, such as ensuring a good biomass is produced, and that the plants are not only finely chopped but pulverised and watered before incorporation, have been found to be key elements in achieving maximum isothiocyanate concentration in the soil (Matthiessen, 2004).

An important point that is often overlooked in green manure or biofumigant studies is whether the cultivation of the biocidal crop itself will serve only to increase levels of pathogens, compared to leaving the land fallow. Certainly, Walker (2004), using pot experiments to investigate the control of root-knot nematodes in carrots, found that some green manure cultivars of sorghum and rapeseed he tested resulted in higher densities of *M. javanica* in soil before planting compared to leaving soil fallow. There also appeared to be differences in the effect of particular green manure crops on different populations of the same species of nematode, thus illustrating the importance of selecting the right cultivar and recording the effects on nematodes at different sites. The susceptibility of some biofumigant cultivars to pest and disease infestation could be overcome by using the most promising candidates, such as lucerne, as a soil amendment in pellet form rather than as a green manure crop. Whilst this would increase costs, the reduced availability of other controls for pest and diseases could make their use a practical proposition. However, the loss of crop cover and biomass would reduce the influence of the crop for weed control and improving soil structure. Certainly the development and marketing of biocidal crops in pellet form is being considered by companies involved in their promotion. Thus whilst green manures may provide other benefits to the soil such as increased levels of organic matter, their use needs to be carefully considered.

Other negative effects of biocidal crops on subsequent cropping also need to be considered carefully. For example, a negative effect of the use of *Brassica napus* (or canola) on the growth of maize has sometimes been reported, and this has been attributed to an effect on vesicular-arbuscular mycorrhizal fungi, thus having a negative effect on uptake of low-mobile nutrients like phosphorous. Work by Pellerin *et al.,* (2004) however, failed to show that the biofumigant had a detrimental effect in this way. Recent research has shown different effects according to the biofumigant crop type on biocontrol agents such as *Trichoderma* spp., used against a range of plant diseases, so information regarding compatibility with such agents could be important (Galletti *et al.,* 2004). Walker (2004) found that carrot emergence was suppressed when amendments were applied 14 days before planting, but not if planting was delayed for at least four weeks.

Buried crop debris has been implicated as a contributory cause of fanging in carrots (Rubatzky *et al*., 1999) but innovations such as the use of pellets, because of their small size, will be less likely to cause problems. As fanging is one of the symptoms used to measure nematode damage (though it can also be a symptom of disease and herbicide damage) care must be taken not to underestimate the effect of biocidal crops in reducing nematode levels.

Whilst many of the potential benefits have been highlighted and are indeed promoted in the commercial literature, there remains a lack of research into the efficacy and consequences of biofumigation. There are risks associated with the development of enhanced degradation (where the continual use of the same fumigant results in a shift in the soil micro-flora to populations that can break down the fumigant so rapidly that the fumigant is not available to destroy the target organisms) (Warton *et al.,* 2001), potential impacts on associated beneficial organisms and the fate of isothiocyanates and other compounds in the environment (Kirkegaard & Matthiessen, 2004). In addition and in contrast to commercial pesticides, biocidal plant tissues also contain other chemicals and contribute large amounts of organic carbon that may positively or negatively influence the toxicity of isothiocyanates. As yet not enough is known about the complex relationships that occur when using biocidal plants to comment on likely efficacy (Morra, 2004).

Research and development of biofumigants in the field is in its infancy in the UK. As more becomes known about the chemical processes involved in the production, release and effectiveness of isothiocyanates, so it becomes essential to obtain specific information on glucosinolate types, levels and profiles in plant tissues of important cruciferous crops and the benefits of using particular cultivars, and even particular parts of these cultivars i.e. the seeds, roots, foliage, etc. The choice of biocidal plant may also depend on the relative importance of its biofumigant action as well as its benefits in biomass production; the latter will thus not only serve to benefit soil structure but may also increase isothiocyanate production.

Although it is unlikely that biofumigation or the addition of amendments will provide a direct replacement for manufactured sterilants or methyl bromide, its integration with other cultural or chemical methods offers an alternative to improve the sustainability of horticulture in general (Bianco *et al.*, 2001). In this survey of the literature it was interesting to note that some of those involved in organic farming did not view biofumigants as an essential tool, except for use in severe pest and disease outbreaks, such as during the first years of conversion from other farming methods (Micheloni & Conte, 2004). An alternative philosophy, however, might be that frequent use of biofumigants might help to keep levels of pests and disease down, providing that this approach did not accelerate the biodegradation process.

Such is the confidence in the role that biofumigant crops can play as part of an integrated control programme for pathogen and weed control that there are several research centres developing cultivars specifically for this purpose, such as the Départ. Agronomie et Environnement, ENESAD, Dijon, France, which is working specifically on *B. juncea* genotypes (Merah *et al.,* 2004) and the *Brassicaceae* breeding group at the University of Idaho (Brown *et al.,* 2004), where breeding efforts have been directed towards two biofumigation systems, namely (1) green manure/incorporation cultivars that have high biomass accumulation and high concentrations of specific glucosinolates in the plant tissue, and (2) cultivars with high concentrations of specific glucosinolates in the seed meal, so that the meal can be used as a soil amendment. The latter might require less interruption in cropping schedules but possible phytotoxic effects of cropping close to incorporation need to be investigated.

One Italian company, Cerealtoscana (www.cerealtoscana.it), is promoting the use of biocidal plants under the brand 'Sovesci Bluformula'. It has worked with the Research Institute for Industrial Crops of (or Instituto Sperimentale Colture Industriali - ISCI) Bologna, to develop a selection of green manures, namely three cultivars of *Brassica juncea* (namely ISCI 20, 61 and 99) and one cultivar of *Eruca sativa* (NEMAT). These are now available in the UK via Plant Solutions Limited (www.plantsolutionsltd.com). The crops ISCI 99 and NEMAT have been selected for the joint VCS/CSL proposal to investigate the potential of such plants to reduce pests, diseases and weeds and improve soil nutrition.

The use of many different *Brassica* cultivars for biofumigation is cited in the literature because of their well-known properties of the production of glucosinolates (e.g. *Brassica napus* and *Brassica campestris* (Bianco *et al*., 2001). However, the cultivars currently being offered commercially in the UK include *B. juncea* and *E. sativa* and it is the use of these that have been particularly investigated in this review.

Many of the papers referred herein have not been published in English, and in the time available it has not been possible to obtain complete translations but wherever possible English summaries have been obtained. In addition, there is much information available only as locally produced reports or Conference proceedings, which are also not immediately available to examine in detail.

Brassica juncea (ISCI 99)

B. juncea is grown in several countries for oil production (e.g. Canada, China, India) and in Burgundy, France where it forms the basis for the famous 'Dijon' mustard used as a table condiment (Lionneton *et al.,* 2004). *B. juncea*, sometimes known as 'Indian' or 'Brown' mustard, has been highlighted in several research projects as one cultivar having particularly high levels of glucosinolates. In particular it contains the glucosinolate sinigrin (which interestingly, is also responsible for the flavour of Dijon mustard), and work with this group has shown that organic matter and glucosinolate yield is highly dependent on plant type and cultivation time.

Researchers have investigated *B. juncea* but it is not always clear in the literature which cultivar has been used, thus some comments refer to the characteristics of the species in general, although it is clear that cultivars of the same species can have very different properties. *B. juncea* Czern et Coss, for example, has been shown to significantly suppress multiplication of the root-knot nematode *Meloidogyne incognita* on tomato roots and thus increase the crop yield (D'Addabbo *et al.,* 2004), but it would be unsafe to assume that the same effect would be achieved by all other cultivars of this species.

Work in Italy has included the use of the cultivar ISCI 20 (Lazzeri et al., 2003a). It is claimed to be a robust producer of glucosinolates, is adaptable to many soil types and climates and is easy to manage in the field. It is reported to produce up to 138 tonnes dry matter (DM) per ha, which may contain more than 1.6% Nitrogen. Reported variations in DM production are said to be due to differences in cultivation, including a failure to sow at the optimal time. Strawberry crops succeeding *B. juncea* in the rotation have shown no adverse reaction to glucosinolates and are said to have given results comparable those following sterilisation with methyl bromide, but no details are available. ISCI 20 is also being investigated by Applied Plant Research Flower Bulbs, Lisse, in The Netherlands as a control for plant-parasitic nematodes and soil borne fungal diseases (van Bruggen, 2004; van Os, 2004). Results for nematodes were not available, but the cultivar did produce a reduction in the incidence of *Rhizoctonia solani* on lily, resulting in a significant increase in bulb yield compared to other green manure crops. Variable results were obtained in similar work with tulip.

In France, trials have investigated an unknown cultivar of *B. juncea* amongst a range of potential biofumigants in the control of soil-borne pathogens in vegetable crops. Whilst, unfortunately, the specific effectiveness of *B. juncea* was not revealed, a general conclusion was that the biofumigants tested were effective against *Rhizoctonia solani* and *R. solani*, but not against *Phytophthora cactorum* (Villeneuve, *et al.,* 2004). The activity against this disease is another example of how in-vitro studies seem to provide little indication as to the effectiveness of biofumigant crops in practice; laboratory work by Dunne *et al* (2003) suggested that *B. juncea* was very effective against *P. cactorum*. However, the laboratory work had demonstrated that there was also significant variation in the sensitivity of the *Phytophthora* species to the suppressive effects of the biofumigants. Experience gained during the trials work found that success was dependent on many factors, including the plant species used, the quantity of fresh organic matter ploughed in, the soil temperature during the period of coverage with plastic (with lower efficacy at lower temperatures) and the type of plastic used (important in their ability to reduce vapourisation rates, maintain temperature and modify the soil atmosphere).

There are several instances of conflicting information being published concerning the effectiveness of this group of green manure crops against pathogens. In laboratory work using pure isothiocyanates, it was predicted that *B. juncea* would be one of a group of *Brassica* plants containing high concentrations of propenyl isothiocyanates most likely to control *Fusarium oxysporum* isolates obtained from forest tree nurseries in Idaho and Washington (Smolinska *et al.,* 2003). However, at a USDA Forest Service Nursery in Idaho, seedling production was not improved by incorporating brassica green manure crops, compared to the use of dazomet or fallowing, and in some cases large increases of potentially pathogenic *Fusarium* spp. were recorded (James *et al*., 2004). Soil *Pythium* levels were reduced when plastic tarpaulins were used to reduce losses of decomposition products, but this did not result in improved seedling production. The laboratory work had noted that only a fraction of the isothiocyanate potentially available from the glucosinolate within the tissues is actually released and available for pathogen inhibition; this combined with other factors that are necessary to maximise the fumigation effects illustrates the care and preparation necessary when investigating the use of biofumigants.

In Washington State, USA, *B. juncea* and *Sinapis alba* were incorporated into soils in the autumn and planted with potatoes the following spring. No information is available for the effects of *B. juncea* only, but the green manures were considered an effective replacement for manufactured soil fumigants (McGuire, 2004b).

Work in the USA has recently evaluated the herbicidal properties of seed meal of *Brassica* plants in glasshouse tests, including that produced from *B. juncea* under the name 'Pacific Gold'. The results varied according to cultivar, with 'Pacific Gold' having good herbicidal activity on wild oat seeds, but less activity compared to other Brassicaceae on wild mustard and pigweed. It did significantly reduce weed biomass overall. In subsequent field trials in strawberry crops 'Pacific Gold' did not perform as well as other seed meals, but in common with others was responsible for high phytotoxicity on first year strawberry transplants. However, yields from the crops were not significantly lower than those from the standard chemical treatment. Further research will determine efficacy rates and the timing of incorporation for maximum effect and productivity (Brown *et al.,* 2004). No phytotoxicity was reported when a crop of *B. juncea* was incorporated into soil to be cropped some 2-3 months before planting with strawberries (Lazzeri *et al.,* 2003b). In this trial weed control was not necessary, yields of strawberries were not compromised, but the effect on pests and diseases was unclear.

In Southern California, the incorporation of the formulation 'Pacific Gold' or the plant mulch was investigated for nematode, disease and weed control, but it is not clear whether the two types of amendment were used separately or jointly (Daugovish *et al.,* 2004). A reduction of 92% in nematode numbers was achieved, but the growth of sclerotia of *Sclerotinia minor* (leaf drop of lettuce) was not affected except when used in combination with plastic covers, when a 75% reduction in sclerotial growth compared to the control was achieved. Such work highlights the requirement for a good seal to maximise the effect of the biofumigation. Colony development of *Phytophthora cactorum* (crown rot of strawberries) from biofumigated plots was inhibited by 90% or more, but intensive growth of *Pythium* spp. was also observed, leading to suggestions that the lack of growth of *P. cactorum* may not have been due to biofumigation but perhaps more likely a change in the microbiological environment that favoured the development of *Pythium* spp.

‘Pacific Gold’ seed meal resulted in 100% mortality of vine weevil larvae when incorporated into compost for potting, but resulted in phytotoxic effects on certain nursery tree species in the glasshouse or field. Also in the USA, glasshouse tests have been evaluating the effectiveness of *B. juncea* as chopped residues for weed control, but the results were very variable. Although in laboratory work germination of all weed species was completely inhibited, in the field trials there was no such inhibition in weed seed emergence. This again illustrates that whilst it is important to collect data from laboratory work where the effect of the biofumigants can be studied, it is also important to investigate their effects in the field to provide more lines of enquiry.

Some work has been done on the environmental effects of *B. juncea.* In Italy this has concentrated on investigating laboratory findings that glucosinolates and their hydrolysis products inhibit soil nitrifying bacteria communities, but in the field contradictory results have been obtained, requiring further work to determine the fertiliser value of this green manure crop (Marchetti *et al.,* 2004).

Eruca sativa cv. NEMAT

Compared to the work that has been done on *B. juncea,* relatively little information is available specifically for *E. sativa* cv. Nemat, perhaps because it has shown more selective action against pathogens in trials. The genus is part of the *Brassicaceae* and *E. sativa* is more commonly known as garden or salad Rocket, whilst the common name given to the one under investigation here is 'wild Rocket'. The main class of glucosinolates produced by this crop appears to be those containing glucoerucin, in contrast to the sinigrin found mainly in *Brassica* plants.

There is a lack of scientific data for the effect of this crop on nematodes. Hydrated, defatted seed meal (the product remaining after oil has been extracted) of *E. sativa* was tested for its efficacy in controlling *Sclerotinia* species (Marciano *et al.,* 2004). In common with other seed meals it greatly reduced the viability of sclerotia of *S. minor*, but did not affect those of *S. sclerotiorum.* It did have some effect on the antagonistic activity of biocontrol fungi, whereas other seed meals did not, so overall it did not rate highly in selections for disease control. Confirmation of this selective action raises an important point when choosing a biofumigant crop – if biofumigants have selective action then integrating them into control programmes will require specialist advice.

No phytotoxic symptoms were seen in strawberries planted after a crop of *E. sativa* had been incorporated into the soil prior to planting (Lazzeri *et al.,* 2003b). The overwintering crop cover so provided eliminated the need for weed control, and yields of strawberries were better than in untreated plots, but the effect on pests and diseases was unclear.

In Italy laboratory tests using a seed meal formulation of the product resulted in high mortality of wireworms, but insecticidal activity ceased after 2-3 days illustrating typical problems in persistence with brassicas that could cause practical problems (Furlan *et al.,* 2004).

Some work has been done on the fertilising effect of the defatted meal of this crop in soil. The meal is rich in organic nitrogen (4-7% N), phosphorous (2-3% P) and sulphur (2-3% S). All these elements have to be mineralised to be available to plants. Meal was found to have potential as a good organic fertiliser (Cavani *et al.,* 2004).

Conclusions

With reform of the Common Agricultural Policy in the EU the basis upon which farm economics operates has been changed quite radically. Whilst the use of plants as whole crop biofumigants may become an economic option there is a need to ensure that truly repeatable results in terms of performance are shown by biofumigants (Askew, 2004).

The promotional literature produced by the commercial companies now marketing biocidal plants tends to present a simplistic picture of their use but evidence for consistent results with pest, disease or weed control is somewhat lacking. For instance, this relatively brief search and report on the available literature concerning *B. juncea* (ISCI 99) and *E. sativa* (Nemat) could obscure the fact that both crops may have a similar effect on levels of plant-parasitic nematodes in the soil. However, both companies have taken an active interest in research projects and in practical trials to gain further knowledge of the effectiveness of these crops in reducing pathogens and weeds. Their accumulated field experience must be utilised to the full when planning to use biocidal crops.

Future challenges for researchers are to identify ways of maximising the release of isothiocyanates from incorporated biofumigants into soil. This might include breeding for higher levels of glucosinolates, improved agronomic practices and understanding the interaction of biofumigation with the soil environment.

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