POSTER SESSION 5B

NON-CHEMICAL CROP PROTECTION

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Posters Papers: 5B-1 to 5B-9

Arbuscular mycorrhizal fungi: their role in the ability of crops to cope with stress

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ABSTRACT

Arbuscular mycorrhizal fungi (AMFs) are responsible for a common and long-standing symbiosis with plants. Although thought to have a role in adapting to water stress, the nature of the role and potential mechanisms are unclear. This study explored the role of AMFs in the plant response to changing soil water potential. The study used hybrid black poplar as the test plant and *Glomus intraradices* and *Glomus mosseae* as the test AMF species. Water use in AMF-infected plants seemed to be more closely coupled to falling soil water potentials. Experiments using a rhizobox, which separated AMFs into root plus hyphae, and hyphae only compartments, suggested that the principal mechanisms for the AMF effect were likely to be through acting as a biosensor for changes in soil water potential, and via some direct uptake of water. The study indicated the importance of AMFs in plant adaptation to stress and consequently the necessity of their not being adversely influenced by agrochemicals in sustainable production systems.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMFs) have long been known to benefit plant growth under conditions of limited availability of mineral nutrients with low soil mobility such as phosphorus (Harley & Smith, 1983). More recently, it has become clear that AMFs influence plant performance in soils with a wider range of limitations (Van der Heijden & Sanders, 2002) and that they are critically important in low input and organic agriculture, both in respect of nutrient supply but also to crop protection (Atkinson *et al*., 2002). With the exception of plant genera such as the *Cruciferae* and the *Chenopodiaceae*, most plants, including crops, are usually infected by AMFs or other mycorrhizas. The symbiosis is of substantial antiquity, around 5M years, and was a major factor in the ability of plants to grow on land. This suggests that the normal state for plants is to be mycorrhizal and that plants and crops in which a normal AMF relationship does not develop are at a disadvantage. This leads to a view that rather than research focusing on situations where the presence of AMFs is advantageous, e.g. phosphorus deficiency, we should be identifying situations where the absence of AMFs makes crops less well adapted to environmental stresses than would otherwise be the case. In crop production this is important because many current crop protection practices reduce mycorrhizal infection (Atkinson, 1983).

Water stress significantly limits crop production on a world-wide scale. The ability of AMFs to increase a plant's ability to abstract water either directly through the hyphae or through other mechanisms has been the subject of a number of studies (Safir *et al*., 1972; Miller *et al*., 1997). Fewer studies have considered the influence of AMFs on water use and its regulation under conditions of water stress. The study detailed here aimed at examining the impact of AMFs on water use under conditions of decreasing water availability and the ways in which AMFs might impact on this situation.

MATERIALS AND METHODS

Two series of experiments were carried out. The first assessed impact of a number of AMF species on plant growth and water use under drought conditions while the second assessed mechanisms by which AMFs might influence plant response to water supply. Both studies employed cuttings of hybrid black poplar (*Populus x canadensis* cv. 'Robusta') which were planted in sterilised soil and grown in a glasshouse which provided temperature in the range 8-17°C and light intensity between 243 and 548 μmol/m^2/s.

In Study 1, plants were grown in a Craibstone series soil and inoculated with a number of AMF species. Only data for the effects of the *Glomus* species, *G. intraradices* and *G. mosseae* are presented here. Each treatment was replicated 6 times. Infection was obtained through the incorporation of infected cucumber root pieces into the soil. Water use was assessed by weighing pots at midday and calculating daily values. Response to drought was assessed on the basis of a 5 day drying cycle after which pots were rewatered. Root length and leaf area were assessed by image analysis. Soil water potential was estimated by relating the amount of water in the soil to the volumetric water content and its known relationship to the water release curve for a Craibstone soil (Dunsiger, 1999).

In Study 2, plants were grown in a rhizobox similar to that described by Faber *et al.* (1991). This provided two environments, one populated by both roots and hyphae and another containing hyphae but to which roots did not have access. This study used a sterilised Corby series soil. Plants were infected with *G. intraradices*. Measurements of gas exchange and stomatal conductance, using an ADC LCA5 IRGA, began when the plants were four months old and continued at intervals over the next 18 months. Changes in soil water potential in the two sections of the rhizoboxes were measured with an electrical resistance meter (ELE International).

RESULTS AND DISCUSSIONS

The effect of the AMF inoculation in the early stages of Study 1 is shown in Table 1.

Table 1. The effect of AMF infection on the growth of hybrid black poplar (NS = not significant, * = $P < 0.05$)

Treatment	% Infection	Leaf area (cm^2)	Shoot dry weight (g)	Root dry weight (g)	Root length (m)
Control	0	93	1.5	2.3	446
G. intraradices	31	101	1.9	2.7	447
G. mosseae	23	117	1.5	2.6	495
Significance		NS	*	NS	NS

The analysis of variance included other AMF treatments not detailed here. The absence of major effects of AMFs on growth, which were consequent on the significant nutrient status of the soil, meant direct comparison could be made of the impact of AMFs on water use without the need to allow for the effects of plant size which have confounded a number of earlier studies.

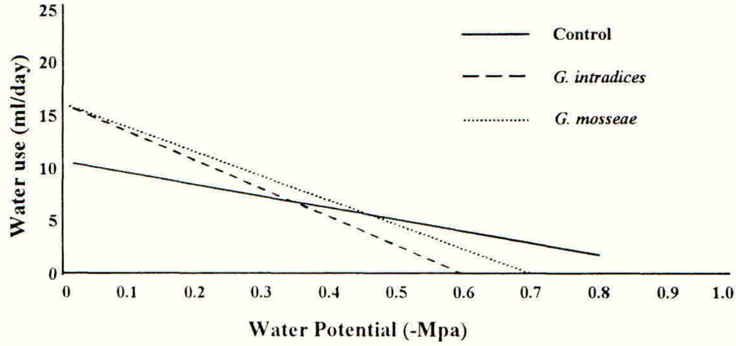

Figure 1. Plant water use compared with soil water potential when inoculated with different mycorrhizal species – one month after inoculation. (Trend lines are fitted by linear regression).

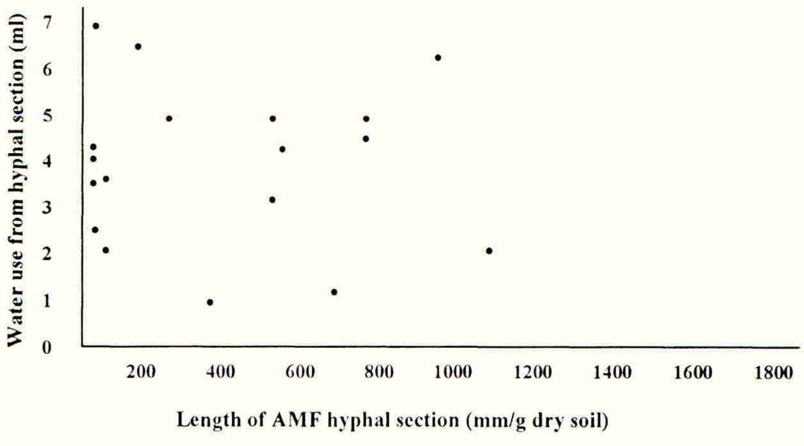

Figure 2. Relationship between AMF hyphal length in the hyphal section of rhizoboxes and water loss from hyphal sections over five days.

Infection with AMFs did not change the relationship between leaf area and water use. Water use increased with increasing leaf area under conditions of good soil water supply but was independent under stressed conditions. Despite this, stomatal conductance was generally higher in control plants. Over the period of measurement not all leaves within a treatment showed the same stomatal conductance. For each treatment, conductance measurements were classified into a range of five intervals. Treatments were compared using a Kolmogorov-

Smirnov analysis following conversion to percentages. Plants infected with *G. intraradices* and *G. mosseae* showed a high proportion of stomata with low conductances; 90% and 70% respectively compared to a control value of 45% for conductances in the 0 to 0.04 mol/m^2/s range. Differences were significant at $P = 0.01$.

For periods during the growth of the plants, total water use was related to soil water potential in the 0 to 0.5 MPa range. When the plants were young, and in summer (August), and within a few weeks of infection being established, there were differences between the control and inoculated plants (Figure 1). In the control plants, water use did not decrease significantly with a decreasing soil water potential. In the AMF-infected plants, water use, although higher than for control plants at a high water potential, decreased significantly with a declining water potential. In the autumn (October/November), when the rate of drying was low, water use within the above range was little influenced by soil water potential for any treatment. In the summer of the plants' second season, water use was again well coupled and again decreased with decreasing soil water potential.

The impact of AMF infection on water use thus was at its greatest in the summer months when the rate of water loss was high, a 15 ml/d maximum compared with 10 ml/d. This reduction was a consequence of a higher proportion of the stomata showing relatively low conductances. How AMFs influence stomatal conductance and water uptake are thus key issues.

AMFs may influence plant response to an increasing water stress and a decreasing soil water potential in three major ways:

a) There may be no major effect directly relating to the AMF hyphae. Any effects may be due to more general changes in growth regulation. In this case, shoot response will be independent of the conditions around external hyphae i.e. variation in the hyphal environment will have no impact on response to water potential.
b) There may be direct transport of water from the soil via hyphae. In this case removal of soil water should be related to hyphae length. If hyphae are removed then this should be associated with reduced transpiration.
c) Hyphae may signal changing soil water potential to the plant shoot, and shoot water potential will then be influenced by the soil water potential being sensed by the hyphae i.e. the soil water potential in the hyphal section will have a disproportionate impact on plant water regulation.

These factors may all be operational in parallel. In Study 2, the uptake of water from the section of the rhizobox containing hyphae only was compared with the quantity of hyphae in that compartment. No clear relationship between the length of hyphae, assessed using the method of Miller *et al.* (1995) and the volume of water removal from the compartment was found although a high hyphal length was commonly associated with higher water use (Figure 2). Variation in the soil water status of the root plus hyphal, and hyphal only boxes seemed to have little influence on the rate of transpiration. This suggests that at least some direct hyphal uptake may occur.

Stomatal conductance was however significantly ($P < 0.01$) affected by the soil water potential in the root plus hyphal section. Severing the hyphae which connected the root plus hyphal and hyphal sections for 4 replicate plants produced a variety of effects. Prior to severing, conductances across the range 0 to 0.12 mol/m^2/s were recorded. After severing, there was an increase in low conductance values and the range decreased to 0 to 0.08 mol/m^2/s. Conductance had returned to the original mean value 48 h after severing, and there was an increase in high conductance values. Assessed with the Kolmogorov–Smirnov test, differences were significant ($P < 0.05$). The relationship between stomatal conductance and soil water potential was positive and significant, for both the root plus hyphal (0.47, $P < 0.01$) and hyphal compartments (0.63, $P < 0.01$). The relationships were less strong and non-significant ($P = 0.80$) after severing the hyphal links between compartments. This suggests that AMF hyphae can have an additional role as biosensors.

CONCLUSIONS

The relationships between crop plants and their stomata, and soil water potentials have always been complex (Hsiao *et al.*, 1976; Jarvis & Davies, 1998). The relationship between stomatal function and transpiration is influenced by atmospheric conditions. Accordingly, the results presented here do not show a simple relationship between infection with AMFs and plant functioning under conditions of low soil water potential. The results presented suggest however that in AMF-infected plants when transpiration stress is high that an increased proportion of stomata will show a reduced conductance compared with the situation in control plants. Both of these changes suggest that infection with AMFs confers a greater general ability to manage water loss under conditions of stress. In addition, in infected plants, total water use has a stronger negative relationship with decreasing soil water potential (Figure 1).

The results presented here also suggest that the linkages between this increased ability to regulate water use and AMFs may be contributed to by three possible mechanisms, which may operate in parallel. Firstly, as suggested above, AMF-infected plants seem to be generally more robust. Secondly, while the relationship between hyphal length and soil water depletion is inexact it suggests that higher hyphal densities can be associated with increased soil water use. Thirdly, in addition, the changes in water use which were associated with the severing of the hyphal network suggest that the role of the hyphae may go beyond the mere provision of water. This suggests the possibility that they might act, at times, as biosensors. Davies & Zang (1991) have reviewed the role of signals originating in the root system in influencing plant growth in drying soils. They did not consider a biosensor role for AMFs. An increase in the ability of the root system to absorb water, which would be a consequence of the second mechanism, would not explain a reduced water use as a consequence of an increasing soil water deficit (decreased soil water potential). AMFs acting as biosensors and reducing water potential more rapidly than in a control plant would be consistent with this effect. The loss of this source of information would be expected to cause a reduced water use as a response to a loss of information and this was obsessed. This role for AMFs needs further exploration.

The role of AMFs in influencing water use emphasises the importance of AMFs and their role as an integral part of the plant, especially when the plant is under stress. This suggests that improved crop production, especially under the more varied conditions expected to be consequential upon climate change will benefit from significant levels of AMF infection. Maintaining significant levels of AMF infection and good levels of inocula in the soil will be important. In sustainable production systems, disease control strategies which do not adversely influence infection of crop roots with AMFs are needed as are weed control programmes which do not adversely influence the levels of infective propagules in the soil (Atkinson, 1983). Maintaining the crop with an effective AMF symbiosis is important to sustainable production systems. Such systems need crop protection programmes which do not adversely influence the plant's AMF status (Atkinson *et al.*, 2002).

REFERENCES

Atkinson D (1983). The growth, activity and distribution of the fruit tree root system. *Plant and Soil* **71**, 23-36.

Atkinson D; Baddeley J A; Goicoechea N; Green J; Sanchez-Diaz M; Watson C A (2002). Arbuscular mycorrhizal fungi in low input agriculture. *Mycorrhizal technology in agriculture,* eds S Gianinazzi, H Schuepp, J M Barea & K Haselwandter, pp. 211-222. Birkhauser, Basel.

Davies W J; Zhang J (1991). Root signals and the regulation of growth and development in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 55-76.

Dunsiger Z (1999). *The influence of arbuscular mycorrhiza on the water relations of trees.* PHD Thesis, University of Edinburgh.

Faber B A; Zanoski R J; Munns D N (1991). A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. *Canadian Journal of Botany* **69**, 87-94.

Harley J L; Smith S E (1983). *Mycorrhizal symbiosis.* Academic Press, London.

Hsiao T C; Acevdeo E; Fereres E; Henderson D W (1976). Water stress, growth and osmotic adjustment. *Philosophical Transactions of the Royal Society, London* **273,** 479-500.

Jarvis A J; Davies W J (1998). Modelling stomatal responses to soil and atmosphere drought. *Journal of Experimental Botany*, **49**, 399-406.

Miller R M; Reinhardt D R; Jastrow J D (1995). External hyphal production of vesicular arbuscular mycorrhizal fungi in pastures and tall grass Prairie communities. *Oecologia* **103**, 17-23.

Miller R M; Hetrick B A D; Wilson G W T (1997). Mycorrhizal fungi affect root stele tissue in grasses. *Canadian Journal of Botany* **75**, 1778-1784.

Safir G R; Bayer J S; Gerdemann J W (1972). Nutrient status and mycorrhizal enhancement of water transport in soybean. *Plant Physiology* **49**, 700-703.

Van der Heijden M G A; Sanders I R (2002). *Mycorrhizal Ecology.* Springer, Berlin.

The effect of increased crop diversity on colonisation by pest insects of brassica crops

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ABSTRACT

Many researchers have shown that the numbers of pest insects found on crop plants are reduced considerably when the crop is allowed to become weedy, when the crop is intercropped with another plant species, or when the crop is undersown with a living mulch. Laboratory and field-cage experiments were done to determine how undersowing brassica plants (*Brassica oleracea* spp., cabbage, cauliflower, Brussels sprout and *Brassica chinensis*) with subterannean clover (*Trifolium subterraneum*) affected host plant selection by eight pest insect species of brassica crops. Experiments were done also to determine the effect on egg-laying by the cabbage root fly (*Delia radicum*) on brassica plants surrounded with plant species other than clover. A total of 24 'companion' plant species were tested, including 1) bedding plants, to provide a different range of plant architectures and leaf colours; 2) weeds; 3) aromatic plants such as rosemary and thyme and 4) companion plant species such as marigold. After studying how pest insects behave in undersown brassica crops, we suggest it is simply the number of green objects surrounding the host plants that is the major factor that prevents the pest insects from finding their host-plants. Hence, increasing plant diversity within brassica crops should help considerably to reduce pest insect numbers.

INTRODUCTION

The numbers of pest insects found on crop plants are often reduced considerably when the crop is allowed to become weedy, when the crop is intercropped with another plant species, or when the crop is undersown with a living mulch (Andow, 1991). Examples of this effect in horticultural crops include: 1) the influence of undersowing with clover on the numbers of pest insects infesting cabbage (Theunissen *et al.*, 1995); 2) the use of clover mulches to reduce *Thrips tabaci* infestations in leeks (Theunissen & Schelling, 1996) and 3) the manipulation of weeds to reduce aphid colonisation in red beet (Wnuk & Pobozniak, 1999).

It has been suggested that when diverse backgrounds 'disrupt' (Vandermeer, 1989) insects from selecting otherwise acceptable host plants, the action is mediated through 1) physical obstruction (Perrin, 1977); 2) visual camouflage (Smith, 1969; 1976); 3) 'masking' of host-plant odours (Tahvanainen & Root, 1972); 4) 'deterrent' or 'repellent' chemicals (Uvah & Coaker, 1984), or through 5) the non-host plants altering the physiology of the host plants (Theunissen, 1994). Two other suggestions, involving the 'Resource Concentration Hypothesis' and the 'Enemies Hypothesis' (Root, 1973), have also been made to explain why fewer phytophagous insects are found on cultivated host-plants growing in diverse backgrounds than on similar host-plants growing in bare soil. This paper summarises the results of recent studies to determine how increased crop diversity disrupts host-plant finding by pest insects of brassica crops, and uses the information to develop a theory to explain how phytophagous insects find their host plants.

MATERIALS AND METHODS

The initial laboratory and field-cage experiments were done to determine how undersowing brassica plants (cabbage, cauliflower, Brussels sprout and Chinese cabbage) with subterranean clover (*Trifolium subterraneum*) affected plant colonisation by eight pest insect species of brassica crops. In each experiment, the insects were given a choice between brassica plants surrounded by living clover or similar plants surrounded by bare soil. Full experimental details are given in Finch & Kienegger (1997).

Field cage experiments were done also to determine the effect on egg-laying by the cabbage root fly (*Delia radicum*) on cauliflower plants surrounded by plant species other than clover. A total of 24 'companion' plant species were tested, including 1) bedding plants, to provide a different range of plant architectures and leaf colours; 2) weeds; 3) aromatic plants such as thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) and 4) companion plant species such as marigold (*Tagetes* spp.). This was followed by laboratory experiments using large cages where the behaviour of female flies was observed in both 'choice' (companion plant and host plant) and 'no choice' (companion plant only) situations. Full experimental details are given in Finch *et al.* (2003).

RESULTS

The data from the first series of experiments using clover showed that undersowing had a similar effect on all eight of the pest species tested, even though they were from four different insect orders (Hemiptera, Diptera, Coleoptera, Lepidoptera) (Table 1; Finch & Kienegger, 1997). However, the size of the effect varied between species, and the effect on the diamond-back moth (*Plutella xylostella*) was less pronounced than that on the cabbage root fly and the cabbage aphid (*Brevicoryne brassicae*).

In the second series of experiments with the cabbage root fly, host plant finding was disrupted to differing extents by the 24 plant species tested (Figure 1). The four least disruptive species were (in increasing order of disruptiveness) the weed *Sallopia convolvulus* and the bedding plants *Cineraria maritima*, *Lobelia erinus* and *Lobularia maritima*. All of these were relatively low-growing species. The five aromatic plants tested, *Thymus vulgaris*, *Rosmarinus officinalis*, *Helichrysum bracteatum* (curry plant) and two species of mint, (*Mentha piperita* × *citrata*; *Mentha pulegium*) were no more disruptive than the non-aromatic plants, and the three 'companion plants' *Tagetes patula*, *T. tenuifolia* and *T. erecta* came 13th, 12th and 5th in the overall rankings. The four plants that were most effective at disrupting oviposition, *Chenopodium album* (weed), *Daucus carota* (carrot), *Chamaenerion angustifolium* (weed) and *Pelargonium x hortorum* (bedding plant) (1st – 4th in the overall rankings), were all relatively tall, but equally important, their foliage surrounded the complete circumference of the cauliflower host plant.

In the 'choice' and 'no-choice' laboratory experiments, the flies stayed 2 – 5 times as long on the leaves of non-host plants as on those of cauliflower plants.

Table 1. Percentage reduction in the numbers of eggs laid on various types of brassica plant surrounded by clover instead of bare soil. N.B. * data represent totals of aphids rather than eggs.

Insect species	Insect order	Host plant	Percentage reduction in egg numbers when plants surrounded by clover, compared with bare soil
*Brevicoryne brassicae**	Hemiptera	Cabbage	95, 78
Delia radicum	Diptera	Brussels sprout	69, 85
Phaedon cochleariae	Coleoptera	Chinese cabbage	80, 95, 85, 81
		Brussels sprout	96
Evergestis forficalis	Lepidoptera	Brussels sprout	65
Mamestra brassicae	Lepidoptera	Brussels sprout	94
Plutella xylostella	Lepidoptera	Brussels sprout	46, 30
		Cabbage	42
Pieris brassicae	Lepidoptera	Brussels sprout	100, 92
		Cauliflower	71
		Cabbage	100
Pieris rapae	Lepidoptera	Brussels sprout	66, 75, 81, 55, 60, 47, 66

DISCUSSION

In our experiments, the numbers of pest insects found on crop plants were reduced considerably when plant diversity was increased, confirming the results of several previous studies (Andow, 1991). The effect was produced also when the host plants were surrounded by sheets of green paper or plant models made from green card, neither of which were releasing plant chemicals (Kostal & Finch, 1994). Therefore, it seems that the disruption to host-plant selection is caused simply by providing the insects with a greater number of green surfaces on which to land. Our observations lead us to develop the 'appropriate/inappropriate landing' theory (Finch & Collier, 2000), which is based on the fact that herbivorous insects land indiscriminately on green surfaces and involves the following sequence of events:

1. The first phase of host-plant finding consists of the searching insects being stimulated to land on green surfaces by odours that characterise the host plant. The odours do not give the insects sufficient directional information to discriminate between host plants and other green surfaces. However, they avoid landing on brown surfaces such as bare soil. When the insects land on the leaf of a host plant, we describe the landing as 'appropriate'. When they land on any other leaf or green surface, we describe the landing as 'inappropriate'. Those insects that make 'inappropriate' landings fly off the plant and repeat the process, or simply leave the area.

2. The second phase of host-plant finding starts as soon as an insect lands on a host plant. Once this occurs, the insect makes short flights from leaf to leaf to assess the overall suitability of the plant. The actual number of flights depends on the insect species involved and the amount of chemical stimulus the insect receives when making contact with each plant leaf. To be successful, the insect must make a certain number of

consecutive 'appropriate' landings. However, if the insect makes an 'inappropriate' landing, the process is terminated and the insect has to start again.

In effect, therefore, herbivorous insects have to find and re-find their host plants. On plants surrounded by soil, most insects land back on the same plant. On plants surrounded by other plants, some insects land on the other plants and then fly off.

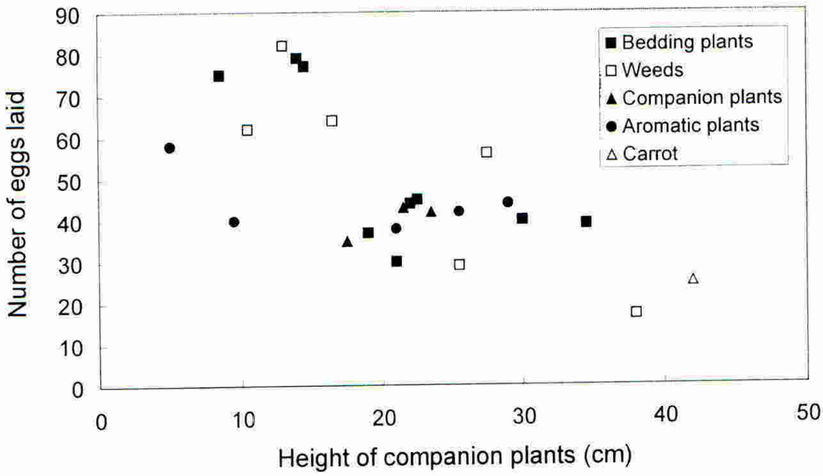

Figure 1. The numbers of cabbage root fly eggs recovered from around cauliflower plants, each surrounded by four plants of one of 24 non-host plant species, versus the average height of the non-host plant species. The numbers of eggs recovered are expressed as a percentage of the numbers of eggs recovered from around cauliflower plants surrounded only by bare soil.

The several studies made on the effects of intercropping on pests of brassica crops indicate that it has a broad-spectrum effect that can reduce colonisation, although by differing amounts, by a wide range of pest species from different insect orders. In our experiments with the cabbage root fly, the effect of increased plant diversity was pronounced. It is likely that if oviposition could be reduced sufficiently when leafy brassica plants are small and susceptible, then there would be no need for additional control measures against this pest. The situation would be somewhat more complex on root brassica crops, such as swede, where cabbage root fly damages the marketable part of the plant and where the crop may be exposed to at least two periods of colonisation (generations), several weeks apart. The effect of undersowing on the cabbage aphid was very pronounced and it is likely that this technique could be used to reduce colonisation by this pest. However, aphids can multiply directly on the crop and may migrate into a crop throughout the summer. Hence, supplementary means of control may be required, particularly when the plants are large and close to harvest. The effect of undersowing on the diamond-back moth was the least pronounced and it may not be possible to provide adequate control of this pest, particularly as this moth is a migrant species in the UK (Chapman *et al.*, 2002) and it is not possible, at present, to predict when migrations will occur. Thus it may not be feasible to use the companion planting strategy to produce the maximum effect against all pests, particularly if the effect of a companion plant diminishes as the crop grows. However, the technique could be combined with other control measures such as host-plant resistance, trap crops, or biological control, to reduce the numbers of pest species to acceptable levels.

These experiments give clues about the ideal characteristics of a companion plant species. It was thought previously that herbivorous insects that landed on aromatic plants found the surfaces distasteful and soon flew off the plant. This is the basis of the 'companion planting' approach of organic growers in which, for example, onions and marigolds, chosen for their pungent odour and taste, are grown interspersed with crop plants to deter pest insects, such as the carrot fly (*Psila rosae*). Our results have shown, however, that plants such as onions and marigolds disrupt host plant finding by insects simply by being green. The disruption has nothing to do with their odour or taste. Of the twenty-four plant species tested, the aromatic plants were no more disruptive than non-aromatic plants. From a practical point of view, therefore, growers could use any non-host plant to prevent insects from finding their crop plants. At present, there are indications that the characteristics required to reduce colonisation of the host plant are related to companion plant architecture and factors such as the relative heights of the companion and host plants and the proportion of the host plant circumference filled (Morley, 2001). Further detailed experiments are required to determine precisely what these characteristics are, and how best to arrange the companion plants around host plants.

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REFERENCES

Andow D A (1991). Vegetational diversity and arthropod population response. *Annual Review of Entomology* **36**, 561-586.

Chapman J W; Reynolds D R; Smith A D; Riley J R; Pedgley D E; Woiwod I P (2002). High-altitude migration of the diamondback moth *Plutella xylostella* to the UK: a study using radar, aerial netting, and ground trapping. *Ecological Entomology* **27**, 641-650

Finch S; Collier R H (2000). Host-plant selection by insects – a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. *Entomologia experimentalis et applicata* **96**, 91-102.

Finch S; Kienegger M (1997). A behavioural study to help clarify how undersowing with clover affects host-plant selection by pest insects of brassica crops. *Entomologia Experimentalis et Applicata* **84**, 165-172.

Finch S; Billiald H; Collier R H (2003). Companion planting - do aromatic plants disrupt host-plant finding by the cabbage root fly more effectively than non-aromatic plants? *Entomologia experimentalis et applicata*, accepted.

Kostal V; Finch S (1994). Influence of background on host-plant selection and subsequent oviposition by the cabbage root fly (*Delia radicum*). *Entomologia Experimentalis et Applicata* **70**, 153-163.

Morley K E (2001). *The effect of undersowing on host-plant finding by the cabbage root fly (*Delia radicum*).* PhD Thesis, Imperial College of Science, Technology & Medicine, London, 191 pp.

Perrin R M (1977). Pest management in multiple cropping systems. *Agro-ecosystems* **3**, 93-118.

Root R B (1973). Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monograph* **43**, 95-124.

Smith J G (1969). Some effects of crop background on the populations of aphids and their natural enemies on Brussels sprouts. *Annals of Applied Biology* **63**, 326-330.

Smith J G (1976). Influence of crop backgrounds on aphids and other phytophagous insects on Brussels sprouts. *Annals of Applied Biology* **83**, 1-13.

Tahvanainen J O; Root R B (1972). The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta crucifera* (Coleoptera: Chrysomelidae). *Oecologia* **10**, 321-346.

Theunissen, J (1994). *Effects of intercropping on pest populations in vegetable crops.* IOBC/WPRS Bulletin **17 (8)**, 153-158.

Theunissen J; Booij C J H; Lotz L A P (1995). Effects of intercropping white cabbage with clovers on pest infestation and yield. *Entomologia Experimentalis et Applicata* **74**, 7-16.

Theunissen J; Schelling G (1996). Undersowing crops of white cabbage with strawberry clover and spurrey. *IOBC/WPRS Bulletin* **19 (11)**, 128-135.

Uvah I I I; Coaker T H (1984). Effect of mixed cropping on some insect pests of carrots and onions. *Entomologia Experimentalis et Applicata* **36**, 159-167.

Vandermeer J (1989). *The Ecology of Intercropping.* Cambridge University Press: Cambridge, 237 pp.

Wnuk A; Pobozniak M (1999). Influence of weeds on occurrence of bean aphid (*Aphis fabae* Scop.) and aphidophagous (*Syrphidae*) on red beet (*Beta vulgaris* L.). *Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture, Horticulture and Vegetable Growing* **18**, 266-275.

The efficacy of high temperature and diatomaceous earth combinations against adults of the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) and the grain weevil *Sitophilus granarius* (Coleoptera: Curculionidae)

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ABSTRACT

Recent research has shown that diatomaceous earth (DE) can be used to augment the effect of high temperature (48°C) to control stored product invertebrate pests in flour mills. Laboratory bioassays were set up against adult *Sitophilus granarius* and *Tribolium castaneum* under conditions simulating stages of heating from 25°C / 70% r.h. to 40°C / 30% r.h.. Humidity was decreased for each step-wise increase in temperature, as calculated using a psychrometric chart. The DE formulation was applied as a dry dust to glass Petri dishes at doses of 1 – 10 g/m^2, and the insects exposed for 48 hrs, followed by a 7-day recovery period on whole wheat. All doses achieved 100% mortality against both species at or above 35°C, with 89% of *S. granarius* surviving in untreated controls at 35°C, and the more heat tolerant *T. castaneum* all surviving 35°C and 40°C. At the lower temperatures there was no significance difference in efficacy for doses at or above 5 g/m^2, suggesting this to be a threshold for maximum DE pickup. It is proposed that DE is used to treat crevices and voids as part of an integrated pest management (IPM) strategy for flour mills.

INTRODUCTION

Diatomaceous earth (DE) is a naturally occurring substance, mined from geological deposits of fossilised diatoms. Diatoms are water-borne, single celled chrysophyte phytoplankton, of which there are 5000 species. DE dust is composed of over 90% silica and has a wide range of applications, including use as a filtration agent, tooth-paste ingredient and in invertebrate pest control.

The DEs marketed for invertebrate pest control in the UK are composed of amorphous, rather than crystalline silica (<1%). The former is safer to the operator, and is only classed as a lung irritant, with protection ensured by use of a dust mask. DEs contain no chemical insecticide or knock-down agents, work by physical action, have low mammalian toxicity, are persistent but do not leave harmful residues, are effective against chemical-resistant species, and are stable at high and low temperatures (Subramanyam *et al.*, 1994; McLaughlin, 1994). The mode of action is through gentle abrasion and sorption of cuticular waxes, stripping away the insects' water-proofing and resulting in desiccation when insects crawl among dust-coated kernels (Ebeling, 1971). DEs can be admixed to stored grain or applied to storage structures to control a wide range of invertebrate pests (Golob, 1997), and despite being less effective at high humidities, previous CSL research has shown efficacy under damp UK conditions (Cook & Armitage, 2002). Three DE products are available in the UK and have been actively marketed since autumn 2001.

In addition to protecting the stored crop, DE can be used to augment the effect of high temperature (>48°C) to control stored product invertebrate pests in flour mills as an alternative to fumigating using methyl bromide (Fields *et al.*, 1997). DE treatments are targeted at inaccessible areas such as crevices and voids, where the high temperature may not penetrate as easily. This research has shown that dry dust treatments offer better efficacy than slurry applications and treatments need to be effective within 48 hours to meet industry expectations. A recent LINK study has considered the suitability of heat disinfestation for UK flour mills. As part of this larger study, the laboratory bioassays reported on here provide efficacy data for the UK DE product 'Silico Sec' against two species of stored product insects, under simulated conditions of heating. These heating scenarios aimed to represent areas of a mill where a high target temperature of >48°C may not have been achieved. An additional combination at high temperature and elevated humidity simulated the presence of local free water.

MATERIALS AND METHOD

For each heat scenario, bioassays were run in controlled environment rooms at exposure conditions of either 25°C / 70% r.h., 30°C / 50% r.h., 35°C / 40% r.h. or 40°C / 30% r.h.. These were decided upon by using increasing temperature increments of +5°C, from the assumed ambient of 25°C (Bell, pers. comm.). The corresponding drop in r.h. with increasing temperature was calculated from a psychrometric chart. A higher r.h. of 40°C / 50% r.h., simulated the nearby presence of free water.

The dry dust treatments were achieved by sieving sufficient quantity of DE through a 250µm wire sieve to give an even coating on 14 cm diameter glass Petri dishes. The DE was applied at doses of 1, 5, 7.5 and 10 g/m^2 (n=5). Untreated dishes were used to show the effect of heating alone. For each temperature / humidity combination, an additional set of untreated dishes at 25°C / 70% were set up as controls. The dishes were held overnight in controlled environment rooms at each condition before exposure of the pest species. Batches of 25 two to four week old adults of *Sitophilus granarius* and *Tribolium castaneum* were added to each separate dish. These had been conditioned overnight by moving cultures to 30°C and 70% r.h. for the tests at 30°C and above. Mortality was assessed 24 and 48 hrs after treatment. After the final assessment, the insects were transferred to jars containing wheat at 15.5% moisture content and recovery was assessed after a further 7 days.

There was no need to correct the data for control mortality since less than 5% died in all cases. There was a broad range of proportions in the data sets, due to one or two insects escaping from the bioassays, therefore comparisons were made on the % mortalities, the data having been subjected to angular transformation ($\sin^{-1} \sqrt{p}$). The data was then statistically compared using analysis of variance (ANOVA) at the 5% probability level, with individual comparisons made using Tukey's pair wise comparisons.

RESULTS AND DISCUSSION

Efficacy against *S. granarius* adults after 24 and 48 hrs

Heating alone for 48 hrs, only achieved 100% mortality against *S. granarius* at the highest temperature scenario of 40°C (Table 1). For the lower temperature scenarios, the DE treatments significantly enhanced the effects of heating alone ($P > 0.05$). At 25°C / 70% r.h., mortality was <50% for all DE doses after 48 hrs, but rose to over 90% for all doses when heating took place. Although there were significant differences between the doses at 25°C, there was no significant difference at the higher temperatures ($P < 0.05$).

Table 1. Mean % mortality for *S. granarius* after exposure to DE at 5 temperature /humidity combinations, simulating mill conditions during heating (range in parenthesis; n=5; * n=25).

Dose	24 hrs exposure					48 hrs exposure				
	25°C 70%	30°C 50%	35°C 40%	40°C 30%	40°C 50%	25°C 70%	30°C 50%	35°C 40%	40°C 30%	40°C 50%
No DE	0 * *a*	0 *a*	0 *a*	80 *a*	74 *a*	0 * *a*	0 *a*	21 *a*	100 *a*	100 *a*
	(0)	(0)	(0)	(60-92)	(64-84)	(0)	(0)	(16-28)	(100)	(100)
1 g/m^2	0 *a*	6 *b*	50 *b*	100 *b*	100 *b*	16 *b*	94 *b*	100 *b*	100 *a*	100 *a*
	(0)	(0-8)	(28-84)	(100)	(100)	(4-24)	(88-100)	(100)	(100)	(100)
5 g/m^2	0 *a*	14 *b*	91 *c*	100 *b*	100 *b*	40 *c*	99 *b*	100 *b*	100 *a*	100 *a*
	(0)	(8-16)	(84-100)	(100)	(100)	(36-44)	(96-100)	(100)	(100)	(100)
7.5 g/m^2	0 *a*	13 *b*	94 *c*	100 *b*	100 *b*	39 *c*	99 *b*	100 *b*	100 *a*	100 *a*
	(0)	(4-20)	(84-100)	(100)	(100)	(20-48)	(96-100)	(100)	(100)	(100)
10 g/m^2	0 *a*	11 *b*	94 *c*	100 *b*	100 *b*	33 *c*	98 *b*	100 *b*	100 *a*	100 *a*
	(0)	(0-16)	(84-100)	(100)	(100)	(20-44)	(92-100)	(100)	(100)	(100)

Proportions in the same column followed by the same letter are not significantly different at $P < 0.05$.

Efficacy against *T. castaneum* adults after 24 and 48 hrs

T. castaneum adults were shown to be far more tolerant to heat alone than *S. granarius*, since no mean mortality was observed in any of the heating scenarios after 48 hrs (Table 2). Despite this, the DE treatments were very effective against this species. At 25°C, mean mortality was >87%, with no significant difference between any of the doses ($P < 0.05$). Complete mortality occurred, at every dose, for every other heating scenario after 48 hrs.

Efficacy after seven day recovery, both species

After the seven-day recovery period on food, mortality rose to 100% for all DE doses against both species, for all heating scenarios > 25°C (Table 3). Even at the assumed ambient condition of 25°C, with no heating, mortality rose to >95%, with the exception of the lowest DE dose against *S. granarius*.

Table 2. Mean % mortality for *T. castaneum* after exposure to DE at 5 temperature/humidity combinations, simulating mill conditions during heating (range in parenthesis; n=5; * n=25).

Dose	24 hrs exposure					48 hrs exposure				
	25°C 70%	30°C 50%	35°C 40%	40°C 30%	40°C 50%	25°C 70%	30°C 50%	35°C 40%	40°C 30%	40°C 50%
No DE	0 * *a* (0)	0 *a* (0)	0 *a* (0)	0 *a* (0)	0 *a* (0)	0 * *a* (0)	0 *a* (0)	0 *a* (0)	0 *a* (0-4)	0 *a* (0)
1 g/m^2	34 *b* (20-52)	88 *b* (80-92)	100 *b* (100)	100 *b* (100)	99 *b* (96-100)	89 *b* (76-96)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)
5 g/m^2	20 *bc* (4-29)	90 *b* (84-96)	99 *b* (96-100)	100 *b* (100)	100 *b* (100)	87 *b* (78-96)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)
7.5 g/m^2	19 *bc* (12-24)	85 *b* (80-88)	100 *b* (100)	100 *b* (100)	100 *b* (100)	90 *b* (80-96)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)
10 g/m^2	16 *c* (8-20)	82 *b* (76-96)	100 *b* (100)	100 *b* (100)	100 *b* (100)	87 *b* (77-96)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)

Proportions in the same column followed by the same letter are not significantly different at $P < 0.05$.

Table 3. Mean % mortality for both species after 7 days recovery on wheat at 25°C and 70% r.h. (range in parenthesis; n=5; * n=25).

Dose	*S. granarius*					*T. castaneum*				
	25°C 70%	30°C 50%	35°C 40%	40°C 30%	40°C 50%	25°C 70%	30°C 50%	35°C 40%	40°C 30%	40°C 50%
No DE	1 * *a* (0-4)	2 *a* (0-4)	42 *a* (28-64)	100 *a* (100)	100 *a* (100)	0 * *a* (0-4)	0 *a* (0)	4 *a* (0-8)	19 *a* (8-40)	9 *a* (0-16)
1 g/m^2	82 *b* (72-96)	100 *b* (100)	100 *b* (100)	100 *a* (100)	100 *a* (100)	99 *b* (96-100)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)
5 g/m^2	96 *bc* (92-100)	100 *b* (100)	100 *b* (100)	100 *a* (100)	100 *a* (100)	98 *b* (96-100)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)
7.5 g/m^2	97 *c* (92-100)	100 *b* (100)	100 *b* (100)	100 *a* (100)	100 *a* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)
10 g/m^2	94 *c* (88-100)	100 *b* (100)	100 *b* (100)	100 *a* (100)	100 *a* (100)	98 *b* (96-100)	100 *b* (100)	100 *b* (100)	100 *b* (100)	100 *b* (100)

Proportions in the same column followed by the same letter are not significantly different at $P < 0.05$.

Implications for integrated pest management (IPM) in flour mills

DE has the potential to be an excellent subsidiary treatment to the heating of mills. A high level of control was achieved for each of the simulated heating scenarios after 48 hrs. In all cases, mortality for heat + DE was greater than additive, compared to the mortalities for heat alone and for DE treatments at 25°C. This suggests the combined treatments had a synergistic effect, which has also been found by other workers. Dowdy (1999), exposed *T. castaneum* adults on glass Petri dishes to temperatures of 34 or 50°C, with and without DE. In these tests, adults were exposed to four different DE products at 0 or 5 g/m^2 for 15 or 30 minutes and mortality assessed after recovery periods of 24 and 7 days. He suggested "as the DE absorbs cuticular lipids from the insect's cuticle, water regulation is compromised which results in an increased rate of desiccation due to the heating". DE can therefore be used to supplement heat disinfestation to give control in the cooler areas where the target temperature of >48 °C cannot be achieved. These are most likely to be voids and crevices in the mill structure. The increased mortalities in the recovery data, compared with the 24 and 48 hr data suggests, even if the adult insects move away from DE treated harbourages after the 48 hr heat treatment has finished, they are unable to recover. The higher mortalities in the recovery data also gives a truer picture of the success of the treatment, and suggests complete control of these two species can be achieved at a dose as low as 1 g/m^2, providing the temperature can be raised to 30°C; this concurs with other published work. In a trial in Canada, a mill was heated to c. 50°C and DE treatments were evaluated on the floor surfaces (Dowdy & Fields, 2002). A DE dose of 0.31 g/m^2 was considered to give no advantage over heating alone, in contrast to a dose of 1 – 2 g/m^2, which was considered appropriate in their previous trial. Given that DEs are known to provide long periods of protection and since good control was shown at 25°C, these treatments would also give added value by continuing to prevent large populations building up in these hidden pockets after the heat treatment.

Concurrent work within the larger LINK project also compared the suitability of different dusters for this application. Initially, three different dust applicators were evaluated when used to treat a steel structure (Cook *et al.*, 2003). An electric powered duster proved too powerful for targeted treatment and the hand-operated model was very flexible since the speed of treatment could be easily adjusted to aid gentler application to vertical sides. The smaller gas powered applicator was deemed best suited for treating small areas and the narrow nozzle and extendable lance made it useful for treating less accessible 'dead-spaces'. This latter device, the 'GPS Gaspot' CO_2 powered duster (Killgerm Chemicals Ltd, West Yorkshire, UK) was further evaluated in two subsequent mill treatments, as a proof-of-principle validation. In the first trial (2002), DE was applied to the dead space under 3 rows of flour rollers that had a history of infestation from beetles and moths. Holes were pre-drilled into the wooden plinths at c. 2.5 m intervals. DE was applied at either 5, 10 or 20 g/m^2. Heating was only to 24.6 °C, since the main form of disinfestation in this trial was by the novel fumigant, sulphuryl difluoride. The gas powered duster, proved easy to use and penetrated into the space readily. The effect of the DE could not be quantified since no before and after assessments were made on the populations. However a year later, the mill owner has confirmed that these areas have not been re-infested, suggesting the DE is giving good residual protection. In the second trial (2003), a flour mill was heated to a target temperature of 48°C. This time, the dead space was much more difficult to reach, consisting of a 5 mm gap between a 36m^2 chimney breast shaped wall lining and the wooden storage bin behind. Holes were again drilled at 2–3 m intervals and the dust injected as before. Despite the large area, and such a thin gap, the DE gave good penetration and was seen to blow out from injection points up to 3 m away.

In conclusion, the use of targeted DE treatments to supplement heat for UK mills is showing promise. Future studies need to include a wider range of insect species and investigate effects against different stages. Since there can be variation in efficacy between different DEs (Dowdy, 1999), these studies should also include other DE products. Finally, further laboratory studies should also be supported by more detailed quantitative field evaluations.

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REFERENCES

Cook D A; Armitage D M; (2002). Integrated pest management for stored grain in the UK incorporating diatomaceous earths to prevent surface infestations of insects and mites. In: *IOBC Bulletin, Proceedings of Integrated Protection of Stored Products*, Lisbon, Portugal. 2-5 September, 2001, eds C Adler, S Navarro, M Scholler & L Stengard-Hansen, **25 (3)**, 221-229.

Cook D A; Collins L E; Armitage D M (2003). Diatomaceous earth structural treatment against *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) under fluctuating UK conditions. In: *8th International Working Conference on Stored-products Protection*, York, UK 2002, eds P F Credland, D M Armitage, C H Bell, P M Cogan, & E Highley, pp. 675-679. CAB International, Wallingford, UK.

Dowdy A K (1999). Mortality of red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to high temperature and diatomaceous earth combinations. *Journal of Stored Products Research* **35**, 175 - 182.

Dowdy A K; Fields P G (2002). Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill. *Journal of Stored Products Research* **38**, 11 - 22.

Ebeling W (1971). Sorptive dusts for pest control. *Annual Review of Entomology*, **16**, 123-158.

Fields P; Dowdy A; Marcotte M (1997) Structural Pest Control: The use of an enhanced diatomaceous earth product combined with heat treatment for the control of insect pests in food processing facilities. *Report; Agriculture and Agri-food Canada/United States Department of Agriculture.* 25 pp.

Golob P (1997). Current status and future perspectives for inert dusts for control of stored product insects. *Journal of Stored Products Research* **33**, 69-79.

McLaughlin A (1994). Laboratory trials on desiccant dust insecticides. In: *6th International Working Conference on Stored-products Protection*, Canberra, Australia 1994, eds E Highley, E J Wright, H J Banks & B R Champ, **2**, 638-645. CAB International, Wallingford, UK.

Subramanyam B; Swanson C L; Madamanchi N; Norwood S (1994). Effectiveness of Insecto, a new diatomaceous earth formulation, in suppressing several stored-grain insect species. In: *6th International Working Conference on Stored-products Protection, Canberra, Australia 1994*, eds E Highley, E J Wright, H J Banks & B R Champ, **2**, 650-659.

Seed consumption by ground beetles

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ABSTRACT

Preferences and consumption rates in polyphagous ground beetles (Coleoptera: Carabidae) feeding on seeds of herbaceous plants were investigated in laboratory and field-based studies. The seeds were mounted into plasticine in small tin trays and exposed to beetle 'predation' at the ground surface. In laboratory studies, feeding preferences for the seeds of 64 species of herbaceous plants were influenced by ground beetle body size. The small *Harpalus affinis* (body mass 13 mg) preferred smaller seeds than the large *Pseudoophonus* (=*Harpalus*) *rufipes* (29 mg). In the field, seeds were exposed in stands of different crops. Vertebrate predation was excluded by using wire mesh cages and arthropod activity-density was assessed by pitfall trapping. Ground beetles were the only seed-eating predators captured, of which *H.affinis* and *P.rufipes* comprised an average of 78%. Within crop, rates of seed removal of six weed species varied with crop type, season, seed species and site. The field preferences (established in 43 species of seed) were subsequently correlated with the laboratory preferences. In the two ground beetle species studied, consumption of seeds on the ground in arable fields was estimated to be as high as 1000 seeds/m^2/day and may selectively influence the quantity of seed of particular herbaceous species that enter the soil seedbank. Therefore, seed 'predation' by seed-eating ground beetles may be an effective component of weed control on arable land, particularly at low densities of weed seed.

INTRODUCTION

Small seeds of herbaceous plants are eaten by both vertebrate and invertebrate predators (e.g. Westerman *et al.*, 2003). After ants, probably the most important seed eaters in the temperate zone, are ground beetles (Carabidae: Coleoptera). Although most species of field ground beetle accept animal prey, the adults and larvae of some species also eat plant materials (Johnson & Cameron, 1969).

Observations on ground beetle diet made in the field and laboratory have distinguished two categories of phytophagous ground beetles, those that are normally carnivorous but supplement their diets with the vegetative parts of plants, and those that mainly eat seeds (Brandmayr, 1990).

Although eating of seeds by ground beetles (granivory) was recorded more than 100 years ago (Forbes, 1883) and confirmed for adults (e.g. Goldschmidt & Toft, 1997) and larvae (Hurka, 1998), there are few studies of seed consumption in the field. Here we report parallel studies of seed preferences in laboratory and field tests, and seed consumption under field conditions.

MATERIAL AND METHODS

Seeds of broad-leaved herbaceous species, particularly weeds of agricultural crops, were collected in 1996 and stored at 24 – 26 °C and 40% relative humidity until required. *Harpalus affinis* and *Pseudoophonus* (= *Harpalus*) *rufipes* were collected in the field using pitfall traps (plastic cups 7 cm in diameter and 8 cm deep). Following collection, the ground beetles were stored at 5 – 7 °C for 1 – 4 days, then used in laboratory-based feeding preference experiments with seeds of 64 species of plant (Honek *et al*., in press). The tests were made in Petri dishes (250 mm diameter; 50 mm deep) containing a 2 cm layer of sieved soil, a piece of moist cotton wool and 10 seed trays, each tray containing the seed of a different species. The trays and the arrangement of seed were identical to those described in the field experimentation below. In the laboratory experiments using *P. rufipes*, one of the 10 trays contained the seeds of *Cirsium arvense* as a 'reference species'. The other seed species were presented in seven groups, each group consisting of nine seed species. Each seed combination was replicated three times. The removal of seed from each tray was recorded daily, for seven days, without replacing seed. In laboratory tests with *H. affinis*, the reference weed species was *Capsella bursa-pastoris*. We subsequently calculated the time taken in removal of 50% of the offered seeds (CT_{50}).

Field experiments on seed removal were made at Praha-Ruzyne (50° 06′ N; 14° 16′E; 350 m altitude), in crops of winter wheat, winter oilseed rape, soybean, maize, millet and in abandoned arable land. To assess field 'predation' by ground beetles, the seeds were exposed in tin trays (28 mm diameter; 6 mm deep) filled with white plasticine (JOVI®, Barcelona). Each tray contained 15 or 30 seeds. The trays were placed on the ground so that the plasticine surface and seeds were level with the soil and exposed to 'predation' by beetles walking across the surface. The trays were exposed in groups of six and each group was covered with a 18 × 18 × 9 cm wire mesh cage (9 mm mesh), whose sides were let into the soil. Seasonal and between-crop variation in seed removal rates were studied by exposing seeds of *C. bursa-pastoris, C.arvense, Descurainia sophia, Lepidium ruderale, Sisymbrium loeselii* and *Taraxacum officinale*. Four, six or eight cage assemblies were placed simultaneously in a crop and exposed to seed 'predation' for different periods of time. Nine such assemblies were exposed during July 19 – September 14, 1999 and 32 assemblies were exposed during April 18 – October 11, 2000. The length of exposure was 3 – 20 days, depending on the intensity of seed removal. In the seed preference experiments, seeds of 46 species of herbaceous plants were exposed during July 14 – 22, 1999 in a crop of winter wheat; during August 5 – 18, 1999 in a crop of millet, and during July 30 – August 10, 2000 in a crop of soybeans (Honek *et al*., in press).

Ground beetles were collected in the seed exposure field experiments to reveal their activity-density around the cages where seeds were exposed. One or two pitfall traps were placed within 1 m distance of each cage. The traps were emptied at two or three day intervals. The beetles were determined to species, counted and immediately released in the same vicinity.

In the field experiments, we calculated the rates of seed removal (no. seeds/day/trap). Differences in seed removal rate were related to seed species or position of the exposed seeds within the crop. The differences were tested using one-way analysis of variance (ANOVA). The combined effects of seed species and cage position were tested by analysis of covariance (ANCOVA), with cage and seed species as factors, average seed consumption in each particular period of seed exposure as covariate (to compensate for changes of 'predation' in time), and seed removal from particular trays as the response variable.

To compare the field and laboratory preferences, the field results of different experiments were expressed as a fraction of the difference between the rejected (the least consumed) and preferred (the most consumed) of the seeds. Laboratory preferences were calculated as arithmetic means of the CT_{50} values for *P. rufipes* and *H. affinis*. The preference for each kind of seed was expressed as a fraction of the difference between the most rejected (longest CT_{50}) and the most preferred (shortest CT_{50}) seed species. The standardised preferences, calculated from seed consumption rate and CT_{50}, then fell between 0 (rejected) and 1 (preferred).

RESULTS

In the laboratory experiments, both ground beetle species preferred particular species of seed. A large proportion of the variation in ground beetle preferences was explained by variation in seed size. *P. rufipes* readily consumed medium sized seeds (calculated preferred seed size was 1.00 mg) but was more reluctant to eat the smaller and larger seeds. *H. affinis* preferred smaller seeds (0.32 mg) than *P. rufipes*.

Table 1. The effect of the position of the experimental seed in the crop ('cage'), and seed species, on the rate of seed removal in crops of winter wheat (June 2 – July 28, 2000) and soybean (Aug. 8 – Oct. 11, 2000).

	Effect		Error			
	df	MS	df	MS	F	P
Winter wheat						
cage	3	20.838	215	6.414	3.249	0.0228
species	5	15.246	215	6.414	2.377	0.0399
cage × species	15	1.684	215	6.414	0.263	0.9977
Soybean						
cage	7	8.233	431	2.823	2.917	0.0054
species	5	22.873	431	2.823	8.103	0.0000
cage × species	35	1.684	431	2.823	0.597	0.9685

Table 2. The differences in overall weed (*C. bursa-pastoris, Lamium amplexicaule, Stellaria media, Veronica arvensis, Viola arvensis*) density (plants/m^2); overall seed-eating ground beetle (*Amara aenea, A. aulica, A. familiaris, A. littorea, A. similata, Calathus ambiguus, H. affinis, H. atratus, H. distinguendus, H. signaticornis, H. tardus, Ophonus azureus, P. rufipes*) activity-density (individuals/trap/day) and average seed removal rate (seeds/day) in crops of rape and wheat.

	Winter oilseed rape Mean±SE	Winter wheat Mean±SE
Weed density	92.9±14.7	0.0±0.0
Ground beetle activity-density	3.75±0.87	0.27±0.07
Seed removal rate	0.71±0.09	3.81±0.51

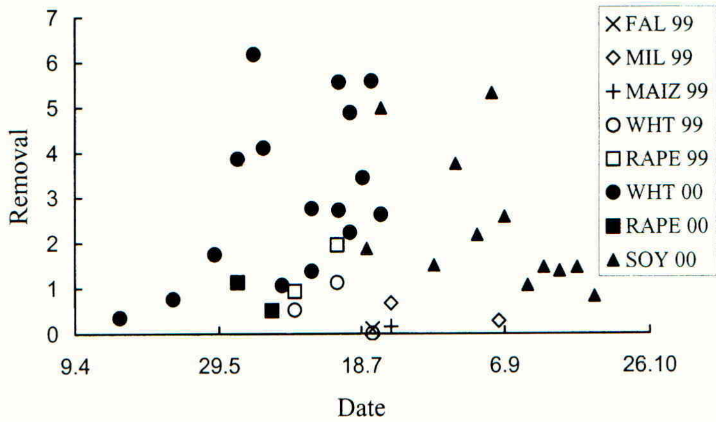

Figure 1. The average rate of seed removal (seeds/day/tray) from field cages containing seed of six herbaceous species of plant, placed in different crops (MAIZ – maize; MIL – millet; RAPE – winter oilseed rape; SOY – soybean; WHT – winter wheat) and a fallow field (FAL), in 1999 (99) and 2000 (00). Each point represents a mean value for a series of cages exposed for a period of 3 – 20 days. On the abscissa, the points are placed in the middle of the exposure period.

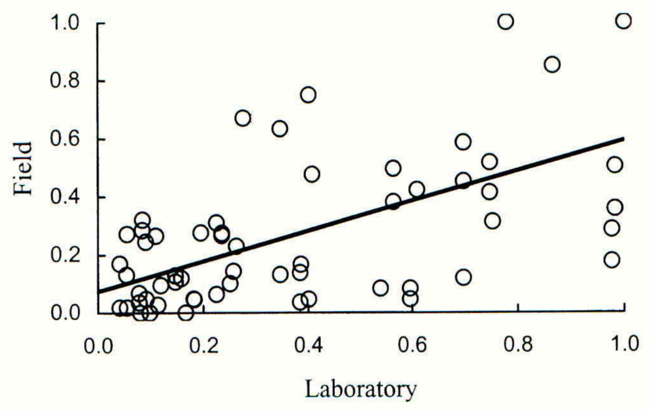

Figure 2. The relationship between standardised field preferences and standardised laboratory preferences for seeds of 42 weed species (some species are shown more than once because of their repeated exposure in the field). $R^2 = 34.85$ %, d.f.=58, $P < 0.001$.

In the field, the rates of removal of six test species of seed from the ground under different crops varied with season, crop, cage location within the crop, and seed species. There was a marked seasonal variation in the overall rate of seed removal (Figure 1). It was low (1.1±0.2 seeds/day) before Julian day 150 (May 29) and after Julian day 250 (September 6). In between these dates, the average rate of seed removal was significantly greater (2.5±0.3 seeds/day). The annual average was significantly greater in 2000 (3.2±0.4 seeds/day) than in 1999 (0.6±0.2 seeds/day). The seeds of different species were removed at different rates. The differences between rates of removal of different seeds were statistically significant in two of the three cases analysed (Table 1), where seed removal rates were high. Location of a cage within a field significantly affected seed removal rates. There were thus statistically significant differences in rates of seed removal between the microsites within crops.

Differences in seed removal rates parallel to the availability of naturally occurring seed were established during June 2 – 26, 2000 in adjacent crops of winter oilseed rape and winter wheat (Table 2). Weeds that produced seed at this time were present in winter rape but virtually absent in winter wheat. The seed eating ground beetles were accordingly more abundant in the winter rape than in the winter wheat. Despite the high activity-density of ground beetles in the winter rape, the rate of seed removal was lower than in the wheat, probably because the naturally occurring seed in the rape stand satiated the ground beetles.

Differences in removal of particular seed species were statistically significant despite the different times and crops where the experiments were replicated. The field and laboratory preferences for the seeds of particular herbaceous species were significantly ($P<0.001$) correlated (Figure 2). The scatter in the data increased with increasing preference. The increase in scatter means that seeds of species rejected in the laboratory were also rejected in the field but consumption of preferred seeds in the field and in the laboratory varied largely.

DISCUSSION

In the two harpaline species of ground beetle studied, the most important character determining the consumption of a particular kind of seed was its mass, which explained 25% of the variance in seed consumption by *P. rufipes* and 33 % variance in *H. affinis*. The high residual variation may be due to morphological and/or biochemical differences between the seeds, e.g. the thickness and/or consistency of the seed testa and the hardness of a seed's contents (cotyledons).

This study revealed that a high proportion of the small seed of herbaceous plants present on the ground in arable fields in central Europe may be eaten by invertebrates, specifically ground beetles. Pitfall traps revealed the activity-density of the arthropod fauna in the vicinity of the cages containing seed. Of the arthropods caught in the pitfall traps, only the ground beetles are likely to eat seed. Of other established seed-eating predators, ants (*Lasius* spp.) were scarce (<1% of the catches of adult ground beetles) and crickets were not present. Millipedes (*Blaniulus* spp.; *Polydesmus* spp.) and beetles (*Silpha* spp.), abundantly present in pitfall catches, did not eat seed (Honek, unpubl.). The importance of granivory for polyphagous ground beetles may be greater than previously assumed (Thiele, 1977). It was not possible to decide what proportion of the seeds was removed by ground beetle larvae. The larvae were rare in pitfall catches but these traps underestimate their densities (Adis, 1979). However, in parallel laboratory experiments, larvae of several *Amara* species were unable to remove the seeds mounted on plasticine (Pavel Saska, pers. comm.). However, there were significant correlations between the rates of adult ground beetles trapped and seed removal rates (Honek *et al.*, in press), and between adult preferences for certain seeds in the laboratory and in the field. The preferences were similar because *H. affinis* and *P. rufipes* were the dominant species and represented, at the time of field preference experiments, 56 – 99 percent of all seed eating ground beetles.

Our data may be used to estimate seed removal in the field. A minimum estimate is that obtained for the winter rape crop in 2000, where seeds produced by natural weed stands were available in excess. In this case, the ground beetles were satiated, so finding experimental seeds was as easy as finding naturally occurring seeds and both kinds of seed were probably removed at similar rates. From the area of the trays (6.2 cm^2) and the daily removal rates (0.7 seeds/tray) the daily removal rate can be calculated and is c. 1150 seeds/m^2. This figure may decrease when the activity-density of seed eating ground beetles decreases; in our case it was rather high at 3.7 individuals/trap/day. On the other hand, consumption rates may increase along with increase in ground beetle hunger.

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REFERENCES

Adis J (1979). Problems of interpreting arthropod sampling with pitfall traps. *Zoologischer Anzeiger* **20**, 177-184.

Brandmayr T Z (1990). Spermophagous (seed eating) ground beetles: First comparison of the diet and ecology of the Harpaline genera Harpalus and Ophonus (Col., Carabidae). *The Role of Ground Beetles in Ecological and Environmental Studies*, ed. NE Stork, pp. 307-316. Intercept: Andover.

Forbes S A (1883). The food relationships of the Carabidae and Coccinellidae. *Illinois State Lababoratory of Natural History Bulletin* **1**, 33-64.

Goldschmidt H; Toft S (1997). Variable degrees of granivory and phytophagy in insectivorous carabid beetles. *Pedobiologia* **41**, 521-525.

Hurka K (1998). Larval taxonomy, development and diet of Amara (Amara) famelica, A. (A.) littorea and A. (A.) proxima (Coleoptera: Carabidae: Amarina). *Acta Societatis Zoologicae Bohemohemicae* **62**, 105-113.

Johnson N & Cameron R S (1969). Phytophagous ground beetles. *Annals of the Entomolomological Society of America* **62**, 909-914.

Thiele H U (1977). *Carabid Beetles in Their Environments*. Springer: Berlin.

Westerman P; Hofman A; Vet L E M; van der Werf W (2003). Relative importance of vertebrates and invertebrates in epigaeic weed seed predation in organic cereal field. *Agriculture Ecosystosystems and Environment* **95**, 417-425.

Reduction of invertebrate contamination of salad crops using directed airstreams

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ABSTRACT

Directed airstreams, at velocities up to 50 m/sec, may remove up to 61% of potential contaminant invertebrates from baby-leaf salad crops in the field at the point of harvest without damaging the crop. Crop type has an influence on efficiency, invertebrates being most difficult to remove from densely-sown crops with a convoluted leaf form.

INTRODUCTION

Processed 'ready-to-eat' bagged fresh salad products continue to increase in popularity with consumers. The quality standard required of such products by retailers and consumers is very high, but there is a consistent problem with foreign body contaminants, including stones, twigs, weed leaves and invertebrates, which results in a significant number of customer complaints (50 contaminants in 1,000,000 packs of salad is considered unacceptable). Many of the invertebrate contaminants are not strictly pests in that they do not feed on the crop plants – but they are common in the field environment. These have come to be known as 'casual intruders' or 'vagrants'.

Management of casual intruders is problematic because of the very wide range of invertebrates involved and their transient nature in the crop. Nonetheless, they only become a problem when they become ensnared in the harvested produce. Many are probably removed during the washing, sorting and packing process but a few remain to act as a source of complaints. Contamination rates can only be reduced by ensuring that fewer foreign bodies are harvested with the produce and/or by improving the efficiency with which they are removed during processing.

Insecticides can be important tools in the management of pest species, but are less appropriate for the management of casual intruders. This work was done in order to test the hypothesis that mechanical removal of invertebrates from a crop at the point of harvest could significantly reduce the number of potential contaminants in the harvested produce. Machines have previously been devised that were intended to remove and destroy pests from crops (Boiteau *et al.*, 1992; Khelifi *et al.*, 1995; Pickel *et al.*, 1994; Walklate, 1994; Weintraub *et al.*, 1996; Weintraub *et al.*, 1999), but none have previously been intended simply to flush invertebrates whilst harvesting takes place.

MATERIALS AND METHODS

There were two phases to the work.

1. Design and construction of a test-rig capable of producing airstreams that could be directed at or through baby-leaf salad crops growing in the field.
2. Field-testing of the rig in commercial crops.

Rig design & construction

The basis of the rig was a frame welded from rectangular-section steel tubing. This was supported on two pneumatic-tyred, 200mm diameter, implement wheels and was guided by a pair of castor wheels, also pneumatic-tyred but smaller. Both sets of wheels were adjustable along stub axles so that the rig could be made compatible with the wheeling widths used on different commercial farms, which do not use standardised widths for the beds on which they grow their baby-leaf salad crops. Two T-handles were fitted so that the machine could be pushed and steered simultaneously by two operators walking in the wheelings.

Airflow was supplied by a pair of garden leaf-blower machines (McCulloch Ltd) powered by two-stroke petrol engines. The outputs from these machines were both taken into a fabricated steel manifold that had eleven 38 mm cylindrical outlets. Baffle plates were introduced into the manifold in such a way that the airflow in all of the outlets was as far as possible equalised. From the outlets the air was directed to a pulsing device via 38 mm diameter reinforced flexible plastic ducting. This pulsing device consisted of a box containing a vane rotated at approximately 60 rev/min by an electric motor. Rotation of this vane temporarily interrupted the flow of air from the outlets at each revolution, producing a pulsed airflow through each of the eleven outlets. This airflow was then taken via more ducting to final output jets made from plumbers' copper fittings. A variety of interchangeable final outlets was designed – 22 mm circular, 15 mm circular and flat fan, the latter made from partially-flattened 15 mm copper tube. These were adjustable for height, angle and separation.

Airflow through these outlets was measured using a vane anemometer and was found to reach speeds of up to 50 m/second (112 m.p.h.), depending on nozzle configuration.

Field testing in commercial crops

The rig was used to test the effect of airstreams on invertebrates in six experiments on four commercial holdings in the south and east of England (Table 1). Work was done exclusively in baby-leaf salad crops, including spinach, red chard, lollo rosso lettuce and red oak-leaf lettuce. These crops are typically grown in multiple close rows (between 18 and 22 rows, with as little as 60 mm separation, on raised beds 1.2 – 1.6 m wide) and are normally only 4 – 6 weeks old when cut, so lack the structure of more mature plants.

The same methodology was used in each experiment. The wheels of the rig were first adjusted on the stub axles so that they would run down the centre of the wheelings at the test site. The selected nozzles were then fitted to the outlets and their separation adjusted so that they would pass between the rows of crop. The height and angle of the nozzles were then adjusted. Preliminary trials had shown that the best set-up was to position the nozzles below crop height and angled slightly upwards, so that the airstreams from them would tend to lift invertebrates (and other debris) up and out of the crop. In spinach, red chard and oak-leaf lettuce this was appropriate, since the airstreams tended to part the crop and allow the nozzles to pass through without causing crop damage. Lollo rosso lettuce however has to be drilled densely, otherwise it tends to have a sprawling habit which makes it difficult to harvest. In the tests on this crop the nozzles had to be positioned just above crop height in order to avoid unacceptable levels of crop damage.

Table 1. Location of experiments on the efficacy of the blower.

Trial ref.	Date	Site	Crop	Nozzle type	Plot length (m)	No. of blocks
A	25/06/02	Deal, Kent	Spinach	22 mm round	10	12
B	26/06/02	Deal, Kent	Spinach	22 mm round	8	13
C	16/07/02	New Alresford, Hants	Lollo rosso	15 mm round	8	13
D	16/07/02	New Alresford, Hants	Lollo rosso	Flat fan	8	13
E	17/07/02	Pallington, Dorset	Red chard	15 mm round	5	13
F	12/09/02	Shouldham Thorpe, Norfolk	Red oak-leaf lettuce	15 mm round	5	13

Experiment design

Each experiment was done in a single baby leaf salad bed. There were only two treatments in each experiment:

1. Pushing the blower the length of the plot with the motors running and therefore producing an airstream through the crop.
2. Pushing the blower the length of the plot with the motors switched off.

To ensure sufficient degrees of freedom to facilitate viable statistical analysis of the data, a minimum of 12 blocks, each consisting of two randomised treatments, was used. The bed of salad crop chosen for an experiment was marked out into 24 (12 blocks) or 26 (13 blocks) equal-length plots. The length of a single plot varied at different sites depending on total length of a bed. The longest used was 10 m, the shortest 5 m. All plots in any one experiment were the same length.

Assessments

In each experiment, the insect fauna present in the salad crop immediately after the passage of the blower was sampled using a D-vac vacuum device (Dietrick, 1961). This consists of a knapsack-mounted two-stroke engine driving an impeller that removes air from a large-diameter hose, producing a suction effect at the nozzle (which is approximately 35 cm diameter). A net stretched over the nozzle intercepts items picked up by the airstream entering the hose. In use, the nozzle is briefly pressed down over the crop before lifting and moving on. At least 10 such 'dabs' were used in sampling each plot and the number used per plot in each experiment was consistent. D-vac catches were transferred from the net to labelled plastic bags to be returned to the laboratory. There they were frozen to kill the invertebrates present before identifying, counting and recording them.

RESULTS AND DISCUSSION

The invertebrates found most commonly in the D-vac samples in the majority of the experiments were small flies (Diptera) under 3 mm long. In all of the experiments but one, these flies formed more than 90% of the catch, with spiders comprising the majority of the remainder. The exception was Experiment F, where small flies formed 70% of the catch, the bulk of the remainder being (in descending order) spiders, springtails and parasitic wasps. For the purpose of analysis, the total number of all invertebrates caught in the D-vac has been included.

The results of the six experiments with the blower fall into two distinct categories (Table 2). In three experiments (A, B and E), the number of potential contaminant invertebrates was reduced by 45 – 61% when the blower was in operation, compared to the number present when it was switched off. In the other three experiments (C, D and F) there was no reduction in the number of invertebrates when the blower was operating compared to when it was not. Since the blower machine and the techniques used in all six experiments were the same, it is most likely that the explanation for the observed differences in efficacy of the method are due to the nature of the crops on which the machine was operating.

Table 2. Mean number of invertebrates in D-vac samples at six experimental sites.

	Site & crop					
	A Spinach	B Spinach	C Lollo Rosso	D Lollo Rosso	E Red Chard	F Red Oak Leaf
Blower on	42.3	78.9	22.5	81.1	31.3	79.7
Blower off	104.3	142.9	25.4	67.5	81.3	90.2
F ratio	55.14	42.67	0.63	1.52	30.90	2.10
df	11	12	12	12	12	12
P	<0.001	<0.001	n.s	n.s.	<0.001	n.s.
LSD	37.27	42.06	-	-	38.68	-
P	<0.001	<0.001	-	-	<0.001	-

The three crops on which useful reductions in invertebrate contaminants were observed consisted of spinach (two crops) and red chard. Both of these crops are in the plant family Chenopodiaceae. These have relatively smooth, flat leaves on moderately long petioles. The young plants grown for salad leaf production have an upright and loose habit. These features of the plants seem to make them suitable for efficient operation of the blower. The nozzles of the machine can be set very low in these crops yet do not cause physical damage to the harvestable produce because the individual leaves are readily parted by the airstream coming from the nozzles. Airstreams produced by low-set, upward-angled nozzles seem to be particularly efficient in removing invertebrates and other debris (loose leaves, feathers, wind-blown downy seeds etc.) from the crop.

The crops in which the blower did not demonstrate a decontaminating effect were lollo rosso lettuce (two crops) and red oak-leaf lettuce. Lollo rosso lettuce, if left to grow normally, has a low, rosette-type growing habit. This would make it a difficult subject for mechanical harvest as a baby-leaf crop. The tendency, therefore, is to sow the rows of lollo rosso very closely so that adjacent plants crowd together and are thereby forced to grow vertically rather than horizontally. Mechanical harvesting becomes a more practical proposition as a result of this. However, when the blower was deployed in such a crop it was found that the plants were so densely packed that the airstreams could not part the crop and allow passage of the nozzles. Therefore, the nozzles could not be set low down without causing unacceptable levels of crop damage and they had to be set at crop height. In this position, the airstreams produced by the nozzles failed to penetrate the densely-packed plants very effectively and tended to knock escaping invertebrates back into the crop. Lollo rosso is also a curly-leaved lettuce whose form seems to 'hold' invertebrates when compared to spinach and chard. The red oak-leaf lettuce was neither as densely-sown as the lollo rosso nor as curly-leaved, but it had already been harvested twice and had been allowed to regrow by the time that it was used for the experiment and this had modified its habit, producing a relatively dense crop that was again difficult for the airstreams to penetrate and which was more prone to physical damage if the outlet nozzles of the test rig were set at a low level.

Although this did not specifically form part of the experimental work, it was possible to make some subjective assessments of the relative efficiency of the airstreams in removing certain types of insect from the crop. For instance, there was a small number of seven-spot ladybirds (*Coccinella septempunctata*) present in the spinach crops used for experiments A and B. There were too few of these to form a statistically viable sample, but it was possible to observe individuals as the blower passed through the area of crop on which they were sitting, and this confirmed that these insects were relatively difficult to remove using airstreams. Conversely, some other insects such as crane flies (Tipulidae), moths (Lepidoptera) and lacewings (Neuroptera), which were also present only in low numbers, seemed to be readily picked up by the airstreams and removed from the crop. The difference in susceptibility to airstream removal between ladybirds and the other insects may be the result of differences in their aerodynamic drag factors. Ladybirds are compact, smooth, shiny insects, flat on the underside and with appendages that are either relatively short (e.g. antennae) or can be hidden (e.g. legs, hind wings). Whilst sitting on a leaf their drag factor is relatively low and this gives them a reduced susceptibility to being entrained and transported by airstreams. Crane flies, moths and lacewings, however, have relatively long, exposed appendages that give them a high drag factor compared to ladybirds and this makes them much more susceptible to removal by airstreams. These latter insects are important contaminants because their long appendages become tangled with harvested produce and this makes them relatively difficult to remove during post-harvest processing.

CONCLUSIONS

This work has demonstrated that directed airstreams are capable of temporarily removing a large proportion of invertebrates from baby-leaf salad crops prior to harvest without causing physical damage to the marketable produce. However, crops with an upright habit and flat leaf form are much more suited to this approach than those with a recumbent habit and/or a convoluted leaf form. It may also be the case that some invertebrates are more readily removed from growing plant material by airstreams than others, as a result of differences in aerodynamic drag factors.

Whilst the blower machine used in these experiments was a test rig rather than a prototype, it should be relatively easy to develop a commercial machine to perform a similar task. Used in approprate crops, significant reductions in invertebrate contaminants in the harvested produce should result.

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REFERENCES

Boiteau G; Misener G C; Singh R P; Bernard G (1992). Evaluation of a vacuum collector for insect pest control in potato. *American Potato Journal* **69**, 157-166.

Dietrick E J (1961). An improved back pack motor fan for suction sampling of insect populations. *Journal of Economic Entomology* **54**, 394-395.

Khelifi M; Lague C; Lacasse B (1995). Potato plant damage caused by pneumatic removal of Colorado potato beetles. *Canadian Agricultural Engineering* **37**, 81-83.

Pickel C; Zalom F G; Walsh D B; Welch N C (1994). Efficacy of vacuum machines for *Lygus hesperus* (Hemiptera, Miridae) control in coastal California strawberries. *Journal of Economic Entomology* **87** (6), 1636-1640.

Walklate P J (1994). Aerodynamic methods for controlling insects. *Vine Weevil Workshop Conference Proceedings*, 6 June 1994, 1-6, Rochester, Kent, UK.

Weintraub P G; Arazi Y; Horowitz A R (1996). Management of insect pests in celery and potato crops by pneumatic removal. *Crop Protection* **15** (8), 763-769.

Weintraub P G; Horowitz A R (1999). Management of the whitefly *Bemisia tabaci* (Genn.) on melon by vacuum removal. *Insect Science and Its Application* **19** (2/3), 173-178.

Use of *Salix* genotype mixtures for the control of rust in short rotation coppice willow

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ABSTRACT

Twenty genotypes of willow (*Salix*) were grown in mono-plots and as part of five, ten, fifteen and twenty-way mixtures. Rust (*Melampsora epitea*) development was recorded on each genotype, whether growing in mono-plots or as a component of a mixture, throughout six growing seasons, 1996–2001. Three genotypes, *S. schwerinii* × *viminalis* × *dasyclados* 'V7531', *S. schwerinii* × *aquatica* 'V7533' and *S. viminalis* 'Gigantea' remained virtually rust free every year. When rust susceptible genotypes were included in mixtures the general trend was for the first appearance of rust to be delayed and for the cumulative rust score towards the end of the growing season to be significantly reduced. Diverse willow mixtures offer the greatest opportunity to reduce the impact of rust disease on short rotation coppice (SRC) willow and maintain the sustainability of the plantation over its 25- to 30-year life.

INTRODUCTION

Many of the willow (*Salix*) genotypes grown in short rotation coppice (SRC), for biomass, as a renewable energy source, are susceptible to rust caused by *Melampsora epitea*. Rust reduces the growth and consequently the yield of infected plants. On some occasions, particularly when grown in mono-plots, infections may be so severe and occur so early in the season that they result in heavy premature leaf fall, followed by infections of the stems by secondary pathogens, resulting in major stool death and almost total crop loss (McCracken & Dawson, 1992). A number of new *Salix* genotypes become commercially available each year, from breeding programmes in Sweden and the UK. These tend to have a relatively high level of rust resistance. However, experience in N. Ireland, where the disease pressures are particularly high, has shown that previously resistant genotypes have become susceptible after eight to ten years, especially when they have been grown in mono-plots (Dawson & McCracken, 1994). The predicted life of a SRC willow plantation for biomass is up to thirty years.

SRC willow rust can be controlled by the intensive use of fungicides (Dawson & McCracken, 1994). Application of chemicals is, however, not a viable disease control strategy. Chemicals represent high cost, relative to the low return from the crop. Furthermore, the intensive use of agrochemicals on a crop, which is being grown as a 'green' source of renewable energy, is unacceptable. It was therefore essential to develop a lost cost, but effective disease control strategy that protected the crop from the effects of rust and ensured the sustainability of plantations over their thirty-year life.

Varietal mixtures have been used successfully to control rust and powdery mildew in cereals (Wolfe, 1985). It is vital that the components of a cereals mixture are compatible in terms of factors such as maturation date, end use etc. No such characteristics affect willow where the end product is wood. The trials described in this paper investigate the effectiveness of genotype mixtures containing different numbers of willow components, in reducing the impact of rust

MATERIALS AND METHODS

Twenty *Salix* genotypes (Table 1) were planted in large mono-plots or incorporated into random intimate mixtures containing five, ten, fifteen or twenty components. All plots were cut back at the end of their establishment year and hence in 1996 were in their first year of regrowth after coppicing. The planting and design of the trial are described in detail elsewhere (McCracken *et al.*, 2001). In each growing season, leaf samples were taken from all genotypes growing in mono-plots or from where they appeared in a mixture. Sampling began in mid-May and continued at fortnightly intervals until mid-September. Leaves were assessed for rust, a cumulative rust score calculated (McCracken & Dawson, 1992) and disease progress curves constructed. This was carried out in each of the six years 1996–2001.

Table 1. Twenty *Salix* genotypes planted in mono-plots and incorporated into five, ten, fifteen or twenty-way random intimate mixtures.

Included in 5, 10, 15, 20-way mixtures	**Included in 15, 20-way mixtures**
S. burjatica 'Germany'	*S. viminalis* × *caprea* 'V789'
S. mollissima-undulata 'SQ83'	*S. viminalis* '77683'
S. dasyclados × *aquatica* 'V7511'	*S. viminalis* '78101'
S. viminalis '77082'	*S. viminalis* '78195'
S. dasyclados × *caprea* 'V794'	*S. schwerinii* × *aquatica* 'V7534'
Included in 10, 15, 20-way mixtures	**Included in 20-way mixture**
S. viminalis × *aquatica* 'V7503'	*S. viminalis* '77699'
S. viminalis '78118'	*S. viminalis* 'Gigantea'
S. viminalis '78183'	*S. viminalis* 'Gustav'
S. schwerinii × *viminalis* × *dasyclados* 'V7531'	*S. schwerinii* × *aquatica* 'V7533'
S. viminalis '870146 ULV'	*S. viminalis* '870082 ORM'

Comparisons were made between the levels of rust, on the sampling date closest to 14th September, on individual *Salix* genotypes grown in mono-plots and when they were incorporated into a mixture, although only the differences from the 20-way mixture are presented. These differences were subjected to Analysis of Variance within each year, making it impossible to make statistical comparisons between years. The number of days, from 1st January, until the first record of rust was also calculated. The differences between days till first rust on genotypes growing in mixtures and mono plots were calculated. These were meaned across all six years, and analysed using years as replicates.

RESULTS

Rust development in mono-plots

Three genotypes, *S. schwerinii* × *viminalis* × *dasyclados* 'V7531', *S. schwerinii* × *aquatica* 'V7533' and *S. viminalis* 'Gigantea' remained virtually free of rust throughout the six years of the trial. At most, only a few pustules were observed, usually towards the end of the growing season. In contrast, the level of rust on *S. dasyclados* × *caprea* 'V794', *S. dasyclados* × *aquatica* 'V7511', *S. viminalis* 'Gustav' and *S. burjatica* 'Germany' was relatively high in each of the six years. By comparing the rust scores on genotypes growing in mono-plots in each of the six years it was possible to construct a list of the relative susceptibilities of the twenty genotypes included in the trial (Table 2).

Table 2. Relative susceptibility to rust of the twenty *Salix* genotypes growing in mono-plots

Rank	Genotype	
1	V7531	Most resistant
2	Gigantea	↓
3	V7533	
4	V7534	
5	78195	
6	77683	
7	ORM	
8	V7503	
9	77699	
10	ULV	
11	78101	
12	77082	
13	78118	
14	78183	
15	V789	
16	SQ83	
17	Germany	
18	Gustav	
19	V7511	
20	V794	Most susceptible

Effect of mixtures on days to first observation of rust

When meaned across the six years, in most cases the time to the first appearance of rust was delayed when genotypes were incorporated into the twenty-way mixture (Figure 1). The differences were significant for genotypes, *S. mollissima-undulata* 'SQ83', *S. viminalis* × *aquatica* 'V7503' and *S. viminalis* × *caprea* 'V789'. For genotypes, *S. schwerinii* × *viminalis* × *dasyclados* 'V7531', *S. viminalis* 'Gigantea', *S. viminalis* '78101' and *S. viminalis* '78195' the first appearance of rust was apparently later in mono-plots i.e. positive figure (Figure 1). It should be noted however that in these genotypes, in particular, the levels of rust were very low. Similar trends were observed when *Salix* genotypes were incorporated into mixtures containing fewer components (data not presented).

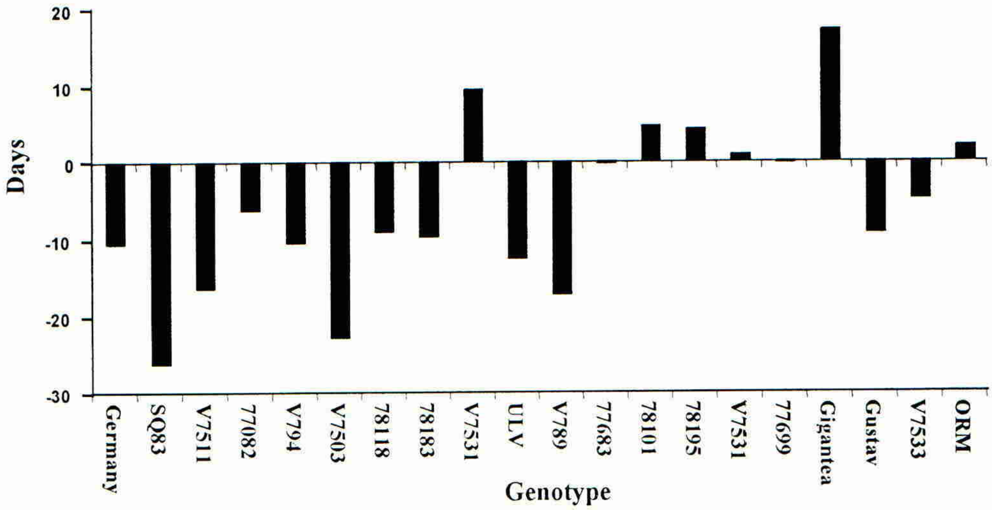

Figure 1. Differences in the days to first record of rust, meaned across six years, 1996–2001, on twenty *Salix* genotypes when grown in mono-plots or incorporated into twenty-way mixtures. NB a negative bar indicates a delay. The line represents the Least Significant Difference at $P = 0.05$.

Effect of mixtures on rust development

For six genotypes, *S. schwerinii* × *viminalis* × *dasyclados* 'V7531', *S. viminalis* '77683', *S. viminalis* '78195', *S. schwerinii* × *aquatica* 'V7534', *S. viminalis* 'Gigantea' and *S. schwerinii* × *aquatica* 'V7533' the level of rust was so low throughout the season that no significant differences were observed between rust score in mono-plots or any of the mixtures. Inclusion of rust susceptible genotypes in the twenty-way mixture consistently resulted in reduced rust scores towards the end of the season (14th September). Of those genotypes included in the five, ten, fifteen and twenty way mixtures only *S. viminalis* '77082' had higher levels of rust in two years, when growing in any of the mixtures compared to the mono-plots (Figure 2). For the remaining eleven genotypes the effect of inclusion in mixtures was variable. However for most there was less rust on plants in mixtures compared to mono-plots the years following coppicing, 1996 and 1999 (Table 3).

In general, with the exception of the five-way mixture, these trends of reduced rust scores were observed in all of the mixtures (data not presented).

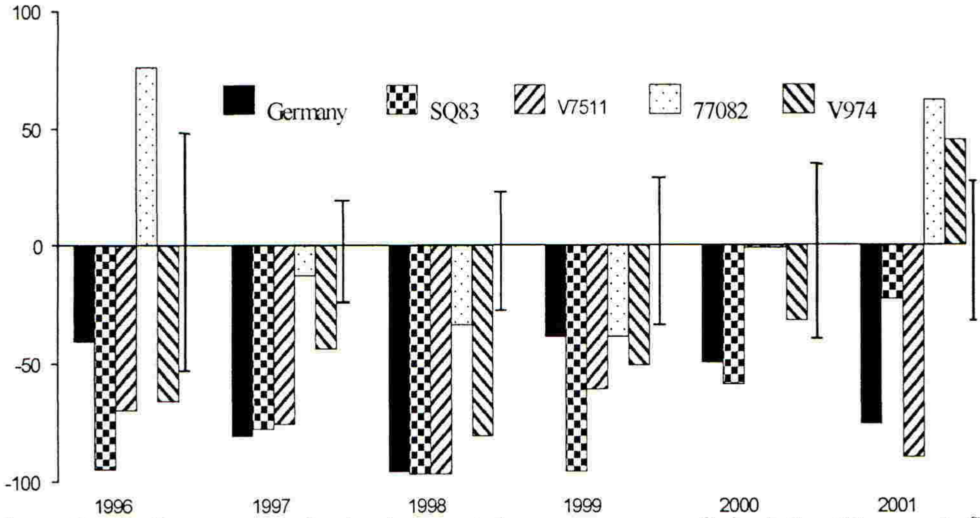

Figure 2. Percentage reduction in cumulative rust scores on *S. burjatica* 'Germany', *S. mollissima-undulata* 'SQ83', *S. dasyclados* × *aquatica* 'V7511', *S. viminalis* '77082' and *S. dasyclados* × *caprea* 'V794' when incorporated into twenty-way mixture, compared to mono-plots. (Bars represent LSD: $P = 0.05$, within years)

Table 3. Percentage reduction in cumulative rust scores on eleven *Salix* genotypes growing in 20-way mixture compared to growing in mono-plots.

Genotype / Year	1996	1997	1998	1999	2000	2001
S. viminalis × *aquatica* 'V7503'	-56	-94	-81	-49	-99	-72
S. viminalis '78118'	-42	10	-25	44	103	18
S. viminalis '78183'	-58	51	-57	-62	-17	-20
S. viminalis '870146 ULV'	-56	-21	30	-51	-20	157
S. viminalis × *caprea* 'V789'	-80	-56	-35	-84	-94	-57
S. viminalis '78101'	-67	175	26	254	15	-46
S. viminalis '77699'	-21	175	6	-34	-21	180
S. viminalis 'Gustav'	-63	101	-4	-26	-1	-51
S. viminalis '870082 ORM'	-52	267	195	-34	135	6
LSD ($P = 0.05$)	69.7	208.9	152.3	108.6	131.8	197.5

DISCUSSION

When SRC willow is grown in mixtures, the impact of *Melampsora epitea* rust is dramatically reduced. The onset of rust on moderately to highly susceptible genotypes incorporated into mixtures is delayed, often by two to three weeks. In addition, the cumulative rust score towards the end of the growing season on such genotypes is similarly significantly reduced. The yield from genotypes included in mixtures was normally higher than when they were grown in monoculture (McCracken *et al.*, 2001). This was due, at least in part, to the delay in onset of disease, along with reduced disease levels. In some cases however, when disease pressure was high on individual genotypes e.g. *S. burjatica* 'Germany', *S. dasyclados* × *aquatica* 'V7511' and *S. dasyclados* × *caprea* 'V794' they have largely died out of mixtures and made little contribution to the final three year harvest yield (McCracken & Dawson, 2001).

Diverse mixtures of predominantly rust resistant *Salix* genotypes provide the best opportunity for sustainability of SRC plantations over 25–30 years. Where individual components of mixtures have been severely affected by rust the remaining components within a mixture compensate for their loss (McCracken & Dawson, 2001). In a mixture, the selection pressures on resistant genotypes should be significantly lessened, to the extent that resistance should not breakdown, as has happened in genotypes growing in monoculture (McCracken & Dawson, 1994). Concern has been expressed that in multiclonal plantations where there may be increased diversity of *Melampsora epitea* there is the potential for the development of 'super-races' or 'super-pathotypes' which may attack a wider range of *Salix* genotypes. Recent work carried out in N. Ireland has indicated that in the cool maritime climate typically found in western Ireland the sexual phase of the pathogen's life cycle was not obligatory and that populations were more stable than in other parts of Europe (Samils *et al.*, 2003).

Much of the new SRC willow planting material becoming available from European breeding programmes are *S. viminalis* or *S. viminalis* hybrids. It is not clear therefore, whether a mixture comprised entirely of *S. viminalis* or *S. viminalis* hybrids would behave in a similar way to more diverse mixtures. Preliminary results from a trial at the Northern Ireland Horticulture and Plant Breeding Station have indicated that these more uniform mixtures are still effective in reducing the impact of rust (McCracken & Dawson, 2001).

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REFERENCES

Dawson W M; McCracken A R (1994). Effect of *Melampsora* rust on the growth and development of *Salix burjatica* 'Korso' in Northern Ireland. *European Journal of Forest Pathology* **24**, 32-39.

McCracken A R; Dawson W M (1992). Clonal response in *Salix* to *Melampsora* rusts in short rotation coppice plantations. *European Journal of Forest Pathology* **22**, 19-28.

McCracken A R; Dawson W M (2001). Disease effects in mixed varietal plantations of willow. *Aspects of Biology* **65**, 255-262.

McCracken A R; Dawson W M; Bowden G (2001). Yield response of willow (*Salix*) grown in mixture in short rotation coppice (SRC). *Biomass and Bioenergy* **21**, 311-319.

Samils B; McCracken A R; Dawson W M; Gullberg U (2003). Host-specific genetic composition of *Melamposra larici-epitea* populations on two *Salix viminalis* varieties in a mixture trial. *European Journal of Plant Pathology* **109**, 183 –190.

Wolfe M S (1985). The current status and prospects of multilane cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology* **23**, 251-273.

Integrated biological control of powdery mildew and grey mould of cucumber and tomato using *Brevibacillus brevis* combinations

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ABSTRACT

Powdery mildew of cucumber caused by *Sphaerotheca fusca* and grey mould (*Botrytis cinerea*) of tomato, two important diseases of greenhouse crops, were tested for suppression of disease using integrated biological control. *Brevibacillus brevis* wild type (WT), a bacterial spore-former and well established biological control agent (BCA) with broad spectrum activity, was used as the main BCA for both disease systems. All agents were tested singly and in combination and also included: i) a plant extract of *Reynoutria sachalinensis* that induces resistance mainly against powdery mildew and ii) *Pseudomonas antimicrobica*, a non spore-forming bacterial BCA active against *B. cinerea*. *B. brevis* WT reduced disease by both fungal plant pathogens. Moreover, significant and heightened control of *S. fusca* was achieved using the *B. brevis* WT/*R. sachalinensis* combination in whole plant systems. *Ps. antimicrobica* also reduced disease incidence when tested using a detached tomato leaf bioassay system which was further enhanced, again significantly, when combined with *B. brevis* WT. These studies indicated that *B. brevis* is well suited to combination with other biological disease control agents to offer increased efficacy for integrated biological control.

INTRODUCTION

Sphaerotheca fusca (formerly *S. fuliginea*), the causative organism of powdery mildew of cucurbits and *Botrytis cinerea* (the causative agent of grey mould disease) are two of the most important aerial pathogens of cucumber and tomato respectively and are of particular importance in Mediterranean countries. Both pathogens cause severe yield reduction and serious economic losses (Elad *et al.*, 1996) and hence have been a target for alternative methods of disease control including biological control. Control of both pathogens has been achieved using individual biological control agents (BCAs) often characterised by a single mode of action (Seddon *et al.*, 1997). Single BCAs are limited by the possibility of resistance development in the pathogen, the range of environments in which they are active and lower efficacy of disease control. Integrated biological control against plant pathogens is a strategy which combines different BCAs and/or natural products with different modes of action, thus aiming at higher levels of disease suppression over a wider range of environmental conditions with reduced risk of resistance development (Seddon *et al.*, 2000).

Brevibacillus brevis wild type (WT), a well established BCA against a range of pathogens (Seddon *et al.*, 1997; Walker *et al.*, 1998) has been well characterised for the control of *B. cinerea* (Edwards *et al.*, 1994). It has two modes of action against this pathogen. It produces i) gramicidin S (GS), an antifungal metabolite which inhibits conidial germination and hyphal growth (Edwards & Seddon, 2001) and ii) a biosurfactant which reduces periods of leaf wetness that are supportive of infection by *B. cinerea* (Edwards *et al.*, 1994). Recent *in vitro* studies have shown that GS inhibited conidial germination of *S. fusca* to a level of 80-90 % and when *B. brevis* WT sporulating cultures (known to contain GS) were applied to young cucumber plants a 40 % reduction of disease intensity was achieved (Schmitt *et al.*, 1999).

In the work presented here, *B. brevis* WT was used in combination with i) a plant extract of *Reynoutria sachalinensis* against *S. fusca* of cucumber and ii) *Ps. antimicrobica* against *B. cinerea* on tomato. *R. sachalinensis* plant extract acts indirectly by inducing resistance in the plant against the invasive stage of powdery mildew (Daayf *et al.*, 1995) whereas *B. brevis* WT acts directly by inhibiting conidial germination (Edwards & Seddon, 2001) prior to invasion. Since two different stages of the life cycle of the pathogen are affected by this combination, a higher level of disease suppression is expected than when individual components are used. Similarly, for the control of *B. cinerea* on tomato plants, *B. brevis* WT was combined with *Ps. antimicrobica* which is known to inhibit *B. cinerea* conidial germination and germ tube extension (Walker *et al.*, 2001).

MATERIALS AND METHODS

Powdery mildew of cucumber

Cucumber plants cv. Chinesische Schlange were grown in multi-purpose compost (Levington, UK) in 10 cm diameter pots in a growth cabinet (23°C, 70 % r.h., 16 h light). A total of 24 plants was used with 4 plants per treatment. The third true leaf of each plant was cut off after its development (approximately 25 d after sowing) to enhance the growth of the remaining two leaves, and one day later the plants were transferred to a growth room (25°C, 70 % r.h., 16 h light). *S. fusca* conidia were harvested from two diseased plants with powdery mildew. The plants were dusted off, using an air compressor (Millipore®, XX5522050, U.S.A), in order that older, non-viable, conidia were removed. Two leaves from each plant were excised 24 h later and put in a beaker with 200 ml sterile distilled water (SDW) containing 0.0125 % (v/v) Tween 80 (Koch-Light Ltd, England) for 10-15 min. The suspended conidia, adjusted to a concentration of 1 x 10^4/ml, were sprayed onto the upper surface of 28 day old plants with an atomiser until total coverage of leaf surface was achieved (day 0 of infection). The BCA *B. brevis* WT and *R. sachalinensis* plant extract (Milsana VP 2002, Dr. Schaette AG, Germany) (0.05 % v/v aqueous solution) (Schuld *et al.*, 2002) were applied singly and in combination to the upper surfaces of the leaves until run off. *R. sachalinensis* plant extract was applied 3 d before inoculation with *S. fusca* to allow induced resistance to occur. *B. brevis* WT was cultivated in Tryptic Soy Broth [TSB (Sigma, UK)] at 37°C in an orbital incubator at 150 rev/min for 48 h. The culture was diluted 1:3 (final concentration 1.2 x 10^8 cfu/ml) with SDW and applied as above at 1, 8 and 15 d after the application of the pathogen or only once at 1 d after inoculation with the pathogen. On the first signs of disease (8 d after inoculation with the pathogen) measurements of the percentage leaf area covered by the pathogen were made at 2/3 d intervals until the 20th d after treatment. Data were analysed by Analysis of Variance (ANOVA) and Bonferroni Post hoc test [SPSS for Windows Release 11.0.1 (15 Nov 2001)].

Grey mould of tomato

For experiments with *B. cinerea*, leaves from tomato plants cv. Moneymaker grown for 25 d under the same conditions as described above for cucumber, were used in a detached tomato leaf bioassay (Tsomlexoglou *et al.*, 2000).

B. brevis WT and *Ps. antimicrobica* were cultivated as described above except that *Ps. antimicrobica* was grown at 28°C. The bacterial suspensions were diluted 1:100 with SDW (final concentrations: *B. brevis* WT, 5 x 10^6 cfu/ml; *Ps. antimicrobica*, 3 x 10^7 cfu/ml) prior to inoculation. The upper surfaces of freshly detached leaves were inoculated with 50 μl drops (Gilson pipetteman) containing bacterial suspension (*B. brevis* WT and *Ps. antimicrobica* individually and in combination), *B. cinerea* conidia (5 x 10^5/ml) and double strength glucose peptone broth [2 GPB (Oxoid, UK)] in the ratio 1:2:1. In all cases the two youngest leaves from each plant were taken and 6 replicates were made for each treatment (3-5 drops per leaf). Disease symptoms were evaluated daily for 7 d according to an 8-score index of symptoms from 0 = no symptom to 7 = rot exceeding droplet diameter by more than 3 mm (Tsomlexoglou *et al.*, 2000). Data from the detached tomato leaf bioassay were analysed using the Kruskal-Wallis H Test for more than two independent samples and the Mann-Whitney U Test for treatments in pairs [SPSS for Windows Release 11.0.1 (15 Nov 2001)].

RESULTS

Powdery mildew of cucumber

In the powdery mildew experiments, all the treatments resulted in a much lower percentage leaf area infected than those that were not treated (Figure 1). Indeed, significant differences were observed between the control and all other treatments ($P < 0.001$). When applied separately, both the *R. sachalinensis* plant extract, applied once before inoculation, and a single application of *B. brevis* WT, applied after inoculation, gave significant disease suppression of about 40-50 %. This level was further enhanced, to give 73 % disease control, when *B. brevis* WT was applied weekly. The combination of *R. sachalinensis* plant extract and *B. brevis* WT gave even better disease control than the above treatments especially when *R. sachalinensis* plant extract was combined with weekly applications of *B. brevis* WT where disease control approached 97 %. The single treatments of *R. sachalinensis* plant extract and that of *B. brevis* WT (1 application) differed significantly from all other treatments ($P < 0.001$) but not from each other ($P = 0.073$). There was no significant difference between the two combination treatments ($P > 0.999$). Also, the combination of *R. sachalinensis* plant extract and *B. brevis* WT (1 application of *B. brevis* WT) did not give significantly greater control than the treatment of *B. brevis* WT (weekly applications) ($P = 0.270$) although there was significant difference between *R. sachalinensis* plant extract combined with weekly applications of *B. brevis* WT and the treatment of *B. brevis* WT (weekly applications) ($P = 0.002$).

Grey mould of tomato

There was a significant difference ($P < 0.002$; Kruskal-Wallis H test) between all data values (which included control) from day 2 of the bioassay, with the control treatment having much higher median disease indices than any of those treated with BCAs (Figure 2). A significant difference in disease control was also found between the individual BCAs and their combination at 6d (P = 0.004, 0.015) (*B. brevis* WT and *Ps. antimicrobica* respectively) and at 7d (P = 0.002, 0.002) (Mann-Whitney U test). No significant difference was found between single treatments.

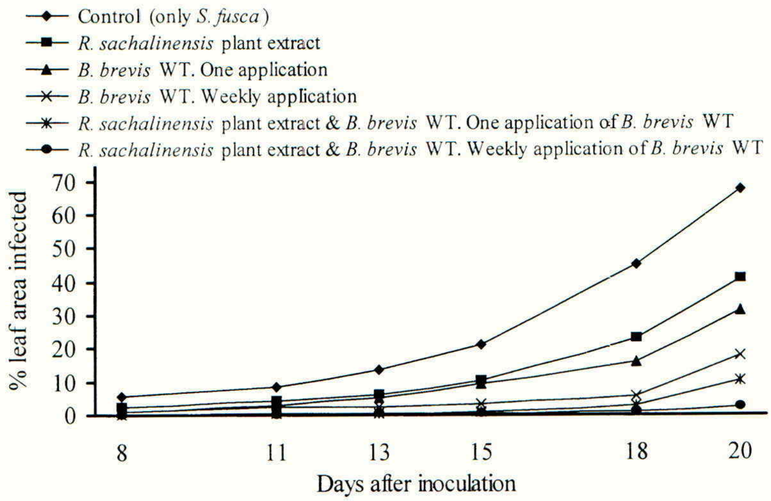

Figure 1. Effect of *B. brevis* WT, *R. sachalinensis* plant extract and combinations against *S. fusca* on young cucumber plants.

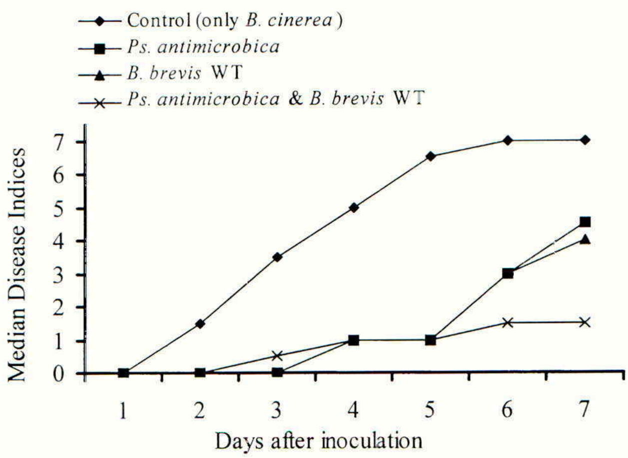

Figure 2. Effect of *B. brevis* WT, *Ps. antimicrobica* and combinations against grey mould disease on detached tomato leaves.

DISCUSSION

The economic losses caused by *S. fusca* and *B. cinerea* have led to the overuse of chemical fungicides resulting in environmental pollution as well as the development of resistance in the pathogens (Seddon *et al.*, 2000). Biological control is an alternative to chemical control with the main advantage being low/no impact on the environment hence offering sustainability. *B. brevis* WT is considered to be an important BCA against *B. cinerea* (Edwards *et al.*, 1994; Walker *et al.*, 1998) and *S. fusca* (Schmitt *et al.*, 1999) although low efficacy is occasionally observed when used alone. Since *B. brevis* WT has broad-spectrum activity (Seddon *et al.*, 2000) the potential exists for its combination with other BCAs with different modes of action in a range of crops for increased efficacy of biological disease control. Combination of *B. brevis* WT with *R. sachalinensis* plant extract was based on the different modes of action of each component with the objective of lowering the concentration of the individual BCAs used, yet still achieving high levels of disease control, thus lowering environmental impact. Indeed, in the work presented here, the two BCAs i.e. *B. brevis* WT and *R. sachalinensis* gave higher disease control against *S. fusca* when combined than when used singly. It may therefore be possible to reduce their concentration even more and still maintain good disease control. It is also of interest that the combined treatment gave a high level of disease control that was not significantly different when *B. brevis* WT was applied either once or at weekly intervals. With similar reasoning, the combination of *Ps. antimicrobica* with *B. brevis* WT used against *B. cinerea* also increased efficacy. This combination may also reduce the possibility of such a ubiquitous plant pathogen developing resistance to one of the BCAs as combined protection is afforded by more than one antifungal component. This study now needs to be extended to whole plants and, as with the powdery mildew work, to glasshouse trials.

Recent studies have shown *B. brevis* and *R. sachalinesis* plant extracts to be safe and harmless against beneficial insects (Schuld *et al.*, 2002) and their extended use for integrated pest management with insect biocontrol systems looks promising for use in glasshouses. There is concern over the use of *Ps. antimicrobica* since it has recently been reclassified as *Burkholderia gladioli*, some strains of which have been implicated in secondary infections in patients with cystic fibrosis and chronic granulomatuous disease (Coenye *et al.*, 2000). Consequently, the organism will not be used for further studies unless it can be clearly differentiated from these pathogenic strains with no potential harmful effects.

Results clearly show that an integrated approach to biological control using different combinations of agents is worthwhile as it offers advantages of increased disease protection and reduced usage of biocontrol agents. Further work is needed to explore whether, as predicted, this strategy will reduce resistance development and broaden the efficacy of biocontrol over a wider range of environmental conditions and plant pathogens. It would at least seem possible that *S. fusca* and *B. cinerea* may in the future both be controlled by suitable combination of *B. brevis* with other BCAs in a system of integrated biocontrol.

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REFERENCES

Coenye T; Gillis M; Vadamme P (2000). *Pseudomonas antimicrobica* Attafuah and Bradbury 1990 is a junior synonym of *Burkholderia gladioli* (Severini 1913) Yabuuchi et al. 1993. *International Journal of Systematic and Evolutionary Microbiology* **50,** 2135-2139.

Daayf F; Schmitt A; Bélanger R (1995). The effects of plant extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long English cucumber. *Plant Disease* **79,** 577-580.

Edwards S G; Seddon B (2001). Mode of antagonism of *Brevibacillus brevis* against *Botrytis cinerea in vitro*. *Journal of Applied Microbiology* **91**, 652-659.

Edwards S G; McKay T; Seddon B (1994). Interaction of *Bacillus* species with phytopathogenic fungi - methods of analysis and manipulation for biocontrol purposes. In: *Ecology of Plant Pathogens*, eds J P Blakeman & B Williamson, pp. 101-118. British Society for Plant Pathology, CAB International, Wallingford, UK.

Elad Y; Malathrakis N E; Dik A (1996). Biological control of *Botrytis* - incited diseases and powdery mildews in greenhouse crops. *Crop Protection* **15,** 229-240.

Schmitt A; Eiseman S; Mackay N; Seddon B (1999). Antifungal activity of Gramicidin S and use of *Bacillus brevis* for control of *Sphaerotheca fuliginea*. In: *Modern Fungicides and Antifungal Compounds* II, eds H Lyr; P E Russell; H-W Dehne & H D Sister, pp. 419-422. Intercept: Andover.

Schuld M; White D; Seddon B; Ernst A; Schmitt A (2002). Milsana and *Brevibacillus brevis* are harmless for the beneficial organisms *Typhlodromus pyri* (Acari, Phytoseiidae) and *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). In: *Modern Fungicides and Antifungal Compounds III,* eds H-W Dehne; U Gisi; K H Kuck; P E Russell & H Lyr, pp. 411-417. AgroConcept: Bonn.

Seddon B; Edwards S G; Markellou E; Malathrakis N E (1997). Bacterial antagonists-fungal pathogen interactions on the plant aerial surface. In: *Multitrophic Interactions in Terrestrial Systems*. The 36th Symposium of the British Ecological Society, eds A C Gange & V K Brown, pp. 3-25. Blackwell Science: London.

Seddon B; McHugh R C; Schmitt A; (2000). *Brevibacillus brevis* – a novel candidate biocontrol agent with broad spectrum antifungal activity. In: *Brighton Crop Protection Conference. Pests & Diseases,* Vol. 2, pp. 563-570. British Crop Protection Council: Surrey.

Tsomlexoglou E; Allan E J; Seddon B (2000). Biocontrol strategies for *Bacillus* antagonists to tomato grey mould (*Botrytis cinerea*) based on the mode of action and disease suppression. In: *Brighton Crop Protection Conference. Pests & Diseases,* Vol. 3, pp. 1037-1042. British Crop Protection Council: Surrey.

Walker R; Powell A A; Seddon B (1998). *Bacillus* isolates from the spermosphere of peas and dwarf French beans with antifungal activity against *Botrytis cinerea* and *Pythium* species. *Journal of Applied Microbiology* **84,** 791-801.

Walker R; Innes C M J; Allan E J (2001). The potential biocontrol agent *Pseudomonas antimicrobica* inhibits germination of conidia and outgrowth of *Botrytis cinerea*. *Letters in Applied Microbiology* **32,** 346-348.

Comparison of brassica tissues for control of soil-borne and tuber diseases *in vitro*

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ABSTRACT

Eleven brassica species/tissues were screened for control of four fungal pathogens; *Rhizoctonia solani*, *Fusarium solani*, *Phoma exigua* and *Gaeumannomyces graminis*. Different brassica species/tissues controlled individual pathogens. The tissues differed in their fungicidal activity, with root tissue, blanched and non-blanched rapeseed meal the most active, controlling pathogens by 40-85%. *G. graminis* was the most sensitive of the pathogens. Very high levels of brassica tissue were calculated as required (0.59-535 t) to give biofumigation in the field equivalent to that of commercial products.

INTRODUCTION

Incorporating of brassica tissues into the soil as green manures is known to reduce soil-borne diseases such as *Gaeumannomyces graminis*, the causal organism of take-all in cereals (Kirkegaard *et al.*, 1998). Control is achieved by hydrolysis of glucosinolates in the plant tissue by the enzyme myrosinase, to give toxic breakdown products including a number of isothiocyanates, many of which have fungicidal and insecticidal activity and can act as natural biofumigants. Brassica tissues used as green manures have primarily been from mustard plants although oilseed rape has been used. The aim of this paper is to report on the use of tissues from a number of brassica species, selected for their different glucosinolate profiles, to control soil-borne and tuber-borne diseases *in vitro*.

MATERIALS AND METHODS

Three brassica species, (*Brassica juncea* (brown mustard), *Sinapis alba* (white mustard) and winter oilseed rape (cv. Synergy and cv. Debut)) were grown in John Innes No. 2 compost, six plants per 50 cm diameter pot, in a polytunnel under ambient temperature and watered regularly. Pre-flowering (GS 3.5-4.0), the above and below ground parts of plants were removed, washed, frozen, freeze-dried, milled using a hand blender and the milled material stored at -4°C prior to use. Oilseed rape meal, cv. Express, was obtained from CETIOM, France. The meal was produced by three oil extraction methods: 1) direct, cold pressed extraction (non-blanched), 2) blanched and extracted, and 3) extruded extracted (Burghart & Evrard, 1999).

The fungal pathogens *Rhizoctonia solani* (black scurf/stem canker of potatoes), *Phoma exigua* var. *foveata* (gangrene of potatoes), *Fusarium solani* (dry rot of potatoes) and *Gaeumannomyces graminis* (take-all of cereals) were isolated from infected plant material and maintained on potato dextrose agar (PDA), 39 g/litre at 16-18°C. Isolates of all four fungi were inoculated onto fresh PDA and grown at 16-18°C for several days prior to use in experiments.

PDA was autoclaved at 121°C for 15 minutes, cooled to hand-hot, poured into sterile 9 cm diameter, triple vent, plastic petri-dishes and allowed to set. Isolates were removed from 4-7 day old fungal colonies using a 10 mm sterile cork borer and placed in the centre of agar plates, 1 isolate per plate. Brassica tissue was weighed out into small vials (5.5 x 3.7 x 0.5 mm), the vials placed into the inverted lid of the petri-dishes (Figure 1) and sterile water added to the brassica tissue at a rate of 6 µl/mg tissue (Kirkegaard *et al.*, 1996). The bases of plates were inverted over the lids, plates double sealed with parafilm and incubated at 18°C for several days. The diameter of each colony was measured; two measurements were taken at right angles and the average taken. Control was calculated as the percent reduction in mean colony diameter by comparison with the untreated. This method was used to screen all brassica tissues at a rate of 500 mg/plate to determine the most effective dose of brassica tissue and to compare the brassica tissues against the soil fumigant dazomet (97% w/w). For *F. solani* and *P. exigua* the commercial fungicide imazalil + thiabendazole (100:300 g a.i./litre) was used. PDA was prepared as described above, with the fungicide added to the hand hot agar at rates of 1, 10, 50 and 100 ppm imazalil prior to pouring into sterile petri- dishes. The brassica tissues were applied as per Figure 1.

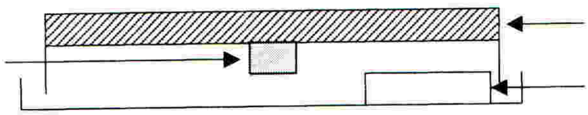


Figure 1. System for testing fungicidal activity of brassica tissue.

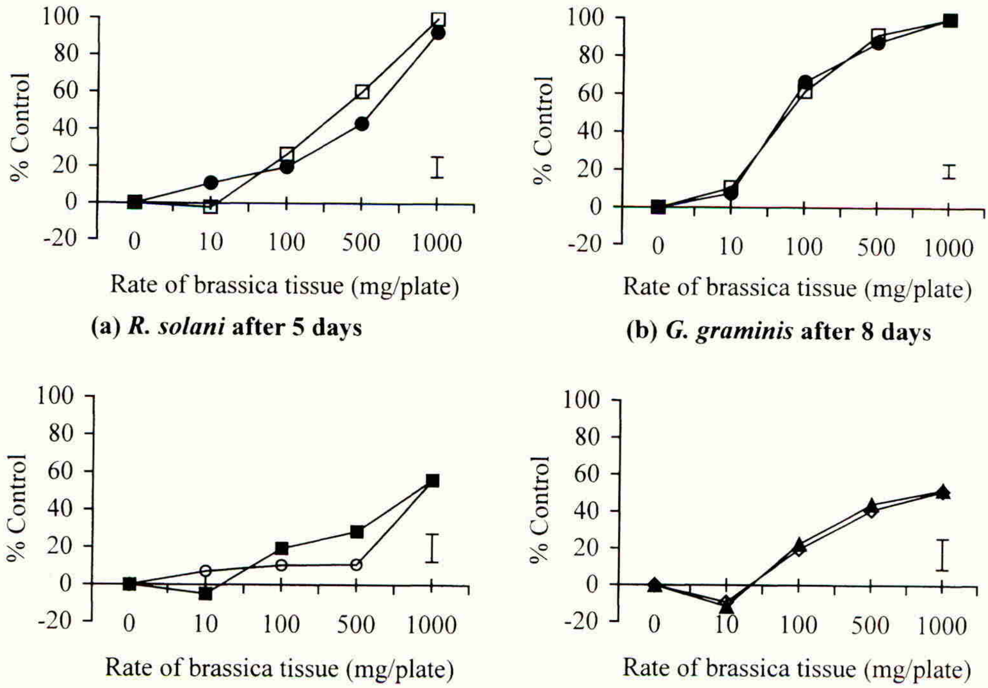
RESULTS

Brassica tissues varied in their levels of control of fungal pathogens (Table 1). Seven of the 11 tissues tested significantly reduced growth of *R. solani*, with Synergy root and Debut root giving in excess of 90% control of the fungus. Synergy shoot material, however, significantly encouraged growth of *R. solani*. Three tissues, Synergy roots, Debut roots and blanched rapeseed meal gave 100% control of *G. graminis*. All but one of the tissues tested gave some control of *F. solani*, with the most effective the non-blanched and blanched oilseed rape meals (69.7 & 77.8% control) followed by the *B. juncea* and *S. alba* root tissues (46.6 & 53.8% control). Growth of *P. exigua* was reduced to some extent by all of the brassica tissues, but the greatest control was achieved with the blanched rapeseed meal, extruded rapeseed meal and Debut shoots (50-80% control).

Of the pathogens tested, *G. graminis* was the most sensitive to the brassica tissues, with an average 89% control (Table 1). The average control of the other three pathogens was < 50%. The two brassica tissues giving the best and most consistent control of the four pathogens were the blanched oilseed rape meal (85.5%) and the non-blanched oilseed rape meal (67.7%). Two of the most promising brassica tissues were tested against each pathogen to determine the rate of material required to give the greatest control *in vitro*. In all cases, as the rate of tissue increased from nil to 1000 mg/plate control of the pathogen improved (Figure 2).

Table 1. Effect of brassica tissues on control of four fungal pathogens *in vitro*.

Treatment (500 mg/plate)	% Control				
	R. solani 3 days	*F. solani* 12 days	*P. exigua* 7 days	*G. graminis* 8 days	Mean
Untreated	0	0	0	0	0
B. juncea - shoots	55.0	-6.0	7.0	79.3	33.8
B. juncea - roots	70.5	46.6	38.8	98.9	65.7
S. alba - shoots	37.5	24.4	8.8	71.1	35.4
S. alba - roots	7.4	53.8	8.6	90.7	40.1
Synergy - shoot	-50.7	11.1	26.4	54.1	10.2
Synergy - roots	99.5	12.4	36.1	100.0	62.0
Debut – shoots	10.9	43.6	50.1	93.0	49.0
Debut – roots	90.5	17.1	28.4	100.0	59.0
Non-blanched meal	64.5	69.7	38.8	97.8	67.7
Blanched meal	84.5	77.8	79.6	100.0	85.5
Extruded meal	-4.6	19.2	50.1	96.7	40.3
LSD ($P \leq 0.05$)	34.15	17.07	19.32	11.01	



(a) *R. solani* after 5 days

(b) *G. graminis* after 8 days

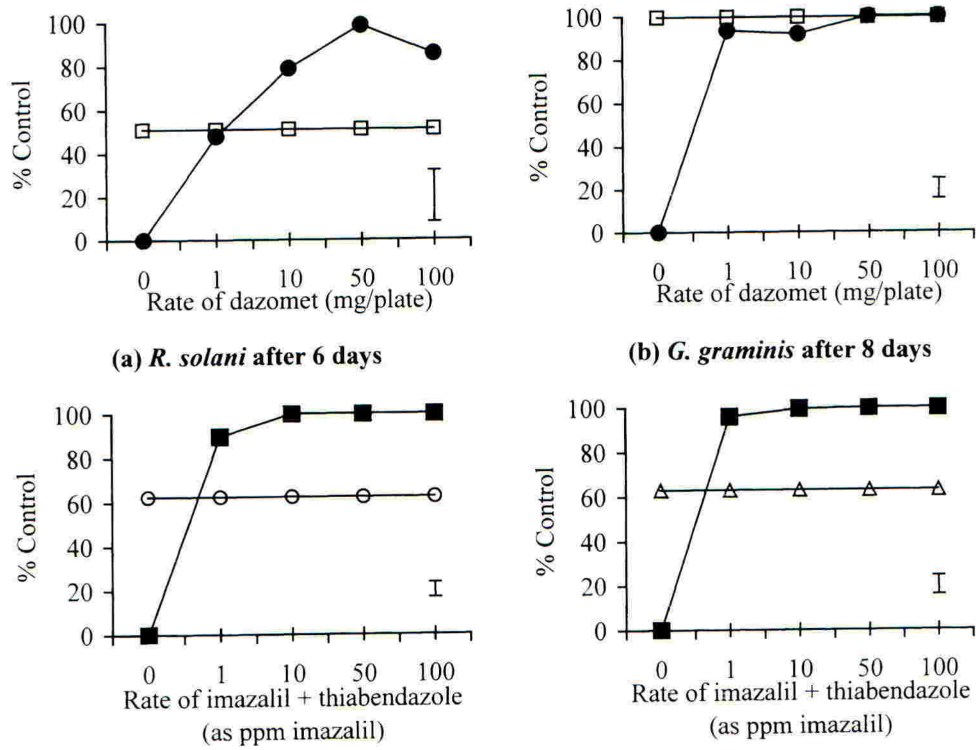
(c) *F. solani* after 14 days

(d) *P. exigua* after 8 days

Figure 2. Effect of rate of Synergy root (•), Debut root (□), *B. juncea* root (■) and *S. alba* root (○) tissues, blanched rapeseed meal (▲) and non-blanched rapeseed meal (◇) on control of fungal pathogens *in vitro* (bars represent LSD for all points on a figure).

R. solani and *G. graminis* were the pathogens most sensitive to the brassica tissues, both were controlled 100% with 1000 mg/plate of Synergy and Debut roots. Control of less than 60% was achieved with *F. solani* and *P. exigua*, even with the highest rate of brassica tissue used. Results suggest that if more than 1000 mg/plate of tissue was used, greater control of *F. solani* and *P. exigua* may have been achieve. However, 1000 mg tissue was the maximum that was practical to use in the test system. Results also suggest that at very low rates of brassica tissue, growth of fungal pathogens could be encouraged.

The efficacy of brassica root tissues and rapeseed meal applied at 1000 mg/plate was compared with a standard fumigant/fungicide. The soil fumigant dazomet gave good control of *R. solani* after 3 days, with 50 mg a.i./plate giving 98.6% control (Figure 3). The effective dose of dazomet to give 50% control (ED_{50}) was calculated at 1.2 mg/plate (Table 2). Synergy and Debut root tissue controlled *R. solani* by an average of 51% (Figure 3), equivalent to 1.35 mg dazomet. Dazomet is applied in Scotland at a field rate of 570 kg/ha. The amount of brassica tissue required to give similar soil fumigation to that of dazomet was calculated as 422 t/ha (Table 2).



(a) *R. solani* after 6 days

(b) *G. graminis* after 8 days

(c) *F. solani* after 14 days

(d) *P. exigua* after 8 days

Figure 3. Effect of fungicides dazomet (●) and imazalil + thiabendazole (■) on control of fungal pathogens in vitro compared with 1000 mg/plate of Synergy & Debut root (□) and *B. juncea* & *S. alba* root (o) tissues, and blanched & non-blanched rapeseed meal (△) (bars represent LSD for all points on a figure).

G. graminis was controlled by low levels of dazomet (Figure 3), the ED_{50} calculated as 0.53 mg a.i. (Table 2). 1000mg Synergy and Debut root tissue gave similar control to that of 1.06 mg dazomet and the amount of root material required to control *G. graminis* in the field was calculated as 538 t/ha (Table 2).

F. solani and *P. exigua* were both controlled by low levels of imazalil + thiabendazole (imaz:thiab), the ED_{50} was calculated as 0.56:1.68 ppm imaz:thiab and 0.52:1.56 ppm imaz:thiab respectively (Table 2). 1000 mg of brassica tissue was equivalent to 0.69:2.07 ppm imaz:thiab for *F. solani* and 0.66:1.98 ppm imaz:thiab for *P. exigua*. The quantity of brassica tissue required to fumigate potato tubers and control these two diseases compared with the fungicide spray was calculated at 0.59 t root tissue/t potatoes for *F. solani* and 0.61 t meal/t potatoes for control of *P. exigua*.

Table 2. Amount of brassica tissue required for biofumigation compared with a standard fumigant/fungicide.

	R. solani	*G. graminis*	*F. solani*	*P. exigua*
ED_{50} dazomet (mg)	1.2	0.53	-	-
ED_{50} imazalil:thiabendazole (ppm)	-	-	0.56:1.68	0.52:1.56
Amount of fungicide equivalent to 1000 mg brassica tissue	1.35 mg	1.06 mg	0.69:2.07 ppm	0.66:1.98 ppm
Field rate of brassica tissue required	422 t/ha	538 t/ha	0.59 t/t	0.61 t/t

Field rates: dazomet = 570 kg/ha, imazalil + thiabendazole = 10:30 g/t potato tubers.

DISCUSSION

Incorporation of fresh brassica tissues into soils has been shown to improve yield and control soil-borne diseases in cereal crops such as winter wheat (Kirkegaard *et al.*, 1999). The present study showed that freeze-dried brassica root tissue of brown and white mustard and oilseed rape and also blanched and non-blanched rapeseed meal had promising fungicidal activity against the fungal pathogens *R. solani*, *F. solani*, *P. exigua* and *G. graminis*. Shoot material had poorer fungicidal activity in most cases. This is in contrast to work carried out by Kirkegaard *et al.* (1996) where brown mustard shoots gave the best control of a similar set of pathogens, with root tissues of brown mustard and oilseed rape less effective. Results confirmed the findings of Kirkegaard *et al.* (1996) that *G. graminis* was consistently sensitive to all brassica tissues. *R. solani* and *G. graminis* are important pathogens that are difficult to control in the soil, although seed treatments are available. *F. solani* is a field/storage disease and *P. exigua* a storage disease of potatoes, both of which are reduced by conventional agrochemicals applied to tubers in-store. If the activity of brassica tissues against these fungal pathogens can be transferred to a field situation these natural fungicides could benefit both conventional and organic growers.

Kirkegaard *et al.* (1996) found rapeseed meal applied at 500 mg/plate reduced fungal growth of *Rhizoctonia*, *Fusarium* and *Gaeumannomyces* by <20% whereas brown mustard meal reduced growth by >90%. Results from the present study showed blanched rapeseed meal gave the most consistent control over the four pathogen species tested, with an average control of >85%, suggesting this product could be taken forward to develop as a commercial biocide.

Biocidal activity of rapemeal is the result of breakdown of glucosinolates, by the enzyme myrosinase, releasing isothiocyanates and other products that fumigate the soil, so controlling fungi. However, blanching through heat treatment destroys most of the myrosinase activity within the meal and isothiocyanates are not released. It is suggested that other biochemical processes, ones not destroyed by heating, are active within the rapeseed meal and that it is by-products of these processes that are providing much of the biocidal activity rather than the glucosinolate/myrosinase system. This would need investigating before the meal could be developed further as a biocide.

The quantities of brassica tissues required (0.59-535 t) to give equivalent control of pathogens were >1000 times higher than the quantities required of commercially available fungicides. This could prevent the development of brassica tissues as biocides at present. Such large volumes of tissue applied to soil could influence the nutritional status of soil, affect soil structure and influence crop growth. Indeed, previous work showed that incorporation of large quantities of rapeseed meal to *R. solani* infected soil prevented emergence of potatoes by causing shoots to rot and producing a physical hard barrier in the soil (Sutherland *et al.*, 2003). This would not be acceptable to growers.

In conclusion, a number of brassica tissues investigated had potential as biocides against fungal pathogens, in particular *G. graminis*. However, further investigations and field studies are required to determine suitable application rates.

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REFERENCES

Burghart P; Evrard J (1999). CETIOM's oil mill pilot plant. In: *Proceedings 10th International Rapeseed Congress*, Canberra, Australia. CD rom.

Kirkegaard J A; Matthieson J N; Wong P T W; Mead A; Sarwar M; Smith B J (1999). Exploiting the biofumigation potential of brassicas in farming systems. In: *Proceedings 10th International Rapeseed Congress*, Canberra, Australia. CD rom.

Kirkegaard J A; Sarwar M; Wong P T A; Mead A (1998). Biofumigation by brassicas reduces Take-all infection. In: *Agronomy – growing a greener future. Proceedings 9th Australian Agronomy Conference,* eds D L Michalk & J E Pratley, pp. 465-468.

Kirkegaard J A; Wong P T W; Desmarchelier J M. (1996). In vitro suppression of fungal root pathogens of cereals by brassica tissues. *Plant Pathology* **45**, 593-603.

Sutherland K G; Booth E J; Walker K C (2003). The use of oilseed rape meal for control of *Rhizoctonia solani* in potatoes. In: *Proceedings 11th International Rapeseed Congress*, Copenhagen, Denmark, pp 1129.

Strategies to control *Cirsium arvense* in organic farming systems

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ABSTRACT

On a 10 ha long-term organic farming experiment at the Institute for Weed Research, Federal Biological Research Centre for Agriculture and Forestry (BBA), *Cirsium arvense* spread in only six years across almost the whole area, although in some patches shoot density decreased. Seed production was not correlated with vegetative growth in the first 4 years and remained low, but increased in the fifth year. Neither yearly ploughing nor intense stubble tillage reduced the rate of spread. The reasons are typical for the situation on organic farms in Germany and other European countries. Spread is enhanced by low crop competition due to a high proportion of spring-sown crops in the rotation and a lack of competition in autumn, post-harvest. Harrowing in growing crops was ineffective against *C. arvense*. The results show that only a long-term and site-specific combination of tillage and crop rotation measures will achieve a sustainable control effect.

INTRODUCTION

In organic farming systems, a significant increase in *Cirsium arvense* is often a problem, especially during the conversion period from conventional to organic. *C. arvense* is characterised by a high competitiveness based on high adaptability, a dense and expanding root system and an ability to regenerate (Mitchell & Davis, 1996). This encourages spread, especially in situations with low or no crop competition, e.g. during a retarded early crop development or after harvest. Also, mechanical control measures are often ineffective (Häni & Zürcher, 2002). Therefore, development of improved measures to control *C. arvense* are aimed at crop rotation, crop management and soil tillage in order to disrupt vegetative growth and nutrient storage in the roots. A long-term control strategy, however, depends on the ideal combination of these measures and must take into account the specific conditions of the farm and the site. This paper reports the rate of spread of *C. arvense* on a long-term organic farming experiment and control options are discussed.

MATERIAL AND METHODS

The site ('Ahlum, BBA') is located south of Braunschweig, Lower Saxony, and consists of homogenous sandy loam with a high nutrient level, an annual precipitation of 579 mm and an annual mean temperature of 9.3°C. Trials on organic farming have been conducted since 1996 on an experimental area of 10 ha certified according to EC Regulation No 2092/91. The trial was conducted under an arable farming system based on ploughing and a crop rotation with a high proportion of cereals (Table 1).

Table 1. Soil tillage and crop rotation, 1996-2001.

	1996	1997	1998	1999	2000	2001
tillage	plough	plough	plough		plough	
crop	*spring wheat*	*spring wheat*	*field beans*	*winter barley*	*spring wheat*	*winter rye*
tillage	chisel plough	disc harrow	mulching	chisel plough	chisel plough	
tillage		plough	plough	chisel plough	plough	
intercrop		clover-grass (mulched)		clover-grass (mulched)		

The shoot density of *C. arvense* was assessed in a grid of 24 x 24 m. The same points were also used for soil samples (to 30 cm) to determine the weed seed bank by germination tests. Because of the initially low abundance of *C. arvense*, direct and indirect control measures were focussed mainly on annual weeds. Therefore harrowing was done at least once within each crop. The soil was ploughed to a depth of 20–25 cm in spring and in autumn. Stubble cultivation was mainly done with a wing share cultivator (cultivation depth 10 cm). There was no manual control of *C. arvense*, e.g. by pulling or cutting.

From 2001, the experiment design was changed by separating the area into 8 plots with a more mixed crop rotation (including e.g. peas and potatoes). Results from this phase are not presented in this paper.

RESULTS AND DISCUSSION

Spatial distribution of *C. arvense*

Since starting the project in 1996, *C. arvense* has spread continuously over almost the whole area (Figure 1). During the six years of the study, there was an increase in the number of *C. arvense* patches and also in the mean number of shoots per patch (Table 2). Whereas in 1996 only 11 of 205 measuring points (5 %) were infested with *C. arvense*, by 2001 the weed was found at 129 points (63 %).

Table 2. Shoot density of *C. arvense* grouped for all measuring points, 1996-2001.

Shoot density	1996	1997	1998	1999	2000	2001
number/m²	number of measuring points					
> 20	1	2	6	15	14	33
11-20	3	2	8	13	19	35
1-10	7	7	12	34	21	61
0	194	194	179	143	151	76

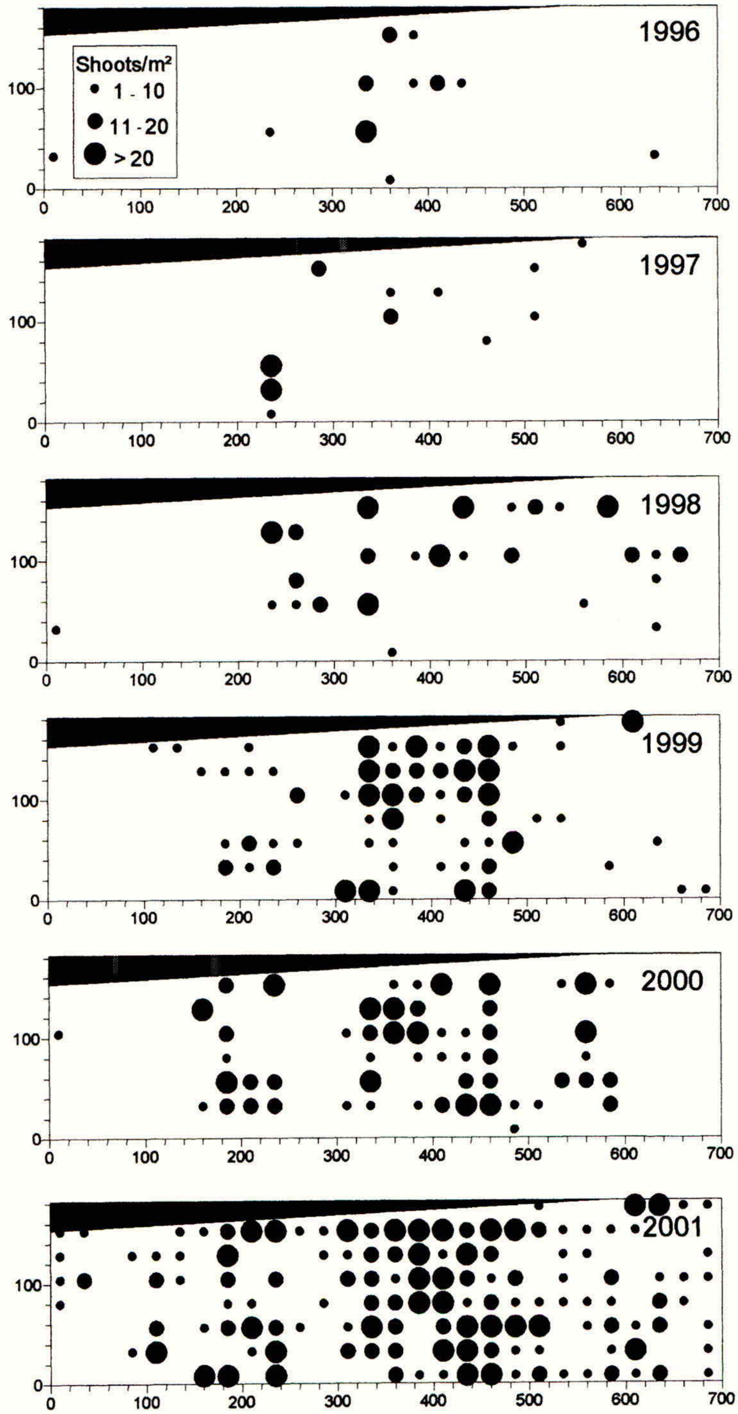

Figure 1. Spread of *C. arvense*, 1996-2001 (numbers on axes are distances (m)).

However, after a few years, the shoot density of *C. arvense* on certain patches decreased. This was also found by Eber & Brandl (2003). This reduction might be due to intraspecific competition or self-exhaustion, but at the moment there is no clear proof for these hypotheses. In contrast to the widespread distribution of *C. arvense* in the field, the seed bank remained very low during the first years, but increased markedly in 2001 (Table 3). There was no correlation between shoot density in the field and numbers of seeds in the soil.

Table 3. Seeds of *C. arvense* and other weed species in soil samples, 1996-2001.

	1996	1997	1998	1999	2001
		Number per m² to 30 cm soil depth			
C. arvense	2	7	9	5	53
Monocotyledonous species	479	924	686	442	286
Dicotyledonous species	3709	6376	6171	4444	9068

This is congruent with the observation that very few seedling plants were found, even with the marked increase in seeds in 2001. More detailed investigations are ongoing in order to estimate the impact of seed production and seedling plants on weed distribution in the field. Recent findings regarding this topic are still contradictory (Hettwer & Steinmann, 2002). A significant passive dispersal of root and shoot parts by means of tillage was not observed. The effect of mechanical spreading by cultivations is probably marginal in comparison to spread due to the high growth rate of *C. arvense* roots (Meyer & Albrecht, 2003).

Effect of crop rotation and soil tillage on abundance of *C. arvense*

Neither the effect of the crop rotation nor the intense cultivations inhibited the spread of *C. arvense*. The main reason was probably that the weed could profit from the low competition from the spring-sown crops grown between 1996 and 1998 (summer wheat - summer wheat - field beans). Once greater than a critical density, *C. arvense* could not be reduced even by more competitive crops like winter rye. Field beans also did not, contrary to other experiences, suppress *C. arvense*. Growing grass-clover mixture as an intercrop in 1997 and 1999 with mulching afterwards, did, however, appear to reduce the rate of spread.

The study showed no effect of harrowing in growing crops on *C. arvense* or other perennial species. Annual weeds were controlled moderately, but results were variable (Table 4). Over the 6 years, densities of emerged weeds did not rise although the seed bank of annual weeds increased.

Table 4. Changes in weed densities (%) 3-5 weeks after harrowing, 1996-2001.

	1996	1997	1998	1999	2000	2001
C. arvense	+ 33	+ 67	- 7	+ 829	+ 109	+ 110
Monocotyledonous species	- 46	- 8	- 29	+ 130	- 39	+ 50
Dicotyledonous species	- 30	- 27	- 49	+ 8	- 56	- 31

Tillage measures alone cannot interrupt or stop regeneration of *C. arvense* because of it's lateral root system below tillage depth. Furthermore, tillage can lead to an advantage for *C. arvense*, especially in times without crop competition, since more mineralised nitrogen is available in the deeper soil layers (Hartl, 2003). Because of the high portion of cereals in the crop rotation, other options for mechanical weed control were not available, e.g. the use of hoeing in row crops like potatoes. This also encouraged the spread of *C. arvense*.

Summarising these findings, the following reasons for an increasing infestation of *C. arvense* can be considered typical for organic farms in Germany and other European countries:

- A high portion of cereals in the crop rotation,
- Little alternation of summer and winter cropping,
- A small portion of hoed crops.

When changing the experiment design in 2001, the focus was on the control of *C. arvense*. The results make clear that an effective strategy can only be accomplished with an ideal combination of crop rotation and tillage measures. Therefore, other crops with higher competitiveness like peas or oilseed-rape were grown combined with a higher intensity of soil tillage and hoeing. The grass-clover mixture now growing as a main crop and mulched several times has controlled *C. arvense* successfully. Although initial results show temporarily good effects, a clear conclusion will be possible only in the longer-term.

In principle, the control methods aim to reduce and exhaust root reserves of *C. arvense*, whereas direct reduction of plants or seeds has a minor priority. However, there is still little knowledge of the biology and physiology of root reserves and regeneration behaviour. In order to optimise control strategies there is also a need for further research on other approaches:

- Knowledge of the regeneration behaviour of *C. arvense* and root growth in the subsoil,
- Relevance of the spread by seed,
- Estimation of the 'critical density' at which *C. arvense* becomes difficult to control,
- Biological control by fungal plant pathogens and herbivorous beetles.

Consequently, the experiments at the BBA are ongoing in order to control *C. arvense* by optimising both crop competition and tillage measures.

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REFERENCES

Eber S; Brandl R (2003). Regional patch dynamics of *Cirsium arvense* and possible implications for plant-animal interactions. *Journal of Vegetation Science* **14** (2), pp. 259-266.

Häni F; Zürcher J (2002). Vermehrung, Ausbreitung und Regulierung der Acker-Kratzdistel *Cirsium arvense* – Ökoflächen im Focus. *Streifenförmige ökologische Ausgleichsflächen in der Kulturlandschaft – Ackerkrautstreifen, Buntbrachen, Feldränder*, ed. N Nentwig, pp 93-112. Verlag Agrarökologie vaö Berlin, Hannover.

Hartl W (2003). Evaluierung verschiedener nichtchemischer Regulierungsmaßnahmen bei der Acker-Kratzdistel mit besonderer Berücksichtigung der Wurzelökologie. In: *Strategien zur Regulierung von Wurzelunkräutern im ökologischen Landbau – ein Verbundprojekt im Rahmen des ökologischen Landbaus*, ed. H Böhm *et al.*, Landbauforschung Völkenrode Braunschweig.

Hettwer U; Steinmann H-H (2002). Genetische Diversität in *Cirsium arvense* (L.) Scop. Proceedings 21st German Conference on Weed Biology and Weed Control, *Journal of Plant Diseases and Protection, Special Issue XVIII*, pp. 75-82.

Meyer F; Albrecht H (2003). Ausbreitungsbiologie der Acker-Kratzdistel (*Cirsium arvense* (L.) Scop.). In: *Strategien zur Regulierung von Wurzelunkräutern im ökologischen Landbau – ein Verbundprojekt im Rahmen des ökologischen Landbaus*, ed. H Böhm *et al.*, Landbauforschung Völkenrode Braunschweig.

Mitchell R B; Davis L T (1996). Effect of control treatments on the root reserves of Californian Thistle (*Cirsium arvense*). *Proceedings of the 49th New Zealand Plant Protection Conference*, pp. 229-233.