BioBoost

Accelerating biobased horticulture

Pilot green pesticides

(WP5)

Final report, December 2019

NIAB

Eastern Agri-Tech Innovation Hub

BioBoost – Final Report Green Pesticides

Authors
Lydia Smith, Ralph Noble and Denise Elliott (2019)

Disclaimer
This report is a summary of the experiments carried out in the framework of the BioBoost project. This project has received funding from the Interreg 2 Seas programme 2014-2020 co-funded by the European Regional Development Fund under subsidy contract No 2S01-038, the province of West Flanders and the province of South Holland.

Not all raw data are included in this report, but they are available for interested stakeholders upon request, if non-confidential.

Contact info
BioBoost coordinators: JCAJStraver@gemeentewestland.nl; gerrit.walstra@haute-equipe.nl
Project website: https://www.BioBoosteurope.com
Authors NIAB: lydia.smith@niab.com

Contents

Summary

NIAB has led the green pesticide trials as part of the BioBoost project in the UK. In order to ensure economic outcomes and continuity of the initiative beyond the scope and lifetime of the project, NIAB has worked very closely with them in our (Eastern Agritech Innovation Hub) incubator north of Cambridge. We have consequently worked with several SME's to deliver the following data. NIAB has also identified and collaborated with the commercial growers to carry out field-based trial work on behalf of BioBoost including the following: Sunclose Farm, Simpsons Nurseries, Peter Beales Roses and Matthews Plants.

Organic by-products from crop and insect production were used to enhance the performance of biocontrol agents and arbuscular mycorrhizal fungi (AMF). The inoculum was sourced from PlantWorks Ltd Kent UK. The following headline results were obtained in conjunction with commercial partners and nurseries:

- Incorporation of 10% v/v spent mushroom compost sustained an inoculum of entomopathogenic fungus in growing media resulting in improved control of vine weevil larvae
- Frass, when used as a potting material amendment repelled vine weevil and reduced egg laying in Heuchera plants.
- Frass, when used as a pot topping material for Euonymus plants reduced weeds compared with using peat or coir as pot topping materials.
- Frass incorporated at 10% v/v into the soil planting hole improved the growth and flowering of roses; spent mushroom compost incorporated at the same inclusion rate slightly improved plant growth.
- Amendment of peat-based growing medium with spent mushroom compost improved root colonisation with AMF and growth of Fatsia plants.
- Fermented fruit waste liquor used as an attractant bait for spotted wing drosophila improved the control efficacy of low doses of insecticides.

1. Control of soil-dwelling insect pests using chitinous wastes and entomopathogenic fungi

Chitin is a polysaccharide and a primary component of arthropod exoskeletons and fungal cell walls. Plants growing in soil or potting compost detect chitin by plant chitosan receptors, therefore plants perceive the presence of chitin as potential signs of invertebrate pest attack. This causes plants to up-regulate their defence mechanisms, which can include production of thicker leaf cuticles and release of chitinases and metabolites into the soil. Chitin can also provide entomopathogenic fungi (EPF) with an alternative nutrient source; these fungi kill insects present in soil or growing media. Sources of these feedstocks are available as by-products from black soldier fly larvae production (frass, @14% chitin) and spent mushroom compost (SMC, @ 5% chitin). See Figures1 a and b.

In BioBoost, we have used these by-product feedstocks in crop pest control (1) as a nutrient source for entomopathogenic fungi (EPF) which can be used as a bio-control agent when they attack soil-dwelling pests (2) by systemic uptake by plants making them unpalatable to pests and (3) through a pest repellent effect when used as an amendment in potting material. The target pests in this work have been western flower thrips (WFT) (*Frankliniella occidentalis*) which are foliar pests that pupate in the growing medium, and black vine weevil (*Otiorhynchus sulcatus*), the larvae of which attack the roots of plants.

Fig. 1 (a) AgriGrub frass, (b) spent mushroom compost, SMC, (c) SMC colonised with Metarhizium.

1.1. Chitinous wastes as a nutrient source for entomopathogenic fungi

Commercial growers are concerned that entomopathogenic fungi (EPF) are not currently as effective as chemical pesticides; but are keen to find an alternative as these chemical options become less acceptable in fresh produce. Incorporating SMC into a peat based potting medium has been shown to increase the efficacy of the commercial formulation of the entomopathogen *Metarhizium anisopliae* Met52, against vine weevils in experiments on strawberry plants. In BioBoost, we tested this technique against western flower thrips WFT, in a coir-based growing medium, and subsequently in our pilot study on a commercial scale nurseries horticultural stock and fruit crops.

1.1.1. Control of western flower thrips

Preliminary tests showed that although *M. anisopliae* Met52 colonised sterile frass, it did not colonise pasteurised frass. Further experiments were therefore conducted using SMC (Fig. 1c). A small-scale experiment was undertaken to test the effect of coir growing medium applied SMC + EPF treatments on pupae of WFT and subsequent population levels of adults.

The treatments added to wetted coir were as follows:

(a) No amendment = control
(b) SMC 10% v/v
(c) SMC 10% v/v + Met52 0.05 g/l
(d) Met52 0.5 g/l
(e) SMC 10% v/v + Met52 0.05 g/l

The coir-based compost and the different amendments were well mixed and then dispensed into small plant pots 7 x 7 x 8 cm. Each pot was enclosed in a plastic box to prevent losses of WFT and to prevent cross contamination among treatments. A piece of green bean pod was placed on the surface of the growing medium in each pot as a food source for WFT. There were 5 replicates of each treatment. Pots were held at 20°C for one week prior to infestation with WFT. Late instar WFT larvae (20) were introduced on to the bean pod in each box and the pots were returned to 20°C 14L:10D conditions.

The adults that had emerged from larvae that had pupated in the compost were recorded; bean pods and the outside of the pots plus the inside of the boxes were inspected under a stereo microscope. Any adults found were removed on each recording occasion. Populations of *Metarhizium anisopliae* (as colony forming units; cfu) present in the growing medium were assessed before inoculation of the plants and at intervals during the experiment.

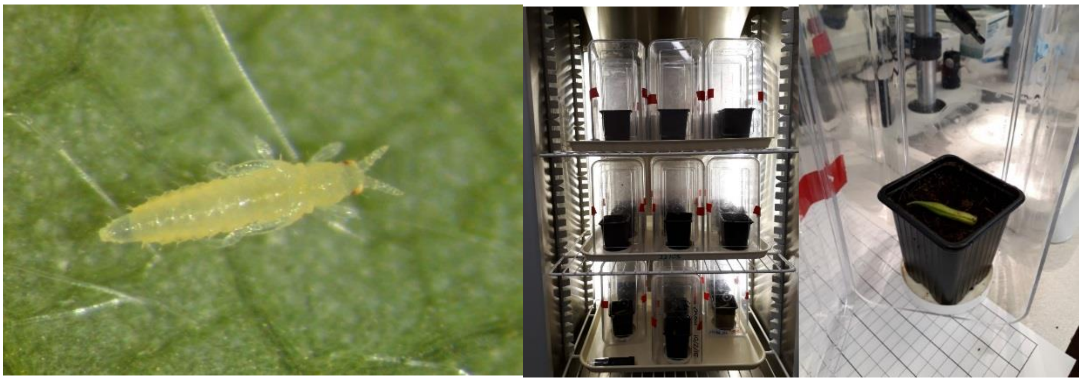

Fig. 2 (a) Prepupae of western flower thrip, (b) incubator and (c) pot bioassay for WFT.

No *Metarhizium* was detected in the coir control and 10% SMC treatments. The population of *Metarhizium* in the Met52 inoculated coir declined during the experiment (Fig.3a). The addition of 10% SMC and Met52 to the coir resulted in a sustained population of *Metarhizium* for over 60 days.

There was a trend for the combined SMC + Met52 treatments to produce the fewest WFT adults, although the difference between treatments was not statistically significant (Fig.3b).

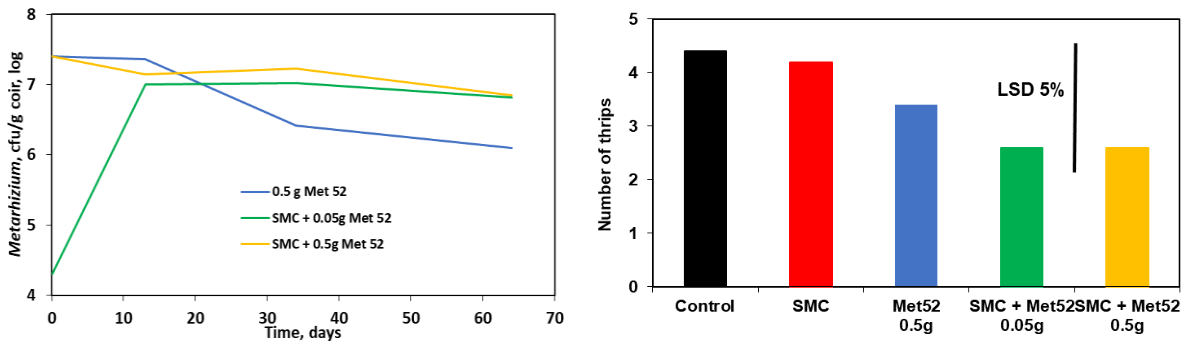

Fig. 3 (a) Population of Metarhizium in coir and (b) emergence of adult thrips in different SMC and Met52 treatments.

1.1.2. Control of black vine weevil on a commercial nursery

Following the preliminary lab-based trials the experiment was then up-scaled at commercial nursery sites. A trial was set up at a commercial nursery (Matthews Plants, Roydon, Essex) to compare the efficacy of the combined SMC + EPF treatment against the individual SMC or EPF treatments for control of black vine weevil in ornamental nursery stock. Heuchera was chosen as a test species since it is highly susceptible to vine weevil attack. Live healthy vine weevil larvae (3) were introduced into some of the pots.

In addition to the inoculated treatments, an area of known natural infestation of vine weevil on the nursery was used to determine whether differences in vine weevil source would impact on the treatment.

The following treatments and/or vine weevil larvae were added to a peat based growing medium (Sinclair Professional):

(a) None, Control
(b) Control + vine weevil
(c) 10% SMC
(d) 10% SMC + vine weevil
(e) Met52, 0.5g/l
(f) Met52, 0.5 g/l + vine weevil
(g) 10% SMC + 0.5g/L Met52
(h) 10% SMC + 0.5g/L Met 52 + vine weevil

There were 15 replicate pots of each treatment and 30 replicates of the controls (a and b) (Fig. 4 a and b). The experiment was set up in July 2017 and assessed for live and dead vine weevil larvae in March 2018 (Fig. 4c). Samples of growing medium were taken at intervals and assessed for *Metarhizium* populations.

Fig. 4 (a) General view of Heuchera nursery trial, (b) potted Heuchera plants and (c) vine weevil larvae infected with Metarhizium.

No *Metarhizium* was detected in the unamended peat or 10% SMC treatments. Where *Metarhizium* was added without SMC, there was a gradual decline in the *Metarhizium* population in the growing medium and it could not be detected after 35 days (Fig. 5a). The addition of 10% SMC with Met52 resulted in a sustained *Metarhizium* population in the growing medium and a reduction in the number of live vine weevil larvae.

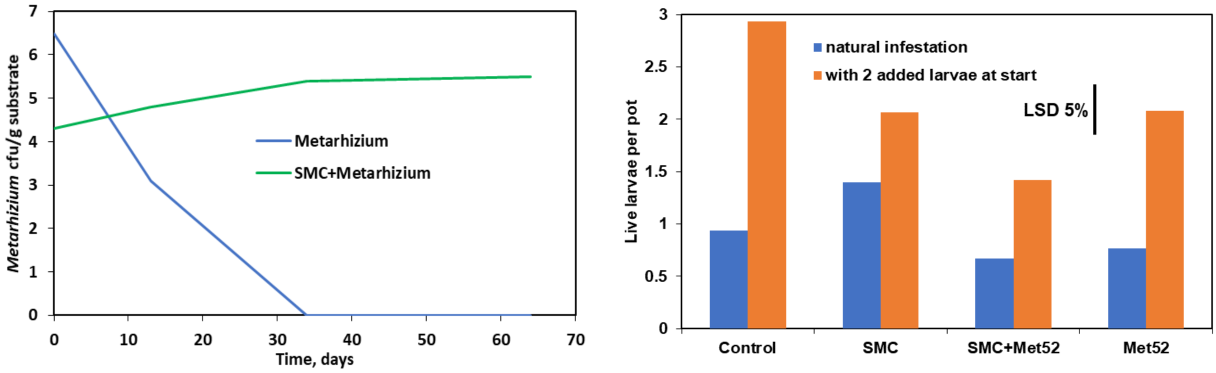

Fig. 5 (a) Population of Metarhizium in peat and (b) and live vine weevil larvae in different SMC and Met52 treatments.

1.1.3. Control of black vine weevil in raspberry

A second pilot trial was set up at Sunclose Farm, Milton, Cambridgeshire to investigate the use of SMC+Met52 and frass for control of black vine weevil. The following treatments have been applied to each of 20 pots of raspberries grown coir in 10 litre pots:

(a) No amendments = control
(b) 10% v/v frass
(c) 10% SMC + 0.05g/l Met52.

The experiment was set up in May 2019 and was assessed in November 2019.
No vine weevil infestation occurred on this section of the farm. Plant height measurements showed that the organic amendments had no significant effect on the growth of the plants. Mean height values of 20 plants (±SD) were control 184±24 cm, SMC 193±48 cm, frass 189±23 cm.

Fig. 6 Raspberries at Sunclose Farm used for an experiment on control of vine weevil larvae using an amendment of the growing medium with spent mushroom compost or black soldier fly frass.

1.2. Chitinous wastes as a pest-repellent material

In the second part of the NIAB pilot, we investigated the use of chitinous wastes and other organic amendments as potting materials to repel the activities of egg-laying vine weevil adults. This is an additional treatment where the initial activities of the adult are impacted, rather than treating the destructive larvae once they have been introduced to the host plant.

1.2.1. Comparison of potting materials for Heuchera

An experiment with *Heuchera* grown in peat and bark medium (Levington) was set up in August 2018 with the following potting material treatments:

(a) Control, peat and bark medium
(b) AgriGrub frass
(c) 'Zee No Weevil', a pest repellent product formulated by Celbius Ltd from olive industry by-product and aromatic plant extracts

The potting materials amendments were applied as a 1 cm layer to *Heuchera* planted in 1.5 litre pots. The pots were set out with *Euonymus* plants which were heavily infested with vine weevil adults and left outside for two months, where the adults were free to move from pot to pot. In October 2018, the pots were brought into an unheated glasshouse and assessed for vine weevil larvae in February 2019. The fresh weight of plants with and without larvae at the end of the experiment was also determined.

Fig. 7 (a) Potting material experiment and (b) vine weevil larvae in Heuchera pot.

The frass potting material resulted in significantly fewer vine weevil larvae per pot than both the control and olive by-product (Fig. 8a). The olive by-product had significantly fewer larvae than the control. The frass potting material resulted in a small reduction in *Heuchera* plant weight in uninfected pots (Fig. 8b).

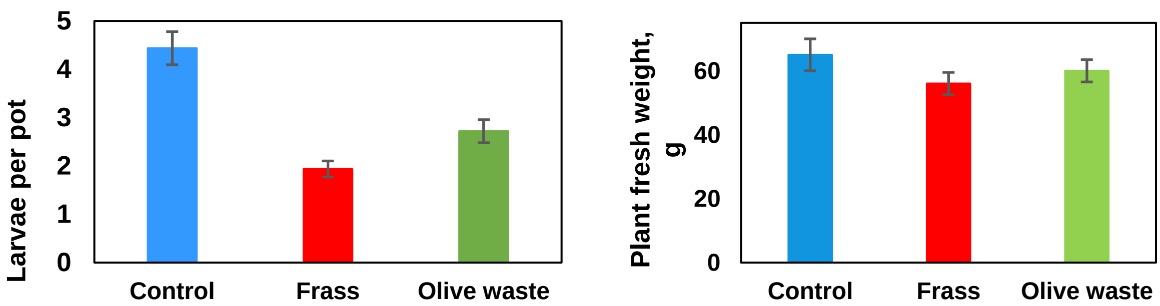

Fig. 8 (a) Vine weevil larvae per pot and (b) Heuchera plant fresh weight at the end of the experiment.

1.2.2. Comparison of potting materials for red robin (Photinia) and Euonymus shrubs

An experiment was set up at Simpsons Nurseries, Fordham, Cambridgeshire to compare chitinous wastes as potting materials for vine weevil and weed control. The experiment was set up in May (*Photinia*) and August (*Euonymus*) 2019 with the following treatments:

(a) Coarse bark, control
(b) Frass
(c) 10% SMC + 0.05g/l Met52.

The potting materials were applied as a 1 cm deep mulch. The pots were assessed for weeds at monthly intervals and for vine weevil larvae in November 2019.

Fig. 9. Pot topping materials at Simpsons Nurseries (left) weeds in standard coir topping (right)

Fig. 10. Pot topping materials on Euonymus plants at Simpsons Nurseries

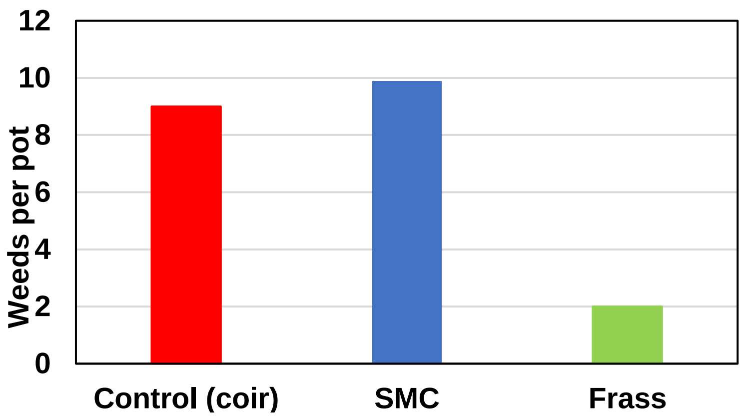

Fig. 11. Effect of pot topping materials at Simpsons Nurseries on weeds in Euonymus

There were fewer weeds in *Euonymus* pots topped with frass than with coir or spent mushroom compost (Fig. 10). There was no effect of the pot topping materials on the growth of the *Euonymus* plants (Fig. 11) After three months, *Photinia* plant height was slightly lower where a frass pot topping was used (55±12 cm) compared with coir or spent mushroom compost (65±11 cm). No vine weevil infestation occurred on either the *Euonymus* or *Photinia* plants at the nursery.

2. Improving the establishment of arbuscular mycorrhizal fungi with chitinous wastes

Arbuscular mycorrhizal fungi (AMF) form a mutually beneficial association with plant roots; enabling the plant to benefit from the ability of the fungal symbiont to access nutrients from the soil, especially phosphate. AMF colonised plants have been shown to suffer from reduced pathogenic attack and from better water use efficiency; hence, a good symbiotic association with these fungi can also improve plant tolerance to drought and enhance plant growth. The work in BioBoost aimed to determine whether the addition of a chitinous waste to the soil or growing medium can improve the beneficial colonisation of plants by AMF and whether this improves plant growth.

2.1. Effect of arbuscular mycorrhizal fungi and spent mushroom compost on the growth of *Fatsia*

A trial was set up at a commercial nursery (Matthews Plants, Roydon, Essex) to compare the efficacy of the combined SMC + AMF treatment with the individual SMC and AMF treatments for establishment of AMF and plant growth. AMF inoculum was provided by Plantworks Ltd, Sittingbourne, Kent.

The following treatments were set up in 3 litre pots containing a peat-based growing medium (Sinclair professional):

(a) None, Control
(b) 10% SMC
(c) 30 ml AMF inoculum in planting hole
(d) 10% SMC in growing medium, 30 ml AMF inoculum in planting hole.

The experiment was set up in July 2017 and assessed in March 2018 for AMF in the growing medium and for plant growth.

The addition of SMC or AMF inoculum alone did not result in a significant increase in the percentage of roots colonised with AMF (Fig. 12). The combined SMC + AMF treatment resulted in a significantly higher percentage of AMF colonised roots and in greater plant weight than the control or individual SMC or AMF treatments (Fig. 13).

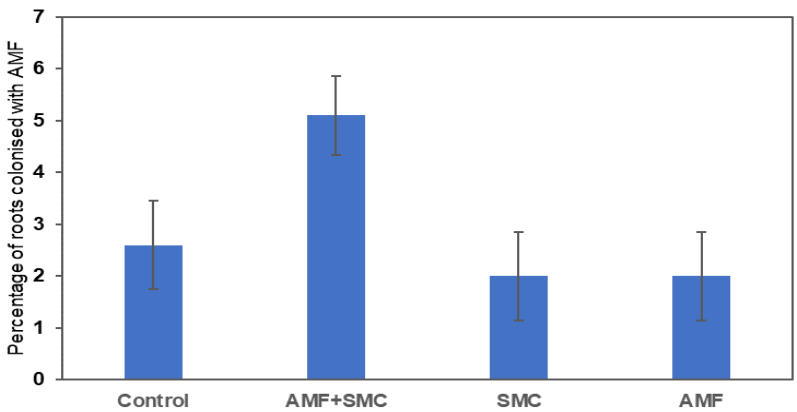

Fig. 12 Percentage of Fatsia roots colonised with arbuscular mycorrhizal fungi

Fig. 13 Fatsia plants growing in (a) unamended peat and in peat amended with (b) 10% SMC (c) AMF and (d) AMF + SMC.

2.2. Effect of arbuscular mycorrhizal fungi and spent mushroom compost on the growth of pot roses

An experiment was set up at NIAB Innovation Farm in April to determine the effects of AMF inoculum and spent mushroom compost on the growth and AMF root colonisation of pot grown roses. Bare root hybrid tea rose (cv Ruby Wedding) and dog rose (*Rosa canina*) plants were potted in 10 litre pots containing a peat+bark growing medium (Levington) with the following amendment treatments:

(a) None
(b) AMF inoculum, 40 ml per pot
(c) 10% v/v Spent mushroom compost
(d) 10% v/v Spent mushroom compost + AMF, 40 ml per pot.

Replicate pots (16) of each treatment were prepared. The plants were grown on Mypex matting with drip irrigation (Fig. 14). Plants were assessed for height and number of shoots and flowers in July 2018 and November 2018. Samples of root/growing media were taken for AMF measurement in July, and at the end of growing season in November 2018.

Fig. 14. (a) Dog roses (left) and hybrid tea roses (left) in July 2018 and (b) overwintered roses in February 2019 growing in pots containing different AMF and SMC treatments.

The addition of AMF inoculum to the pots resulted in a measurable level of root colonisation when assessed in November 2018 and January 2019. The addition of SMC had no significant effect on the percentage of roots colonised with AMF (Table 1). There were no significant differences in flowering or growth between the treatments.

The very low levels of AMF colonisation seen in this experiment may have been due to the optimised irrigation and high levels of nutrition present in the potting compost within the pot treatments; which tends to reduce rates of colonisation. The hybrid tea roses were used for the soil plot experiment (see below). The dog roses were assessed again in June 2019.

		%AMF colonisation of roots			
Date	Rose Type	Control	+AMF	+SMC	+SMC+AMF
November 2018	Hybrid T	0	22	0	0
	Dog	0	4	0	24
January 2019	Hybrid T	0	5	0	4
	Dog	4	7	8	7

Table 1. Percentage of roots colonised with arbuscular mycorrhizal fungi. Values are means of between 10 and 16 replicate samples.

Measurement	Rose Type	Control	+AMF	+SMC	+SMC+AMF
Flower number	Hybrid T	41.6	39.0	38.7	39.7
Prunings weight, g	Hybrid T	47.5	48.5	45.6	70.4
	Dog	244.1	230.9	259.9	257.1
Height, cm	Hybrid T	100.7	96.1	101.9	90.4
	Dog	175.0	155.1	180.8	166.1

Table 2. Rose flowering and growth assessments. Values are means of between 10 and 16 replicate plants.

2.3. Effect of arbuscular mycorrhizal fungi (AMF) and organic amendments on the growth of roses in soil

An experiment was set up with Peter Beales Roses Ltd, Attleborough, Norfolk to determine the effects of organic amendments and AMF on the growth of roses in soil. Pot grown hybrid tea roses (cv. Ruby Wedding) and bare root floribunda roses (cv. Queen Elizabeth) were planted into soil plots at two sites. The plots were planted in February and were assessed for flowering and plant growth during the summer and autumn of 2019.

Fig. 15. Roses planted into soil plots at Peter Beales Roses Nursery, Norfolk.

Treatment	Shoot weight, g	Height, cm	Stem diameter, mm	Flowers
Control	125	67	12.3	20
Organic A	145	69	12.0	18
Organic B	169	72	12.0	23

Table 3. Effect of organic amendments on plant growth and flowering of roses, cv. Queen Elizabeth planted as bare root roses. Values are means of 12 replicate plants grown on each of two sites.

Rose (cv. Queen Elizabeth) shoot weight was significantly increased by amending soil with 10% v/v of organic material B (Table 3). Organic material B also slightly increased plant height and flowering. Organic material A had no significant effects on plant growth of flowering.
Growth and flowering of roses (cv Ruby Wedding) planted as pot grown plants were unaffected by AMF with organic amendments added to the growing medium (Table 4). The AMF did, however have a small positive impact on growth of the rose plants in the absence of other amendments. If time allowed, it would be interesting to reassess this treatment, but using a lower level of nutrition in the potting compost, combined with alternative base medium (coir), since peat based media are less amenable to colonisation rates

Treatment	Shoot weight, g	Height, cm	Stem diameter, mm	Flowers
Control	198	73	12.8	21
AMF	226	81	14.5	19
SMC	208	74	14.5	22
AMF + SMC	201	77	14.3	21

Table 4. Effect of AMF and spent mushroom compost on plant growth and flowering of roses, cv. Ruby Wedding planted as potted roses. Values are means of 6 replicate plants grown on each of two sites.

3. Use of fruit waste liquor as a bait attractant for spotted wing drosophila

Spotted wing drosophila (*Drosophila suzukii* - SWD) is a major pest of fruit crops. Soft fruit growing produces around 5 tonnes of waste fruit per hectare (Fig. 16). Without treatment, this waste material becomes a potential source of infestation for subsequent crops. The waste fruit is being fermented in sealed pallet bins to destroy any larvae of the pest that may be present in the waste. The fermented liquor is attractive to SWD and can be used as an attractant bait. Experiments reported here, as part of the BioBoost biopesticides pilot, investigated the use of the bait in combination with low, sub-lethal concentrations of insecticides in an 'attract and kill' control strategy.

Fig.16 Soft and stone fruit wastes – about 15-20% of the harvested crop.

3.1. Production of an attractant bait for SWD control from fruit waste liquor

Fruit waste consisted of soft fruit (strawberries or raspberries) or stone fruit (plums or cherries) kept at ambient temperatures (12–19 °C) for up to two days or at 4 °C for up to 10 days from harvesting. The majority of the fruit was undamaged but classed as unmarketable due to 'over-size' or 'under-size', uneven shape or ripening, or with blemishes; less than 5% of the fruit in any of the batches was mechanically damaged or rotting.

Fruit waste was inserted into 615 litre pallet boxes (460 kg soft fruit or 410 kg stone fruit) to within 100 mm of the top. The pallet boxes were then sealed with a single layer of polyethylene stretch shrink wrap, with an additional covering of a plastic lid. The lids were then sealed on to the pallet boxes around the rim with a further layer of shrink wrap. Once the bins were sealed, rapid depletion of oxygen and an increase in carbon dioxide concentration occurred due to the fermentation of the fruit, this resulted in death of the eggs, larvae, pupae and adults of SWD.

After 10 days, the fermented waste was separated into solid and liquid fractions, which is then filtered and can be used as a bait for the SWD adults. This method is now used commercially on several farms including Langdon Manor Farm, Faversham Kent (see Fig.17).

Fig. 17 Preparation of sealed pallet bins for fermentation of fruit waste to produce an SWD attractant

3.2. Testing waste fruit liquor for use as a SWD bait attractant

A jar bioassay was used to test the effect of baits and different insecticides at sub-lethal concentrations (when used without amendments) on *D. suzukii*. Baits and/or insecticides were pipetted as droplets on to blackberry leaves in jars which contained 12 SWD adults (see Fig. 18). The following bait treatments were compared:

(a) Water control
(b) Fruit strawberry juice waste liquor (FSJ)
(c) Yeast, *Hanseniaspora uvarum*
(d) Yeast, H. uvarum + FSJ
(e) Combi-protec; a commercial SWD bait

The treatments (b) to (e) above were used in combination with insecticides at dilute doses: spinosad, cyantraniliprole, thiacloprid, lambda-cyhalothrin and water (control).
After 3 days, the jars were assessed for SWD mortality.

Without baits, the dilute doses of insecticides were ineffective in killing SWD, with similar numbers of live SWD to the water control after 3 days (Fig. 19). All of the bait treatments were effective in increasing the SWD toxicity of the insecticides. There were no significant differences in efficacy between the different bait treatments, including fermented waste strawberry juice.

Fig. 18 Jar bioassay for testing the attractiveness of fruit waste as an SWD bait

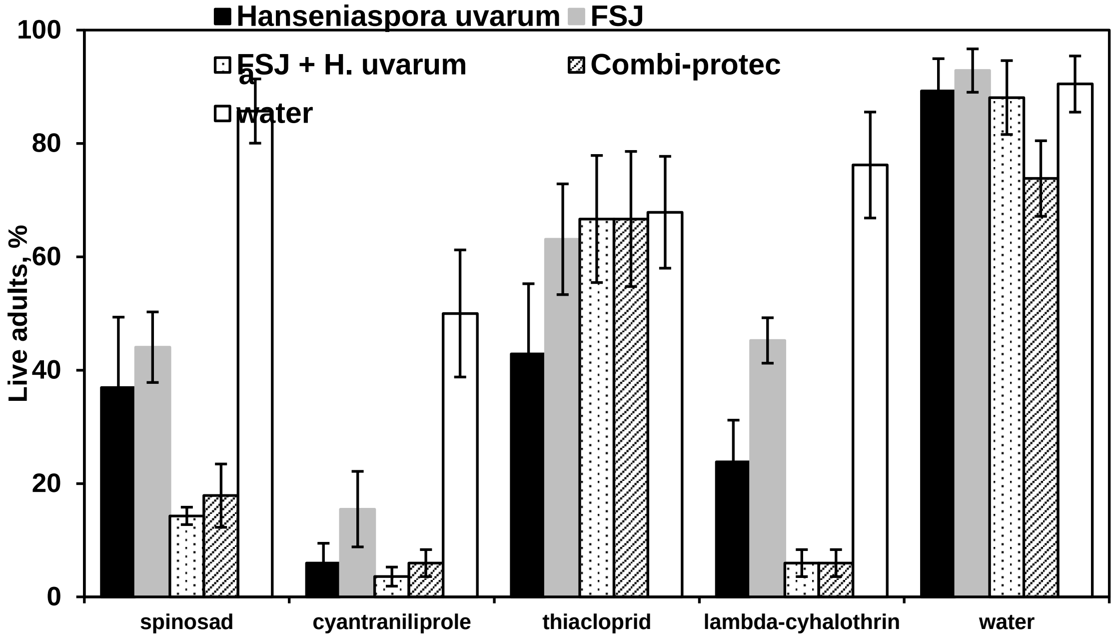

Fig. 19. Effect of bait and insecticide treatments on mortality of spotted wing drosophila

A commercial scale trial with fruit waste as a SWD bait is now being set up at Sunclose Farm, Milton, Cambridgeshire. Final comments and application of results will be discussed in 2020.

4. Commercial development and opportunities from work in BioBoost on green pesticides

The following commercial developments and opportunities have arisen as a result of the work on green pesticides in the 2 Seas Interreg project, BioBoost:

- The marketing of insect frass as a growing medium component and pot topping material that improves the control of pests and reduces weeds
- Frass can be used as an organic fertiliser that improves the vegetative growth and flowering of roses; and potentially of other plants and crops
- The combined use of arbuscular mycorrhizal fungi (AMF) with organic amendments (spent mushroom compost) thereby improve the growth promoting effect in plants such as *Fatsia*
- Combining the use of entomopathogenic fungi (EPF) with spent mushroom compost prolongs their longevity and improves their control effect on soil-dwelling pests
- The use of baits for the control of spotted wing drosophila in fruit crops has been shown