Potential use of mealworm (*Tenebrio molitor)* frass in fruit tree pest management

Master of Science in Biodiversity, Ecology, and Evolution

by

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Abstract

As the world population grows, the food supply and demand are expected to rapidly increase. Insect production is a growing industry producing sustainable protein for food and frass as a byproduct, all while effectively recycling organic waste. The utility of frass has received increasing attention the past years. Its most common use today is as a fertilizer but has been suggested in recent studies to potentially reduce insect pest pressure in plants. Sustainable agriculture is key in limiting the environmental effects of increased food demand. Investigating the possible use of frass as an alternative to pesticide is thus of scientific importance and could potentially increase the economic value of the frass byproduct. This is the first study on the effect of frass on insect pests in perennial fruit trees. Frass from *Tenebrio molitor* was applied to apple and plum trees to investigate the effect on the aphid pest species *Hyalopterus pruni*, *Aphis pomi*, and the mite pest specie *Aculus fockeui* in fruit trees in Norwegian orchards. Contradicting studies suggesting the promising effect of frass on chitin-containing organisms, frass from T. *molitor* did no cause a reduction in growth and development or population size of the insect pests in apple and plum trees in this study. Observed changes in distribution and population size of aphids could not be explained by frass application. In addition, mite population size did not differ between treated and nontreated plum trees. Continuing the study by measuring the pest pressure in frass-treated trees next summer should be conducted to investigate if a priming-effect of frass occurred. Future studies on the effect of frass on insect pests in fruit trees should also be investigated using frass from other insect species.

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Introduction

Global perspective

As the world population is increasing to an expected 9.4 to 10.1 billion by 2050 (United Nations, 2019) the demand for food and the environmental impact of food production will increase. Excessive use of agrochemicals is one of the challenges that comes with an increasing food demand (Maluin & Hussein, 2020). Along with the controversial use of pesticides and their benefits in agriculture comes the negative impacts and hazards on the surrounding environment and on human health (Maluin & Hussein, 2020; Sharma et al., 2019). Sustainable agriculture, in which nonchemical fertilizer and pesticides is used instead of chemical ones, is suggested to limit the environmental effects that increased agricultural output in relation to population growth proposes on the environment (Farooq et al., 2019; Jorge Poveda, 2021).

Insect farming

Insect production is being suggested as a more efficient way of producing protein for both human consumption and animal feed than conventional production animals such as chickens, cattle, and pigs. This is mainly due to the equal or higher feed-to-biomass conversion rate of insects (Oonincx et al., 2015; Ramos-Elorduy, 2008), their lower environmental impact due to less greenhouse gas (GHG) emission, significantly less land use, and similar energy consumption (Oonincx et al., 2015; Oonincx & de Boer, 2012; Y.-S. Wang & Shelomi, 2017). In addition, insects can transform organic side streams, producing high-value protein from low-value organic waste and by-products (van Huis & Oonincx, 2017). For these reasons, insect production is being hailed as a potential savior in a food-insecure world.

Black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*), from now on referred to as BSF and TM (figure 1) respectively, are two of the most common insects species used in insect production for human food and livestock feed (Cortes Ortiz et al., 2016). BSF has a higher biomass conversion rate than TM, and has conventionally been considered more as feed while TM is considered safe for human consumption (Oonincx et al., 2015).

The life cycle of TM consists of four stages: egg, larvae, pupa, and adult, and the whole cycle is variable in length; 280 to 630 days depending on the environmental factors (Makkar et al.,

2014). The adult female beetle lay on average about 400-500 eggs during their lifetime, which hatch 10-14 days after depositing. The larval stage lasts from 3 to 8 months depending on the conditions. In this stage the larvae undergo multiple molting stages; ranging from 8 to 20 (Ghaly & Alkoaik, 2009; Makkar et al., 2014). The pupal stage lasts from 7-9 days and the emerging adult beetles live for 2-3 months (Makkar et al., 2014).

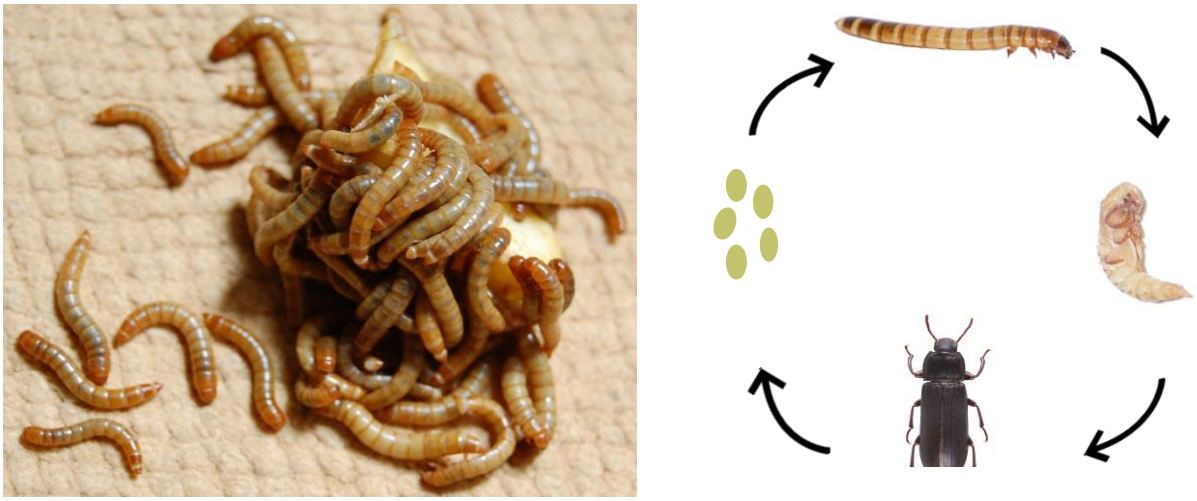

Figure 1. Picture of Tenebrio molitor and illustration life cycle. *"Mealworm" – by MarioM. Licensed under CC by 2.0*

Frass as byproduct

In addition to being highly efficient at converting biowaste into biomass (Wang et al., 2017), the insect' own waste, in form of excrements, skin residue and left-over feed, is a valuable resource in itself (Houben et al., 2020; Schmitt & de Vries, 2020). This waste is often referred to as frass (Schmitt & de Vries, 2020) and has been a subject for research over the past years, with increasing number of publications, due to its properties and in relation to an increased focus on circular economy and reducing the ecological and environmental footprint of synthetic fertilizers and pesticides on agricultural crops (Poveda, 2021; Tilman et al., 2011; van Huis & Oonincx, 2017; Wang et al., 2017).

In order to visualize the potential for using frass as a resource, the amount of frass one can obtain from the production by TM has been determined from a study by Wang et al (2017). The study found that 1700 TM larvae can consume 220 g of food (corn stover and carrots)

supporting an insect biomass production of 4 g and 180 g of frass and residue, both in dry weight (Wang et al., 2017). The frass production occurs during the molting stage in which the larvae produces 2-3 times the amount of frass in relation to biomass growth (Yang et al., 2019).

Frass as fertilizer

The insect industry produces frass in large amounts, up to 40 times larger than the animal biomass produced, showing that frass can be produced on an industrial scale. Due to the nutrient composition of frass, it's use as an organic fertilizer instead of conventional fertilizer is being considered a viable alternative in light of sustainable agriculture and insect production (Poveda et al., 2019; Poveda, 2021; Schmitt & de Vries, 2020)

The chemical and microbial properties of insect frass makes it an efficient natural fertilizer that promotes plant growth (Houben et al., 2020; Poveda, 2021). Frass contains a high amount of labile carbon and has readily-available nutrients in the form of Nitrongen (N), Phosphorus (P) and Potassium (K) that is comparable to that of mineral NPK fertilizers (Houben et al., 2020; Kagata & Ohgushi, 2012). In addition to the chemical properties, microbiota in frass also play an important role in promoting plant growth. The frass from TM, depending on their diet, was found to contain several plant growth promoting microorganisms, generating interest in the use of frass as a biofertilizer (Poveda et al., 2019). As both the interest and production of frass increases, it is of benefit to discover possible uses of frass in order to fully utilize it as a resource.

Frass to protect against disease/pests

One intriguing area of research is that of the stimulating effects of insect chitin and metabolites on reducing pest pressure in ecological systems (Schmitt & de Vries, 2020). The use of frass is argued to be considered a promising alternative to pesticides (Poveda, 2021).

In terms of using frass for defense against pest and pathogens, there are multiple studies on both the direct effects of frass, such as oviposition inhibition, and on plant defense response activation by cellular receptors in the presence of chitin or microorganisms (Poveda, 2021; Tanaka et al., 2019; Xu et al., 2006). One such effect of frass which has been documented in several studies is oviposition inhibition in adult females. Frass from a wild population of desert locus (*Schenistocera gregaria*) showed oviposition inhibitory properties when mixed in sand and presented in cups to female adults, significantly reducing the number of deposited eggs.

Both frass from the desert locus and two other locus species had an inhibitory effect on oviposition in desert locus (Tanaka et al., 2019).

In potato plants, frass from black cutworm (*Agrotis ipsilon*) reduce oviposition of potato tuber moth (*Phtorimaea operculella*) caused by phenols and flavonoids in the frass, when potato tubers were dipped in frass extract (Ahmed et al., 2013). Frass from cotton bollworm (*Helicoverpa armigera*) is shown to have both specific and non-specific oviposition deterrence in bioassays which can reduce inter-specific oviposition (Xu et al., 2006), and larval frass from the Japanese pine sawyer (*Monochamus alternatus)* reduced oviposition in mature females when pine bolts were directly covered with the frass extract (Anbutsu & Togashi, 2002).

Frass can also activate defense responses in plants due to eliciting molecules and microorganisms which are present in the frass. Quilliam et al. (2020) verified that frass from black soldier fly (*H. illucens*) was able to activate defense responses in cowpea plants that reduced wilt disease due to the presence of chitin (Quilliam et al., 2020). Another study by Ray et al. (2016) shows that chitinase enzymes elicited from the frass from *Spodoptera frugiperda* is, while suppressing herbivory defense, able to induce pathogen defense against *Cochliobolus heterostrophus* in maize plants (Ray et al., 2016).

Chitin is an important polysaccharide that is the primary component of the exoskeleton in insects (Steinfeld et al., 2019). As chitin is not a natural component in plants, it is recognizable by the plant and a known elicitor involved in triggering defense pathways in plants (Kombrink et al., 2011). In addition to chitin, the detection of Herbivore-associated molecular patterns (HAMPs), found in frass, can lead to a recognition of the herbivore by the plant (Ray et al., 2016). The HAMPs, compounds found in frass and insect saliva, are cues indicating an insect attack. These compounds can lead to recognition of an insect attack and activate an insect directed defense response (Blakstad, 2021; Ray et al., 2016).

Priming, the physiological process in which plants' readiness to future biotic or abiotic stress is increased, can be any environmental cue that indicate the presence of herbivores (Frost et al., 2020). Chemical compounds can also act as the priming stimulus (Mauch-Mani et al., 2017). Frass can potentially act as one of these stimuli for priming. A primed plant can activate defenses more rapidly or strongly against pest attacks (Pastor et al., 2013). This is shown in De Tender et al' study (2021) where chitin caused a clear priming effect in strawberry leaves

(Tender et al., 2021). Sensing the presence of chitin can trigger different responses within the plant that can potentially repel chitin-containing organisms (Steinfeld et al., 2019; Zogli et al., 2020).

Aphids and mite

Aphids are a serious problem to fruit production and to agriculture in general, damaging the crops and reducing the yield resulting in economic damage. Around 60 of the 4000 aphid species are considered a pest to deciduous fruit trees (Barbagallo et al., 2007). Aphids have two major types of life cycles depending on how they use their host, dioecious (host-alternating) and monoecious (non-host alternating). Dioecious species are the most studied of the two due to the secondary host in these species often being an herbaceous crop plant. In the species that affect crop plants, spring migrants migrate to the crop plants during spring and reproduce throughout the summer before migrating to the primary host in autumn. Here overwintering eggs are produced that hatch the next spring, or in some cases, the stem mothers survive the winter and start reproducing in the spring (Williams & Dixon, 2007). In deciduous fruit trees, the woody fruit tree itself is the primary host. In spring, aphids reproduce asexually on the fruit trees, and in the summer, the dioecious species develop wings and migrate to their secondary hosts before returning to the primary host to lay eggs. The monoecious species stay on the fruit trees the whole period (Rousselin et al., 2017).

There are several ways in which aphids can weaken or harm their host plant. Being sap-sucking insects, they feed on the phloem, diverting nutrients the plant needs for growth and reproduction. Twig stunting, aborting of flowers, and smaller fruits are also common reactions in fruit trees due to aphid nutrient diversion. Additionally, aphids can inject phytotoxic saliva into the host plant, inflict injury by vectoring plant pathogens, and reduce photosynthesis due to sooty molds growing from the aphids' honeydew (Dedryver et al., 2010; Quisenberry & Ni, 2007). In apple and pear trees, direct damage by leaf deformation and shoot distortion is the most common type of damage, along with phytotoxic saliva, honeydew excretion, and fruit damage (Barbagallo et al., 2007).

The mealy plum aphid *(Hyalopterus pruni)* is one of two the aphid species used for investigating the effect of frass on aphids in this experiment. It is a dioecious aphid species

common in plum trees. If left unattended they can form large populations that can halt growth in young trees and directly damage fruit quality (Lozier et al., 2008). Infestation of Mealy plum aphid can cause severe contamination of fruit and foliage due to sooty molds growing on the honeydew produced by the aphids. (Gratwick, 1992). This aphid specie shows little apparent symptoms on the trees in the early stages of infestation. Glistening honeydew on the surface of the leaves and signs of sooty mold growing on the honeydew are the first visual signs of infestation which can lead to this aphid species being overlooked early on (Gratwick, 1992).

The green apple aphid (*Aphis pomi*) is the other aphid specie used in the experiment. It is the most common type of harmful aphid in Norwegian apple orchards. This monoecious aphid species causes stagnation in growth and development and the curling of leaves, but little damage, except for sooty molds, on the fruits. The species has the most potential economic damage on young trees where growth is halted (Røen et al., 2008).

The Plum rust mite *(Aculus fockeui)* is one of the most important mites found in plum trees in conventional Norwegian orchards. Adult females overwinter under leaf buds and in crevices and emerge when the leaves start budding to feed and reproduce (Røen et al., 2008). Reduced sugar content in fruits, lower photosynthetic rates in injured leaves, and reduced growth are all symptoms caused by A. *fockeui* (ASHIHARA et al., 2004).

With an increasing insect farming production industry in relation to increasing food demand, it is important to explore the utility of the resources gathered from the production. Studies has shown that frass can be considered a good alternative to conventional fertilizer, and more recent research has looked at the use of frass in reducing pest pressure. Taking a more general approach on the effect of frass on pest pressure by using frass from T. *molitor*, a specie unrelated to deciduous fruit trees, this study aims to investigate its potential effects and benefits in pest control. The aphid species H. *pruni* and A. *pomi* along with the mite specie A. *fockeui* were chosen as model pest species to see the general effect of frass on insect pests. As this is the first study of its kind on the effect of frass on insect pests in deciduous fruit trees, applying frass by different methods ensures a more thorough exploration of the effect of frass on insect pests in fruit trees. Apple and plum trees treated with frass are hypothesized to result in a lower pest pressure. The expected reduction in pest pressure should be seen in a lower population growth and development, and a lower population size of aphids and mites.

Materials and methods

The experiments in using frass from T. molitor in reducing insect pests were done on two different fruit tree sorts: apple and plum trees. The effect of frass on apple trees were only investigated by spraying trees with liquid frass, while the plum trees were investigated, in different treatments, by both spraying with liquid frass and applying frass in the growth substrate.

Experimental fields

Plum Field

The plum trees used in the experiment were of the species Mallard and were supplied from Jaastad plant nursery AS. The trees had undergone a treatment of lime sulphur when fully dormant to treat for potentially overwintering mites. The trees were planted in 10L pots in accordance with their respective treatments and trimmed to an initial height of 100cm. The planting of the plum trees happened in late April, approximately 1.5 months before aphid infection procedure. Each replica contained three potted plum trees, lined in a Styrofoam box. The boxes were randomly distributed along the two lines, from North to South, and placed in an Mypex covered field with even spacing between them in late April (figure 2).

Figure 2. Layout of the plum field. Photo: Sondre Kaastad Sørsdal

Water and fertilizer were supplied to the trees through drip irrigation. The fertilizer used was Superba red fertilizer, with 9kg mixed with 60l water. At the start of the experiment 0,8 EC

fertilizer (corresponding to 0.5l of water) was used in a 15-minute watering period. From June, 0,4 EC fertilizer (1l of water) was used in a 30-minute watering period. From August, 0,0 EC fertilizer (2l of water) was used in a 1-hour watering period.

Duct tape was placed on the lower part of the stem with the sticky side out, and insect glue was smeared on the tape. This was done in order to stop ants from moving up the trees and interacting with the aphids, as ants have shown to have a positive mutualistic relationship with ants resulting in increased fitness of aphids (Cushman & Addicott, 1989; Renault et al., 2004). Insect glue was also placed on the irrigation pipes in between replicas to stop movement from aphids from one replica to another.

Apple Field

The apple trees used in the trial were from an established natural orchard near NIBIO Ullensvang. The apple trees were imported from the Netherlands and contain a mix of cultivars, namely Summerred, Red Gravenstein, Red Elstar, Red Aroma, and Discovery. The trees were planted in two rows, 50 apple trees in each row with 1m between each tree, and 4.5m between the rows. The apple orchard was planted in May 2020, about a year before the start of the experiment.

The trees were watered through irrigation pipes with a ratio of 1.6l water per tree. During the drought season in June-July, the ratio was increased to 3,2l per tree. The watering happened once a day during a 30-minute period.

Figure 3 - Layout of the apple field. Photo: Gunnild Jaastad

Frass

Frass from T. *molitor* was delivered from Invertapro - an insect rearing, and breeding company based in Norway. The larvae were reared on a diet containing wheat bran and food waste in 70% air humidity and 25-27 °C. The frass has an NPK of 2,6–1,8–2,8 according to Invertapro. While not the exact frass used in the thesis, the full nutrient composition of T. molitor frass produced at Invertapro can be seen in Appendix 1.1.

Frass in soil

For simplicity, the peat substrate used in the plum tree experiments is referred to as soil hereafter when treatment is involved. The content composition of the peat substrate can be seen in table 1.

Table 1 – Contents of peat substrate used in in the experiment

Type	Spahgnum peat
Added pr. m^2	4,0 kg Limestone flour
	1,0 kg Dolomite flour
	1,0 kg Multimix with micronutrients
Dry matter	70g/l
Organic content	90% of dry matter
Acidity	pH 5,5-6,5
Phosphorus	50 mg/L
Potassium	250 mg/L
Total-N	850 mg/L

Investigating the effect of frass by applying frass in the soil was only done on plum trees. 100 grams of frass was used per tree for a total of 3 kg of frass for the 30 plum trees in the frass in soil experiment. Each tree was planted in a 10l pot, giving a ration of 100g/10l. The required amount of peat substrate and frass were placed in a large tractor bed and the mixture was thoroughly mixed before hydrating with water, seen in figure 4. The mixture was then added to the 10l pots along with the plum saplings. In the control treatments, peat mixture without frass was used in the pots.

Figure 4 – Frass and peat substrate being mixed in a large tractor bed. Photo: Sondre Kaastad Sørsdal

Spray application

The frass extract used in spray application of trees was prepared accordingly to methods in Tanaka et al., 2019. Instead of a ratio of 10g/100ml as in their study when mixing frass in 270g of sand, 2 grams of frass per 100ml water was soaked and left at room temperature for 24 hours in 25 l plastic barrels. The 2g/100ml solution was hand shaken and filtered through a large piece of cloth. The frass extract solution was prepared 24 hours before planned spray application on the plum trees, ensuring that the applied solution was fresh.

A Solo 425 comfort backpack sprayer with a Hypro F110-04 flat nozzle was used in the spray application on the plum trees. Frass from TM was sprayed directly on the plum trees from both sides until the solution began to drip from the leaves. A plastic tarp was used to cover the sprayed area in order to not contaminate other treatments, as seen in figure 5.

Figure 5 – Second frass spraying event on apple trees using a Solo 425 comfort backpack sprayer. A plastic tarp was used to not contaminate non-sprayed treatments. Photo: Gunnhild Jaastad

There were two separate spraying events – one set of trees were sprayed two weeks before aphid infection and the other set the day before infection. On average for both spraying events, 108 ml of liquid frass solution was sprayed on each plum tree. An average of 328 mL was sprayed on each apple tree.

Insects

Aphids

Mealy plum aphid (*Hyalopterus pruni*) and the apple aphid (*Aphis pomi*) were selected as the model aphid species of choice for infection due to a large natural population of this species near the field of the experiment. In addition, these species are common aphid pests in Norwegian orchards, making them good model species for the purpose of this study.

H. pruni and *A. pomi* infected leaves were collected from plum and apple trees growing close to the facilities at the research station at NIBIO Ullensvang, in Norway. The infected leaves

were brought to the laboratory upon where five to ten individual aphids of relevant specie were transferred to clean leaves, collected from the same field, with a painting brush. To ensure the clean leaves were not infected with other aphid species they were submerged in water and scrubbed with a brush before infecting. At least one adult individual was placed on each leaf as to promote a faster development in the aphid populations.

Aphid infection procedure

A shoot near the top of the tree was selected and marked for infection. Two of the infested leaves were placed on two different fresh leaves near the top of the shoot and pinned together with an insect needle, seen in figure 6. Clean uninfected leaves were pinned on trees in the treatments not set for aphid infection. The pinned leaves were removed six days after placement and visual confirmation of aphids successfully transferring to the shoots was conducted.

Figure 6 – Infection procedure of aphids on apple and plum trees. Two infested leaves were pinned with insect needles to the trees as depicted. Leaves and needles were removed six days after infection.

Mite

The population-plum rust mite (A. *fockeui)* was investigated by counting mites inside a 1cm incisions at three different locations along the midrib, using a microscope. The population count consisted of ten leaves per replica. The incisions were located at the bottom part near the stem, in the center, and at the top of leaf near the apex (figure 7). All visible and living mites of the right species in all life cycle stages within the circle were counted. Observation of predatory mites were noted but not counted.

Figure 7. Placement of the three 1cm leaf incisions used for counting mites under the microscope. Photo: Sondre Kaastad Sørsdal

Experimental setup

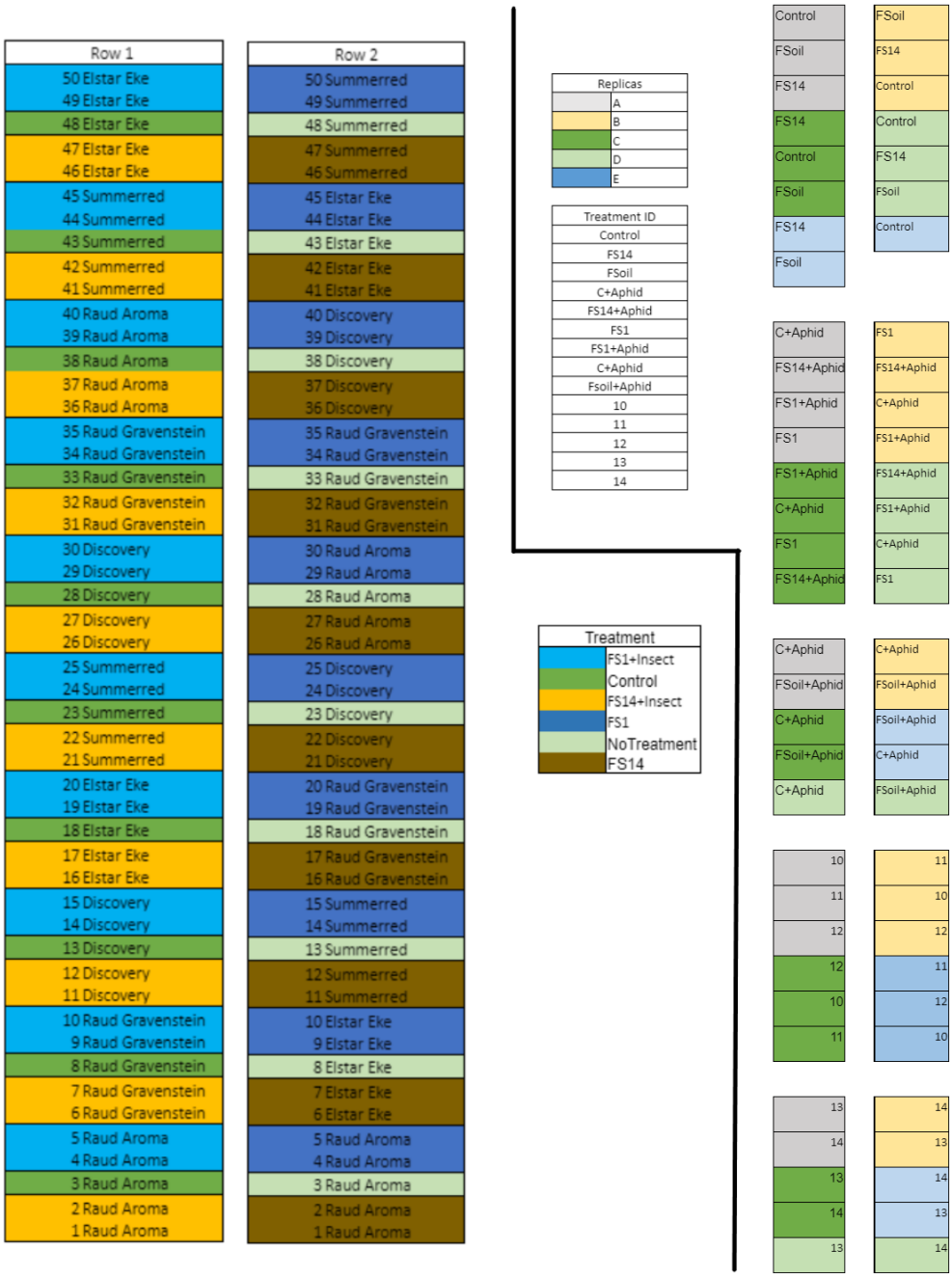

Figure 8 – Layout of apple field (left) and plum field (right). In the apple field (left): 50 trees in each row. Different colours represent different treatments. Tree number and apple variety is displayed within. Only row 1 was infected with A. pomis at start of trial. FS = sprayed with frass, 1 and 14 = days before aphid infection, Insect = infected with A. pomi. - In the plum field (right): Two rows of Styrofoam boxes placed on a Mypex sheet. Horizontal spacing represents different treatment groups. Colour represents replica. Each box represents three plum trees (n=3). The numbered treatments 10-14 are not of relevance as they belong to another experiment but are present in the same plum field. FS = sprayed with frass, FSoil = Frass in soil, 1 and 14 = days before aphid infection, Aphid = infected with H. pruni, C+Aphid = Control infected with aphids.

Apple

The experimental setup of the different treatments on apple trees can be seen on the left in figure 8. The two rows received almost identical treatments, with the exception being that only 1 row was artificially infected with A. *pomi.* This was done to measure the pest-reducing effect of frass on apple trees where A. *pomi* was guaranteed to be present, and to measure the effect of frass in terms of natural infection of A. *pomi.* The liquid frass solution was sprayed on the trees, in different treatments, 1 and 14 days before infecting with A. *pomi,* hence the treatment id's FS1 and FS14 (table 2). There were 10 replicas of each treatment, with FS1 and FS14 treatments having two trees in each replica. The two control treatments had only one tree in each replica.

Table 2 – Table showing the different treatments in the spray application on apple trees and the amount of liquid frass sprayed on each tree in the treatments. Each treatment contained 10 replicas with 2 trees per replica except for treatment "Insect" and "NoTreatment" which had one tree per replica (n = 20, and n = 10).

Treatment	Treatment ID	Infected with aphids	Average amount of liquid frass sprayed
Sprayed with frass 1 day before infection	FS1	No	328 ml/tree
Sprayed with frass 1 day before infection	FS1+Aphid	Yes	328 ml/tree
Sprayed with frass 14 day before infection	FS14	No	328 ml/tree
Sprayed with frass 14 day before infection	FS14+Aphid	Yes	328 ml/tree
Control	NoTreatment	No	0 ml/tree
Control	Control	Yes	0 ml/tree

Plum

The layout of the plum field can be seen on the right in figure 8. The uppermost block consists of treatments not artificially infected with H. *pruni.* The second and third block represents treatments sprayed with liquid frass solution and infected H. *pruni*, and frass-in-soil

treatments infected with H. *pruni* respectively. The numbered treatments in blocks four and five are part of the same plum field but are treatments in another unrelated experiment. The effect of frass from T. *molitor* on H. pruni in plum trees was investigated by both spraying liquid frass solution and by adding frass to the soil, differentiated by the placement in the field and treatment id's FS1 and FS14 for frass spray application (table 3) and FSoil for frass in soil treatments (table 4). These two experiments, as with the spray-application experiment done on apple trees, both had identical artificially infected treatments and treatments where natural infection was investigated.

Table 3 – Table showing the different spray application experiment on plum trees. Each treatment contained 4 replicas with 3 trees per replica (n = 12). All trees were continuously supplied with fertilizer through drip irrigation. 108 ml of liquid frass was sprayed on each tree in the frass-sprayed treatments.

Treatment	Treatment ID	Infected with aphids	Average amount of liquid frass sprayed
Control	Control	No	0 ml/tree
Sprayed with frass 14 days before infection	FS14	No	108 ml/tree
Sprayed with frass 1 day before infection	FS1	No	108 ml/tree
Control	Control+Aphid	Yes	0 ml/tree
Sprayed with frass 14 days before infection	FS14+Aphid	Yes	108 ml/tree
Sprayed with frass 1 day before infection	FS1+Aphid	Yes	108 ml/tree

Table 4 – Table showing the different treatments in the frass-in-soil application experiment on plum trees. Each treatment contained 5 replicas with 3 trees per replica (n = 15).

Treatment	Treatment ID	Infected with aphids
Control	Control	No
Frass in soil	FSoil	No
Control	Control+Aphid	Yes
Frass in soil	FSoil+Aphid	Yes

Registrations

Two methods for measuring population development were used: 1) number of infected shoots over time, and 2) number of aphids on selected leaves (population size)

1) Distribution and population growth - Aphid shoots

In order to follow the establish- and development of the aphid populations on the trees, the number of tree shoots containing one or more aphid individuals were counted on each tree every week after infection for a period of 8 weeks. Previous infected shoots where, if infection subsided due to migration to summer host or other reasons, were not included as an infected shoot in subsequent registrations.

2) Aphid population size – aphids per leaf

The population of aphids for H. *pruni* on the plum trees and A. *pomi* on the apple trees were investigated by counting the number of aphids on 10 leaves per replica every three weeks during the trial period using a magnifying glass. The tree shoots marked for infection were prioritized in counting the population. Leaves were counted evenly from all trees in the replica in order to get an even distribution. In plum tree replicas this would amount to 3-4 leaves per tree. In the apple trees there would be 5 or 10 leaves per tree in each replica. If one of the trees in a replica was not infected with aphids, more leaves were counted on the remaining trees so that total counted number of infected leaves became 10. If a whole replica had less than 10 infected leaves the population on the remaining leaves would be counted as 0.

Liquid frass analysis

Analysis of the liquid frass solution used in spray application of apple and plum trees were done at a laboratory at NIBIO Aas after the experiments ended. Chitin content was measured indirectly by measuring the glucosamine content after acidic hydrolysis (Eikenes et al., 2005). The amount of chitin monomers present in the liquid frass solution used for spray application was 98.0 mg/l.

Statistical analysis

All computation, graphs, and numbers used in this thesis are available to be examined and reproduced by running the markdown files found at https://github.com/SondreSorsdal/Master-Project.

Calculations were done using R, in Rstudio with the package Packman, version 0.5.0, 2018 (R Core Team, 2021; RStudio Team, 2021).

The Shapiro-Wilk normality test was conducted on the data to check for normality (Royston, 1992). As the data was not normally distributed, the non-parametric Kruskal Wallis test (McKight & Najab, 2010) was used to check for significant differences between the treatments for the whole time period. In tests where a significant difference between the treatments was found, Dunn's post hoc test using the Bonferroni method (Dinno, 2015) was used to investigate in which treatment pair the significance lay.

To investigate the development of aphids over time, a general linear model was used for each time period in the data, followed by Tukey's HSD. The glm's were made using Minitab (Minitab, 2021), and the timeseries plots were made in R (RStudio Team, 2021).

Results

Evaluations of frass in plum trees

Experiment 1 – Frass spray application

Aphid distribution and population growth – aphid shoots

The nonparametric Kruskal-Wallis H test for the number of aphid infected shoots on the frass-sprayed treatments for the whole time period had a chi-squared value and p-value of 243 and < 0.0001 respectively, signaling a significant difference between the treatments.

Investigating the development over time, a GLM was used for each week. Significant effect of treatments on aphid shoots was found in week 1 (df = 5 , f = 7.17, p < 0.0001), week 2 (df = 5 , f = 4.99, p = 0.001), week 3 (df = 5 , f = 10.24, p < 0.0001), week 4 (df = 5 , f = 16.19, p < 0.0001), week 5 (df = 5 , f = 9.82, p < 0.0001), week 6 (df = 5 , f = 17.73, p < 0.0001), week 7 (df = 5 , f = 16.31, p < 0.0001), week 8 (df = 5 , f = 8.52, p < 0.0001), shown in figure 9 and table 5.

Table 5 – Spray application of frass. Mean number of plum shoots infested with aphids in each treatment (n = 6) and standard deviation, from week 1 to week 8, after infection with H. pruni. The mean includes all replicas (n=4) of the treatments. Different letters in a row indicate significant differences between treatments (p < 0,05). FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphid at start of trial.

Week	Control	Control+Aphid	FS1	FS1+Aphid	FS14	FS14+Aphid
1	0.00 ± 0 b	1.8 ± 1.9 a	0.1 ± 0.3 b	1.3 ± 0.9 ab	0.08 ± 0.28 b	2.3 ±0.6 a
2	0.00 ± 0 b	1.5 ± 2.2 ab	0.4 ± 0.7 b	0.4 ± 0.7 b	0.08 ± 0.28 b	2.7 ± 3.0 a
3	0.3 ± 0.6 c	3.5 ± 3.1 ab	2.9 ± 1.5 bc	2.8 ± 1.8 bc	0.3 ± 0.6 c	6.1 ± 4.1 a
4	0.4 ± 0.7 b	3.8 ± 1.7 a	3.4 ± 1.4 a	3.3 ± 1.6 a	0.5 ± 0.9 b	4.8 ± 2.3 a
5	0.5 ± 0.8 b	2.8 ± 2.2 a	3.6 ± 1.7 a	3.0 ± 1.7 a	0.3 ± 0.6 b	3.4 ± 2.0 a
6	0.3 ± 0.7 b	3.0 ± 1.6 a	3.6 ± 1.1 a	3.3 ± 1.7 a	0.7 ± 0.8 b	4.0 ± 1.5 a
7	0.4 ± 0.7 b	2.6 ± 1.8 a	3.9 ± 1.3 a	3.3 ± 1.3 a	0.6 ± 1.2 b	3.9 ± 1.5 a
8	0.2 ± 0.4 b	2.3 ± 2.0 a	3.4 ± 1.5 a	2.1 ± 1.6 a	0.2 ± 0.4 b	1.7 ± 1.9 ab

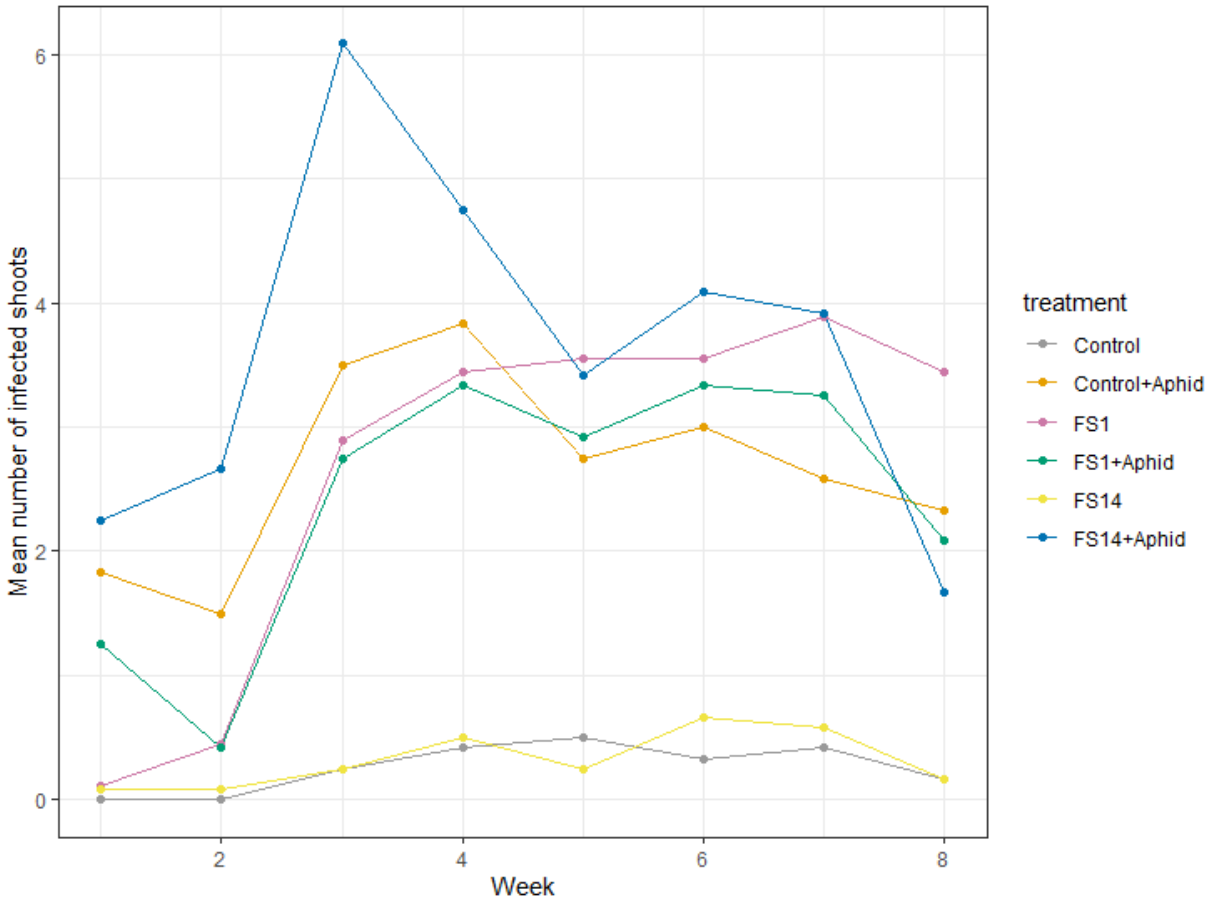

Figure 9 – Timeseries plot showing the mean number of tree shoots infected with H. pruni in each treatment in each treatment at each week in the frass sprayed plum tree experiment. The mean includes all replicas (n=4) of the treatments. FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphid at start of trial.

FS1 had a constant significant higher mean number of infected shoots than Control and FS14 from week 4 and onwards. In this time period, FS1 had a comparable mean to that of the artificially infected treatments. While significantly differing from each other at week 2 and 3, at no point during the trial did FS1+Aphid and FS14+Aphid significantly differ from Control+Aphid (table 5).

Population size – aphids per leaf

The population counts of H. *pruni* on the frass-sprayed treatments for the whole period had a chi-squared value of 279 corresponding with the p-value of $p < 0.0001$.

A GLM was used on each time period in order to investigate the population size. Significant effect of treatment was found in June ($df = 5$, $f = 8.10$, $p < 0.0001$), July ($df = 5$, $f = 20.33$, $p = 0.001$), and August ($df = 5$, $f = 16.37$, $p < 0.0001$), shown in table 6 and figure 10.

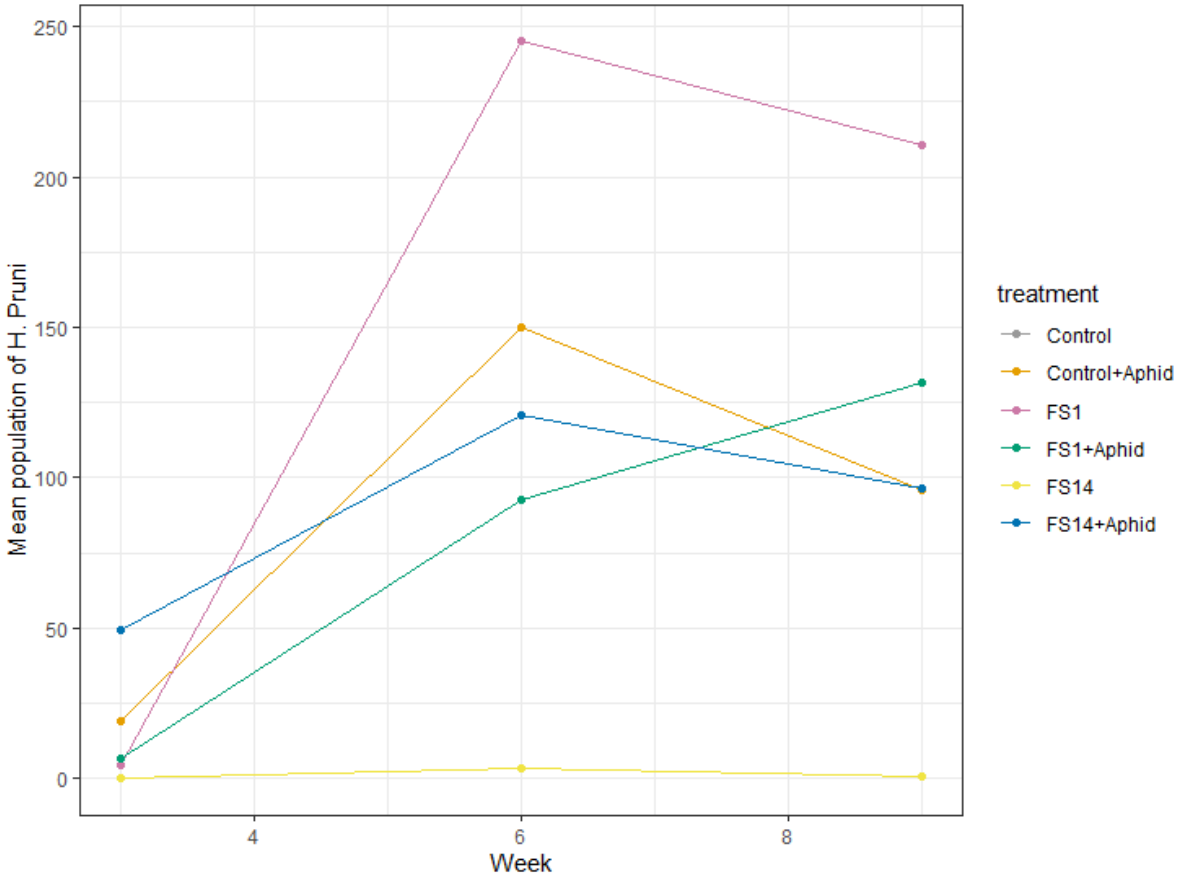

Figure 10 – Timeseries plot showing the mean population of H. pruni in each treatment in each week for the frass sprayed plum tree experiment. The mean includes the population on all leaves (n=10) in every replica (n=4) for each treatment. FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphid at start of trial. Control is not visible since it overlaps with FS14.

Table 6 – Frass in soil. Mean population number of H. pruni in each treatment, including standard deviation, from week 1 to week 8. The mean includes the population on all leaves (n=10) in every replica (n=4) for each treatment. Different letters in a row indicate significant differences between treatments (p < 0,05.) FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphid at start of trial.

Week	Control	Control+Aphid	FS1	FS1+Aphid	FS14	FS14+Aphid
3	0 ± 0 b	19.3 ± 42.2b	4.5 ± 9.2 b	6.6 ± 13.8 b	0.2 ± 1.1 b	49.4 ± 90.2 a
6	3.2 ± 9.3 c	150.0 ± 180.0 b	245 ± 182.0 a	92.9 ± 114.4 b	3.2 ± 9.9 c	120.6 ± 120.0 b
9	0.7 ± 3.2 c	95.7 ± 121.4 b	210.4 ± 175.8a	131.7 ± 148.5 ab	0.5 ± 1.2 c	96.5 ± 142.4 b

The treatment FS1 has a significantly higher population of H. *pruni* than FS14 and control in week 6 and 9. Besides FS14+Aphid having a significantly higher population than FS1+Aphid and Control+Aphid, none of these pairs showed any difference between each other for week 6 and week 9.

Experiment 2 – Frass in soil

Aphid distribution and population growth – aphid shoots

The Kruskal-Wallis H test for the frass-in-soil treatments for the whole time period had a chi-squared of 221 and a p-value of $p < 0.0001$.

A multivariate analysis including time as a factor was not run as aphid populations naturally change with time. The most interesting is if and how treatments differ at each time interval.

To investigate the significant difference of frass applied in the soil of plum trees in terms of the population distribution and population growth, a GLM was done on each time period. Significant effect of treatments was found in week 1 (df = 3 , f = 38.63, $p < 0.0001$), week 2 (df = 3 , f = 6.54, p = 0.001), week 3 (df = 3 , f = 10.54, $p < 0.0001$), week 4 (df = 3 , f = 13.26, $p < 0.0001$), week 5 (df = 3 , f = 14.20, $p < 0.0001$), week 6 (df = 3 , f = 27.96, $p < 0.0001$), week 7 (df = 3 , f = 30.47, $p < 0.0001$), week 8 (df = 3 , f = 38.33, $p < 0.0001$), shown in figure 11 and table 7.

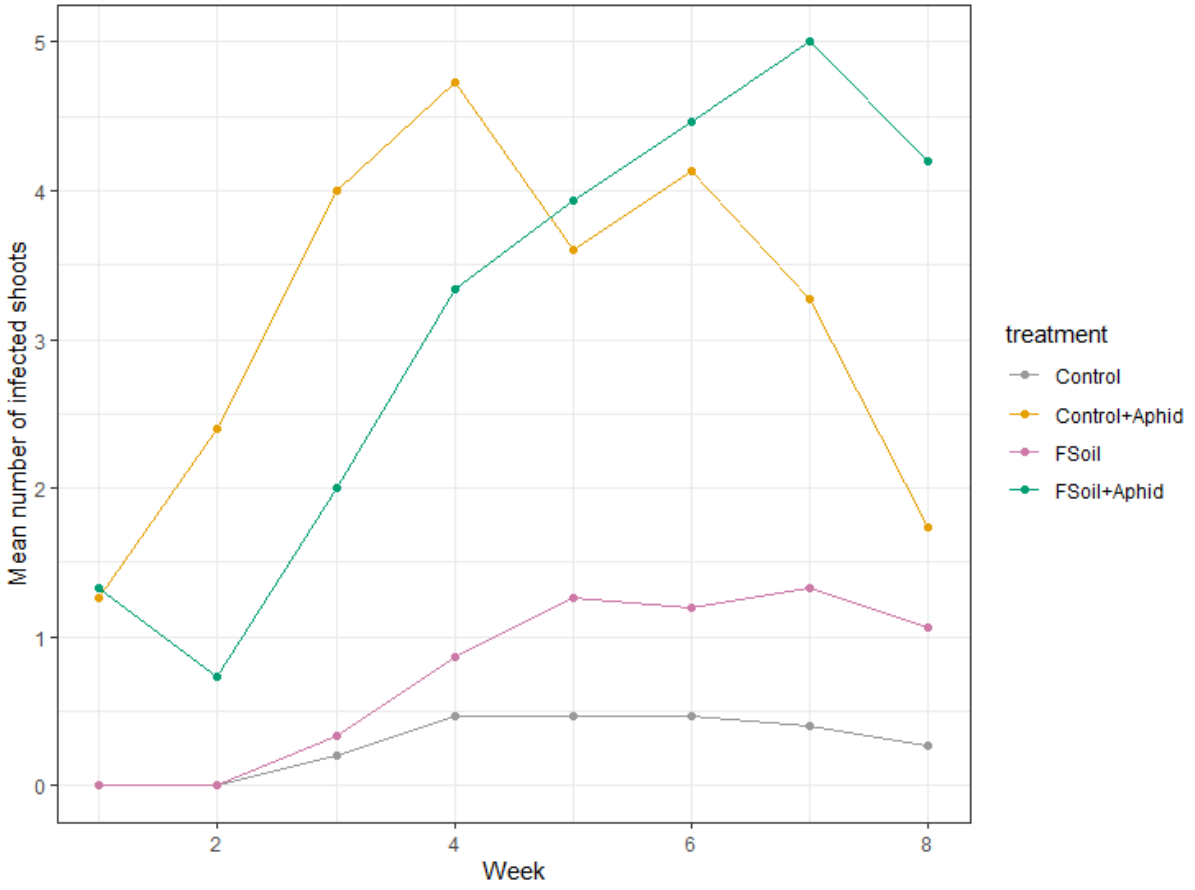

Figure 11 – Timeseries plot showing the mean number of tree shoots infected with H. pruni in each treatment at each week for the frass in soil experiment. The mean includes all four replicas of the treatments. FSoil = frass in soil, Aphid = infected with aphids at start of trial.

Table 7 – Spray application of frass. Mean number of plum tree shoots infested with H. pruni in each treatment, including standard deviation, from week 1 to week 8. The mean includes all replicas (n=4) of the treatments. Different letters in a row indicate significant differences between treatments (p < 0,05). FSoil = frass in soil, Aphid = infected with aphids at start of trial.

Week	Control	Control+Aphid	FSoil	FSoil+Aphid
1	0.0± 0.0 b	1.3 ± 0.7 a	0.0 ± 0.0 b	1.3 ± 0.6 a
2	0.0 ± 0 b	2.4 ± 3.4 a	0.0 ± 0.0 b	0.7 ± 0.5 b
3	0.2 ± 0.6 b	4.0 ± 3.7 a	0.3 ± 0.8 b	2.0 ± 1.7ab
4	0.5 ± 0.7 b	4.7 ± 3.6 a	0.9 ± 1.4 b	3.3 ± 1.8 a
5	0.5 ± 0.7 b	3.6 ± 2.7 a	1.3 ± 1.5 b	3.9 ± 1.5 a
6	0.5 ± 0.7 b	4.1 ± 2.3 a	1.2 ± 1.6 b	4.5 ± 0.8 a
7	0.4 ± 0.6 c	3.3 ± 1.9 b	1.3 ± 1.6 c	5.0 ± 1.4 a
8	0.3 ± 0.5 c	1.7 ± 1.4 b	1.0 ± 0.9bc	4.2 ± 1.3 a

Initially Control+Aphid has a higher mean number of infected shoots than FSoil+Aphid in the first 4 weeks, with week 2 being significantly different. At no other time period was there a significant difference between these two pairs. The treatments Control and FSoil did, while FSoil having a higher mean throughout the whole period, not significantly differ at any time period.

Population size – aphids per leaf

The Kruskal-Wallis H test showed a significant difference between aphid populations in the frass-in-soil treatments for the whole period (chi-square = 260, P-value < 0.0001).

A GLM was used on each time period. Significant effect of treatment in June (df = 3, f = 7,22, p < 0.0001), July (df = 3, f = 45.30, p = 0.001), and August (df = 3, f =57.75, p < 0.0001), shown in table 8 and figure 12.

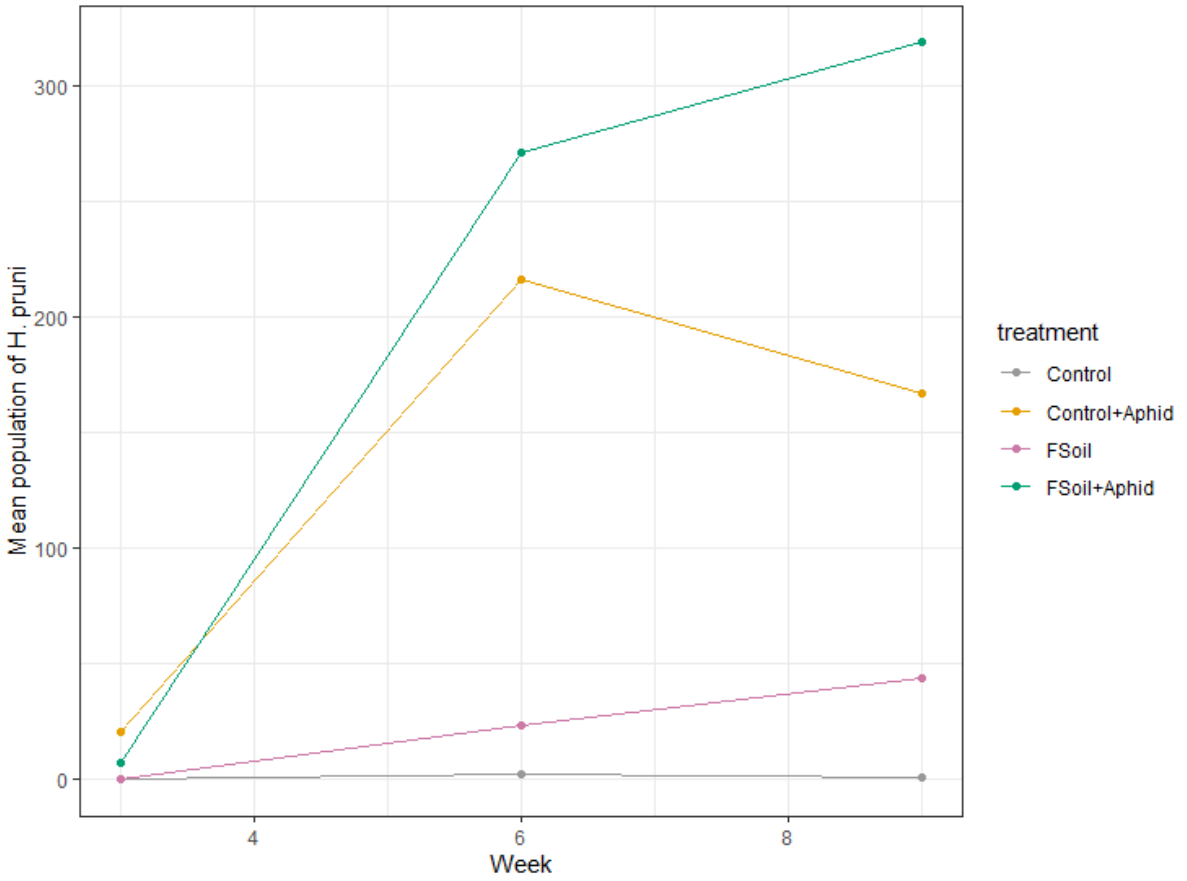

Figure 12 – Timeseries plot showing the mean population of H. pruni in each treatment in each week for the frass in soil experiment. The mean includes the population on all leaves (n=10) in every replica (n=5) for each treatment. FSoil = frass in soil, Aphid = infected with aphids at start of trial.

No significant difference was found between the Control and FSoil pair for the whole time period. FSoil+Aphid ended up with a significantly higher population of H. *pruni* in relation to Control+Aphid in week 9 at the end of the experiment.

Table 8 – Frass in soil. Mean population number and standard deviation of H. pruni in each treatment, from week 1 to week 9. The mean includes the population on all leaves (n=10) in every replica (n=5) for each treatment. Different letters in a row indicate significant differences between treatments (p < 0,05). FSoil = frass in soil, Aphid = infected with aphids at start of trial.

Week	Control	Control+Aphid	FSoil	FSoil+Aphid
3	0 ± 0 b	20.9 ± 50.3 a	0.0 ± 0.0 b	7.3 ± 12.7 b
6	2.5 ± 8.4 b	216.7 ± 187.2 a	23.7 ± 85.6 b	271.5 ± 196.5 a
9	0.8 ± 3.0 c	167.0 ± 191.3 b	43.9 ± 93.4 c	319.0 ± 159.0 a

Mite

Mite population

The nonparametric Kruskal-Wallis H test showed no significant difference (chi-squared =6, p-value = 0.135) in the population of A. *fockeui* between the different treatments, seen in figure 13.

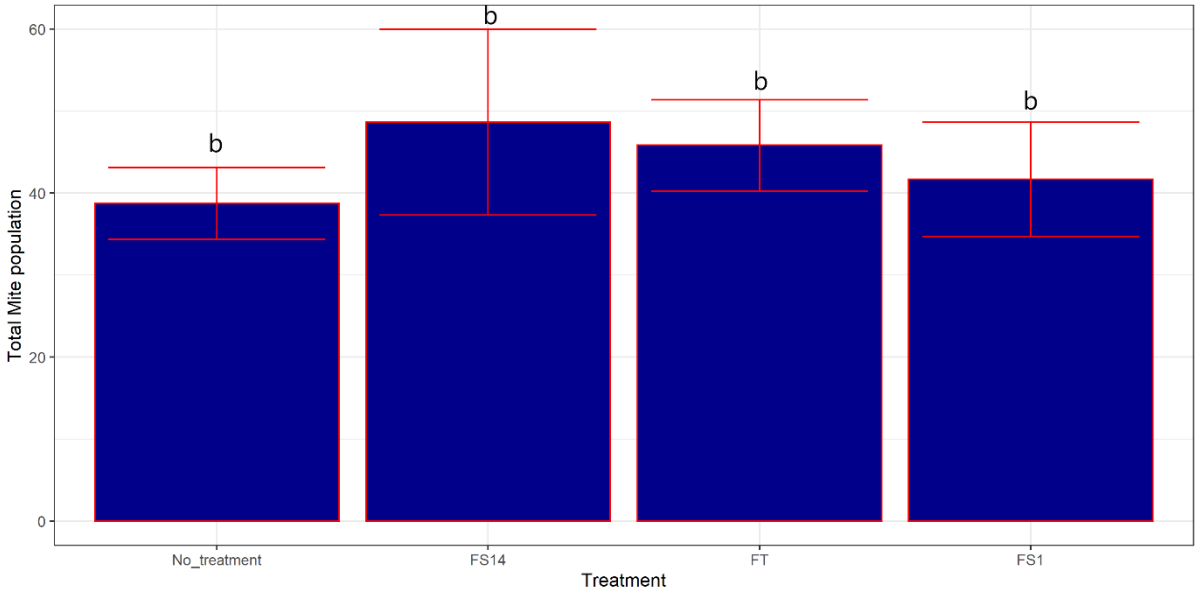

Figure 13 - Histogram showing the mean total population of A. fockeui and SE for each treatment for the whole period. The mean includes the population found on all three incisions on every leaf (n=10) for each tree in in all replicas. Different letters indicate significant differences between treatments (p < 0.05). FS = sprayed with frass, FT = frass in soi, 1 and 14 = days before aphid infection.

Evaluation of frass in apple trees

Experiment 1 – Frass spray application

Aphid distribution and population growth – aphid shoots

A significant difference (chi-squared = 19, P-value < 0.0002) was found in the number of shoots infected with A. *pomi* between the different treatment on the apple trees. Dunn's test using the Bonferroni method was used to check for significance between the different pairs as seen

in figure 14. No significant difference was found between the infected treatments and non-infected treatments.

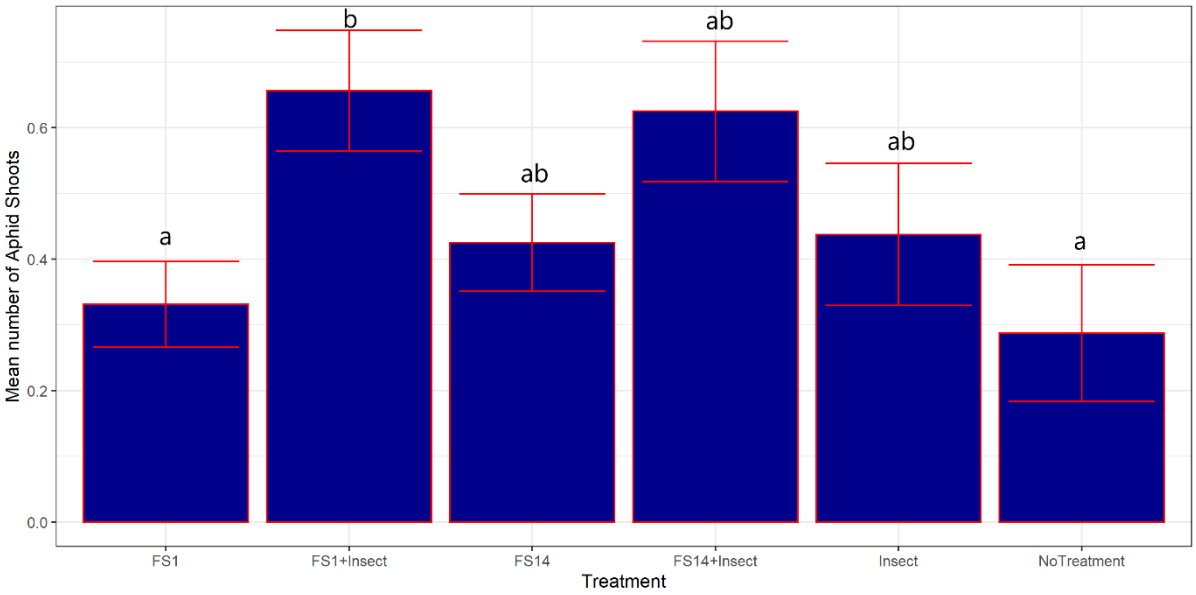

Figure 14 - Histogram showing the mean number of A. pomi infected *shoots in each treatment on the different frass-sprayed treatments over an 8-week period. The mean includes all replicas (n=10) of the treatments Different letters indicate significant differences between treatments ($p < 0.05$).*

A GLM was used on each time period. No significant effect of treatment was found in any time period, shown in table 9 and figure 15. Including apple variety in the equation term resulted in a significant difference between the apple varieties, but the data is not shown.

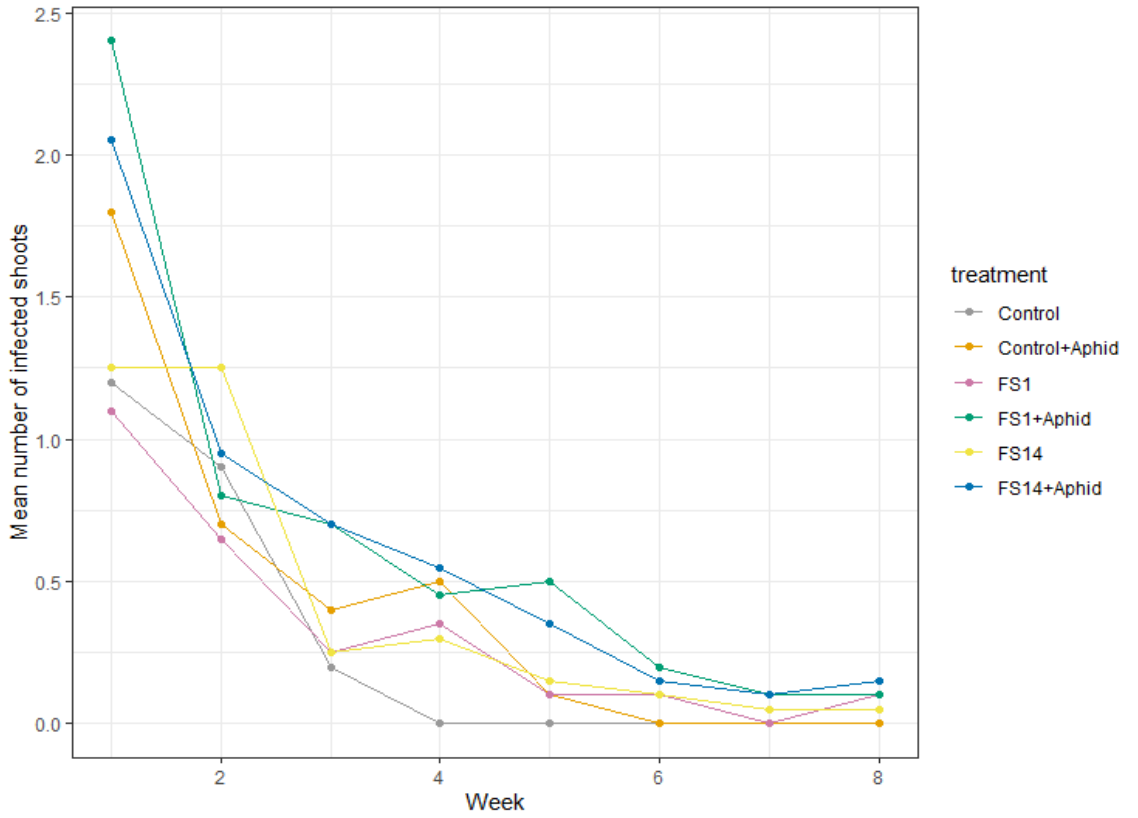

Figure 15 – Timeseries plot showing the mean number of tree shoots infected with A. pomi in each treatment at each week in the frass sprayed apple tree experiment. The mean includes all ten replicas of the treatments. FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphid at start of trial

Table 9 – Spray application of frass. Mean number of apple shoots infested with A. pomi in each treatment, from week 1 to week 8. Different letters in a row indicate significant differences between treatments (p < 0,05). FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphids at start of trial.

Week	Control	Control+Aphid	FS1	FS1+Aphid	FS14	FS14+Aphid
1	1.2 ± 2.0 a	1.8 ± 2.0 a	1.1 ± 1.2 a	2.4 ± 2.0 a	1.3 ± 1.1 a	2.1 ± 2.0 a
2	0.9 ± 1.3 a	0.7 ± 1.3 a	0.7 ± 1.0 a	0.8 ± 0.9 a	1.3 ± 1.8 a	1.0 ± 1.7 a
3	0.2 ± 0.4 a	0.4 ± 0.4 a	0.3 ± 0.6 a	0.7 ± 0.8 a	0.3 ± 0.4 a	0.7 ± 1.4 a
4	0.0 ± 0.0 a	0.5 ± 1.0 a	0.4 ± 0.3 a	0.5 ± 0.8 a	0.3 ± 0.6 a	0.6 ± 1.3 a
5	0.0 ± 0.0 a	0.1 ± 0.3 a	0.1 ± 0.4 a	0.5 ± 0.4 a	0.2 ± 0.4 a	0.4 ± 0.8 a
6	3.0 ± 1.6 a	0.0 ± 0.0 a	0.1 ± 0.3 a	0.2 ± 0.5 a	0.1 ± 0.4 a	0.2 ± 0.5 a
7	2.6 ± 1.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.1 ± 0.3 a	0.1 ± 0.2 a	0.1 ± 0.4 a
8	0.0 ± 0.0 a	0.0 ± 0.0 a	0.1 ± 0.3 a	0.1 ± 0.3 a	0.1 ± 0.2 a	0.2 ± 0.7 a

Population size – aphids per leaf

A GLM was used on each time period. Significant effect of treatment was only found in week 6 (df = 5, f = 6,20, $p < 0.0001$), shown in table 10 and figure 16.

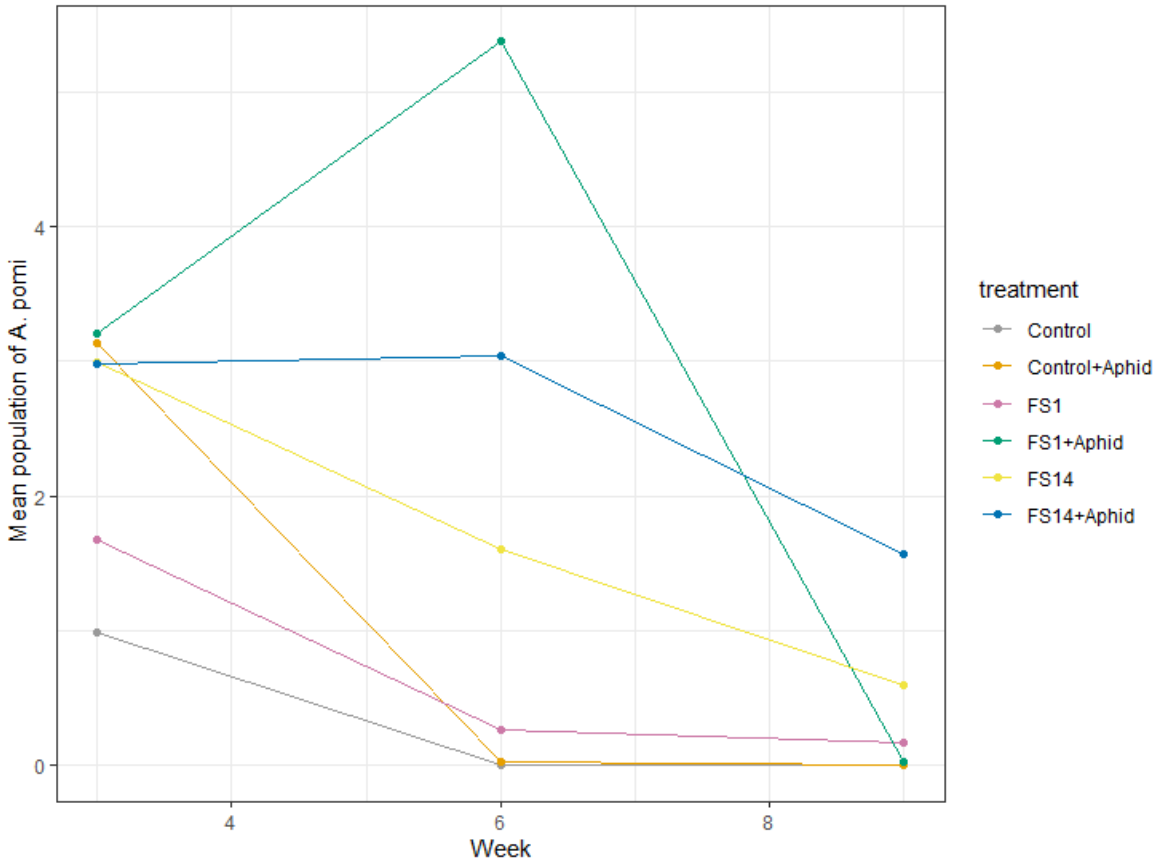

Figure 16 – Timeseries plot showing the mean population of A. pomi in each treatment at each week in the frass sprayed apple tree experiment. FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphid at start of trial.

The FS1+Aphid treatment significantly differed from the Control+Aphid treatment at week 6, having a higher mean population of A. *pomi*.

Table 10 – Spray application of frass. Mean population number of A. pomi in each treatment from week 1 to week 9. Different letters in a row indicate significant differences between treatments (p < 0,05). FS = Sprayed with frass, 1 and 14 = days before infection with aphids, Aphid = infected with aphids at start of trial.

Week	Control	Control+Aphid	FS1	FS1+Aphid	FS14	FS14+Aphid
3	1.0 ± 5.2 a	3.1 ± 10.1 a	1.7 ± 0.5 a	3.2 ± 8.5 a	3.0 ± 7.3 a	3.0 ± 9.3 a
6	0.0 ±0.0 b	0.03 ± 0.3 b	0.3 ± 1.5 b	5.4 ± 16.1 a	1.6 ± 4.8 b	3.0± 12.3 ab
9	0.0 ± 0.0 a	0.0 ± 0.0 a	0.2 ± 1.2 a	0.03 ± 0.2 a	0.6 ± 5.3 a	1.6 ± 8.3 a

While not a focus in this study, including apple variety in the equation term provided a lower AIC for the model. Apple variety was of significance for the whole time period and for week 3 and 6 ($p < 0,05$) Comparing to the F-values of the treatment and variety terms to the population of A. *pomi,* the whole period ($F = 6,23$ vs $F = 17,93$), week 3 ($F = 1,54$ vs $F = 11,39$), and week 6 ($F = 6,42$ vs $F = 6,24$), only week 6 was better explained by treatment than apple variety.

Discussion

The background for this study is rooted in practical and economic value for Norwegian orchard farmers. The practice of applying substances by spraying directly on the trees and into soil of fruit trees is already common to plant nurseries and in orchards. The methods used in applying frass in this study are based on these common practices with equipment already used by orchard farmers. Results yielded in this study would therefore not only be of scientific value but also practical value that could potentially be implemented if positive results were found. Due to the time constraint of my thesis, the study was done by investigating the practical effect of frass from T. *molitor* on insect pests in apple and plum trees in the field. Analyses of the frass was received after the end of the experiments, meaning that the content in the frass could not be compared to other studies before experiments ended. Thus, the experiment setup and methods were focused on investigating the effect of frass on insect pests in fruit trees in the field, and not influenced by lab trials or analyses.

Unfortunately, no clear effect of frass on the population of the fruit tree pests used in the experiments were found. Neither spray application of frass from T. *molitor* nor the addition in soil significantly affected population development and growth or population size of aphids negatively. If frass has the possibility to induce a plant defense response one would assume to see a reduction in population development, growth, and infection by insect pests in frass-treated apple and plum trees.

As this is the first study measuring the effect of frass on insect pests in perennial fruit trees it is contributing to the increasing number of publications on the topic of utilizing frass as both a resource and in terms of plant defense against insect pests. In regards to investigating the effect of frass on mites, in 2013, no information on the effect of chitin-based treatments on mites was yet been published (Sharp, 2013). Through extensive searching, no articles on this topic were found in any databases, meaning little or no information on this topic is still available. As mites have chitinous exoskeletons (Gibbs & Morrison, 1959) and some species are a pest to fruit trees, I wanted to explore how frass would affect the mite population of A. *fockeui.* There was, however, no significant effect of frass from T. *molitor* found on the mite specie A. *fockeui* in this study.

Measuring the population size of aphids in relation to frass has similarly been done in a study by Ray et al. (2020). In their study, aphid populations of corn leaf aphid (*Rhopalosiphum maidis*) were counted on maize plants treated with frass and frass chitinases from S. *frugiperda*. Both the frass and the frass chitinases resulted in a significantly lower aphid population when applied to the maize plants (Ray et al., 2020). The aphid population measurements in this study did not yield similar results. Here, in the frass sprayed plum trees, only at week 3 was there a significant difference in the artificially infected treatments, with FS14+Aphid having a higher mean than its control treatment. Also, in the apple tree experiment, the frass-treated treatment FS1+Aphid had a significantly higher population than the control treatment at week 6. In the frass in soil experiment, while starting out lower, FSoil+Aphid ended up having a significantly higher population mean of H. *pruni* at week 9. These results show that the effect of frass from T. *molitor* on H. *pruni* and A. *pomi* resulted in either no difference in population, or an increase of aphids in frass-treated treatments, opposite of Ray et al' (2020).

From which specie the frass used in the treatments derives from is a key difference between this study and Ray et al' study, in which frass from a pest specie which naturally infects corn was used. Yellow mealworm (T. *molitor*), which the frass used in this study was collected from, is not a natural pest specie of fruit trees. As concluded from their study, Ray et al (2016), proposed that frass-induced defenses in host plants are complex and specific to the host-herbivore system. This could be an explanatory factor of why no reduction in pest pressure was observed not only in the populations of H. *pruni* and A. *pomi,* but also for population growth and development of these species on the plum and apple trees.

In terms of population growth and development over time, measuring the number of infected shoots in plum, at no point in time in the frass-sprayed treatments were the infected FS1+Aphid or FS14+Aphid significantly different from the control treatment. The same results were found for the treatments in the apple trees where no significant difference was found between any treatments. In the frass in soil experiment in plum there was a difference. The FSoil+Aphid treatment had a significantly higher infected shoot mean in relation to the control treatment in the last two weeks of the experiment. As H. *pruni* leaves the primary host in late summer, these last two significant weeks can indicate that the aphids in the frass-in-soil

treatments thrived better late in the season and migrated later than the aphids in the control treatment.

Adding frass to the soil or substrate of plants in order to promote growth and increase stress-tolerance has been done in previous studies by Houben et al. (2020), and Poveda et al. (2019). In terms of the chitin present in the frass, a study by Spiegel et al. (1987) showed that adding chitin directly in the soil of was beneficial in reducing the pest nematode specie *Meloidogyne javanica* in beans (Spiegel et al., 1987). Chitin is considered to be a strong elicitor of plant responses, such as activating defense mechanisms by gene alterations (Shibuya & Minami, 2001). By adding frass directly in the soil of the fruit trees it ensured recognition of biomolecules, microorganisms, and chitin present in the frass, which could potentially activate plant defense responses resulting in a lowering of pest pressure of the species tested in this study. Based on their review article from 2020, Zogli et al, states that plants are able to recognize chitin, a process that activates downstream plant defenses (Zogli et al., 2020). Hohenstein et al showed in their study that chitin from an aphid infection from Soybean Aphids (*Aphis glycines*) was indicated to be involved in triggering soybean defense responses as several soybean genes were involved in chitin regulation (Hohenstein et al., 2019). The same was found in a study by Donze-Reiner et al. (2017) on greenbug (*Schizaphis graminum*) feeding on switchgrass, where genes responsible for chitin recognition and degradation were upregulated when chitin was present. In both studies chitin was suggested to functioned as an Herbivore-Associated Molecular Pattern (HAMP) that triggered a defense response in the plants. The results of this study do not match the above-mentioned literature as there was no obvious reduction of H. *pruni* and A. *fockeui* in treatments where frass was added to the soil. Poveda et al. (2019) showed a positive effect of frass from T. *molitor* on stress tolerance in in chard plants (*Beta vulgaris*) when frass was supplied in the growth medium, and the plants were stressed. The apple and plum trees used in this experiment were supplied with optimal nutrition and water and therefore not stressed. It could be that stress is a factor enabling a response, as frass from T. *molitor* already has proved beneficial to induce tolerance to abiotic stress (Poveda et al., 2019). Increasing the stress put on the trees could elicit a positive effect of frass on pest species.

As stated earlier, no reduction of pest pressure was found in frass-sprayed treatments on plum and apple trees for all three species tested. If spraying frass worked in simulating an insect

attack on the trees, both in terms of priming and inducing a defense response, it was assumed that the trees sprayed 14 days before aphid infection would have a lower pest pressure than the trees sprayed 1 day before infection. If the frass had a non-specific oviposition deterrence effect as in Xu et al' study from 2006 (Xu et al., 2006), one could expect the treatments sprayed 1 day before aphid infection to have a lower pest pressure. The reason being that phenols, flavonoids, or other molecules responsible for an inhibitory effect would be exposed to the environment and be less efficient, as reported by Hilker and Klein in 1989 where the larval frass was assumed to lose its efficiency due to evaporation or damage by oxygen or light (Hilker & Klein, 1989).

The chitin monomer content in the liquid frass extract used on the apple and plum trees were found to be at 98.0 mg/L. No previous studies were found where the chitin content in liquid frass was analyzed as done in this study. While it is confirmed that chitin was present in the liquid frass used on the apple and plum trees, it is not comparable to frass solutions used in other studies where the effect of frass on insect pests were investigated. The reason is that those studies did not focus on chitin but rather other components of frass such as phenols and flavonoids.

The treatments not infected with aphids at the start of the trial showed no difference between frass- and non-frass-treated trees when measuring aphid shoots and population in apple trees. The same results were found in the frass-in-soil experiment in the plum trees. In the frass-sprayed treatments in the plum trees however, the FS1 treatment had a constant significantly higher value of infected shoots and population of H. *pruni* than both the control and FS14 treatments from week 4 to the end of the experiment. A reason for the high amount of *H. pruni* in the FS1 sprayed treatment in plum could be a result of the experiment setup where the replicas of the FS1 treatment were placed randomly in between treatments artificially infected at the start of the experiment. Strong winds on some occasions knocked some trees over enabling direct contact between treatments. This means there was a direct contact between treatments which could have resulted in the movement of aphids from artificially infected treatments to the FS1 treatment. Also, around week three of the experiment, aphids in heavily infested trees tried to flee by moving down the stem and along the water irrigation pipes (Appendix 1.2 and 1.3). Most aphids got stuck on the insect glue placed on the trees, but

an unknown number could have managed to migrate to other treatments. With the results from the artificially infected treatments in plum and the overall results found in apple trees, both showing no significant effect of frass from T. *molitor* on the insect pest species tested for, weighs up for abovementioned potential source of error.

For future studies

The aphids present on the apple and plum trees did not have a choice of where to lay their eggs. In terms of oviposition inhibition, an interesting question is if there could be an effect of frass on the fruit trees next year. If frass triggered a defense response within the trees, could there be a reduction in females laying eggs on frass-treated trees or a reduction in spring hatchlings the next year? The dioecious specie H. *pruni* returns to the main host to lay overwintering eggs. Looking at the population development of H. *pruni* in spring next year is an interesting aspect for future research in order to investigate if the frass from T. *molitor* had an effect. Continuing or doing a similar experiment on the same trees next summer would be of interest to see if the frass had a priming effect, reducing the insect pressure of the tested insect species the subsequent year.

In terms of experiment setup, apple variety was found to be of significance when investigating the effect of frass on the population size of A. *pomi* over time. While an interesting factor that could be investigated, I would recommend using only one apple variety in order to exclude it as an influencing factor. In addition, the experiment setup in the plum field could be altered to further separate infected and non-infected treatments to limit the movement of aphids between treatments.

As no reduction in pest pressure was found when using frass from T. *moltior*, future research on the effect of frass on pest pressure on fruit trees could instead use frass from another specie, either a non-host insect species or a specie naturally infecting fruit tree. In addition, instead of providing optimal nutrition and watering, increasing the stress put on the fruit trees when treating with frass in future studies is a variable to be explored.

Conclusion

The results from all experiments indicate that the addition of frass from *Tenebrio molitor* to plum and apple trees did not explain the variation in aphid and mite population size or aphid distribution. The most important explanatory variable in terms of aphid distribution and population growth was found to be if the trees were artificially infected or not. No reduction in in growth and distribution or population size due to frass was found, contradicting recent studies on the topic of frass on insect pests and questions the use of frass from T. *molitor* produced in industrial insect production as a general utility in reducing insect pests in perennial fruit trees in Norwegian orchards. Further studies are needed to more thoroughly investigate the effects of frass in fruit tree pest management.

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Appendix

A 1.1 – Nutrient composition of T. molitor frass from Invertapro

Table A1.1 – Nutrient composition of T. molitor produced at Invertapro. Produced frass with a similar profile was used in this thesis. From Blakstad 2021

Nutrient	mg/kg dry weight	% of dry weight
NPK		
Total Nitrogen (N)	28 000	2.8
Ammonium (NH_4^+)	1 800	0.18
Nitrate (NO_3^-)	100	0.01
Total Phosphorus (P)	19 000	1.9
Available P	13 000	1.3
Total Potassium (K)	30 000	3
Available K	23 000	2.3
Other macronutrients		
Calcium (Ca)	6 400	0.64
Magnesium (Mg)	8 700	0.87
Sulphur (S)	3 900	0.39
Micronutrients		
Sodium (Na)	500	0.05
Boron (B)	11	0.0011
Cobalt (Co)	n/a	n/a
Copper (Cu)	18	0.0018
Iron (Fe)	380	0.038
Manganese (Mn)	230	0.023
Molybdenum (Mo)	1.9	0.00019
Zinc (Zn)	150	0.015
Heavy metals		
Chromium (Cr)	< 0.5	≈ 0
Nickel (Ni)	1.3	≈ 0
Cadmium (Cd)	0.26	≈ 0
Mercury (Hg)	< 0.05	≈ 0
Lead (Pb)	< 0.5	≈ 0
Other properties		
Organic content		43.6
Dry matter		93.2
Water content		6.8

A 1.2 – Aphid movement

Figure A1.1 – Pictures showing H. pruni stuck on insect glue (left) and the movement of individuals along the irrigation pipe (right). Photo: Sondre Kaastad Sørsdal