Development of an integrated pest management strategy for the control of insect pests of apple orchards in collaboration with growers, advisors, pheromone producers and researchers

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Background
The apple fruit moth, the codling moth and the leafrollers are amongst the most damaging pests in Swedish apple production. Control by chemical insecticides has been the only remedy (Sjöberg et al., 2015). However, the chemical repertoire available for pest control in marginal crops, such as Fennoscandian apple production, is being reduced and public awareness of ecological issues are increasing. Residues from pesticides and their metabolites are an environmental issue and can be present in natural waters and fruits. The chronic effects of pesticides from food intake on human health are not well understood, but there is increasing evidence of carcinogenicity and genotoxicity, as well as disruption of hormonal functions. Accordingly, the use of pesticide will be strongly restricted through the mandatory conversion of the EU apple cultivations into integrated production (91/414/EEC).

Growers, advisors and researchers identified the following insects among the major challenges to meet the EU requirement on integrated production: (1) the apple fruit moth (*A. conjugella*) and (2) a group of tortricid moths including codling moth (*C. pomonella*) and 5 leafrollers (*A. orana*, *A. podana*, *A. rosana*, *P. heparana*, *S. ocellana*) (Äppelträffan, 2011). According to their suggestions, we identified the following as relevant issues to be taken into consideration to meet their requirements:
-Immigration of mated apple fruit moths from rowan forests cannot be avoided and it is extremely difficult to predict
-Only in years when rowan does not yield berries, an insecticide treatments to avoid a damage from apple fruit moth larvae is required; however, in some of these intermasting years, only a low damage on apple is reported and the treatment may be avoided with a significant positive effect on the environment; therefore, a monitoring tool that can precisely point out the migration of female moths into the orchards will lead to a significant optimization of the control of this pest
-Unexpected outbreaks of leafrollers occurred in orchards treated with mating diruption against codling moth; the possibility of formulating and implementing a device that successfully control all of these pests will be of great practical interest for the growers and will lead to a significant reduction in pesticides use.
Research on chemical ecology has revealed environmentally low-impact and effective ways to control pest insects, which may be implemented in integrated pest management programs (Thomson & Jenkins, 2014). Semiochemicals, such as sex-pheromones and kairomones, play a major role in the behavioural manipulation of these pest insects.

Mating disruption by the release of sex pheromones has proven to be effective against a number of lepidopteran pests, including codling moth and leafrollers, and it is nowadays adopted on more than 600000 hectares worldwide. By permeating the atmosphere of the crop with synthetic pheromones, the location between sexes and thus mating is both prevented and/or delayed (Mori & Evenden, 2013). As a natural consequence, the population of the target insect is, over time, subjected to a major contraction, which cannot be achieved with the application of conventional insecticide regimes. Populations of pest insects decrease to such a level that curative interventions with insecticides are, in most cases, no longer necessary (Koul et al., 2008).

The mating disruption technique is species specific and non-target animals, such as predators and parasitoids, remain unaffected. In addition, pheromones are non-toxic for humans and for the environment (Witzgall et al., 2010). Their very low release rate (in the range of 10-30 mg/ha/h) along with their rapid natural degradation, designate them as one of the most promising methods for an economically, environmentally and socially sustainable agriculture. Accordingly, pheromones are admitted in both organic and integrated production.

A successful use of pheromones cannot be achieved without taking into consideration the peculiar attributes of these compounds. Among them, it is known that pheromones affect male behaviour only, and do not target ovipositing females. For the AFM, for example, sex-pheromone could not thus be used as a direct control method (mating disruption), since moths mate in the forest before migrating into the apple orchards where oviposition and damage will occur. In this case, plant-derived kairomones such as rowan volatiles may be used instead of pheromone to monitor the insect during its host-shift. Kairomones are semiochemicals acting as behaviour modifying volatile signals between individuals of different species. In contrast to sex-pheromones, kairomones attracts both females and males. In the case of apple fruit moth, kairomones identified from rowan are a promising tool to monitor the migration of insects from the forest to the orchard (Bengtsson et al., 2006).

Another limitation is that pheromones are usually species specific (i.e. only one species can be controlled at a time) and the formulation of multipurpose mating disruption devices is a current challenge. For example, in orchards where codling moth is controlled by mating disruption, unexpected outbreaks of leafrollers cannot be avoided by the codling moth pheromone alone, which has a behavioural effect only on the target species (Deland et al., 1994).

Through this project we aimed at developeing an integrated management strategy to overcome the above-described limitations of the mating disruption technique. This strategy provides new field-devices for (1) detecting and monitoring the apple fruit moth flight into the orchards and (2) for the simultaneous control of codling moth and leafrollers.

Material and methods

Mating disruption. Field experiments were conducted in the Skåne County, in Southern Sweden. A mating disruption plot with Isomate CLS (1.89-2.70 ha) and a control plot (0.91-3.12 ha) were selected as a single replicate of the experiment in each of the 6 orchards included in the study.

Ethylene-vinyl-acetate twin tubes (Isomate CLS; C. pomonella: (E,E)-8,10-dodecadien-1-ol (34.9%), 1-dodecanol (5%), 1-tetradecanol (1.1%); leafrollers: (Z)-11-tetradecen-1-yl-acetate (24.1%) and (Z)-9-tetradecen-1-yl-acetate (5%); *S. ocellana*: (Z)-8-tetradecen-1-yl-acetate (29.9%); total pure active substances: 364 mg/dispenser) were manufactured by Shin-Etsu Ltd. (Tokyo, Japan) and provided by CBC Europe (Milan, Italy). In 2014 a new component of *S. ocellana* was added to the dispenser with the aim to increase the efficacy of the formulation towards this pest. Dispensers were hand applied in apple orchards at a rate of 800 per ha. Dispensers were hung out in mid-April, prior to the onset of the first flight of the earliest target pests (codling moth).

White cardboard delta traps with a replaceable sticky base were used to monitor the flight activity of the six pests (*A. orana, A. podana, A. rosana, C. pomonella, P. heparana and S. ocellana*). Traps were placed inside each plot, within 10 m from the upwind and the downwind borders and 40 m inside the crop (Figure 1). Captures were recorded weekly from the onset of apple flowering to the end of the season, i.e. when no captures were observed for at least two weeks after the last record.

Since damage to apple inflorescence by *H. nubiferana* are common in Sweden, monitoring traps lured with the corresponding sex-pheromone were also set in the experimental orchards with the aim to measure a possible effect of the synthetic pheromone formulation on this non-target species.

The difference in captures between the untreated and the pheromone-treated plots (trap shutdown) was taken as an estimation of the disruption effect.

Two cages (one in the mating disruption and one in the control plot) were assembled to enclose three apple trees in two of the six orchards. A delta trap baited with two 48 hr-old virgin females, was hung on a branch of an apple tree inside each cage. At the same time, 10-30 virgin males of the same species (*C. pomonella* or *A. orana*) were released inside the cage. The effect of communication disruption was estimated by calculating the difference in male captures between the untreated and the pheromone treated plot and dividing it by the number of males caught in the control (see Porcel et al. 2014 for additional details).

Assessment of larval infestation was carried out at an interval of 7-14 days from July 1st to harvest. In each of the plots, a minimum of 15 trees was randomly selected for this purpose. Within each tree, 30 apples were randomly checked along the entire profile of the plant. When a threshold of 0.7% apples with very fresh larval entries (stage L1) was exceeded in any of the assessed plots, growers were recommended to stop the infestation with insecticide application. In some cases, growers applied additional sprays according to their experience.

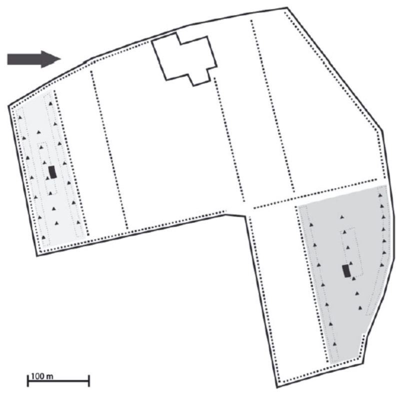

Figure 1. Schematic representation of an orchard used in this study. Control (light grey) and mating disruption plot (dark grey) were equipped with a field cage (black rectangle) and with pheromone monitoring traps hang at the upwind, centre, and downwind side of each plot (black triangle). A dotted line inside the plots comprises a damage inspection area. Dashed lines represent plant hedges and the arrow indicates the direction of the prevailing wind.

From May to September 2012 and 2014, five dispensers were collected every ca. 30 days from an apple orchard situated in Alnarp (Skåne County) and stored at -18 °C until analysis. The orchard was equipped with a climate station. In order to verify whether the remaining pheromone at the end of the first year could be released in the coming season, the release of the dispensers applied in 2012 was monitored from April 2012 to September 2013. Active pheromone ingredients were solvent extracted and analysed through gas chromatography following a published protocol.

<u>*Apple fruit moth.*</u> Wind tunnel experiments were carried out to identify the most behaviourally active compounds to be evaluated in the field as attractants or repellents to egg-laying apple fruit moth. To do this, we measured the attraction of the female moth to rowan, apple and pear. We carried out field trapping experiments in Norway and Sweden during 2012-2014. Both apple orchards and rowan forests (with rowan as the major species) were included in the tests. Commercial apple orchards (0.5-3 ha; cv. Aroma, Discovery, Katja, Ingrid Marie, Rubinola, Gloster, Greensleeves, Elstar) were selected every year according to pest infestation reported in previous years and annual forecasts for the apple fruit moth in Norway reported through VIPS (Forecasts of plant diseases, pests and weeds).

In 2012 we tested blends of rowan volatiles with a different complexity within a rowan and an apple background odour. At the same time, a prototype for a low-release device was developed in collaboration with Shin-Etsu (Tokyo, Japan). The attraction of this

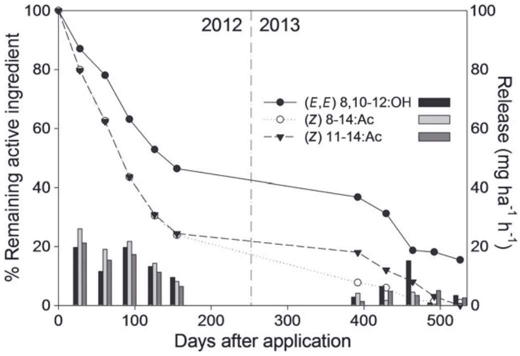

Figure 2. Release (mg/ha/hr) of the main three components from the mating disruption dispenser Isomate CLS during 2012-2013.

dispenser was evaluated in Norway in comparison to those with different composition or material (see Knudsen and Tasin 2015 for additional details).

In 2012-2014 we tested if cumulative catches of the optimized blend within 550-day degrees (DD) in delta traps were correlated with damage at harvest. Day-degrees are measured from full bloom in rowan and 550 DD is currently used in forecasting models to indicate the start of apple fruit moth larvae hatching from the egg. A total of 10 traps per orchard were hung in Sweden and in Norway. In each plot 900 fruits were checked at harvest to establish the injury caused by apple fruit moth larvae. Norwegian experiments were conducted in conjunction with the local field advisory personnel. Prior to commencement of the experiment advisors were trained on how to mount and place the monitoring traps within experimental plots. In addition they were given instruction in adult recognition and diagnosis of characteristic damage caused by target species.

Results

Mating disruption. A significant shutdown of moth catches in traps was recorded for all of the tested pests durinbg 2012-2014, although populations of *A. orana*, *A. rosana* and *P. heparana* were represented by low numbers.

The ability of males to locate calling codling moth females was decreased by the pheromone treatment. A significantly lower communication disruption rate (±SEM) was measured in 2012 (66±13.7 %) compared to 2013 (98.7±1.3%; Figure 3). A high degree of communication disruption was measured for *A. orana* during both 2012 and 2013 (88.1±7.9 and 100±0 (b) in 2012 and 2013, respectively).

The mating disruption treatment had a significant effect (P<0.01, generalized linear mixed model) on damage by leafroller/*S. ocellana* during 2012-2014. The majority of the larvae retrieved on fruits or leaves damaged by leafrollers belonged to *S. ocellana* (41%) or to *A. podana* (29%). Results from the dispenser analysis showed a constant release during the whole season under the climatic conditions of Alnarp (Figure 2).

Apple fruit moth. Volatiles from from rowan were attractive as such and on the top of an apple background (Figure 4). A 7-component blend mimickin the rowan headspace was capable of attracting moths within an apple background in the field. The field experiment in Norway (2012) showed a high activity of a 7-component blend of rowan volatiles. This blend was capable of attracting female moths in both rowan and apple background and was therefore included in the prototype releasing device. The prototype dispenser was able to attract significant numbers of apple fruit moths (Knudsen et al., 2014; Tasin et al., 2013).

In 2013 we measured a damage in Norway but not in Sweden (Knudsen & Tasin, 2015). In 2014 we carried out experiments in 19 sites, 11 in Sweden (10 in Skåne and 1 in Bläkinge) and 8 in Norway. We calibrated our system with a threshold of 0,4 moths per trap within 500 degree days. This value correspond to a damage of 2,6% at harvest. Out of the 19 sites monitored in 2014, 15 were in line with our predicted model. We registered 11 sites with less than 0.4 captures per trap and less than 2% of damage and 4 sites with more than 0.4 captures and a damage greater than 2%. However, we had 4 cases where the captures did not match the damage level. In 1 case we measured less than 0.4 captures and a damage of 3,5% and in 3 cases more than 0.4 captures with a damage <2%. Although the model has

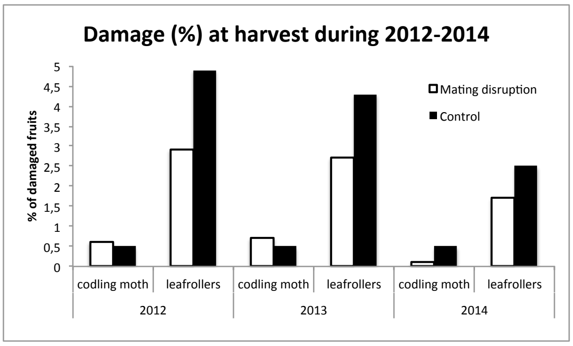

Figure 3. Damage (as % of injured fruits) in treated and control plots by codling moth and leafrollers during the three season of the study.

overestimated the damage in 3 orchards and underestimated in one of them, such an error may still be considered acceptable in the practice. Overall, the output from the model allowed for a reduction of 58% in insecticide compared to the Norwegian forecasting system VIPS. Over the period 2012-2014 the correlation between captures in the kairomone trap and the injury at harvest had a R squared coefficient of 0.90 (Figure 5). This corroborates the validity of the forecasting model.

During 2012-2014 we registred a damage also on the pollination trees. We observed a higher damage on these plants compared to nearby apple trees. We argue that pollination trees could either be more attractive to egg-laying females or that the larvae of the pest may have a higher survival, since fruits are smaller than ordinary apple. Pollination trees may be use as a trap crop for the apple fruit moth in future experiments.

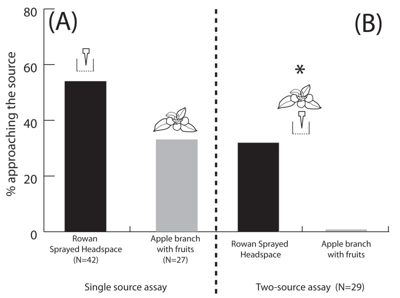

Figure 4. Laboratory response of female apple fruit moths to a headspace collection from rowan and to an apple branch (A) or to the same headspace with the branch in the background (B). Bars capped with an asterisk differ significantly.

A mass-trapping experiment was carried out in Norway during 2012 using the prototype dispenser. In this experiment, we had plots with and without traps and we measured the damage inflicted by the larvae. Traps were hung at a density of 1000 per hectare. Although traps caught a part of the incoming apple fruit moths, the remaining population was capable of damaging the fruits and no differences was found between the two treatments. According to this result, a decision was taken to focus our effort exclusively on the development of the kairomone trap as a monitoring tool (Knudsen et al., 2013).

Discussion

Mating disruption. A substantial inhibition of the communication between females and males was achieved in the experiment with cages. While the communication was completely inhibited in *A. orana*, a lower effect of the synthetic formulation was measured in *C. pomonella*. The new formulation reduced the number of males caught in pheromone monitoring traps for the whole range of tested species during 2012-2014. While a total inhibition of catches was measured for the majority of the leafroller moths, a partial effect was observed in the case of *C. pomonella* and *S. ocellana.* We recorded a significant decrease of injured apple at harvest over the period of the study. The tested formulation had a comparable or better efficacy than the sprayed control. The reduction of the damage at harvest was however variable within years and species, with leafrollers such as *S. ocellana* and *A. podana* being among the most damaging larvae. Due to a low population density in the experimental plots, additional experiments are needed to evaluate the efficacy of the product against *C. pomonella.* The release from the twin with a partial release in the following year. A higher release of acetates was measured in comparison with alcohols. Once the technical efficacy of a mating disruption product has been established, its implementation becomes often associated with a number of supplementary issues, i.e. the social context, the regional values and the commitment of the involved stakeholders to make it work. In the Skåne

County, where the present study was conducted, the protection of the watershed and the preservation of the natural ecosystems are issues currently under societal debate. The local communities perceive an interconnection between these subjects and their life quality in addition to the touristic image of the area. Local stakeholders may thus share their vision and their perspectives with the aim to evaluate the advantages that the implementation of mating disruption would offer over conventional insecticides, as a social and environmental investment in the region.

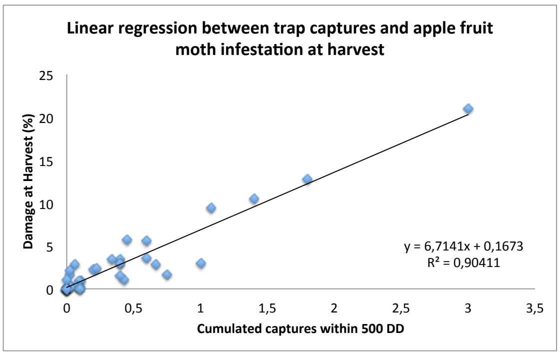

Figure 5. Linear regression on which we based the forecasting system to predict apple fruit moth attack in orchard. Each point (N=42) represents an area of ca. 500 m² at a border or at the center of an orchard. Each area was equipped with 5-10 kairomone traps. Experiment were carried out in Sweden and Norway during 2013-

Apple fruit moth. The forecasting system based on the kairomone (rowan volatiles) trap is a valid tool to predict apple fruit moth damage at harvest. Although additional experiment will be needed to acquire further knowledge, this monitoring system reflected the real infestation in the field during two years of experiments in both countries. The kairomone developed in the project represents a novel tool for pest monitoring. Kairomones offer the advantage over sex-pheromones of being bisexual attractants. This is especially important in species such as the apple fruit moth, where males and females have different activity patterns and females migrate after mating from the forest to the orchards to find an oviposition substrate. By catching females, an accurate prediction of the damage was accordingly possible.

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Conclusions (use of the results by the sector)

According to our results, we recommend the use of the new mating disruption product as a tool to significantly reduce the field population of lepidopteran pests as well as to diminish the load of chemical insecticides in orchards. Due to the variability of the results in relation to single orchard features, field scouting needs to be considered as a necessary support to growers. Through an active co-operation between growers and the advisors belonging to Äppelriket, the use of pheromone disruption has already replaced conventional insecticides over an area of ca. 70 ha. The company CBC Biogard/Shin Etsu (Milan, Italy) is currently dealing with the registration issue at the EU level. The company will send in the application at the Belgian Ministry of Agriculture with the aim to first register the product in this country, which should be relatively quick in handling the documents. Upon approval, the registration of the product will be extended to additional interested countries, in accordance with local dealers. Because of the current narrow market, in Sweden it is still not clear whether or not a company will take the cost for this process. However, we will continue to facilitate a dialogue between stakeholders, including Äppelriket, CBC Europe Biogard and Kemi, with the aim to find possible ways to register the product in Sweden.

Data about the forecasting system for the apple fruit moth are published in both popular science and scientific journals and currently used in the field both in Norway and Sweden. We are currently buying the kairomone lures from Shin-Etsu and delivering it to growers and advisors along with the respective traps. Nibio/Bioforsk has financed the patent of the lure, which is nowadays approved (Knudsen & Tasin, 2014). A company has been created in Norway to take care of the product, together with other innovative tools for pest control (AdeoScent Technology, www.adeoscent.no). Because the product is currently used exclusively for monitoring, a registration is not required.

Communication with the sector

Results of the project were presented and discussed at the following meetings:

-IOBC (International organization for biological control) meetings: "Semiochemicals: the essence of green pest control", Bursa (Turkey, 2012.10.1-5)
-Annual meeting (Äppelträffan) with apple stakeholders (2013.01.21, Alnarp, organized by SLU)
-IPM Future (PURE Conference), Riva del Garda (Italy) (2013.03.19-21)
-Meeting with growers and advisors at Äppleriket (2013.11.11, Kivik)
-Annual Meeting of LRF Trädgård (2014.04.04, Åkarp)
-IPM days (2014.11.4-5, Örebro, organized by the Swedish Board of Agriculture)
-IPM days (2014.12.18, Kristianstad, organized by the Swedish Board of Agriculture)
-Meeting with advisors at Äppleriket (2015.02.17, Kivik)
-IPM days (2015.03.19, Hallsberg, organized by the Swedish Board of Agriculture)
-Organic fruit seminar and workshop (2015.04.21, Alnarp, organized by EPOK-SLU)
-Working Group on Control of Apple Fruit moth (7 meetings via telephone with the aim to share knowledge on the pest and organize a field experiment; participants: Henrik Stridh (Äppelriket, Kivik), Sanja Manduric (Swedish Board of Agriculture), Nina Trandem (Bioforsk, Ås, Norge), Karin Jahr and Christina Marmolin (LRF), Pernilla Gabrielsson (Ålands Landsbygdscentrum) and Fredrik Bjöklund (FHS, Finnland).