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Biological control of tortricids and aphids in strawberries

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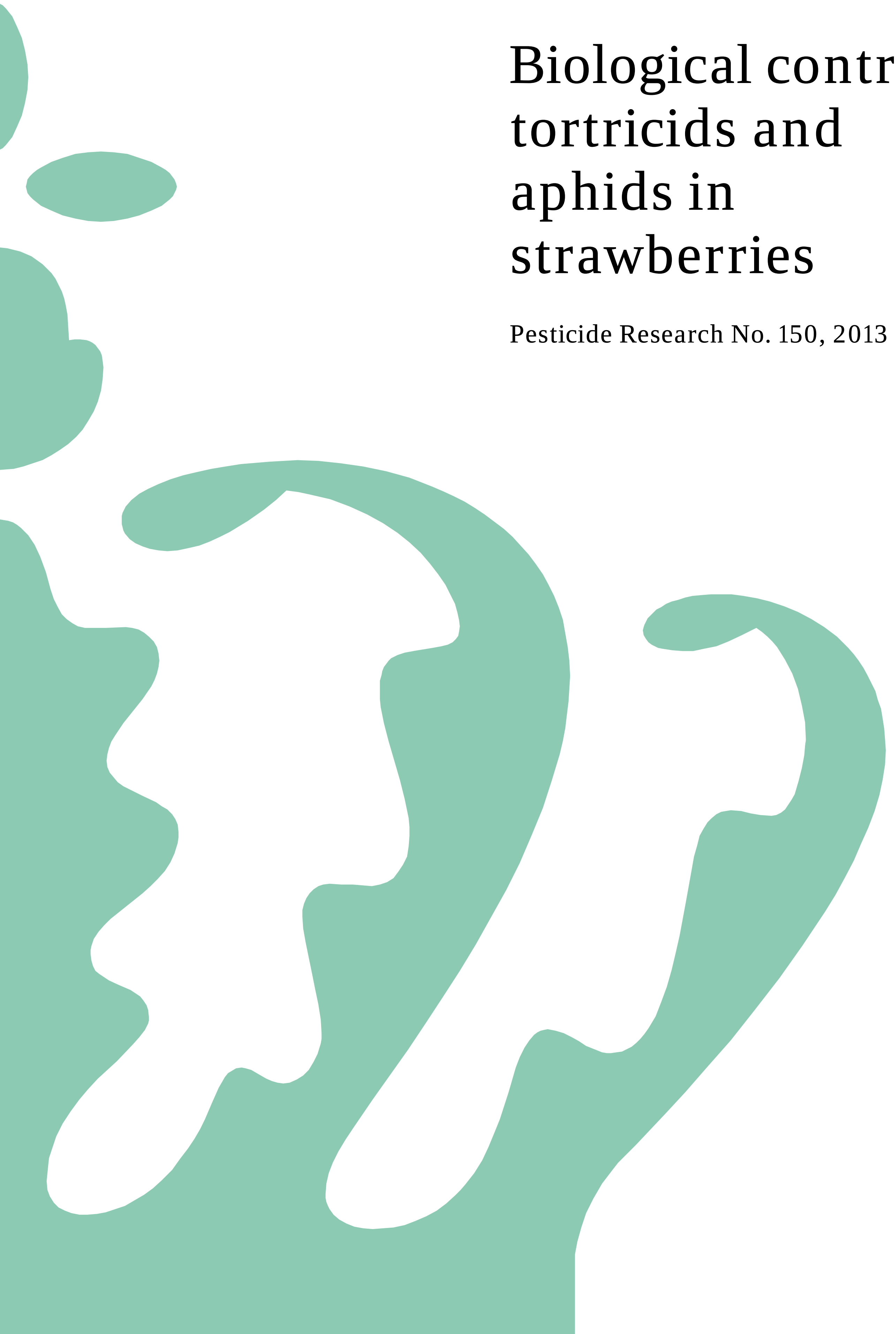

Title:

Biological control of tortricids and aphids in strawberries

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Indhold

Forord

Adgang til alternativer til insekticider er en væsentlig kilde til at nedbringe pesticidforbruget i jordbær. Her er biologisk bekæmpelse og fremme af naturlig regulering (funktionel biodiversitet) centrale elementer. Fremme af funktionel biodiversitet (funktionel biologisk bekæmpelse/ *conservation biological control*) omfatter håndtering af såvel afgrøde som habitater uden for afgrøden. Funktionel biodiversitet kan give et væsentligt bidrag til skadedyrsregulering og kan kombineres med andre typer biologisk bekæmpelse og indgå i strategier til integreret skadedyrsregulering (IPM).

Jordbær, der er den vigtigste danske bærafgrøde, angribes af en række skadedyr, blandt andre jordbærvikleren. I fremtiden forventes nye skadedyrarter at gøre deres entré i danske jordbær, fx løgbladlusen. Insekticidforbruget i danske jordbær er højt og hindrer i vid udstrækning udnyttelse af biologisk bekæmpelse over for jordbærskadedyr.

Denne rapport følger et for MST-rapporter nyt format, idet videnskabelige artikler udgør rapportbilagene I-III, mens et strategipapir for øget brug af biologisk og integreret bekæmpelse i jordbær udgør bilag IV. Af ophavsretsmæssige hensyn er artikler submitted til Open Access Journals.

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Sammendrag

Jordbær, der er den vigtigste danske bærafgrøde, angribes af en række skadedyr, blandt andre jordbærvikleren. I fremtiden forventes nye skadedyrarter at gøre deres entré i danske jordbær, fx løgbladlusen. Insekticidforbruget i danske jordbær er højt og hindrer i vid udstrækning udnyttelse af biologisk bekæmpelse over for jordbærskadedyr. Dette projekt har afklaret forhold af betydning for valg af dyrkningspraksis - hhv. økologisk og konventionel praksis og praksis' virkning på jordbærvikleren og dens naturlige fjender (snyltehvepse og insektpatogene svampe) samt på nematoder (plantepatogene og insektpatogene). Projektet har også set på udnyttelse af funktionel biodiversitet i form af blomsterbræmmer som kilde til føde (nektar og pollen) for jordbærviklerens naturlige fjender. Desuden har projektet belyst muligheder for biologisk bekæmpelse af løgbladlus. Projektets resultater og strategiske overvejelser bidrager til videreudvikling og øget anvendelse af biologisk og integreret skadedyrsbekæmpelse i jordbær.

1.1 Jordbær er Danmarks vigtigste bærafgrøde.

Jordbær er den vigtigste bærafgrøde i Danmark til friskkonsum. Danske jordbær, der dyrkes på ca. 1051 ha, er en højværdiafgrøde med et dækningsbidrag på 128.000 kr. pr. ha (2004). Med en gennemsnitlig produktion på 4624 tons og en pris på 14,6 kr. pr. kilo giver dette en årlig værdiskabelse på 67 mio. kr.

1.2 Det høje insekticidforbrug hindrer udbredt brug af biologisk bekæmpelse

Der er et betydende insekticidforbrug i danske jordbær, der angribes af en række skadedyr, hvoraf jordbærvikleren, *Acleris comariana* (Lienig & Zeller) (Lepidoptera: Tortricidae), er blandt de mest alvorlige. Arten er udbredt, og stort set alle avlere oplever årlige angreb med følgende pyretroidbehandlinger ved konstateret angreb (1-3 behandlinger pr. sæson). Endvidere er nye skadedyrarter, der kan optræde som alvorlige skadedyr i varmere egne, begyndt at dukke op – et af disse nye skadedyr er løgbladlusen, *Myzus ascalonicus* Doncaster (Homoptera: Aphididae), der med de globale klimaforandringer kan forventes at blive et stigende problem i Danmark. Det eksisterende pyretroidforbrug mod jordbærviklere og det forventede forbrug af insekticider mod løgbladlus er en betydelig hindring for implementering af biologisk bekæmpelse over for jordbærskadedyr, da insekticiderne er skadelige over for mange nyttedyr, såvel de naturligt forekommende som arter udsat som led i biologiske bekæmpelsesstrategier. Så længe viklere og bladlus bekæmpes kemisk, vil brug af insekticider således som oftest også være nødvendig mod andre skadedyr.

1.3 Hvordan kan insekticidforbruget nedsættes?

Adgang til alternativer til insekticider er en væsentlig kilde til at nedbringe pesticidforbruget i jordbær. Her er biologisk bekæmpelse og fremme af naturlig regulering (funktionel biodiversitet) centrale elementer. Fremme af funktionel biodiversitet (funktionel biologisk bekæmpelse/ *conservation biological control*) omfatter håndtering af såvel afgrøde som habitater uden for afgrøden. Funktionel biodiversitet kan give et væsentligt bidrag til skadedyrsregulering og kan kombineres med andre typer biologisk bekæmpelse og indgå i strategier til integreret skadedyrsregulering (IPM). Forudsætningen for udvikling af biologisk bekæmpelse og IPM-strategier er et øget kendskab til skadedyr og deres naturlige fjenders biologi, og hvordan de påvirkes af dyrkningspraksis.

Projektets mål var at a) afklare betydningen af dyrkningspraksis herunder økologisk eller konventionel driftsform for jordbærvikleren og dens naturlige fjender, b) afklare metoder til fremme af naturlig regulering af jordbærvikleren ved øget funktionel biodiversitet, i dette projekt en undersøgelse af værdien af forskellige blomster for snyltehvepse og forsøg med udsåning af blomsterbræmmer i jordbærmarker, og c) identificere naturlige fjender til biologisk bekæmpelse af løgbladlusen. Projektet skulle desuden d) sikre, at de opnåede resultater viderebringes til jordbæravlerne som led i hurtig implementering. Til dette formål identificerede projektgruppen i forbindelse med projektansøgning tre områder, hvor der var særlig mangel på viden om biologisk bekæmpelse og udarbejdede i projektperioden et såkaldt strategipapir, som beskriver eksisterende viden med inddragelse af den nye viden fra projektet. Strategipapiret skal fremadrettet bidrage til at sikre forskning, udvikling og implementering af biologisk bekæmpelse i jordbær. Projektet er derfor udført i samarbejde mellem forskere ved Københavns Universitet og Aarhus Universitet og GartneriRådgivningen. Dialog med jordbæravlere er sikret blandt andet gennem deres deltagelse i projektworkshops undervejs i projektet.

Projektet har været struktureret i fire dele I, II, III, IV (nedenfor). Resultaterne af de enkelte projektdele er afrapporteret i tre videnskabelige artikler samt strategipapiret, der dels er vedlagt som bilag til rapporten, dels kort refereres og perspektiveres i det følgende.

I. Indvirkning af dyrkningspraksis på jordbærvikler, nematoder og deres naturlige fjender
II. Metoder til fremme af funktionel biodiversitet i jordbær
III. Identifikation af naturlige fjender til bekæmpelse af løgbladlus
IV. Strategipapir

1.4 Jordbærvikleren og dens fjender er påvirket af dyrkningspraksis

For jordbærvikleren har projektet afklaret sammenhængen mellem dyrkningspraksis, omfang af angreb og forekomst af viklerens naturlige fjender med henblik på udvikling af funktionel biodiversitet til biologisk bekæmpelse. Vores resultater viser, at jordbærviklerangreb ved konventionel dyrkningspraksis er væsentligt højere end i tilsvarende økologisk praksis. Med hensyn til virkning af dyrkningspraksis på naturlige fjender er der ikke nogen sammenhæng i samlet parasitering, når vi alene ser på økologisk versus konventionel dyrkningspraksis, men i økologiske brug er den mest almindelige snyltehveps *Copidosoma aretas* (Walker) (Hymenoptera: Encyrtidae) mindre dominerende. Der var en signifikant effekt af markalder med større angreb i ældre marker. Desuden faldt viklerangreb med 7 % pr km fra syd mod nord (en afstand af 30 km). Der var også signifikant større angreb med flere år med jordbær på bedriften. Vi fandt dog ikke nogen signifikant effekt af markalder på angreb af jordbærvikler i en sammenligning af 1.-års og 2.-års jordbær i 2010.

For entomopatogene svampes vedkommende kan vi konkludere, at naturligt forekommende svampeangreb på jordbærvikleren er på så lavt et niveau, at det ikke har betydning for naturlig regulering af jordbærvikleren. Dette udelukker dog ikke, at entomopatogene svampe kan anvendes til inundativ biologisk bekæmpelse. Undersøgelsen peger endvidere på en højere forekomst af entomopatogene nematoder i økologiske dyrkningssystemer og lidt mindre forekomst af planteparasitiske nematoder.

Den nye viden, der er opnået gennem projektet, peger på, at økologisk dyrkningspraksis kan bidrage til betydeligt reduceret angreb af jordbærvikler ned til et niveau, hvor yderligere bekæmpelse sjældent er nødvendig. Ligeledes peger undersøgelserne på, at ældre jordbærmarker vil have større jordbærviklerangreb. Det højere angreb mod syd vurderer vi kan have sammenhæng med, at der dyrkes mest jordbær her, med medfølgende større risiko for angreb af jordbærvikler, men det vil kræve yderligere undersøgelser at verificere dette.

1.5 Boghvede har højest fødeværdi for jordbærviklerens snyltehveps

Projektet har undersøgt om blomstrende planter kan fremme forekomst af nyttedyr. Specielt er der fokuseret på betydningen af blomstrende planter som fødekilde for jordbærviklerens snyltehveps, *C. aretas*. Hvis parasitering kan fremmes ved hjælp af blomstrende planter, så kan det forventes, at udsåning af blomsterbræmmer kan bidrage til at regulere jordbærviklere og herved indgå i en strategi rettet mod at nedbringe det høje insekticidforbrug mod dette skadedyr. Undersøgelser af blomstrende planters værdi som føde for *C. aretas* samt for jordbærvikleren tjente til at udvælge den bedst egnede plante til forsøg med blomsterbræmmer.

Projektet har påvist, at boghvede var den blomstrende plante, der bedst fremmede jordbærviklerens snyltehveps, mens den for voksne jordbærviklere ikke var bedre end andre blomstrende planter. Hermed favoriseres nyttedyret over skadedyret. I undersøgte afstande fra blomsterbræmmerne (1-11 m) var der ikke forskel i parasiteringsgrad, men øget larvemortalitet nær blomstrende boghvedebræmmer. Dette peger på, at blomsterbræmmer fremmer andre naturlige fjender, og viser behov for yderligere undersøgelser af blomsterbræmmers bidrag til funktionel biodiversitet i jordbær.

1.6 Mariehøns mod løgbladlus ser lovende ud

For løgbladlus har projektet afklaret, hvilke kommercielt tilgængelige bladlusfjender (prædatorer, snyltehvepse), der er virksomme over for dette skadedyr, og hvorledes en bekæmpelsesstrategi kan baseres på de mest effektive fjender. Vores resultater viser, at ellers almindeligt anvendte bladlussnyltehvepse (*Aphidius colemani* Viereck, *A. ervi* Haliday (Hymenoptera: Aphidiidae), *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) ikke er egnede til bekæmpelse af løgbladlus. Blandt de undersøgte prædatorer har den toplettede mariehøne *Adalia bipunctata* L. (Coleoptera: Coccinellidae) derimod vist sig lovende med en god prædationskapacitet og vilje til at lægge æg i jordbær inficeret med løgbladlus. Ved allerede på nuværende tidspunkt at udvikle biologiske bekæmpelsesmetoder mod løgbladlus, kan det stigende insekticidforbrug, der vil være et resultat af artens hyppigere optræden i danske jordbær, imødegås.

For løgbladlus er der med projektet opnået vigtig viden om, hvilke kommercielt tilgængelige naturlige fjender det kan være værd at satse på ved udvikling af biologiske bekæmpelsesstrategier baseret på udsætninger af nyttedyr. Den toplettede mariehøne er lovende, men der er behov for yderligere undersøgelser af artens biologi i jordbær inficeret med løgbladlus.

1.7 Hvad med fremtiden?

Projektet har bidraget til udvikling og implementering af biologisk bekæmpelse på friland med henblik på en reduktion af pesticidforbruget og deraf følgende gevinster for miljø, arbejdsmiljø og forbrugersikkerhed. Projektet har skabt grundlag for en øget produktion af økologiske jordbær samt af danske jordbær med et minimum af pesticidrester.

De strategiske overvejelser for videreudvikling og implementering af biologisk og integreret skadedyrsbekæmpelse i jordbær viser, at der er gode muligheder for at anvende biologisk bekæmpelse i danske jordbær, især ved dyrkning i væksthuse. For biologisk bekæmpelse i frilandsjordbær er der færre erfaringer. EU's IPM-politik sammenholdt med gode afsætningsmuligheder for økologiske produkter forventes imidlertid at kunne virke fremmende for udvikling og implementering af biologisk bekæmpelse i frilandsjordbær, især når dette forstærkes af yderligere uddannelse af jordbæravlere. Udvikling af moniteringsværktøjer samt danske skadetærsker for skadedyr inden for de forskellige dyrkningssystemer vil også kunne styrke anvendelsen af biologisk bekæmpelse og dermed bidrage til nedsættelsen af pesticidforbruget. Udvikling af populationsdynamiske modeller vil yderligere kunne forbedre mulighederne for optimal rådgivning inden for området.

Summary

Strawberry, the most important berry crop in Denmark, is prone to infestation with a number of pest species, including the strawberry tortricid. In the future new pest species are expected to appear in Danish strawberries, e.g. the shallot aphid. The considerable use of insecticides in Danish strawberries is obstructive to implementation of biological control against strawberry pests. This project has clarified aspects of importance for choice of cropping practice – conventional and organic, respectively, and its influence on the strawberry tortricid and its natural enemies as well as on nematodes (plant parasitic and insect parasitic nematodes). The project has also assessed the use of functional biodiversity – in this case the use of flower strips - as a source of food (pollen and nectar) for the natural enemies of the strawberry tortricid. In addition the project has investigated the possibilities for biocontrol against shallot aphids based on releases of beneficials. The results and strategic considerations of the project contribute to continued development and implementation of biological and integrated pest control in strawberries.

1.1 Strawberry is the most important berry crop in Denmark

Strawberry is the most important berry crop in Denmark for fresh consumption. Danish strawberry, grown on approx. 1051 hectares, is a high value crop with a contribution margin of 128,000 DKK per hectare (2004). At an average production of 4624 tons and a price of 14.6 DKK per kilogram this generates an annual value of 67 million DKK.

1.2 The considerable use of insecticides is an obstacle to increased use of biocontrol

There is a considerable use of pesticides in Danish strawberries, which are prone to infestations with a number of pest species of which the strawberry tortricid moth, *Acleris comariana* (Lienig & Zeller) (Lepidoptera: Tortricidae), is one of the most important. The strawberry tortricid is widespread and the majority of strawberry growers experience annual attacks with ensuing pyrethroid applications (1-3 treatments per season). In addition, new pest species known to cause serious problems in warmer climates are beginning to appear – one of these new species is the shallot aphid, *Myzus ascalonicus* Doncaster (Homoptera: Aphididae), which with the global climate changes in view can be expected to become an increasing problem in Denmark. The existing use of insecticides against the strawberry tortricid and the anticipated use against the shallot aphid is a significant obstacle to implementation of biological control against strawberry pests as the insecticides are harmful to many beneficials, both those occurring naturally and species released as a part of inundative strategies. As long as tortricids and aphids need to be chemically controlled, the use of insecticides will therefore usually also be needed against other pest species.

1.3 How can the use of insecticides be reduced?

Availability of alternative methods is necessary to reduce the use of insecticides in strawberries. Central elements are the use of biological control and enhancement of natural regulation (conservation biocontrol, functional biodiversity). Conservation biocontrol which encompasses management of the crop as well as of habitats outside the crop can contribute significantly to pest control and can be combined with other biocontrol strategies and incorporated in integrated pest management (IPM). A prerequisite for development of biological control and of IPM strategies is an increased knowledge of the pests and their natural enemies, including the influence of cropping practices.

The aims of the project were to a) clarify the influence of cropping practice, including organic and conventional production, on the strawberry tortricid and its natural enemies; b) clarify methods to enhance natural regulation of the strawberry tortricid through conservation biocontrol, in this study including increased access to nectar and pollen sources; and c) identify suitable natural enemies for biological control of the shallot aphid. The project further aimed at d) ensuring dissemination of achieved results to strawberry growers with the aim of speedy implementation. In the application process the project group consequently identified three areas with prominent knowledge gaps in relation to biological control and synthetized strategic considerations during the project period to a document describing existing knowledge with the new knowledge from the project integrated. The strategic document will contribute to ensure future research, development and implementation of biocontrol in strawberries. The project was accordingly carried out in collaboration between scientists from Copenhagen University and Aarhus University and HortiAdvice Scandinavia. The dialogue with strawberry growers was ensured through their participation in project workshops.

The project was structured in four parts, I, II, III, IV (below). The results from the different parts are described in three scientific publications and in the strategic document which is attached to this report as an appendix and in addition shortly summarized and put in perspective below.

I. Influence of cropping practice on the strawberry tortricid, nematodes and natural enemies
II. Conservation biological control in strawberry
III. Augmentative biocontrol of shallot aphids
IV. Strategic document

1.4 The strawberry tortricid and its natural enemies are influenced by cropping practice

Regarding the strawberry tortricid, the project has clarified the relationship between cultivation practice, extent of infestation and occurrence of natural tortricid enemies with the aim of exploiting functional biodiversity for biological control. Our results show that strawberry tortricid infestation in conventional production was higher than in organic production. With respect to the effect of organic versus conventional production on natural enemies, no relationship was found for total parasitism but the most dominating/dominant tortricid parasitoid, *Copidosoma aretas* (Walker) (Hymenoptera: Encyrtidae), was less abundant in organic fields. There was a significant effect of crop age, with higher infestation in older strawberry fields. It is normally assumed that problems in strawberry build up over time, but there was only a tendency that years with strawberry production on a farm increased infestation. We did not find an effect of crop age when comparing 1-year and 2-year fields of strawberry in 2010.

Natural mortality of the strawberry tortricid from infection with entomopathogenic fungi are of no practical importance, as we found very low levels of infestation. However, this does not preclude their use as inundative biocontrol agents. In addition, data indicate higher prevalence of entomopathogenic nematodes (EPN) in organic strawberry fields and slightly less plant parasitic nematodes in organic farms.

The new knowledge generated as a result of this project shows that organic cropping practices can contribute substantially to reduce infestations of tortricids to a level where additional control is rarely needed. In addition the study shows that older strawberry fields will have larger infestation with strawberry tortricids. The higher infestation with strawberry tortricids to the south could be an effect of higher production intensity in this part of Zealand with higher risks of infestation as a result, but this would require further studies to verify.

1.5 Buckwheat has the highest nutritional value for the parasitoid of the strawberry tortricid

The project has focused especially on the importance of flowering plants as a nutritional source for *C. aretas*, the parasitoid of the strawberry tortricid. If parasitism can be augmented by flowering plants then it is likely that sown flower strips can contribute to control the strawberry tortricid and in this way be part of a strategy to reduce the high use of insecticides against this pest. Investigations of the nutritional value of flowering plants to *C. aretas* as well as to the strawberry tortricid served to select buckwheat for experiments with flower.

The project has shown that buckwheat was the most suited flowering plant to enhance the parasitoid of the strawberry tortricid, while for the tortricid its value was not higher than other flowers. In this way the beneficial is favored over the pest. In assessed distances of the flower strip (1-11 m) the level of parasitation was not different, but increased mortality of tortricid larvae near flowering buckwheat strips indicates that the strips have enhanced other natural enemies and calls for further investigation of the contribution by flower strips to conservation biological control in strawberry.

1.6 Lady beetles seem promising against shallot aphids

Regarding the shallot aphid, the project has clarified which commercially available biocontrol agents against aphids (predators, parasitoids) are effective against this pest and how a control strategy can be based on the most effective among these enemies. Our results show that frequently used aphid parasitoids (*Aphidius colemani* Viereck, *A. ervi* Haliday (Hymenoptera: Aphidiidae), *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae)) are unsuited for biocontrol of the shallot aphid. However, among the studied predators the lady beetle *Adalia bipunctata* L. (Coleoptera: Coccinellidae) showed promising potential based on a high predation capacity and the ability to lay eggs in strawberries infested with shallot aphids.

Regarding the shallot aphid the project has provided important knowledge on the suitability of commercially available beneficials for use in the development of biocontrol strategies based on releases of natural enemies. The coccinellid *A. bipunctata* is promising but further investigations on its biology in shallot aphid infested strawberry are needed. Development of biological control strategies against the shallot aphid already at this time will forestall the increased use of insecticides that will accompany its increased presence in Danish strawberries.

1.7 The future

The project has contributed to development and implementation of biological control in outdoor crops aiming at a reduction in the use of pesticides with subsequent gains for the environment, working environment and consumer safety. The project has established the basis for an increased production of organic and Integrated Production (IP) strawberries in Denmark.

The strategic considerations of the project have revealed good opportunities for using biological control in strawberry production in Denmark, especially in greenhouses. For biological control in field strawberry, experience is still lacking to a large extent. However, the EU IPM policy as well as the presently good market for organic products is likely to support development and implementation of biocontrol in field-grown strawberry, especially when combined with reinforced education of strawberry growers. Development of monitoring tools and Danish thresholds of pests in the different cropping systems could increase the use of biological control, and thereby contribute to a reduction of pesticide use. Development of population dynamic models could furthermore improve the possibilities for optimal advice in the field.

2. Introduction

2.1 Background and present state of knowledge

Strawberry is the most important berry crop in Denmark for fresh consumption (Danmarks Statistik, 2005). Danish strawberry, grown on approx. 1051 hectares (Danmarks Statistik, 2005), is a high value crop with a contribution margin of 128.000 DKK per hectare (2004) (Nielsen, 2006). At an average production of 4624 tons (Danmarks Statistik, 2004) and a price of 14.6 DKK per kilogram (Fødevareøkonomisk Institut, 2005) this generates an annual value of 67 million DKK. The Danish Horticultural Advisory Service estimates the present yearly use of pesticides in Danish strawberries to be 3-4 treatments with herbicides, 3 treatments with insecticides (including treatments against slugs) and 4 treatments with fungicides (Bodil Damgaard Petersen, Danish Horticultural Advisory Service, pers. comm.). Further statistics are not available.

Danish strawberries are prone to infestations with a number of pest species of which the strawberry tortricid moth, *Acleris comariana* (Lienig & Zeller) (Lepidoptera: Tortricidae), is one of the most serious – other important pest species are the strawberry blossom weevil (*Anthonomus rubi* Herbst (Coleoptera: Curculionidae)), the strawberry mites (*Phytonemus pallidus* (Banks) (Acari: Tarsonemidae)) and the two-spotted spider mite (*Tetranychus urticae* Koch (Acari: Tetranychidae)) (Lindhardt et al., 2003). The strawberry tortricid is widespread and the majority of strawberry growers experience annual infestations with ensuing pyrethroid applications as a result of observed attacks (1-3 treatments per season) (Sigsgaard and Petersen, 2008). In addition, new pest species known to cause serious problems in warmer climates are beginning to appear – one of these species is the shallot aphid, *Myzus ascalonicus* Doncaster (Homoptera: Aphididae), which with the global climate changes in view can be expected to become an increasing problem in Denmark (Bodil Damgaard Petersen, Danish Horticultural Advisory Service, pers. comm.).

The existing use of insecticides against the strawberry tortricid and the anticipated use against the shallot aphid is a significant obstacle to implementation of biological control against these and other strawberry pests as the insecticides are harmful to many beneficials (Linder et al., 2008), both those naturally occurring and species that are released as part of inundative strategies. As long as tortricids and aphids need to be chemically controlled, the use of insecticides will therefore usually also be needed against other pest species.

The strawberry tortricid overwinters as eggs on leaves. The larvae attack the fresh shots in outdoor strawberries in the beginning of May, weaving leaves together and feeding on leaves and flowers, the latter with direct economic consequences. The second generation appearing after harvest is responsible for the production of eggs destined for overwintering. The biology of the strawberry tortricid has been investigated in a Master's study by Hansen (2008), in British studies (Petherbridge, 1920; Turner, 1968), and in an ongoing Danish project (Sigsgaard and Petersen, 2008, Sigsgaard, 2008). Empirical evidence suggests that the extent of tortricid infestation increases with the age of the strawberry field (Bodil Damgaard Petersen, Danish Horticultural Advisory Service, pers. comm.). Proximity to other strawberry field is likewise suspected to increase the severity of infestations (Bodil Damgaard Petersen, Danish Horticultural Advisory Service, pers. comm.). In addition to yearly fluctuations in the level of infestation, variations also occur between different localities (Bodil Damgaard Petersen, Danish Horticultural Advisory Service, pers. comm.; Lene Sigsgaard, pers. obs.). There are indications that the degree of parasitism of tortricids is higher in organic and Integrated Production (IP) strawberry compared to conventional production

(Hansen, 2008). The present project has contributed to a better understanding of the biology of the strawberry tortricid and how its natural enemies can be enhanced.

Aphids have not previously posed a serious problem in Danish strawberries grown in open fields or in tunnels. However, in 2008 infestations with the shallot aphid were observed in Denmark and Sweden in plastic-covered beds of strawberries (Petersen et al., 2008; Bodil Damgaard Petersen, Danish Horticultural Advisory Service, pers. comm.). The species is anholocyclic (only parthenogenetic reproduction; males and oviparous females unknown) and strongly polyphagous attacking plant species from a number of different families including Liliaceae, Compositae, Cruciferae, Gramineae, Rosaceae (Stenseth, 1989). It overwinters outdoors or in protected places, e.g. in greenhouses or in storage piles of onions or bulbs (Heie, 1961). The shallot aphid, known to attack strawberry in for instance Germany (Martin Hommes, BBA, pers. comm.) and the UK (Cross et al., 1994), can inflict serious damage to this crop due to its induction of malformed leaves, stunted growth and shortened and distorted flower stalks (Petersen et al., 2008). Experience from abroad indicates that shallot aphids can become especially problematic in strawberries when temperatures in February and March are above normal (Hurst, 1969). The future mild winters in Denmark, anticipated as a consequence of global climate changes combined with elevated temperatures during production due to the ongoing shift in strawberry cultivation methods (from open field to plastic-covered beds or in tunnels) (Bodil Damgaard Petersen, Danish Horticultural Advisory Service, pers. comm.), are likely to increase the importance of the shallot aphid as an pest in Danish strawberries. To forestall the accompanying increase in the use of insecticides, it is important already now to develop biological control methods against this pest. In this way the possibility for using biocontrol against other pest species in strawberry can be maintained.

The project has focused on the strawberry tortricid (part I/ paper I (submitted), part II/paper II (in press)) and shallot aphids (part III/ paper III (in press) – a present and an expected pest problem in strawberry, respectively, both of which are now controlled by insecticides incompatible with biological control. Development of better cropping practice and biological control methods against the strawberry tortricid will facilitate biocontrol strategies against the shallot aphid and vice versa, as well as facilitate biological strategies against other pests in strawberries, e.g. tarsonemid mites and spider mites (Enkegaard and Petersen, 2009). Aiming at a speedy implementation of the achieved results by strawberry growers, the project has developed a strategic plan for the continued development and implementation of biological and integrated pest control in strawberry (part IV). This plan is based upon the research achievements of the project as well as on information and experiences gathered at the project workshops for project partners and associated strawberry growers.

Biological control (or biocontrol) is: '*The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be*'. This definition includes living organisms as biocontrol agents: predators, parasitoids, nematodes, fungi, bacteria, virus and protozoa. The definition thus excludes agents consisting of plant extracts and similar. The definition is in accordance with the EU regulations on registration of microbiological control agents.

Biological control can be subdivided into four main strategies:

1) Inundation biological control
2) Inoculation biological control
3) Conservation biological control
4) Classical biological control

The four strategies are outlined in Eilenberg et al. (2001). Often, inundation and inoculation are together called 'augmentation', since both strategies includes the application of commercially available biocontrol agents. Conservation biological control does not include any application, but relies solely on enhancing the effect of naturally occurring predators, antagonistic fungi etc. These three strategies are all a part of the present report. The fourth strategy 'Classical biological control' includes the release of exotic organisms and will not be considered further here.

The term 'Functional biodiversity' is linked to that of conservation biological control. Functional biodiversity can be defined as the part of biodiversity contributing to a given ecosystem service such as biocontrol – or more generally "*that part of the total biodiversity composed of clusters of elements (at the gene, species or habitat level) providing the same (agro)ecosystem service, that is driven by within-cluster diversity*" (Moonen & Barberi, 2008). Conservation biological control basically means keeping alive and enhancing the effectiveness of those natural enemies that are already present. It thus depends on biological insight into functional biodiversity.

3. Part I. Strawberry cropping practice effects on pests and their natural enemies

The research that was conducted in this part of the project is described in the scientific paper "Strawberry cropping practice effects on pests and their natural enemies" (Sigsgaard et al., submitted) included in this report as Journal paper I, Appendix 1. Also see this appendix for references. The paper has been submitted to Journal of Insect Science.

3.1 Introduction

Cropping practice may affect both pests and natural enemies with effects on crop health and yield. A better understanding of the impact of cultivation practice on *A. comariana* numbers and how mortality factors can contribute to control *A. comariana* is fundamental to the development of conservation biological strategies. A three-year study in 7 organic and 7 conventional farms of the strawberry tortricid, *Acleris comariana* (Lienig & Zeller) (Lepidoptera: Tortricidea), of *A. comariana* parasitoids with focus on the major species, *Copidosoma aretas* Walker (Hymenoptera: Encyrtidae), and of entomopathogenic fungi infesting larvae of *A. comariana* was conducted to assess such effects. An assessment of the presence of plant-parasitic and entomopathogenic nematodes in soils of the same farms was conducted to assess the response of another taxonomic group of organisms to cropping practice. Farms were characterized with respect to cropping practice (organic or conventional), cropping history and other parameters including the use of insecticides, the number of years strawberry has been grown on a farm, the crop age (1-3 years), the area with strawberry on the farm and the shortest distance to another strawberry grower.

3.2 Materials and Methods

Tortricid infestation covering five *A. comariana* generations was recorded. Field collected larvae were laboratory reared to assess mortality due to parasitoids and entomopathogenic fungi. In 2010 infestation between a first- and a second-year field in all farms was compared. Also in 2010 a survey of nematodes was conducted. In 2011 yield in experimental fields was assessed.

For further details regarding materials and methods, see Journal paper I, Appendix 1

3.3 Results and Discussion

2743 tortricid larvae were collected. 2584 were *A. comariana*, of these 579 were parasitized by *C. aretas*. Other parasitoids were recovered from 64 *A. comariana*, principally Hymenopterans. Two individual larvae were infected by entomopathogenic fungi, *Isaria* spp. and *Beauveria* spp. (Ascomycota: Hypocreales).

A. comariana densities were significantly higher under conventional practice (Figure 3.1). No significant effect was found of year of study or generation (spring, summer). There was a significant effect of crop age (1, 2 or 3 years) with higher infestation in older fields. Latitude significantly affected density, as numbers of *A. comariana* decreased by 0.07 times per km north. One other explanatory variable, the years a farm had been producing strawberries, was near significant (P = 0.082) with a trend of increasing infestation with years of production.

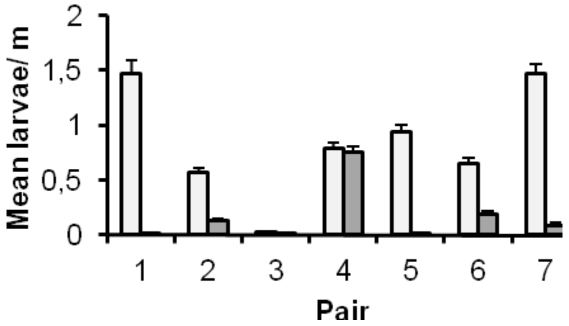

FIGURE 3.1 MEAN NUMBER (± SE) OF *A. COMARIANA* LARVAE SAMPLED PR 1 M STRAWBERRY ROW IN THE SEVEN PAIRS OF CONVENTIONAL (LIGHT GRAY) AND ORGANIC (DARK GREY) FARMS SPRING 2009-SPRING 2011. FARM 7 CONVENTIONAL WAS FIRST SAMPLED SUMMER 2009, FARM 7 ORGANIC WAS FIRST SAMPLED SPRING 2010. FARM 3 ORGANIC STOPPED GROWING STRAWBERRIES SUMMER 2010.

Three conventional farms used insecticides in strawberries (farms 5, 6, 7). Pyrethroid was applied in spring/early summer to control strawberry tortricids and/or strawberry blossom weevils. The remaining conventional farms used herbicides and fungicides, except one (farm 3) which only used fungicides. All conventional farms used fertilizer. A model describing *A. comariana* densities as an effect of cropping practice was not improved by including insecticide use as a co-variable. However, fertilizers, herbicides and fungicides can have an impact on arthropods. Herbicides and fungicides may directly affect arthropods and can also have an indirect effect through changes in flora composition.

A. comariana was the dominant tortricid species in both organic and conventional farms, but less so in organic farms with 14.8% individuals of other tortricid species, but only 3.1% individuals of other species in the conventional fields. Numbers of other tortricid species were comparable between organic and conventional farms, making the proportion of other tortricid species in organic strawberry fields where there were less *A. comariana* significantly higher. These other tortricid species were generalists with a wide plant host range making the surrounding landscape or other crops a likely origin. The higher nutrient levels normally found in conventional fields would have suggested higher densities of generalists in conventional fields (Staley et al., 2010), opposite to findings.

A comparison of the densities of *A. comariana* in a first- and a second-year fields of all farms was done in 2010 to test if infestation was affected by crop age and cropping method. The infestation was higher in conventional fields with on average 4.5 more *A. comariana* in first-year fields and 6.3 times more tortricids in second-year fields. Highest infestation was found in the first generation with 9.1 ± 1.7 larvae/m row, 1.6 times higher than in the second generation. Though we found higher infestation in older fields in the 3-year study, the comparison of *A. comariana* infestation in first- and second-year fields of all farms in 2010 found no difference in infestation levels. It is still practice to plant old and young strawberry crops next to each other, and on the farms young fields were grown near old ones, separated by a distance covering from a few meters on small farms up to a maximum of 100 m, thus facilitating migration of insects between old and young crops, even if effective mobility of *A. comariana* is restricted to a few hundred meters as indicated by literature (Turner, 1968; Jeanneret and Charmillot, 1995).

The successful emergence of adult *A. comariana* in first generations was higher (51.7%) than in second generations (42.8%). There was no difference between total parasitism of *A. comariana* between conventional (26.8%) and organic farms (21.0%), but *C. aretas* share of total parasitism was higher in conventional (93.1%) than in organic farms (74.3%). Parasitism in this study was higher than the below 10% found by Turner (1968) and Vernon (1971) but lower than the 76% found by Alford (1976). Figure 3.2 shows the proportional distribution of *A. comariana* larvae which were healthy and emerge as adults in rearing, of larvae or pupae that died, were parasitized by *C. aretas* and by other parasitoids.

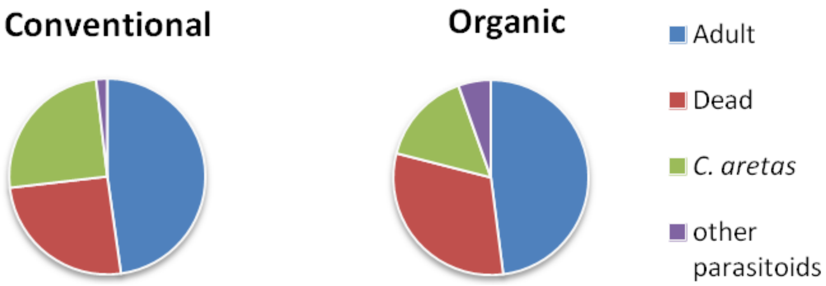

FIGURE 3.2 PROPORTION OF *A. COMARIANA* LARVAE FROM CONVENTIONAL AND ORGANIC FARMS WHICH, WHEN REARED IN THE LABORATORY EMERGED AS HEALTHY ADULTS (BLUE), DEAD AS LARVA OR PUPA (READ), PARASITISED BY *C. ARETAS* (GREEN) AND BY OTHER PARASITOIDS (VIOLET).

A strong correlation between *A. comariana* density and parasitism by *C. aretas* indicates the potential of this parasitoid to regulate *A. comariana* (Sigsgaard et al., *in press*) but can also help to explain the low density of this parasitoid in organic farms where the density of *A. comariana* was low, exposing the specialist *C. aretas* to a higher risk of local extinction. The results indicate that at low densities of *A. comariana*, as found in organic farms, other parasitoids provide supplementary control to that of the specialist *C. aretas*. Other tortricid species present may serve as alternative hosts for these other parasitoids, contributing to conserve them in the habitat (Pfannenstiel et al., 2010).

Unknown mortality was higher in organic farms, and it increased more from first to second generation in organic farms than in conventional farms (conventional, first generation: 20.9%, second generation: 30.7%, organic, first generation: 26.2%, second generation: 39.7%). Mortality differed between years and generations ranging from 6.2% to 33.3%. This calls for further study. One such unknown mortality factor could be non-consumptive predator effects (McCauley et al., 2011). Recent studies demonstrate that killing (i.e., the consumptive effect of predators) is only one component of how predators impact herbivores and plants. A substantial portion of the net predator effect can be attributed to non-consumptive effects which occur when predator presence alone alters the behavior, physiology, or life-history of the surviving prey. These effects can be as important as killing to regulate pests.

Only two larvae turned out to be naturally infected with insect pathogenic fungi. Both were collected in 2011. One was infected by *Beauveria* spp., the other was infected by *Isaria* spp. The very low natural prevalence of entomopathogenic fungal infestation of *A. comariana* larvae indicates that natural regulation by this group of natural enemies is not likely. This may be attributed to the fact that *A. comariana* larvae are not in contact with soil, though entomopathogenic fungi can also be found on the phylloplane (Meyling and Eilenberg, 2007).

Entomopathogenic nematodes were recovered from one conventionally grown field and three organically grown fields (all *Steinernema* sp. (Steinernematidae)). Plant parasitic nematodes were also present. The dominant nematode species were *Pratylenchus* spp. (Pratylenchidae) and

Longidorus spp. (Longidoridae) *Pratylenchus* spp. were present in 7 conventionally grown fields and 6 organically grown fields, *Longidorus* spp. were present in 4 conventionally grown fields and 1 organically grown field. Data are summarized in Appendix I, Table 2. A higher presence of entomopathogenic nematodes in organically grown fields may be linked to greater insect host availability in such fields compared to conventionally grown fields sprayed with pesticides or possibly soil structure or other effects of cropping practice. A comparable Norwegian study found a similar response by these nematodes to cropping practice, and also found lower infestation by plant parasitic nematodes in organic fields (Trandem et al., *in prep*).

Yield in the 14 fields in 2011, excluding organic field 3, which was no longer cultivated in 2011, was assessed as the sum of flowers, fruits and empty stems per m row by late June (Table 1, Appendix I). This sum –representing potential yield – was used as frost damage made a comparison of harvested yield impossible. Yield was not significantly different in conventional and organic fields.
A model including *A. comariana* density as an explaining variable for yield was also not significant.

3.4 Conclusion

Across the three years of study there was a highly significant lower infestation of *A. comariana* in organic strawberry fields. The years for which a farm had been producing strawberries were near significant, showing a trend of increasing infestation with years of production. There was a significant effect of crop age (1, 2 or 3 years) with higher infestation in older fields, and numbers of *A. comariana* decreased by 0.07 times per km north. However, crop age was not significant when we compared first- and second-year fields of all farms in 2010. The practice of planting old and young strawberry crops next to each other only separated by from a few meters on small farms up to a maximum of 100 m, facilitating migration of insects between old and young strawberry fields. This practice may have been the reason why we did not find a significant effect of crop age in this trial. If the effective mobility of *A. comariana* is restricted to a few hundred meters (Turner, 1968; Jeanneret and Charmillot, 1995), a farm design with larger temporal and spatial distance between strawberry crops is likely to reduce this pest.

A strong correlation between *A. comariana* density and parasitism by *C. aretas* underlines the potential of this parasitoid to regulate *A. comariana* (Sigsgaard et al., *in press*) but can also help to explain the low density of this parasitoid in organic farms where the density of *A. comariana* was low, exposing the specialist *C. aretas* to a higher risk of local extinction. For those systems in which extinction probabilities have been quantified, the parasitoid is more prone to extinction than its host, supporting the theoretical expectation that higher trophic levels are at greater risk of extinction than lower trophic levels (Cronin and Reeve, 2005).

4. Part II. Conservation biological control - can access to flowering plants increase natural parasitism?

The research that was conducted in this part of the project is described in the scientific paper "The effect of floral resources on parasitoid and host longevity – prospects for conservation biological control in strawberry" (Sigsgaard et al., in press) and included with this report as Journal paper II, Appendix 2, where also references can be found. The paper is in press by Journal of Insect Science.

4.1 Introduction

In Europe, natural control by *Copidosoma* parasitoids (Hymenoptera: Encyrtidae) is likely to prevent populations of many moth species in agriculture and forestry from reaching economic injury levels (Guerrieri and Noyes 2005). The high natural rate of *Acleris comariana* (Lienig & Zeller) (Lepidoptera: Tortricidea) parasitism by *C. aretas* Walker in Danish strawberry fields (L. Sigsgaard, pers. obs.) makes it a potential candidate for conservation biological control. Flower strips sown in the field can be used to provide natural enemies better access to pollen and nectar (Thompson and Hagen 1999). Selective food plants (Baggen and Gurr 1998), which can be used to augment *C. aretas* in strawberry fields, while not at the same time favoring *A. comariana* would be preferred, as enhancement of pest fitness via the same mechanisms as natural enemies is potentially disadvantageous to conservation biocontrol. The present study examined the dietary value of three flowering plants to both *C. aretas* and its host *A. comariana* in order to identify food plants that might selectively augment *C. aretas* in strawberry fields, while not favoring the pest.

4.2 Materials and Methods

Adults of both *A. comariana* (males and females) and *C. aretas* (females) were held on diets of buckwheat (*Fagopyrum esculentum* Moench) (Polygonaceae), borage (*Borago officinalis* L.) (Boraginaceae), phacelia (*Phacelia tanacetifolia* Bentham) (Hydrophyllaceae), and strawberry (*Fragaria x ananassa* Duchesne) (Rosaceae) and their longevity was compared. Pollen (bee pollen) and sucrose (20% solution) served as positive controls as they were provided freely accessible so insects were unaffected by factors which may affect longevity such as flower architecture or sugar composition. Water was a negative control.

For further details regarding materials and methods, see Journal paper II, Appendix 2.

4.3 Results and Discussion

Longevity of *A. comariana* was highest, 35-39 days, on pure diets of sucrose and pollen, although not significantly different on diets of borage and buckwheat (Appendix 2, Table 1). Longevity on floral diets tested was not significantly different, except for phacelia which was inferior being equivalent to water. The long corolla of phacelia, outward pointing hairs on the style and ovary, and scale-like appendages within corollae, may limit nectar access, as discussed for *C. aretas* below. The high longevity of *A. comariana* on pollen indicates that this species may utilize pollen. However, the use of bee pollen as a pollen diet in this study may overestimate the dietary value of pure pollen, as honey bees mix pollen with regurgitated nectar or honey for transport on their legs.

Highest longevity of *C. aretas* females was found on sucrose (5.8 days) (Appendix 2, Table 1), followed by pollen and buckwheat (3.8 days). Among the floral diets, buckwheat was a superior diet for *C. aretas*, with an early survival distribution equivalent to that found on a pure pollen diet. Borage was also of high quality while intermediate values were found for strawberry and dill, and longevity on phacelia was less than on water (Figure 4.1). As for *A. comariana* the use of bee pollen may have overestimated the dietary value of pure pollen. A higher parasitoid longevity when a floral diet was provided does not necessarily imply higher fecundity but increased longevity can indirectly lead to increased parasitism as the time to encounter and parasitize hosts is increased.

The effect of distance to flower strips on *A. comariana* density, parasitism and mortality was assessed from a total of 663 *A. comariana* larvae, which were collected from strawberry plants at the three conventional strawberry farms at distances of 1, 6 and 11 m from the buckwheat strips (Appendix 2, Table 2). *A. comariana* densities in the three fields averaged 6.7, 0.4 and 3.1 larvae/m row, respectively. Across fields there was a highly significant correlation between the density of *A. comariana* and the proportion parasitized. There was no significant effect of distance from the flower strip on *A. comariana* density and no significant effect of distance on the proportion *A. comariana* parasitized by *C. aretas*. However, unknown mortality was highest in larvae collected near the flower strips (Appendix 2, Table 2), with a significant effect of distance and an estimated mortality by the flower strip. Total *A. comariana* mortality, including parasitism, was highest near the strip, with a highly significant effect of distance.

Among the flowers tested, it was not possible to single out a fully selective floral diet that was beneficial to the parasitoid without at the same time also being beneficial to the strawberry tortricid. However, buckwheat was superior for *C. aretas* while buckwheat, borage and strawberry were not significantly different in quality for *A. comariana*. Therefore we selected buckwheat for flower strip assays. Furthermore, as its seeds do not overwinter, it does not pose a potential weed problem next year in strawberry, a problem which Danish strawberry growers have reported with dill. Other advantages of buckwheat are that it germinates easily, flower shortly after sowing and seeds are inexpensive and readily available (Bowie et al. 1995).

Though no effect on *A. comariana* density or on parasitism of proximity to flower strips could be found, the high level of parasitism by *C. aretas* found in two of the fields and the strong correlation between pest density and parasitism shows the potential of this parasitoid to regulate *A. comariana*. It is possible that larger densities of pest and parasitoid or higher distances than used in our study may be necessary to discover the effects of foraging movements of *C. aretas* on resulting parasitism. Flowering weeds, present in low densities in all fields, may also be utilized by both species, potentially masking the effects of the floral strips. Finally, increased longevity of *C. aretas* under laboratory conditions does not mean that it will preferentially feed on buckwheat in the field.

Higher *A. comariana* mortality from unknown factors near flower strips shows that buckwheat had a positive effect on other mortality factors of *A. comariana* that was measurable at the scale studied. Stress responses induced by exposure to predator cues can increase the vulnerability of prey to other mortality factors, and thus mere exposure to predators may result in significant increases in mortality (McCauley et al., 2011). Syrphid larvae were observed to prey on *A. comariana* larvae inside the rolled strawberry leaves during the study. Other predators observed in strawberry include spiders, anthocorids, coccinellids and carabids. However, for all, their contribution to *A. comariana* mortality is unknown. A positive effect of flower strips with buckwheat on both predators and parasitoids was found in several other studies (Irvin et al., 2006; Nicholls et al., 2000; English-Loeb et al., 2003; Tylianakis et al., 2004; Eggenschwiler et al., 2010).

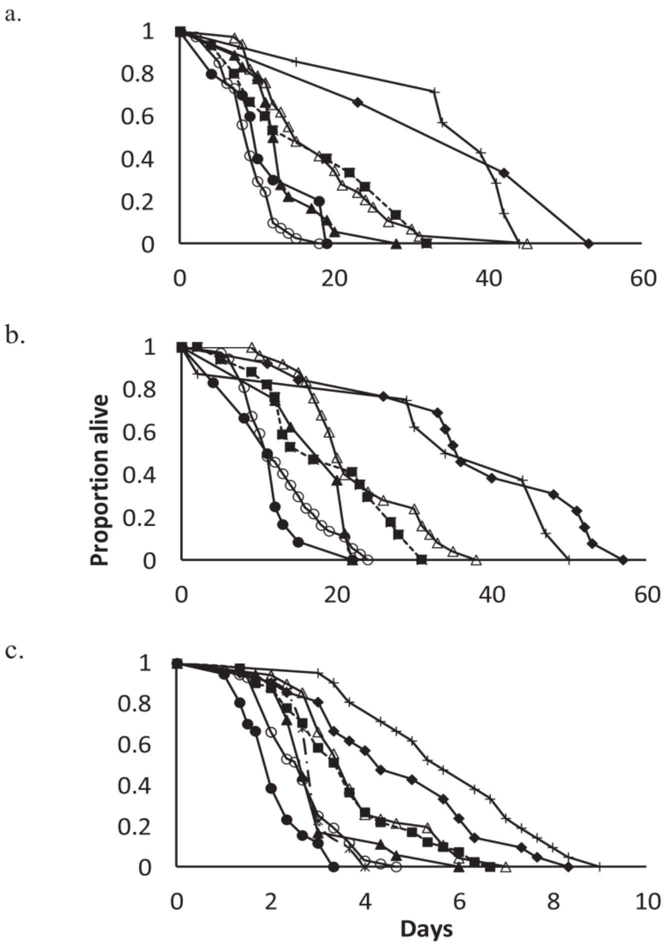

FIGURE 4.1 SURVIVAL OF A) *ACLERIS COMARIANA* FEMALES, B) *ACLERIS COMARIANA* MALES AND C) *COPIDOSOMA ARETAS* FEMALES ON DIETS OF BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH) (Δ), BORAGE (*BORAGO OFFICINALIS* L.) (■), PHACELIA (*PHACELIA TANACETIFOLIA* BENTHAM) (●), DILL (*ANETHUM GRAVEOLENS* L.) (--X--) AND STRAWBERRY (*FRAGARIA X ANANASSA* DUCHESNE) (▲) AS WELL AS ON POLLEN (♦), SUCROSE (+) AND WATER CONTROL (○). SURVIVAL ON DILL WAS ONLY ASSESSED FOR *C. ARETAS*. (FROM SIGSGAARD ET AL., *IN PRESS*, APPENDIX II)

4.4 Conclusion

In conclusion, it was not possible to identify a fully selective plant for the flower strips, but buckwheat was found to be the most optimal. Although buckwheat is of high dietary value to *C. aretas*, and although levels of parasitism in two of the fields tested were high, a positive effect on parasitism could not be demonstrated at the distances tested in the field. Similar numbers of larvae were collected at all distances, but there was a higher unknown mortality of larvae collected near flower strips. As literature indicates that buckwheat for flower strips can augment a more complex suite of natural enemies including predators, one such unknown mortality factor could be non-consumptive predator effects (McCauley et al., 2011). For conservation biological control of *A. comariana* a selective food plant for *C. aretas* remains to be identified. If a positive effect of buckwheat on *A. comariana* control is confirmed, buckwheat may be utilized together with a selective food plant in flower strips, once it is identified.

5. Part III. Augmentative biocontrol of shallot aphids

The research that was conducted in this part of the project is described in the scientific paper “Shallot aphids, *Myzus ascalonicus*, in strawberry – biocontrol potential of three predators and three parasitoids“(Enkegaard et al., in press), included in this report as Journal paper III, Appendix 3.

5.1 Introduction

As mentioned in 2.1 aphids have not previously posed a serious problem in Danish strawberry grown in open fields or in tunnels. However, the shallot aphid (*Myzus ascalonicus* Doncaster (Homoptera: Aphididae)), which is a serious pest in other European countries (Borchardt, 1958; Cross et al., 1994; Anon., 2011; Martin Hommes, BBA, pers. comm.), is likely to become an increasing problem in Danish strawberries in the future due to global climate changes and a shift in strawberry cultivation methods (from open fields to plastic-covered beds or in tunnels) (Daugaard, 2005). Development of biological control methods against the shallot aphid would therefore be prudent already now.

Only few investigations on natural enemies of the shallot aphid are available in the literature (Wahab, 1985; Wahab and Askew, 1989); however, more information exists regarding natural enemies of the strawberry aphid *Chaetosiphon fragaefolii* (Cockerell) (e.g. Easterbrook et al., 2006; Fitzgerald, 2006). In addition, countless investigations have been made regarding the possibilities for biological control of other aphid species in various field and, especially, greenhouse crops. A number of aphidophagous natural enemies have well-documented beneficial effects and are commercially available. However, these natural enemies are not necessarily effective against the shallot aphid – this species may not be included in the host or prey range of the natural enemy, it may not occupy accessible spatial niches or it may display effective defense behavior (Enkegaard and Brødsgaard, 2002). Consequently, this study screened a number of commercially available beneficials for their predation or parasitization capacity towards shallot aphids, and further studies were undertaken with the better suited candidate species.

5.2 Material and Methods

The species studied were 3 parasitoids (*Aphidius colemani* Viereck, *A. ervi* Haliday (Hymenoptera: Aphidiidae), *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae)) and 3 predators (lacewings (*Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae)), lady beetles (*Adalia bipunctata* L. (Coleoptera: Coccinellidae)), gall midges (*Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae))). The experiments were conducted in the lab at light and temperature conditions mimicking those found in spring in Danish tunnel strawberry. Parasitization or predation capacity was evaluated in Petri dish assays with detached strawberry leaves and, for some species, also in separate cage experiments with strawberry plants. Egg laying capacity of *A. bipunctata* was evaluated in Petri dish assays and the olfactory preferences of the lady beetle were evaluated in olfactometer experiments.

For further details regarding materials and methods, see Journal paper III, Appendix 3.

5.3 Results and Discussion

Please refer to Table 5.1 for a summary of the results.

In Petri dish assays both *A. colemani* and *A. ervi* readily stung shallot aphids with no significant difference in stinging frequency between the two species. Not surprisingly, a high proportion of aphids that had been stung by either species died within 1½ week. *A. ervi* induced a significantly higher mortality (79.0%) in stung aphids compared with *A. colemani* (55.3%). However, only a minor fraction (less than 10%) of the killed aphids resulted in formation of mummies, presumably due to a physiological response to parasitism. Parasitization by the two parasitoid species leading to fully developed mummies has been reported to vary up from 40 to 80% (e.g. van Steenis, 1995; Takada and Tada, 2000; Byeon et al., 2010). These rates, expressing mortality among all exposed aphids (i.e. not just stung aphids), are higher than the rates for mummification found here, indicating that the performance of *A. colemani* and *A. ervi* against shallot aphids on strawberry is far from optimal. The low degree of mummification suggests a physiological response to parasitism by *Aphidius* parasitoids in *M. ascalonicus* as demonstrated for other aphid species (Henter and Via, 1995), although the physiological mechanism in our case did not serve as a true defense (i.e. survival) reaction since the stung aphids actually died.

In similar set-ups *A. abdominalis* killed almost half of the exposed aphids through host feeding. In addition about 25% of non-host-fed aphids developed into mummified aphids and about 40% of non-host-fed aphids died from other parasitoid-induced causes. *A. abdominalis* inflicted the same overall mortality on shallot aphids as *A. ervi* although the mortality for the latter was based only on stung aphids. The mortality to be expected among a group of exposed shallot aphids would thus be higher for *A. abdominalis*, which – combined with its ability to reproduce on this aphid species – at first would seem to make it a better candidate for biological control with the potential to be used as an inoculative agent. However, on whole strawberry plants the host feeding rate of *A. abdominalis* was reduced to only about 1% and no significant parasitism mortality was observed suggesting that host plants interfered both with host feeding and parasitism activities of *A. abdominalis*.

Third instars of all 3 predators readily preyed upon the shallot aphid in Petri dish set-ups with significant differences in daily predation (about 35, 25 and 13 aphids/day, respectively). These results are within the range of predation rates reported on various aphid species for all three predators (e.g. Harizanova and Ekbom, 1997; Jalali et al., 2009a; Montoya-Alvarez et al., 2010). Further studies on *A. bipunctata* revealed that the larvae maintained their daily predation capacity when experiments were done on whole strawberry plants. Contrary to the situation with *A. abdominalis*, the change in experimental dimensions was thus not obstructive for the activity of the lady beetle larvae. Between 47 and 67% of already ovipositing *A. bipunctata* refrained from laying any eggs on the first day after transfer to set-ups with combinations of shallot or peach-potato aphids (*Myzus persicae* (Sulzer) (Homoptera: Aphididae)) and strawberry or sweet pepper leaves. The aphid species and the plant species did, however, not have a significant influence on the number of females laying eggs. The results are in accordance with the study by Hemptinne et al. (2000), demonstrating that *A. bipunctata* females in their oviposition site selection use other cues than those associated with aphids or plants. The overall average number of eggs laid during the first day was about 6 per female, which, not surprisingly, is lower than reported in studies where egg production was observed for longer periods (16-20 eggs) (Hamalainen et al., 1975; Ware et al., 2008; Jalali et al., 2009b). Further studies will be needed to examine if the proportion of egg layers as well as the egg laying capacity of *A. bipunctata* will increase after longer exposure to shallot aphids on strawberry. Adult lady beetles had a significant preference for odor from controls without plants over odors from uninfested strawberry or pepper plants but showed no preference between either of the plant species, whether infested with aphids or not.

For more detailed discussion of results, see Journal paper III, *Appendix 3.*

TABLE 5.1. SUMMARY OF THE AVERAGE (± S.E.) VALUES FOR THE DIFFERENT BIOLOGICAL CHARACTERISTICS EXAMINED FOR THE 3 PARASITOIDS (*APHIDIUS COLEMANI, APHIDIUS ERVI, APHELINUS ABDOMINALIS*) AND 3 PREDATORS (*APHIDOLETES APHIDIMYZA, CHRYSOPERLA CARNEA, ADALIA BIPUNCTATA*) WITH SHALLOT APHIDS AS HOST/PREY. NS: MORTALITY WAS NOT SIGNIFICANTLY DIFFERENT FROM THE CONTROLS.

	A. colemani	***A. ervi***
Number of stung aphids	6.8±2.4	6.1±1.2
Mortality induced in aphids stung	55.3±4.1%	79.0±7.2%
Completed parasitization	7.1±3.1%	2.7±1.8%

	set-up	***A. abdominalis***
Mortality due to host feeding after 24 h exposure	detached leaves	49.6±5.3%
Non-host-fed aphids developing to mummified aphids	detached leaves	23.2±7.3%
Non-host-fed aphids dying from parasitoid-induced causes	detached leaves	38.1±13.2%
Mortality due to host feeding after 24 h exposure	whole plants	sweet pepper: 32.1±5.3% strawberry: 1.2±0.8%
Mortality corrected for mortality in the controls	whole plants	sweet pepper: NS strawberry: NS

	A. aphidimyza	***A. bipunctata***	***C. carnea***
Daily predation rates	13.34±1.45	25.25±3.18	34.62±3.45

	prey-plant	***A. bipunctata***
Daily corrected number eaten, whole plants	shallot aphids-sweet pepper	56.3±1.7
	shallot aphids-strawberry	32.0±6.3
Egg laying in 24 h	shallot aphids-strawberry	8.67±3.08
	peach-potato aphids-strawberry	8.07±2.91
	shallot aphids-sweet pepper	5.93±2.59
	peach-potato aphids-sweet pepper	2.8±1.24

5.4 Conclusion

This study has demonstrated that the reproductive success of especially *A. colemani* and *A. ervi* but also of *A. abdominalis* is low on shallot aphids compared to the abilities of these parasitoids on other aphid species. The low reproductive success is not a result of behavioral defense reactions towards the parasitoids. Instead the shallot aphid seemingly responds physiologically to the internal presence of parasitoid eggs or larvae although this reaction does not serve as an effective defense mechanism since the aphids themselves die. The very low reproductive success of the two *Aphidius* species precludes their use in biocontrol against shallot aphids in strawberry except for purely inundative releases. In addition, the reduced host feeding and reproductive capacity of *A. abdominalis* on whole strawberry plants likewise precludes its use even as an inundative agent. Whether other parasitoid species than the three species examined here will be able to parasitize shallot aphids on whole strawberry plants remains to be seen. It likewise remains to be seen if other shallot aphid populations will exhibit the same resilience to parasitism as seen here.

All predator larvae readily preyed upon shallot aphids on strawberry with lacewing larvae being the most voracious. Although *A. bipunctata* seems promising as a biocontrol agent as judged from its predation capacity and its ability (at least for about half of the females) to lay eggs on shallot aphid-infested strawberries, further studies are needed to examine its departing tendencies in strawberry as are further studies on realized fertility, development and survival for individuals feeding continuously on shallot aphids. At the same time it would be interesting to further examine the two other predators, especially the green lacewing, to clarify the realization of their predation potential under more realistic conditions.

6. Part IV: Further development and implementation of biological control in strawberries

The following synopsis is referring to the strategic paper (in Danish, “Strategipapir for skadedyrsbekæmpelse i jordbær - Friland, tunnel eller væksthus“) about pest control of strawberries, grown in open fields, tunnels or greenhouses. The complete paper can be found in Appendix 4.

6.1 Introduction

Work package IV was implemented in order to get project results rapidly integrated into the practical work at the strawberry grower’s farms. The horticultural advisory service, HortiAdvice Scandinavia A/S, has professional and practical experience with a large part of the Danish strawberry growers and was therefore involved in locating the 14 farms participating in the project, supervising about practice-related situations regarding the growing conditions and communicating project results to the growers. During the project advisors from HortiAdvice Scandinavia screened professionally grown strawberry fields in order to identify possible new pests and to monitor changes in occurrence of important pests already present in Danish strawberries. In view of the risk of rapid changes in pest species composition and abundance in strawberries as a consequence of climate changes and import of plants, close crop monitoring is important for timely development of pest control strategies for both present and new pests.

The strategic paper was prepared based on the knowledge and experience by the project partners and based on the results from the project. The strategic paper focuses on further development and implementation of biological control in the Danish strawberry production. Since the strategic paper is targeted to Danish advisors and strawberry growers, it has been written in Danish. Part of the material from the strategy paper and this report will be used in articles in growers’ magazines.

The background for the paper is that the majority of strawberry production is traditional outdoor but in the future more protected production can be anticipated. In such systems placed continually in the same area pest and disease problems may increase, making prevention and biological control even more important. To successfully implement biological control the biology of the beneficials must be known, especially their temperature and humidity requirements. The use of insecticides and some fungicides can result in high mortality of beneficials, and a release limit of 8 weeks or more after their application may be needed. Biological control also requires good timing to be applied at an early stage of the pest attack, which again requires monitoring of the crop.

6.2 Material and Methods

In 2009, data regarding the farms and their growing conditions were collected through questionnaires for all farms participating in the project. In addition, supplementary data concerning pests and control strategies were collected.

HortiAdvice Scandinavia A/S monitored the status of new and already present pests in a number of farms on both Funen and Zealand. The information from the monitoring was used to decide which pests should be described and evaluated in the strategic paper. During the project period topics about pests and biological control were discussed at project workshops for growers and scientists.

6.3 Results

The strategic paper begins with a background description including cropping practice and a general discussion of the current and future opportunities for application of functional biodiversity (conservation biocontrol). Then follows a review of a number of strawberry pests and the current practices for their control in Denmark with descriptions of existing and potential opportunities for implementation of augmentative biological control in strawberry production in Denmark. The paper covers the following pests: strawberry tortricids, blossom weevils, vine weevils, tarsonemid mites, spider mites, thrips, aphids, bugs, slugs and whiteflies.

Conservation biocontrol

Conservation biocontrol – the protection and enhancement of naturally occurring beneficials for example by sowing flower strips or establishing beetle banks or by conserving natural ecological infrastructures such as hedges – is the second line of defense toward pests, once a good cropping practice has been established. According to the IOBC-WPRS at least 5% and better 10% of a farm should be ecological infrastructures (IOBC-WPRS, 2009).

All of EU agricultural production must by 2014 be under IPM (Direktiv 2009/128/EU) and ecological infrastructures are one of the methods, but each country has the freedom to develop its own strategies. In Denmark there are for example demonstrations for flower strips, wildlife strips and beetle banks by the advisory service. For example establishment of flower strips can be supported financially (Ministeriet for Fødevarer, Landbrug og Fiskeri, 2010).

Enhancement of natural control is often an element in wider strategies aimed at increasing the nature-content on a whole farm rather than for a single production line. An example of multifunctional character is the planting and maintenance of hedgerows, which also serve as windbreaks and corridors for wildlife.

The optimal composition, placing and distance between ecological infrastructures is a field where much more knowledge is needed.

Augmentative biocontrol

Augmentative biocontrol is already used to some extent in Danish greenhouse produced strawberry (e.g. the use of predatory mites against tarsonemid mites in tunnels; the use of parasitoids against aphids in greenhouses). It is expected that many of the already commercially available beneficials used in other greenhouse cultures can be of use in strawberry and that the general experience from greenhouse biocontrol relatively easily can be used to develop strawberry-specific biocontrol, for instance against thrips and whiteflies. The fact that troublesome pests such as the strawberry weevil and the strawberry tortricid – the presence of which necessitates biocontrol disrupting use of insecticides in strawberry in tunnels and open field – are absent from greenhouse strawberry in Denmark, adds to the likelihood of successful implementation of biocontrol in this system.

Augmentative biocontrol is also used, albeit to a lesser extent, in Danish tunnel and field production of strawberry, for instance using the entomopathogenic bacteria *Bacillus thuringensis* against strawberry tortricids or using predatory mites against tarsonemid mites. However, knowledge and experience is lacking regarding the possibility for biocontrol of other pests in these system and further research will be needed to improve the present use, to elucidate if beneficials employed in greenhouse cultures may be of use in tunnels or outdoor and to develop biocontrol based on additional beneficial organisms, e.g. microbials against weevils. Exploitation of conservation biocontrol tactics are of outmost importance for pest control in strawberry produced open fields and in tunnels, as already mentioned above. Development of other non-chemical methods such as early warning system based on damage threshold, population models and monitoring systems, exploitation of host plant resistance and mass trapping is likely to increase the possibilities for biocontrol implementation.

Bottlenecks

The results from this part of the project have highlighted an important bottleneck for increased implementation of biocontrol in strawberry in tunnels and open field: troublesome pests which cannot yet be controlled without the use of insecticides. Especially the strawberry weevil are troublesome since no non-chemical solutions are imminent. Further research to find management solutions against the strawberry weevil that are compatible with biological control of other pests should be prioritized. The future emphasis on IPM is expected to increase attention to development and implementation of biocontrol in strawberry in general and in field-grown strawberry in particular.

Dissemination

Results were communicated to the growers in order to allow an early implementation at the farms. This was done through articles in journals such as "Frugt og Grønt" and strawberry newsletters, through oral presentations at strawberry seminars, through workshops and through information to growers' organizations and updates of other consultants on the progress in the project.

6.4 Conclusion

The opportunities for using biological control in strawberry production in Denmark are good, especially for strawberry production in greenhouses. For biological control in tunnels and particularly in field strawberries, experience is still lacking to a large extent. However, with extra focus on IPM in the years to come, biocontrol in field-grown strawberries will hopefully get more attention.

Development of monitoring tools and of Danish economic thresholds of pest infestations in the different strawberry cropping systems could increase the use of biological control and thereby contribute to a reduction of pesticide use. Development of population dynamic models could furthermore improve the possibilities for monitoring and early warning of pests which could help the advisory service to provide growers with optimal advice in the field. Last but not least, the differences between strawberry cultivars in host plant resistance to various pests, reported by growers, needs to be verified and quantified.

7. Discussion

This chapter provides an overall discussion for the whole project. For detailed discussion of the results from each WP, please see chapter 3, 4, 5 and 6, respectively.

The present project was undertaken with the EU IPM Directive in mind, the Directive stipulating that European agriculture from 2014 should comply with IPM strategies (Directive 2009/128/EU) emphasizing preventive methods and non-chemical approaches such as biological control. The results and strategic considerations of the project contribute to continued development and implementation of biological and integrated pest control in Danish strawberries.

The present project has demonstrated that the level of tortricid infestation is greatly reduced in organically grown strawberries thus being in line with other studies demonstrating positive effects of organic cropping practice on lowering pest infestation levels and biodiversity of natural enemies (Bengtsson et al. 2005). There is no known resistance towards pyrethroids in strawberry tortricids, but ineffectiveness of the insecticides applied in conventional strawberry practice may have contributed to the differences observed. The long period over which strawberry tortricids hatch in spring and the fact that larvae most of their lives are protected in rolled-up leaves makes it difficult to optimize timing of pesticide application. Further, direct or indirect side-effects of applied agrochemicals can reduce the numbers of natural enemies (Bueno et al., 2008), or there may be a higher abundance and diversity of natural enemies in organic farms for other reasons. In strawberries the preservation of parasitoids in organic cultivation was demonstrated to contribute to suppression of tortricid populations. Our results indicate the existence of a significant potential for reduction of the use of pesticides and other agrochemicals in non-organic strawberry production. Elucidation of the factors contributing to keeping tortricid infestations low in organic strawberry production requires further studies.

Regarding preventive measures for Danish strawberry production, considerations as to rotation and as to distances to strawberry fields from previous years should be taken into account when IPM programs are designed, as our results have indicated that tortricid populations build up over time on farms with a long history of strawberry production. Many strawberry producers still tend to establish new fields close to older fields for practical reasons though this is not recommended by the advisory service. This is a practice that is likely to increase the risk of young plants being rapidly colonized by tortricids and other pests from the old fields. Studies on the dispersal ability of strawberry tortricids and other strawberry pests are needed to develop guidelines for advisable distances between strawberry fields; these may aid the implementation of better practice. For other pest species in strawberry such as mirids (*Lygus rugulipennis*) the widespread use of June bearers in Denmark is an effective preventive tactics already implemented. Future use of ever-bearers or extended seasons in tunnels may provide problems with the second and much larger generation of mirids in the future. In this respect verification and quantification of possible differences in host plant resistance between strawberry cultivars will be needed to provide a basis for the growers to choose cultivars based on plant management considerations.

This study, as well as previous investigations (Turner, 1968; Alford, 1976; Hansen, 2008), showed that parasitoids, including *C. aretas*, are important natural enemies of tortricids in Danish strawberry worthwhile targeting with conservation biocontrol measures. A frequently used conservation method is the use of flower strips as a source of food, alternative hosts/prey or shelter for natural enemies (Sigsgaard et al., 2007). A flower strip is also an ecological infrastructure. For enhancement of tortricid parasitoids the food quality of potential flower strip plant species is in focus since the mono-/oligophagous parasitoids cannot be expected to be able to exploit other tortricids than those found in strawberries. Plant species used in flower strips should preferably be selective, i.e. favoring only the target natural enemy and not the target pest. Our project was not able to identify a fully selective plant species for *C. aretas* but identified buckwheat as being the optimal for promoting fitness of *C. aretas*, while at the same time only being equivalent to other plant species for the strawberry tortricid. Buckwheat has also been documented as being nutritionally valuable for other natural enemies such as syrphids, green lacewing and various parasitoids (Irvin et al., 2006; Nicholls et al., 2000; English-Loeb et al., 2003: Tylianakis et al., 2004: Eggenschwiler et al., 2010). Although we were unable to demonstrate a positive effect of buckwheat flower strips on tortricid parasitism, the strips had a positive effect on other tortricid mortality factors, most likely through enhancing the presence and fitness of various predators as has also been demonstrated by others (Irvin et al., 2006; Nicholls et al., 2000; English-Loeb et al., 2003; Tylianakis et al., 2004; Eggenschwiler et al., 2010). Further investigations are needed to optimize plant species composition with fully selective food plants for the dominant tortricid parasitoid and to elucidate the effect of flower strips on other naturally occurring predators and parasitoids, not only of tortricid but also of other strawberry pests. In addition, further specific studies on the importance of various conservation tactics for the beneficial fauna in strawberry and the consequences of their increased presence on pest control are needed to build on the knowledge generated in the present project. For other pest species in strawberry such as aphids, thrips and mites, flower strips or other conservation biocontrol may be of value by serving as a source of food, e.g. for syrphids and green lacewing, providing alternative prey, e.g. for predatory bugs (Sigsgaard, 2010), or shelter, e.g. for spiders and predatory bugs (Sigsgaard, 2010).

Although especially conservation biocontrol may contribute to the control of aphids through conservation of for instance hover flies, lacewings and lady beetles, these tactics may not be sufficient for a satisfactory control. Implementation of augmentative biocontrol strategies may therefore be a necessity for an optimal IPM program for Danish field-grown and tunnel strawberry. For strawberry in greenhouses augmentative biocontrol will be the cornerstone in IPM programs. Several aphid parasitoids are used extensively in augmentative strategies in greenhouse crops as well as in some outdoor crops and may be of use also in Danish strawberry against some aphid species. However, when it comes to the possibility for controlling the shallot aphid in strawberry, our results have demonstrated that none of the three examined parasitoid species will be suited, except for purely inundative releases of *Aphidius* species, which is likely to be too expensive. This seems to preclude employment of the well-known strategy combining specialist parasitoids with good searching abilities at low pest densities with more generalist predators directed against higher pest density levels, although further studies on other parasitoid species is needed for a definite conclusion. However, our results indicate that augmentative biocontrol of the shallot aphid may be possible using the lady beetle *A. bipunctata*, although additional studies of the biology of the lady beetle in shallot-infested strawberry are needed, as are studies to determine the economic feasibility of exploitation of the lady beetle in augmentative biocontrol programs. For some pests of strawberries grown in greenhouses or in permanent tunnels augmentative biocontrol is already used (e.g. the use of predatory mites against tarsonemid mites in tunnels; the use of parasitoids against aphids in greenhouses), whereas for other pests it needs to be adapted from biocontrol programs used in other greenhouse/tunnel crops. In field-grown strawberries augmentative biocontrol may be an option for example against thrips and aphids provided that suited beneficials are commercially available and that economically feasible release strategies are established. In some cases inundatively released beneficials may be assisted and further augmented through a combination with conservation biocontrol tactics. Thus, it is for instance likely that *A. bipunctata* released against aphids may benefit from the presence of flower strips.

The strategic considerations of the project have revealed good opportunities for using biological control in strawberry production in Denmark, especially in greenhouses. For biological control in field strawberries, experience is still lacking to a large extent. However, the EU IPM policy as well as the presently good market for organic products is likely to support development and implementation of biocontrol in field-grown strawberries, especially when combined with reinforced education of strawberry growers.

The time perspective for implementation of biological control of pests in Danish strawberries under different types of cultivation is shown in Table 1. The table shows the present options for monitoring, chemical control and biological control of the most important strawberry pests as well as the options to be achieved in the near future. From the table it is evident that especially the strawberry weevil is a troublesome pest since it cannot yet be controlled without the use of insecticides. Solutions based on cropping practice, conservation or augmentative biological control against strawberry weevil are not imminent, but a first step towards integrated pest management of this pest has recently been initiated through a Core Organic project supported by GUDP targeting the strawberry weevil and two other pest species through mass-trapping (Wibe et al, *accepted w. revision*).

Table 1: Overview of present and near-future (< 1 year) possibilities for monitoring (“moni”), as well as chemical (“chem”) and biological (“bio”) control in strawberries grown in fields, tunnels and greenhouses.

Pest	**Field grown strawberries**					**Strawberries in temporary tunnels**					**Strawberries in permanent tunnels and greenhouses**				
	Present			Near-future		Present			Near-future		Present			Near-future	
	Moni.	Chem.	Bio.	Moni.	Bio.	Moni.	Chem.	Bio.	Moni.	Bio.	Moni.	Chem.	Bio.	Moni.	Bio.
Tortricids		X	X				x	x					x		
Strawberry weevils		X		X			x		x						x
Wine weevils		(x)	(x)				(x)	(x)				(x)	x		
Tarsonemid mites		X	X				x	x				x	x		
Spider mites		X	X				x	x				x	x		
Thrips	x	(x)	X			x	(x)	x			x	(x)	x		
Aphids		X	(x)				x	x				x	x		
Flower bugs				X					x					x	(x)
Whiteflies	X					x		(x)			x		x		
Slugs, snails		X	(x)				x	(x)					x		

8. Conclusion

Pest management can be seen as several lines of defenses with cropping practice representing the first line of defense. In this study we have documented that organic cropping practice leads to considerably lower levels of infestations with the strawberry tortricid. This difference was not attributable to increased densities of the tortricid specific parasitoid *C. aretas*, whose abundance is closely linked to that of its host, making it more exposed to local extinction in organic farms where the density of *A. comariana* is low. The lower levels of tortricid infestations in organic fields was rather a results of a higher proportion of other tortricid parasitoids and predators supporting results from other studies that organic cropping practice leads to a more diverse flora and fauna including natural enemies. Additional explanations for the differences in tortricid infestations levels between farm types may be negative impacts of pesticides and other agrochemicals on natural enemies in conventional farms and/ or lack of effect of tortricid insecticides, both leading to increased tortricid infestation in conventional farms. Overall, our study also showed an influence of crop age on tortricid infestation level with increasing levels in older fields. This difference was, however, masked in one observation year, presumably reflecting that the practice of planting old and young strawberry crops in close proximity (1-100 m) facilitates tortricid migration between old and young strawberry fields. As tortricids are poor fliers (100-200 m) changing farm design to include increased temporal and spatial distances between strawberry crops is likely to reduce problems with this pest.

A second line of defense against pests is conservation biological control aiming at increasing functional biodiversity, i.e. increasing the presence and performance of naturally occurring beneficials. In our study buckwheat was found to be the most optimal for the tortricid parasitoid, *C. aretas*, while not being better for strawberry tortricid than other plants. In a trial with flower strips of buckwheat in strawberry field, however, a positive effect on parasitism could not be demonstrated at distances up to 11 m from the buckwheat strips. However, mortality due to other reasons than parasitism was higher among tortricid larvae collected near the flower strips supporting other studies showing an augmentative effect of buckwheat flower strips on a complex suite of natural enemies including predators. It is likely that this higher unknown tortricid larval mortality was due to non-consumptive predator effects. For conservation biological control of strawberry tortricids a selective food plant for *C. aretas* remains to be identified. If a positive effect of buckwheat on tortricid control is confirmed, buckwheat may be utilized together with a selective food plant in flower strips, once it is identified.

Inundative and inoculative biological control is a third line of defense for situations in which cropping practice and conservation biological control are not sufficient to keep a pest under control. When a biological control method is available it can replace the use of pesticides and in this way contribute to reduce pest infestations. Biological control is well developed for protected crops, but less so for semi-protected and field crops. Against new pests new biological control methods need to be developed. The shallot aphid is a pest occurring more frequently in Denmark in recent years and is anticipated to become a more important problem in strawberries in the future. This study has demonstrated that two *Aphidius* parasitoid species frequently used in greenhouses against other aphid pests have a very low reproductive success on shallot aphids which precludes their use in biocontrol against shallot aphids in strawberry except for purely inundative releases. Another parasitoid often applied for aphid biocontrol in greenhouses, *A. abdominalis*, performed poorly in terms of host feeding and reproduction on whole strawberry plants and is therefore not considered suitable either for inoculative or for inundative biocontrol. It remains to be seen if other parasitoid species will be able to parasitize shallot aphids in strawberry. In contrast to the parasitoids, larvae of the three examined predator species (lacewing, gall midge, lady beetle) readily preyed upon shallot aphids on strawberry. The lady beetle, *A. bipunctata* seems promising as a biocontrol agent against the shallot aphid as judged from its predation capacity and its ability to lay eggs on shallot aphid-infested strawberries, although further studies are needed to elucidated its propensity to stay in the crop after releases and its fitness when fed continuously on shallot aphids.

The final line of defense in pest management is the use of pesticides. The current use of pyrethroids against strawberry tortricids and strawberry weevils has a negative impact on beneficials in the field and also limit the possibility for inundative and inoculative biological control. To reduce or completely abandon the use of pesticides in strawberries, preventive and curative non-chemical methods need to be available and applicable in a holistic manner to target not just a few pest species but the entire pest complex. Other alternatives than biological control are physical/ mechanical control measures, pheromone disruption and mass-trapping. Preferably such new methods are also integrating non-chemical measures against diseases and weeds.

As a first step towards a reduction in the use of pesticides in strawberry we have with this project contributed with potential biocontrol strategies against tortricids and aphids in strawberry. In addition, our project has clarified the possibilities for reduction in pesticide use against other strawberry pests, showing that good opportunities exists for increased use of biocontrol in Danish strawberries. This is especially the case for greenhouse production where experience from other greenhouse cultures may be exploited and where many existing biocontrol agents can be expected to be of use. For biological control in tunnels and particularly in field strawberries, experience is still lacking to a large extent. In addition to development of biocontrol methods and strategies for these production systems, development of population dynamic models, monitoring and warning tools and of Danish economic thresholds, as well as exploitation of cultivars with possible host plant resistance to pests would support implementation of biological control.

9. Perspectives

9.1 Scientific perspectives

Cropping practice

Natural regulation of pests is an important ecosystem service, and lower pest infestation and/or higher levels of natural enemies have been demonstrated as a function of cropping practice in several cases. The present study has demonstrated that organic cropping practice can bring down strawberry tortricid infestation to a level where additional control is rarely needed. It also shows that infestations will mostly be higher in older fields. Further studies providing more information on cropping practice factors contributing to natural pest control are needed not only for the strawberry tortrix but also for other major strawberry pests. From the literature it is known, that functional biodiversity can be very important but more research directed to specify practical application is needed. Such research should also include the importance of plant and landscape diversity. For example, to prevent pests to move from old crops to young crops, distance between the fields can be significant. The large variability found in datasets from studies at the farm level, points to the need for more detailed plot trials to examine selected factors further.

Conservation biological control

Danish strawberry farms often have hedgerows, waterholes and similar ecological infrastructures. These ecological infrastructures provide habitats for natural enemies which can reduce pest infestation levels of for example aphids and caterpillars. The proportion of the farm land which they cover and their placement, design, species composition and management will all affect the natural enemies and hence the pest suppression achieved and thereby the value of integrating ecological infrastructures into IPM programs. Further studies are needed on how to optimize the use of natural enemies in pest prevention

The present project has shown that buckwheat was the best food source among the flower tested for *Copidosoma aretas*, the most common parasitoid of the strawberry tortricid. It was further found that flower strips in strawberries can lead to increased mortality of strawberry tortricid larvae near the strips. Neither larval density nor the level of parasitism, nor entomopathogenic fungal infestation was affected by distance to the flower strip, so mortality is possibly caused by non-consumptive predator effects. Further studies to identify selective plants and/or plant mixtures which will favour natural enemies over pests are proposed. In addition, we propose further studies of the generalist predators of the strawberry tortricid to explore how they can be used in conservation biological control. Since it is possible that the distances used in this study (from 1-11 m distance from strip) was too small to detect parasitoid response to the added floral resource, it is considered relevant to examine such effects at larger scales.

Inoculative and inundative biological control

For strawberries in greenhouses, IPM strategies should mainly be based on augmentative releases combined with a possible use of resistant varieties. It is expected that many of the already commercially available beneficials used in other greenhouse cultures will also be of use in strawberry although research may be needed in terms of modifications of frequency of releases and amount of beneficials released.

For strawberries grown in tunnels augmentative biocontrol is already practiced against some pests, for instance predatory mites against spider mites and tarsonemid mites. For other pests, beneficials employed in greenhouse cultures may be of use. Thus, for the new pest, the shallot aphid, our study has identified the two-spotted lady beetle as a promising biocontrol agent, but further studies are needed on its fitness on shallot aphids and its propensity to stay in the crop after releases. Further studies are likewise needed to examine if and how greenhouse-employed beneficials can be of use against other pest species occurring in tunnel strawberry.

Especially for open field strawberries more research is needed on inundation and inoculation biological control.

IPM strategies

Biological control methods or other alternatives to pesticides still remain to be developed for some problematic pests in strawberries. One such pest is the strawberry weevil. Incorporating pest management of strawberry weevils and other pests for which alternatives to pesticides have not yet been developed into IPM-programs requires minimization of the frequency of pesticide applications. This may be achieved through targeted sprayings based on monitoring and damage thresholds. For optimized pest management and to avoid unnecessary sprays monitoring tools, damage thresholds and population models for key pests under Danish conditions should be developed.

9.2 Administrative perspectives

Cropping practice

Development of IPM strategies for Danish strawberries grown in open fields and in tunnels should be founded on prevention, exploiting the natural enemies of insect pests present in the cropping system and applying conservation biocontrol methods to preserve and enhance naturally occurring beneficials. The important contribution of cropping practice to pest management underpins that farm management practices can contribute not only to significantly reduced pesticide input but also to reduced pesticide need, making further encouragement structures for environmentally sound cropping practices advisable.

Conservation biological control

Support for establishment and maintenance of ecological infrastructures such as flower strips and hedgerows can have real positive implications for pest management and for conserving beneficials. These structures also have a biodiversity and landscape preserving effect. The multifunctional role of ecological infrastructures makes the IOBC-WPRS defined minimum of 5% ecological infrastructures in IP a reasonable goal.

Augmentative biological control

Augmentative biocontrol (or pesticides, as a last resort) should be included in IPM strategies to combat pest species for which prevention does not suffice and additional control measures are needed. In cases where there is an existing biological control solution, but an extra cost of biological control for pest management in comparison with pesticides, compensation to the growers, or a higher tax on pesticides may be a way to encourage more use of biological control and a reduced use of pesticides.

IPM strategies

In future IPM strategies it is very important that prevention of pest problems – cropping practice and conservation biological control – plays a central role. This also includes prevention through the use of resistant cultivars, healthy plant material, sanitation etc. Education of growers in IPM, as well as all levels of society from school children to consumers is essential for the uptake and implementation of IPM principles. IPM strategies for Danish strawberries must naturally also include management of weeds and diseases and a holistic approach that ensures that measures aimed at one group of pest organisms do not interfere with the functioning of measures against other pest organism groups.

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Appendix 1 Journal paper I. Strawberry cropping practice effects on pests and their natural enemies

The paper has been submitted to Journal of Insect Science

Strawberry cropping practice effects on pests and their natural enemies

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Abstract

Cropping practice may affect both pests and natural enemies. A three-year study in 7 pairs of organic and conventional farms of the strawberry tortricid, *Acleris comariana*, its parasitoid *Copidosoma aretas*, and its entomopathogenic fungi was conducted to assess such effects. Farms were characterized with respect to cropping practice, cropping history and other parameters. Field collected larvae were laboratory reared to assess mortality due to parasitoids and entomopathogenic fungi. In 2010 a survey of nematodes was done to assess the response of an unrelated taxonomic group to cropping practice. 2743 larvae were collected. 2584 were *A. comariana*, of these 579 were parasitized by *C. aretas*. Other parasitoids were recovered from 64 *A. comariana*. 28% larvae and pupae died from unknown mortality. Only two field collected larvae were infected by entomopathogenic fungi, *Isaria* spp. and *Beauvaria* spp., respectively. Density of *A. comariana* was significantly lower on organic farms, on average four times lower. *A. comariana* was less dominant among tortricids found on organic farms. Crop age (1, 2 or 3 years) was significant, with higher infestation in older fields. Density of *A. comariana* decreased by 0.07 times per km north. No effect of crop age on *A. comariana* infestation was found comparing first and second year fields in 2010. Cropping practice did not lead to significant differences in level of total parasitism and *C. aretas* parasitism, but *C. aretas* parasitized a higher proportion of parasitized larvae in conventional farms (93%) than in organic farms (74%). Unknown mortality of *A. comariana* was higher in organic (33%) than conventional farms (26%) and 2-7 times higher in second generation than in first generation. Entomopathogenic nematodes were found in 3 organic and 1 conventional farms, while plant parasitic nematodes were found in more samples from conventional than organic fields.The low density of *A. comariana* in organic farms would expose the specialist *C. aretas* to a higher risk of local extinction. At low densities of *A. comariana*, as found in organic farms, other parasitoids may provide important supplementing control of *A. comariana* to that of *C. aretas*. Other tortricid species may serve as alternative hosts for these other parasitoids, contributing to conserve them in the habitat. Higher unknown mortality of larvae from organic fields may also be a result of nonconsumptive predator effects. This study provides an example of a considerable effect of cropping practice on an insect pest, with similar effects on nematodes. An understanding of the factors responsible could be used to develop more sustainable cropping systems for the future.

Introduction

Cropping practice may affect both pests and natural enemies with effects on crop health and yield. Organic cropping practice is considered to lead to increased species diversity (Bengtsson et al. 2005) and to increased natural enemy pest control (Crowder et al. 2010), as a result of more semi-natural vegetation and reduced use of agrochemicals on organic farms.

A three-year study in 14 different farms of the strawberry tortricid, *Acleris comariana*, *A. comariana* parasitoids, focussing on its major parasitoid *Copidosoma aretas*, and on entomopathogenic fungi infesting larvae of *A. comariana* was conducted to assess such effects. An assessment of the presence of plant-parasitic and entomopathogenic nematodes in soils of the same farms was conducted to assess the response of another taxonomic group of organisms to cropping practice.

A complex of six species of tortricids (Lepidoptera: Tortricidea) can attack strawberry. By far the most common is the strawberry tortricid *Acleris comariana* (Lienig & Zeller). The other species are *Celypha lacunana* (Denis & Schiff), *Clepsis spectrana* (Treits), *Cacoecimorpha pronubana* (Hübn.), *Cnephasia asseclana* (Denis & Schiffermüller) and *Lozotaenia forsterana* (Bovien & Thomsen 1950, Stenseth 1982, Alford 1984, Cross et al. 2001). *Acleris comariana* is considered one of the main arthropod pests in strawberry, the others being the strawberry weevil *Anthonomus rubi*, spider mites and tarsonemid mites (Lindhard et al. 2003). Moth larvae feed on leaves and flowers of strawberries. Pesticide use for control of moth larvae is harmful to many natural enemies, and may contribute to propagation of other pests such as mites. There is therefore a need to look at alternative methods for moth control (Sigsgaard and Petersen, 2008). A better understanding of the impact of cultivation practice on *A. comariana* numbers and how mortality factors can contribute to control *A. comariana* is fundamental to develop conservation biological strategies.

The main parasitoid of *A. comariana* is the egg-larval parasitoid *Copidosoma aretas* Walker (Hymenoptera: Encyrtidae) (Sigsgaard et al *In press*). This parasitoid has also been found to be the dominant parasitoid of *A. comariana* in the United Kingdom (Turner 1968, Alford 1975, Alford 1976), however, the impact of cropping practice on the parasitoid is not known. Insect pathogenic fungi have not earlier been recorded in *A. comariana*, but are known from other Lepidopterans (Guzmán-Franco et al. 2008), and can be an important mortality factor, of relevance in conservation biological control.

Entomopathogenic nematodes occur naturally in soil (as infective non-feeding juvenile stages) and parasitize soil living stages of their insect hosts. Entomopathogenic nematodes are common in cultivated and uncultivated land and many species occur more frequently in sandy soils (Hominick 2002, Griffin et al. 2005, Haukeland et al. 2006). Studies on natural populations of entomopathogenic nematodes have mostly been concerned with recovery of new species and prevalence in certain habitats or altitudes. Few studies (if any) have compared the presence of entomopathogenic nematodes in different cropping systems.

The study aimed to analyse the impact of organic or conventional cropping practice on *A. comariana* and its natural enemies and how cropping practice may affect their interaction. Nematodes were assessed in the same fields to test if cropping systems effects were reflected in non-related taxonomic groups.

Materials and methods

Farms and fields

The project was designed to have six pairs of farms, which matched as good as possible in terms of geography, area and years with strawberries. Since there are few organic strawberry farms, the ecological farms were selected first and next the conventional farms. Two more farms were subsequently included in the study: in 2009 a large conventional strawberry production (conventional farm 7), and in 2010 an organic farm (organic farm 7), which overlapped with and replaced organic farm 3, on which strawberry production was phased out in 2010. These two latter farms constituted the last pair. The farms were selected to represent a range of sizes and cropping histories (either a long history or on new sites).

One field in each farm was studied. Selected fields in 2009 were 1-year strawberry, indicating that they had been planted the previous year and this was the first year of harvest. In 2010 samplings were continued in the same fields, now 2-year strawberry. However in farm 7 the field in 2009 was a 3-year crop. It was replaced by a 2-year crop in 2010. An assessment in 2010 of tortricid numbers in new 1-y crops compared with the 2-year crops was done in order to provide a separate dataset for analysis of the effect of crop age on tortricid infestation. The new 1-y crops replaced the old fields for samplings of the summer generation in 2010 in those cases, where the 2-year crop had been removed. In spring 2011 conventional farms 2, 3 and 7 and organic farm 7 had 3-y old fields, the remaining fields were 2-y old.

Field assessments 2009-11

Farms were characterized with respect to cropping practice, cropping history, area with strawberry and proximity to other strawberry farms. Recordings of tortricid infestation were done from April 2009 to June 2011 covering a total of five generations of *A. comariana*. In 2009 spring larvae were sampled only once in early May in 1-year fields when larvae were about 3-5 instar. One field was sampled in each farm (Table 1). Fields with the widely used, early variety Honeoye was, as far as possible selected to reduce variation. Thirty random samples were made during a transect walk of the field. In the following samplings two collections were made per generation, when the larvae were about 2-3 instar and again when they reached 4-5 instar. Sampling size in the following samplings was at least 30 samples, and was increased up to a maximum of 60 samples, in case of low larval density. Infestation was recorded by registering the number of larvae per 1 m of row, equivalent to 3-3.5 plants of Honeyoe.

The summer larvae of 2009 were sampled twice with a 10-d interval in late July and early August. Recordings were continued in spring and summer of 2010 and spring of 2011. In 2010 a 1-y field was also sampled in each farm to allow a direct comparison of the effect of field age on infestation.

Parasitism of A. comariana

To assess mortality due to parasitoids and entomopathogenic fungi, larvae were reared singly in 35 ml containers at 20°C ± 1°C. The bases of the containers were covered with 1% water agar. A strawberry leaf was inserted in the agar, to feed the larva. Twice weekly larvae were transferred to a new container with a fresh leaf. Every second day larvae were examined and any mortality recorded.

Emerged parasitoids were conserved for later determination. *C. aretas* was determined to species Guerreri and Noyes 2005), other parasitoids to family. Dead larvae without signs of parasitism were transferred to incubation to screen for insect pathogenic fungi. Field collected larvae that emerged as adults were likewise identified to species. Finally pupae that did not emerge were dissected.

Prevalence of insect pathogenic fungi

Dead larvae without signs of parasitism were set in humid chambers to enhance fungal growth. Spore preparations were made on a microscope slide for diagnosis to species (Lacey 1997). Normally infestation will be clearly visible after two weeks, but to ensure that no pathogens were missed, all samples were retained for 3 weeks, and those where there was some doubt, were retained in humidity chamber for up to 5 weeks.

The influence of cropping practice on nematodes

Mid-September 2010, nematodes were sampled in the same fields used for the insect study providing a total of seven organic and seven conventional samples. Samples were taken using a soil corer. Each sample comprised 20 pooled cores of 2.5 cm diameter taken to a depth of approximately 15 cm. Cores were taken as evenly as possible along a 'W' pattern within each field and mixed to give one sample of around 1 kg per field.

For recovery of entomopathogenic nematodes a subsample from each sample was placed in a 500 ml plastic container and baited with three late instar larvae of *Galleria mellonella* (wax moth) (Kaya and Stock 1997). The containers were place at room temperature and the larvae were examined for mortality and entomopathogenic nematode infection after 1 and 2 weeks. Dead larvae were placed in humid Petri-dish chambers to allow for nematode development. Nematodes were identified to the genus level by morphological examination of the infective juvenile stages (Adams and Nguyen 2002). Free-living plant parasitic nematodes were extracted from a 250 ml sub-sample from each soil sample using the elutriation technique Seinhorst (1962). Nematodes were identified by morphological examination of the adult stages to the genus level.

Yield

Strawberry yield was assessed in all farms in 2011, in the same fields as used in the study. In late June the number of flowers, fruit and empty stems were counted in eight to ten randomly selected 1 m rows, equivalent to 3-3.5 plants. The sum of flowers, fruit and empty stems represented the production potential of the crop. This measure replaced a yield estimate, as frost damage affected fruit formation of several fields in 2011.

Data analysis

Prevalence of A. comariana in the field

The number of observed tortricids was summarized for each field. Prior to analysis the number was corrected by the proportion of *A. comariana* in samples. Data was then analysed in a generalised linear mixed model, where the number of larvae was assumed to be Poisson distributed with a mean that depended of cropping practice, year, crop age and generation as well as interaction between these (as systematic effects). In addition characteristics for the individual farms/fields were attempted to be included as explanatory variables – with the possibility that the effect depended of the cropping practice. The farms were ordered in pairs attempting one organic and one conventional farm in each pair. These pairs and year, field and generation within field were included as a random effect together. Finally a possible over dispersion effect was estimated and taken into account. To accommodate the slightly varying numbers of samples per field the logarithm to the number of samples were included as an offset-variable. The offset-variable also included the correction needed to take into account tortricids sampled which were not *A. comariana*.

Mortality factors

The proportion of *A. comariana* parasitized by *C. aretas* and by other parasitoids as well as the proportion dead and adult emergence were analysed using a generalized mixed linear model assuming parasitism to follow a binominal distribution. In the model the logit of the binomial probability depended on the fixed effects of cultivation method, generation, year and the two-way interactions among these and the random effects of pair and pair within year. However, the non-significant interaction effects of fixed effects were removed from the final model.

Yield

Yield was analysed using a linear mixed linear with cultivation method as fixed effects and pairs together with farms as a random factor.

Results

Effect of cropping practice and other management practices on tortricid infestation of strawberry

During the duration of the experiment a total of 2743 tortricid larvae were collected, of these 1198 adult *A. comariana* emerged. 159 other tortricids were found. Of those that completed development to adult were *Celypha lacunana* (n =40 adults, of which 22 in conventional fields), *Cnephasia asseclana* (n = 49 adults, of which 20 in conventional fields), and *Clepsis spectrana* (n = 2, both in conventional fields). The larvae of these species are normally darker in appearance than *A. comariana* and can be distinguished from *A. comariana* in the laboratory. The larvae of the three other tortricid species feed on a wide variety of herbaceous plants. Densities of *A. comariana* per m of strawberry row, equivalent to 3-3.5 plants for the study period are presented in figure 1, corrected for presence of other tortricids.

Density of *A. comariana*, corrected for presence of other tortricids, depended strongly on cropping practice (organic or conventional) (Figure 1) with higher densities under conventional practice ($F_{1,24.6} = 14.75$, $P = 0.001$). No significant main effect was found of year of study and generation (spring, summer). There was a significant effect of crop age (1, 2 or 3 years) ($F_{2,45.8} = 3.43$, $P = 0.041$) with higher infestation in older fields. Interaction effects were not significant. One explanatory variable was found to be significant and was included in the analysis: UTM North ($F_{1,8.7} = 11.1$, $P = 0.008$), the log(x) estimate being (-0.00007 ± 0.00022), indicating numbers of *A. comariana* decreasing by 0.07 times per km north. One other explanatory variable, the years a farm had been producing strawberries, was near significant ($F_{1,14.7} = 3.48$, $P = 0.082$).

Use of insecticide was not a significant explanatory variable of densities of *A. comariana*. Conventional farms 5, 6 and 7 used insecticides in strawberries. Pyrethroid was applied in spring/ early summer to control *A. comariana* and/or *A. rubi*. The remaining conventional farms used only herbicides and fungicides, except for conventional farm 3 which also not use herbicides. All conventional farms used fertilizer.

Acleris comariana was the dominant tortricid species in both organic and conventional farms, but less so in organic farms with 14.8% individuals of other tortricid species, but only 3.1% individuals of other species in the conventional fields ($F_{1,48} = 31.7$, $P < 0.0001$). The interaction effect of time of sampling (following the two generations of *A. comariana*) and year on proportion of other species was also significant ($F_{1,48} = 4.3$, $P = 0.04$) with seven times more other tortricid species found in the sampling for the first generation *A. comariana* in 2009 compared to the second generation but only the double number in the first generation in 2010 compared to the second generation. In 2009 2.7% individuals of other tortricid species were collected during sampling for the first generation and 0.4% in the sampling for the second generation, in 2010 the corresponding percentages were 6.2% and 3.3%. In 2011 only the first generation was sampled, and there were 11.0% individuals of other tortricid species.

Effect of crop age

In 2010 we sampled new first year strawberry fields at the same time as the second year fields once for both generations (the second sampling) to test whether crop age influenced infestation. An analysis of the density of *A. comariana* as an effect of cropping practice, generation (first or second), crop age and all interaction effects showed that the average number of *A. comariana* per m row found in the first year fields (conventional 0.63 ± 0.14, organic 0.14 ± 0.05) was not significantly different from that in the second year fields (conventional 0.81 ± 0.14, organic 0.11 ± 0.05) ($F_{2,36} = 0.55$, $P = 0.584$), while there was a highly significant effect of cropping practice ($F_{1,21.1} = 11.13$, $P = 0.003$), with higher density of *A. comariana* in conventional fields (lsmeans conventional: 11.9 ± 1.9, organic 2.8 ± 1.9) and a significant effect of generation (lsmeans first generation 9.1 ± 1.7, second generation 5.7 ± 1.7) ($F_{1,23.5} = 4.71$, $P = 0.040$). There were no significant crossed effects.

Natural parasitism of A. comariana

The dominant parasitoid was *Copidosoma aretas*. Of the *A. comariana* larvae collected, 579 were parasitized by *C. aretas*. Other parasitoids were recovered from 64 *A. comariana*. They were principally Hymenopterans (Ichneumonidae (31), Braconidae (15)) but also a few Dipterans (Tachinidae (6)). Dissection of unemerged pupae showed that 12 were parasitized by hymenopterans, which had died prior to emergence. Finally 664 larvae and 122 pupae died from unknown reasons, where neither parasitism nor fungal pathogens could be detected, equivalent of an unknown mortality of 28% (Figure 2).

An analysis of the proportion of *A comariana* adults emerging from collected larvae as a function of cropping practice (organic or conventional), generation (first or second) and year (2009, 2010, 2011) (Proc Glimmix) showed a significant main effect of generation, reflecting that the successful emergence in first generations were higher (51.7%) than in second generations (42.8%) ($F_{1,32.3}$ = 5.83, P = 0.022), and no interaction effects were significant.

The proportion of larvae which died for unknown causes was analysed as above. There was a significant main effect of cropping practice on unknown mortality, with higher mortality in the second generation. In conventional farms it was 20.9% in the first generation and 30.7% in the second generation, in organic farms it was 26.2% in the first generation and 39.7% in the second generation ($F_{1,44}$ = 5.08, P = 0.029). There was also a significant interaction effect of year and generation on mortality. In 2009 it was 6.2% in the first generation and 33.3% in the second generation. in 2010, it was 19.7% in the first generation, and 30.4% in the second. In 2011 where only the first generation was sampled mortality was 26.4% ($F_{1,44}$ = 17.53, $P < 0.001$).

A near significantly higher proportion of larvae were parasitized by *C. aretas* in conventional (24.9%) than in organic farms (15.8%) ($F_{1,46}$ = 3.9, P = 0.054). There was also a near significant difference between the three years studied with 30% individuals parasitized by *C. aretas* in 2009, 18% in 2010 and 21% in 2011 ($F_{1,46}$ = 3.9, P = 0.054).

There was no difference between total parasitism of *A. comariana* between conventional (26.8%) and organic farms (21.0%) ($F_{1,46}$ = 0.04, P = 0.839), the main effects of year and generation also not being significant. However, the proportion of total parasitism of *A. comariana* by *C. aretas* was higher in conventional (93.1%) than in organic farms (74.3%) ($F_{1,34}$ = 15.0, $P < 0.001$).

Natural occurrence of insect pathogenic fungi (prevalence)

Only two of the incubated larvae turned out to be naturally infected with insect pathogenic fungi. One larva, collected from the conventional farm no 6 in 2011, was infected by *Beauveria* spp. The other larva, also collected in 2011 from conventional farm no 7, was infected by *Isaria* spp. Both isolates are kept in the fungal culture collection at the Department of Plant and Environmental Sciences of University of Copenhagen.

Cropping type effect on nematode population

Entomopathogenic nematodes were recovered from one conventionally grown field (*Steinernema* sp.) and 3 organically grown fields (*Steinernema* sp.). Live cultures of these nematodes are kept at Bioforsk in Ås (Norway). Plant parasitic nematodes were also present. The dominating nematode species were *Pratylenchus* spp and *Longidorus* spp. *Pratylenchus* spp were present in 7 conventionally grown fields and 6 organically grown fields, *Longidorus* spp were present in 4 conventionally grown fields and 1 organically grown field. Data are summarised in Table 2.

Yield

Yield in the 14 fields in 2011, excluding organic field 3, which was no longer cultivated in 2011, is presented (Table 1) as the sum of flowers, fruit and empty stems per m row by late June. This number –representing potential yield, was used as frost damages made a comparison of harvested yield impossible. Yield was not significantly different in conventional and organic fields ($F_{1,11}$ = 1.2, P = 0.30). A model including *A. comariana* density as an explaining variable was not significant.

Discussion

Effect of cropping practice and other management practices on tortricid infestation of strawberry

Across the three years of study there was a highly significant lower infestation of *A. comariana* in organic strawberry fields. Also there was a significant effect of crop age. No other factors were significant and there were no interaction effects. A single co-variable, UTM-North, was significant, with infestation of *A. comariana* decreasing from South to North by 0.07 times per km. It is normally assumed that problems in strawberry build up over time, but years with strawberry production on a farm was only a near-significant co-variable of much less significance than cropping practice. Comparisons between organic and conventional cropping practice at farm scale are more heterogenous than studies at plot scale as found in a meta-analysis (Bengtsson et al. 2005). Such heterogeneity can mask an effect of co-variables, but does not exclude an influence by them.

Though the 3-year study found an effect of crop age, when comparing *A. comariana* infestation in first and second year fields within individual farms no difference in infestation level was found. This may be due to field distances between old and young strawberry crops within farms being small. In fact, it is still practice to plant old and young strawberry crops next to each other, and on the farms young fields were grown near old ones, separated by a from few metres on small farms up to a maximum of 100 m, thus facilitating migration of insects between old and young crops. If the effective mobility of *A. comariana* is restricted to a few hundred metres as noted by Turner (1968) and as indicated for tortricids in general (Jeanneret and Charmillot 1995), a farm design with larger temporal and spatial distance between strawberry crops could in all likelihood reduce this pest.

A. comariana was more dominating in conventional farms, so though numbers of other tortricid species were comparable between organic and conventional farms, the proportion of other tortricid species in organic strawberry fields was significantly higher. These other tortricid species were generalists with a wide plant host range making surrounding landscape or other crops a likely origin. The higher nutrient levels normally found in conventional fields would have suggested higher densities of generalists there (Staley et al. 2010), opposite to findings.

While total parasitism of *A. comariana* was not different between the two cropping practices, the proportion of larvae parasitized by *C. aretas* was near significantly higher in conventional farms than in organic farms. Percentage parasitism by *C. aretas* found in this study (organic 16%, conventional 25%) are higher than the below 10% reported by Turner (1968) and Vernon (1971), but lower than the 76% found by Alford (1976).

The proportion of parasitism by *C. aretas* was highest in conventional farms, with a higher contribution to parasitism by other parasitoids in the organic farms. A strong correlation between *A. comariana* density and parasitism by *C. aretas* underlines the potential of this parasitoid to regulate *A. comariana* (Sigsgaard et al., *In press*), but can also help to explain the low density of this parasitoid in organic farms, where the density of *A. comariana* was low, exposing the specialist *C. aretas* to a higher risk of local extinction. For those systems in which extinction probabilities have been quantified, the parasitoid is more prone to extinction than its host supporting the theoretical expectation that higher trophic levels are at greater risk of extinction than lower trophic levels (Cronin and Reeve, 2005).

Results indicate that at low densities of *A. comariana*, as found in organic farms, other parasitoids provide supplementing control to that of the specialist *C. aretas*. Other tortricid species present may serve as alternative hosts for these other parasitoids, contributing to conserve them in the habitat, as found for the parasitoid *Colpoclypeus florus* (Walker) (Hymenoptera: Eulophidae) of leaf rollers in apple (Pfannenstiel et al., 2010).

Higher unknown mortality of *A. comariana* larvae collected in organic farms calls for further study. Identical rearing conditions for all collected larvae exclude the possibility that this was an effect of rearing. One unknown mortality factor could be nonconsumptive predator effects (McCauley et al 2011). Higher unknown mortality in the second generation *A. comariana*, at a time of summer where there are more natural enemies could support this.

The less dominance of *A. comariana* and *C. aretas* in organic fields is in accordance with the findings of a meta-study of the effect of organic farming practice on diversity demonstrating higher diversity in organic farming practice (Bengtsson et al 2005). Likewise Macfadyen et al. (2011) in a study comparing parasitoid diversity between ten organic and ten conventional farms, found significantly greater parasitoid species complementarity on organic farms, with on average more species in each functional group.

The higher diversity of tortricids and parasitoids in organic fields in this study could be a result of a higher floral diversity than in conventional fields. For *A. comariana* and *C. aretas* it is known that flowering plants may increase longevity of both species (Sigsgaard et al *In press*), which in turn may lead to higher densities. Flowering plant species abundance and richness of strawberry fields and hedgerows of conventional farms 1, 2, 3, 5 and 7 and organic farms 1, 2, 4 and 7 according to a study by E. J. Ahrenfeldt et al. (*unpublished*.) are however comparable. Thus richness in conventional fields were12, 12, 7, 5 and 7 species and in organic fields 13, 10, 3 and 10 species, while richness in conventional hedgerows adjacent to these fields were 6, 13, 7, 5 and 6 species and in organic hedgerows: 9, 9, 6 and 6 species. Differences in plant or crop diversity at larger scales such as between fields cannot be excluded.

Organic cropping practice is considered to lead to increased species diversity (Bengtsson et al 2005) and to increased natural enemy pest control (Crowder et al, 2010), as a result of more semi-natural vegetation and reduced use of agrochemicals on organic farms. Though only three of the conventional farms in this study used insecticides, and insecticide was not a significant co-variable, all of the conventional farms used fertilizers, herbicides (except one) and fungicides, of potential impact on arthropods. Herbicides and fungicides may directly affect arthropods, and can have indirect effect through changes in flora composition. Thus noxious effects of herbicides on egg parasitoids have been reported for the encyrtid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) (Bueno et al. 2008). Though parasitism was not higher in organic farms, the higher unknown mortality found in larvae collected in organic farms point to a higher activity of natural enemies as one explaining factor for the much lover infestation by *A. comariana* under organic cropping practice.

Very low prevalence of entomopathogenic fungi on A. comariana

The very low natural prevalence of entomopathogenic fungal infestation of *A. comariana* larvae indicates that natural regulation by this group of natural enemies is not likely. This may be attributed to the fact that *A. comariana* larvae are not in contact with soil, though entomopathogenic fungi can also be found on the phylloplane (Meyling and Eilenberg 2007). No previous studies have been done to assess prevalence of entomopathogenic fungal infection of *A. comariana*, however Entomophtora was reported from the congener *Acleris minuta* (Robinson) (Mcleod and Müller-Kögler 1973), and according to Zimmermann and Weizer (1991) fungi are often found on Tortricid larvae and pupae.

Effect of cropping practice on nematodes

Data may indicate higher prevalence of entomopathogenic nematodes in organic strawberry fields, while plant parasitic nematode levels appear less different between organic and conventional farms.

A higher presence of entomopathogenic nematodes in organically grown fields may be linked to greater insect host availability in such fields compared to conventionally grown fields, if chemical measures are implemented in the latter. A parallel Norwegian study, found a similar response by entomopathogenic nematodes to cropping practice, with a lower infestation by plant parasitic nematodes in organic fields (Trandem et al *In prep*). Further studies to elucidate which factors favour the apparent higher occurrence of entomopathogenic nematodes in organically grown fields is worth pursuing. Nematode data from the present study and from a study in Norway (Trandem et al *In prep*) are suggesting that cropping practices have comparable effects on this unrelated system. Likewise Gliessmann et al. (1996) in a comparison between conventional and organic strawberry production during a 3-year conversion found higher levels of beneficial nematodes in organic fields.

Estimated yield was not different between cropping practices pointing to the possibility for reduced *A. comariana* infestation as well as reduced pesticide and agrochemical input without compromising on yield. In contrast to this study, yields found in the conversion fields studied by Gliessmann et al (1996) were less, possibly due to a conversion decline.

Findings that *A. comariana* numbers are consistently 3-4 times lower under organic than conventional cropping practice encourages further study into the factors responsible. Understanding such factors may provide information which can aid the development of more sustainable cropping practices and conservation biological control strategies. For nematodes findings also suggest benefits from organic cropping practice in terms of higher numbers of entomopathogenic nematodes. For such studies into improved practices plot experiments may be needed, as the effect of organic farming was clearest in studies performed at the plot scale, whereas in studies at the farm scale, when organic and conventional farms were matched according to landscape structure, the effect was significant but highly heterogeneous (Bengtsson et al. 2005). The low prevalence of fungi probably excludes the use of fungi in conservation biological control. However for inundation/ inoculation biological control entomopathogenic fungi may still hold potential, which remains to be tested.

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Table 1: Farms sampled. Cropping practice, number, locality, geographical UTM coordinates (Zealand includes zones 32 and 33, we used the modified map where all of Zealand is in zone 32), area with strawberry, total area belonging to farm, years with strawberry (2009), distance to other farms with strawberry production, use of insecticide (I).

Crop. Practice	#	Locality	UTM-East	UTM-North	strawberry ha.	Tot. ha	Years w. strawberry (‘09)	Soil quality (JB)	Dis. to other grower (km)	I *	stems/m row	SE
Conventional	1	Ringsted	678907	6151417	1	12	8	3-5	5	-	154	8
	2	Klippinge	710005	6139644	2.3	50	40	6	0.5	-	108	11
	3	Tågerup	653826	6158976	1.2	6	23	5	5	-	119	7
	4	Skælskør	649253	6129270	0.75	2	10	11	0.5	-	100	7
	5	Slagelse	647026	6143613	4	40	25	6	5	+	131	8
	6	L. Skensved	700412	6159759	5	30	23	6	4	+	99	3
	7	Skælskør	648014	6127775	21	165	16	4-6	0.2	+	107	8
Organic	1	Ringsted	669561	6151293	1.5	80	5	5-6	1	-	126	11
	2	Klippinge	710043	6140360	1.5	25	11	5-6	0.5	-	132	4
	3	Tågerup	657733	6158619	0.1	55	1	3	5	-	.	.
	4	Skælskør	650313	6129770	0.2	100	2	5	1	-	132	7
	5	Fjenneslev	670332	6146341	0.5	2	12	6	7.5	-	35	4
	6	L. Skensved	697890	6157065	12	75	13	6	5	-	86	4
	7	Lejre	687451	6166198	0.5	18	2	5	0.5	-	82	5

Table 2. Occurrence of entomopathogenic nematodes and plant parasitic nematodes in fields with different cropping systems (conventionally and organically grown strawberry fields)

Nematode species	Conventional field	Organic field
Entomopathogenic nematodes	Number of samples (fields) with nematodes, n=7	
Steinernema spp.	1 (Field: 2K)	3 (Fields: 3Ø, 4Ø, 7Ø)
Plant parasitic nematodes	Mean number of nematodes per 250ml soil (SE), n=7	
Pratylenchus spp.	9.7 (2.3) (range 1-20, nematodes present in 7 fields)	6.4 (2.5) (range 0-19, nematodes present in 6 fields, all but 7Ø)
Longidorus spp.	4.4 (3.4) (range 0-25, nematodes present in 4 fields:K1, K4, K5, K7)	8 (8) (range 0-56, nematodes present in 1 field:7Ø

Figure legends

Figure 1. Field densities of *Acleris comariana* in organic and conventional strawberry fields from 2009 -11. Columns to the left are the conventional fields, columns to the right the organic fields, a) Spring 2009, b) summer 2009, c) spring 2010, d) summer 2010, e) spring 2011.

Figure 2. Mortality factors of *A. comariana*. The stacked columns height shows total number of larvae collected from each field. From below they are: the number emerging as adult *A. comariana* (50% grey), number dead (hatched lower left to upper right), parasitized by *C. aretas* (hatched, bold line, upper left to lower right), Ichneumonid (80% black), Braconid (chequered), Tachinid (zigzag lines). a) 2009 first generation , which was sampled only once, while all subsequent generations were sampled twice, b) 2009, second generation, c) 2010 first generation, d) 2010 second generation, e) 2011, first generation.

Figure 1

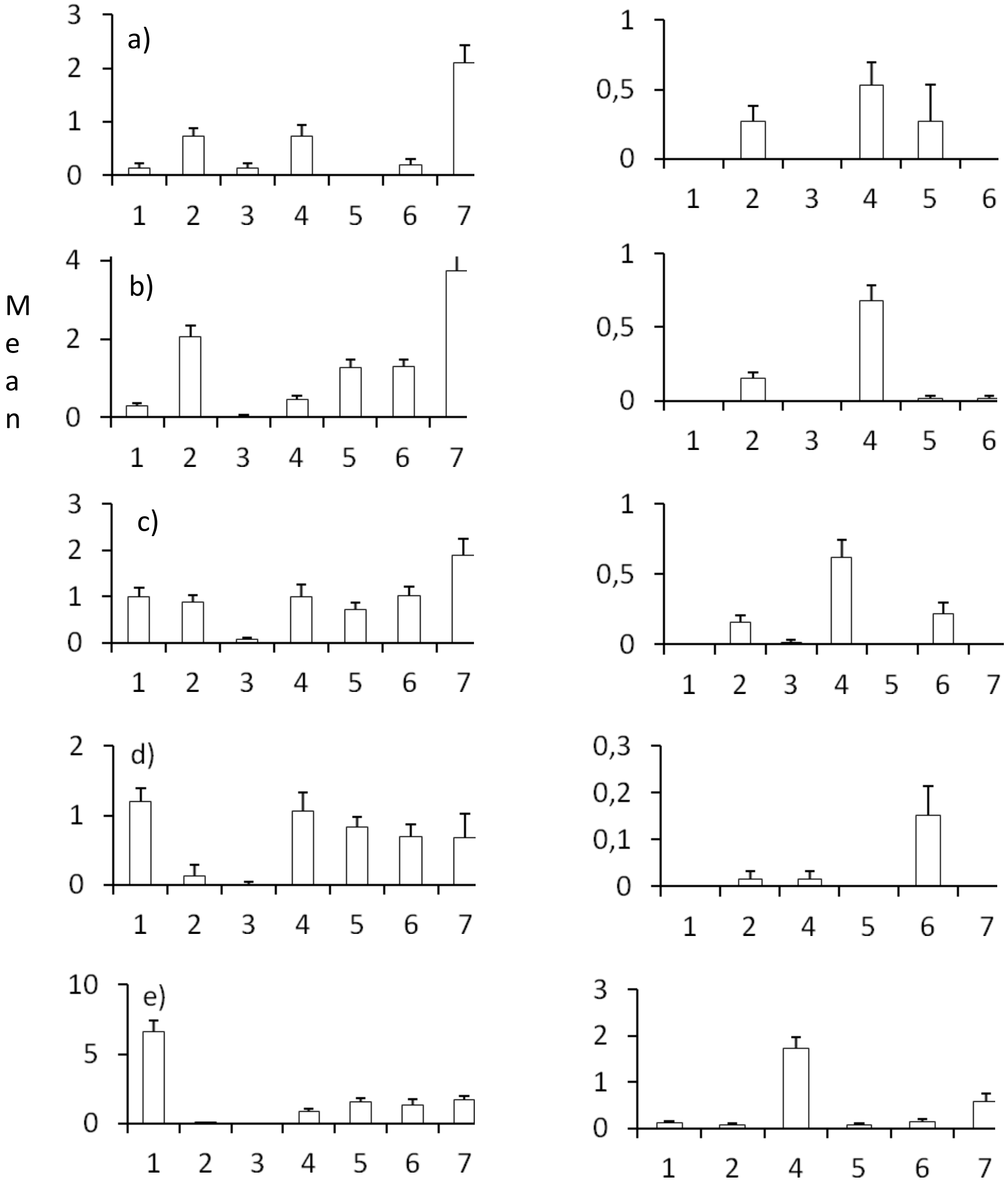
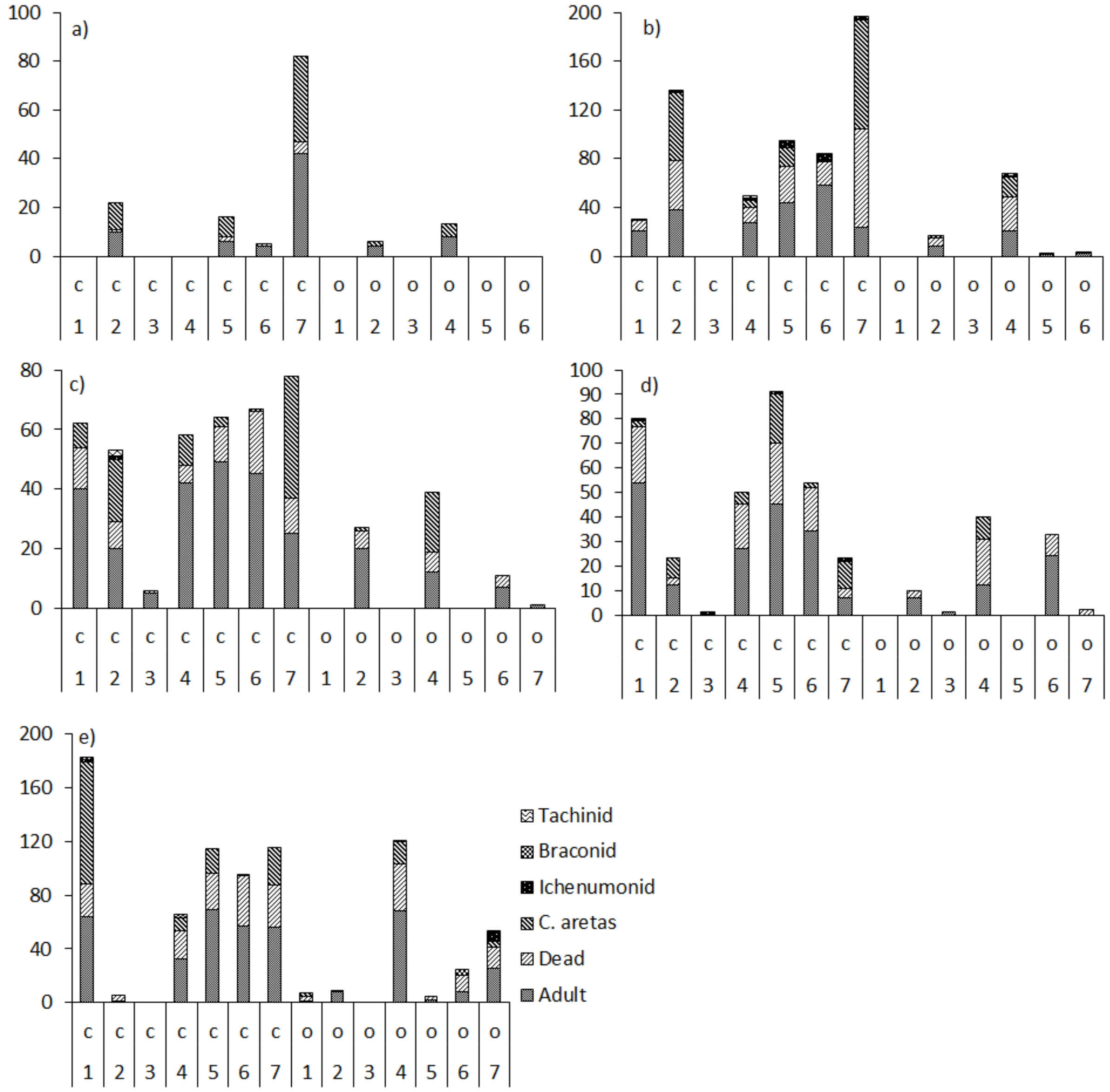


Figure 2



a)
100
80
60
40
20
0
b)
200
160
120
80
40
0
c)
80
60
40
20
0
d)
100
90
80
70
60
50
40
30
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10
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e)
200
160
120
80
40
0
c 1
c 2
c 3
c 4
c 5
c 6
c 7
o 1
o 2
o 3
o 4
o 5
o 6
o 7
▧ Tachinid
▩ Braconid
■ Ichenumonid
▧ C. aretas
▨ Dead
▩ Adult

Appendix 2 Journal paper II: Floral resources affect parasitoid and host longevity – prospects for conservation biological control in strawberries

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Floral resources affect parasitoid and host longevity – prospects for conservation biological control in strawberries

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Abstract

The strawberry tortricid, *Acleris comariana* Lienig & Zeller (Lepidoptera: Tortricidae) is an important pest in Danish strawberry production. Its most common parasitoid is *Copidosoma aretas* (Walker) (Hymenoptera: Chalcidoidea, Encyrtidae). To identify selective flowering plants which could be used to increase functional biodiversity, the longevity of *C. aretas* and it's host *A. comariana* was assessed on five flowering species: buckwheat (*Fagopyrum esculentum* Moench), borage (*Borago officinalis* L.), strawberry (*Fragaria x ananassa* Duchesne), phacelia (*Phacelia tanacetifolia* Bentham) and dill (*Anethum graveolens* L.). Dill was only tested with *C. aretas*. Sucrose and pollen served as positive controls, and water as a negative control. Among the tested floral diets, buckwheat was superior for *C. aretas*, increasing it's longevity by 1.4 times compared to water. Although buckwheat also increased longevity of *A. comariana*, it's longevity and survival on buckwheat, borage and strawberry was not significantly different, so buckwheat was chosen for field experiments. *A. comariana* larval density was assessed at 1, 6 and 11 m distances from buckwheat flower strips in three fields and the proportion of larvae parasitized by C. *aretas* was assessed. *A. comariana* densities in the three fields with buckwheat flower strips were 0.5, 4.0 and 8.3 larvae/m,row of strawberry and a total of 1%, 39% and 65% were parasitized by *C. aretas*, respectively. Densities of *A. comariana* larvae and the proportion parasitized by *C. aretas* were highly significantly correlated. Distance from floral strips had no significant effect on either *A. comariana* larval density or on the proportion of individuals parasitized by *C. aretas*. Few other parasitoids emerged from collected larvae and no larvae were infected by entomopathogenic fungi.

Notwithstanding, total *A. comariana* mortality was significantly affected by distance to flower strips, with the highest mortality in close proximity to them. As no effect of buckwheat proximity on *C. aretas* parasitism was found, the positive effect on *A. comariana* control stemmed from unknown mortality factors. Since the literature indicates that buckwheat can foster a complex suite of natural enemies including predators, one such mortality factor could be nonconsumptive predator effects, but this requires further study. If confirmed buckwheat may be utilized together with a selective food plant, once identified.

Keywords: *Copidosoma aretas*, *Acleris comariana*, diet, functional biodiversity, buckwheat, borage, phacelia, dill

Introduction

The strawberry tortricid *Acleris comariana* Lienig & Zeller (Lepidoptera: Tortricidae) is widely distributed in Europe, North America, China and Japan (Guerrieri and Noyes 2005). It is considered one of the most important pests in Danish strawberry production (Lindhard et al. 2003). The larvae feed on leaves and flowers of wild and cultivated strawberry and also on fruit trees and species of the family Rosaceae (*Malus* spp., *Pyrus* spp., *Potentilla palustris* (L.) and *Geum rivale* L.) (Alford 2007). The common parasitoid of *A. comariana* in Denmark is the polyembryonic egg-larval parasitoid *Copidosoma aretas* (Walker) (Hymenoptera: Chalcidoidea, Encyrtidae), which is also found in the UK (Turner, 1968, Guerrieri and Noyes, 2005).

In Europe, natural control by *Copidosoma* is likely to prevent populations of many moth species in agriculture and forestry from reaching economic status (Guerrieri and Noyes 2005). The high natural rate of *A. comariana* parasitism by *C. aretas* in Danish strawberry fields (L. Sigsgaard, pers. obs) makes it a potential candidate for conservation biological control. Conservation biological control entails modification of the environment or agronomic practices to protect and enhance specific natural enemies or other organisms that reduce the impact of pests (Eilenberg et al. 2001). Flower strips sown in the field can be used to provide natural enemies with pollen and nectar resources (Thompson and Hagen 1999). Selective food plants (Baggen and Gurr 1998), which might augment *C. aretas* in strawberry fields without favoring *A. comariana* would be preferred, as enhancement of pest fitness via the same mechanisms as natural enemies is a potential disadvantage if the wrong food plants are selected (Begum et al. 2006, Zhao et al. 1992, Baggen and Gurr 1998).

Thompson and Hagen (1999) reviewed the effects of natural plant foods on adult parasitic wasps. In most cases, parasitic wasps feed on nectar as well as pollen. This feeding normally increases both female longevity and fecundity, as in *Trichogramma* spp. (Zhang et al. 2004). In predominantly proovigenic species, food resources such as flower strips may increase longevity and, indirectly, rates of parasitism, even if they do not increase fecundity (Thompson and Hagen 1999, Witting-Bissinger et al. 2008).

Pure proovigeny appears to be rare (Jervis et al. 2001), but an unpublished study by J.A. Harvey lists *Copidosoma floridanum* (Ashmead) as proovigenic (Jervis et al. 2001). It is possible that the fecundity of *Copidosoma* spp. may be relatively insensitive to adult food supply, but fecundity of another *C. koehleri* Blanchard increased when fed honey, and its longevity was increased when it had access to flowers (Baggen and Gurr 1998).

No previous studies of floral diets exist for *C. aretas*, but buckwheat and borage were documented to be of high value to *C. koehleri* (Baggen and Gurr 1998). Borage benefitted *C. koehleri* but not its host, the potato tuber moth, *Phthorimaea operculella* (Zell.) (Lepidoptera: Gelechiidae) (Baggen and Gurr 1998). Likewise, phacelia benefitted *C. koehleri* but not its host (Baggen et al. 1999). Buckwheat and phacelia also proved to be a suitable food source for *Diadegma semiclausum* (Hellen) (Ichneumonidae), but of lower value to its host *Plutella xylostella* (L.) (Hyponomeutidae) (Winkler et al. 2004, Lavandero et al. 2005), so these two plants could be considered selective for the parasitoid (Lavandero et al. 2005). Irvin et al. (2006) demonstrated that intersowing buckwheat in apple orchards increased parasitism of light-brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), by *Dolichogenidea tasmanica* Cameron (Braconidae). Tylianakis et al. (2004) found increasing levels of aphid parasitism were associated with shorter distances to buckwheat plantings.

The present study examined the dietary value of three flowering plants to both *C. aretas* and its host *A. comariana* in order to identify food plants that might selectively augment *C. aretas* in strawberry fields, while not favoring the pest.

Materials and methods

Plants, pollen and sucrose

Adults of both *A. comariana* and *C. aretas* were held on diets of buckwheat (*Fagopyrum esculentum* Moench), borage (*Borago officinalis* L.), phacelia (*Phacelia tanacetifolia* Bentham), and strawberry (*Fragaria x ananassa* Duchesne) and their longevity compared. Pollen and sucrose (20% solution) served as positive controls, as they were provided freely accessible so insects were unaffected by factors which may affect longevity such as flower architecture or sugar composition (Lee and Heimpel 2003). Water was a negative control. The pollen was fresh bee pollen from Rosaceae (Percie du Sert (R), France). In addition, *C. aretas* longevity on a diet of dill (*Anethum graveolens* L.) was tested; dill plants designated for a longevity trial with *A. comariana* were lost so this trial was not completed.

Plants were chosen based on a literature search identifying plant species with value to parasitoids. Also agronomic performance was considered (Bowie et al. 1995). Buckwheat and phacelia are sucrose rich (Baker and Baker, 1983, Irvin et al. 2007, Tompkins et al. 2010). Borage has a balanced composition, and a high nectar production (Wykes 1953, Irvin et al. 2007) and strawberry and dill are glucose-fructose rich (Grünefeld et al., 1989, Irvin et al., 2007, Abrol 1992).

Buckwheat, borage, dill and phacelia were grown from seeds sown in trays with 0-35 mm light sphagnum supplied with clay granulate, lime, and an average level of fertilizer, pH 6.0 adjusted with lime (Pindstrup 2 ®). Seedlings were transplanted after one week to 13 cm pots with 10-30 mm light sphagnum with coarser structure, a high contents of air, supplied with clay granulate, an average level of fertilizer, pH 6,0 adjusted with lime (Pindstrup 4 ®) and kept in the greenhouse at normal daylight and temperatures at around 20-22 °C. Seedlings were kept in a greenhouse for the first 14 d at normal daylight and temperatures at around 20-22 °C before being transferred to outdoor netted cages (3 x 1.5 x 1.5 m). A staggered sowing ensured fresh flowers during the course of the experiment. Strawberry flowers were obtained from potted plants of the ever bearing variety Ostara, which were grown in 3.5 l pots with the same fertilizer (Pindstrup 4 ®). All plants were fertilized with irrigation water for a pH of 5.5 and a conductivity of 1.9. Strawberry plants were regularly pruned of developing fruits during the trials to ensure continued flowering.

Insect rearing

A. comariana larvae of various instars were field collected in summer from ten different commercial strawberry fields, both organic and conventional on Zealand, Denmark and reared individually in 30 ml plastic containers with small pinholes to provide ventilation and prevent condensation. A strawberry leaf inserted in water-agar (1 %) at the basis of the container served as diet. The water-agar provided support and moisture to the leaf. Every second day, larvae received a fresh leaf and if necessary they were moved to a new plastic container. When a larva had pupated, it was transferred to an empty container. Pupae were checked daily for emergence of adults. Rearing took placed in a 20 °C climate chamber (± 1 °C) with a 16:8 h photoperiod. Newly emerged adults (0-24 hours old) were used in experiments. Sex is difficult to observe on live individuals, so both males and females were used, and sex was determined at the end of experiments (Vandeurs, 1956).

Adult *C. aretas* were obtained from field-collected *A. comariana* larvae. After the fifth instar, parasitoid pupae form inside the skin of dead larvae. Individual dead parasitized larvae with parasitoid pupae were transferred to empty containers (similar to the above except without water-agar and without ventilation holes) and observed daily for emergence. Live wasps were sexed by antennal shape (Guerrieri and Noyes 2005) and newly emerged females (0-24 h) were used in experiments.

Methods

Longevity of *Acleris comariana*

A. comariana longevity was assessed for moths placed singly in plastic containers (7 cm diameter, 9.5 cm height) with a 2 cm diameter ventilation hole covered with filter paper in the top. Distilled water was provided continuously in an eppendorf vial plugged with a folded piece of filter paper to serve as a wick. Flowers were provided in 30 ml plastic containers (3. 5 x 4 cm) with the stems inserted in 1% water-agar in the base of the container to maintain turgidity and freshness of the flowers. Pollen grains were provided on a small inverted plastic lid (diameter 3.8 cm, lip 0.4 cm) to prevent contact with water. Sucrose was provided in the same way. Distilled water served as a control treatment. Fresh diet and water was provided every morning. Flowers have the most nectar available in the morning (Lee and Heimpel 2003). Excised plants were used for trials, since there is little evidence that nectar content and production of nectar is altered in excised plants (Wade and Wratten 2007). Adults were distributed among treatments as they emerged and sex of *A. comariana* was determined at death. The number of replicates initiated on a given day depended on the availability of fresh flowers; the higher number of replicates with buckwheat and borage reflect higher production of flowers in these two species.

Longevity of *Copidosoma aretas*

Containers (as above) were inverted with cut flower stems fit through a hole in the lid and secured with parafilm. Outside the container, the stem was inserted in a similar container filled with about 20 ml 1% water-agar, providing the flower with water and support. Distilled water was provided on a 1 x 1 cm piece of filter paper in all treatments and food (pollen grains or sucrose) was provided on a 1 x 1 cm piece of filter paper. Distilled water served as a control treatment. In each container, three newly emerged adult females from the same brood (i.e. siblings) were released. Fresh diet and water were provided daily.

Flower strips

Flower strips of buckwheat (1 m x 40 m) were established inside three different strawberry fields (var. Honeoye) at three conventional farms on the island of Zealand, where *A. comariana* had been found the two previous years (Farm 1, near Ringsted (55°28'34''N, 11°49'51''E), Farm 2, near Klippinge (55°21'24''N, 12°18'45''E) and Farm 3, near Skælskør (55°16'27''N, 11°19'48''E)). No insecticide was used in the three fields during the year of the study (2011). Farms 1 and 2 do not use insecticides in strawberry, whereas on farm 3 a pyrethroid treatment is often applied in spring to control *Anthonomus rubi* Herbst (Coleoptera: Curculionidae) and/or *A. comariana*. Strawberry was planted in double rows with 4 plants per m, with inter row distances of 60 cm in the double row and 100 cm between double rows. A split sowing of buckwheat (half the width of the strip by 20-23 April, 2011 and the other half two weeks later) ensured buckwheat flower availability from early June until mid-August or later, throughout the period when *A. comariana* summer eggs and larvae occur. Flower strips replaced strawberry rows, which were planted in the fields' longitudinal direction. At farm 1, the field was 120 m long from north to south and 50 m wide; the buckwheat strip was placed in the centre of the field. To the south and east there was a hedgerow, at least 30 m from the strip, to the west there was a cereal crop, and to the north, farm buildings. At farm 2, the field was 120 m from north to south and 50 m wide. This strip was sown in the full length of the field, 10 m from a hedgerow to the east, separating it from another strawberry field. To the north, south and west there were cereal fields. At farm 3, the field was 120 m long from west to east and 50-80 m wide; the buckwheat strip was 40 m long. To the south of the strip there was a cereal field separated by a grassy field margin with a few bushes. To the north there was a tarmac road and farm buildings, to the west strawberry tunnels and to the east another strawberry field. In all three fields there was a low presence of weeds. In farm 1 the common flowering weeds were *Senecio vulgaris, Tripleurospermum perforatum, Veronica arvensis, Sonchus asper* and *Stellaria media*. In farm 2 common weeds observed were *Tripleurospermum perforatum, Capsella bursa-pastoris, Carduus arvense, Rumex obtusifolius, Veronica persica* and *Lamium purpureum* while in farm 3 common flowering weeds were *Senecio vulgaris, Crepis capllaris, Geraniaceae pyrenaicum* and *Capsella bursa-pastoris*. In spite of a very dry spring, the buckwheat plants established. The density of *A. comariana* larvae was assessed visually once in late July in at least 30 1 m stretches of strawberry plants in each field at 1 m, 6 m and 11 m distance from the flower strip (Table 2). In farm 2, only the part of the field to the West of the strip facing away from the hedgerow was sampled. Field collected larvae were reared as described above in a climate-controlled cabinet to determine parasitism rates. Samples from different fields and distances were all handled identically. Tortricid pupae from which adults had not emerged after two months were dissected. Dead larvae, which were suspected to have died from fungal infection (external symptoms) were placed individually in humid chambers (small plastic containers with moistened filter paper) at 20 °C for at least two weeks to enhance fungal growth.

Data analysis

Longevity was analyzed using mixed models (e.g. West et al. 2007). Before analysis data were log transformed (log10 (x + 1)) to meet the requirements for normal distribution and homogeneity of variances. Models were reduced by removing non-significant higher order interactions (Bibby et al. 2004). Comparisons of individual diets were done by comparing the marginal means (least square means) using two-sided t-tests. The model used for *A. comariana* longevity included 'diet' and 'sex' and their interaction as fixed factors, and 'field' as a random variable. For *C. aretas*, the model included the same fixed effects, with three random variables: 1) the field where the larva had been sampled, 2) the container (there were 3 *C. aretas* per container), and 3) the maternal descent of the *C. aretas* (on average 20-30 *C. aretas* emerged from a single dead larva, and in some cases such identical siblings were used in more than one container). Survival distributions for both species were analysed with a nonparametric analysis using survival distribution plots and product-limit survival estimates (Kaplan and Meier 1958). Tests for homogeneity of survival periods over strata include the log-rank test, which is most sensitive to differences late in the survival periods, and the modified Wilcoxon test, which is most sensitive to differences early.

The effect of distance from flower strip on *A. comariana* larval density (log-transformed), proportion of larvae parasitized by *C. aretas*, proportion parasitized including other parasitoids, and proportion dead from unknown causes before becoming adult as well as total mortality (no transformation needed) was analysed using a mixed linear model with distance as a continuous variable and field as a random effect. Mixed models were performed using PROC MIXED whereas the nonparametric analyses were performed using PROC LIFETEST (SAS 2008).

Results

Longevity and survival of *A. comariana*

The greatest longevity of *A. comariana* was obtained on diets of sucrose and pollen, although these were not significantly different from those on borage and buckwheat (Table 1). Longevity of *A. comariana* (Table 1) was highly significantly affected by diet ($F_{6,238}$ =20.86, $P < 0.001$) but not by sex ($F_{1,238}$ =1.46, $P = 0.23$), and there was no significant interaction effect of diet and sex ($F_{6,238}$ =1.33, $P = 0.246$).

Survival distributions of females on borage, buckwheat and strawberry (Figure 1 a, b) were not significantly different early on (Table 1). According to the survival analysis, phacelia was inferior to buckwheat, but not different from borage and buckwheat early on. There were also significant differences between buckwheat and pollen late on, and between borage and pollen throughout the survival distribution. For males, the value of pollen was superior to buckwheat. The value of floral diets for males was similar overall to that for females, though early on, strawberry was superior to water for males. A comparison of survival distributions of the two sexes showed no differences for diets of phacelia, sucrose or pollen. In three cases, survival of the two sexes differed: males lived longer than females on buckwheat and strawberry early in the survival period (Wilcoxon, $P < 0.012$

and Wilcoxon, $P < 0.030$) and throughout the period males lived longer on water (Wilcoxon $P < 0.001$, Log-Rank, $P < 0.001$).

Longevity and survival of *C. aretas*

Longevity of *C. aretas* was significantly affected by diet ($F_{7,229} = 21.04$, $P < 0.001$). The highest longevity was obtained on a diet of sucrose, followed by diets of pollen and buckwheat, which were not significantly different from the longevity on borage, whereas the longevity on strawberry was equivalent to the longevity on dill. The shortest longevity was on phacelia, which was even shorter than that on water (Table 1).

The survival analysis showed similar trends. For *C. aretas,* the survival on buckwheat (Figure 1 c) early in the survival period was not significantly different from pollen (Table 1). Buckwheat and borage survival periods were not significantly different. Buckwheat was superior to all other floral diets, while borage was not significantly different from dill early in the survival period. The survival distributions on strawberry and dill were not significantly different, but both were superior to the survival on phacelia. Survival on phacelia was inferior to survival on water.

The effect of distance to flower strips on *A. comariana* density, parasitism and mortality

A total of 663 *A. comariana* larvae were collected from strawberry plants at the three farms at distances of 1, 6 and 11 m from the buckwheat strips (Table 2). At the time of sampling, larvae were 1.-3. instar ensuring that the sample would represent the full parasitism by *C. aretas* being an egg-larval parasitoid, but would not represent total parasitism or hyperparasitism of all instars. Four tortricids of species other than *A. comariana* were found and excluded from the analysis. Of the collected *A. comariana*, 307 (46.3%) were parasitized by *C. aretas.* Fifteen larvae were parasitized by ichneumonids (2 in farm 2, 13 in farm 3). No hyperparasitoids emerged. 107 larvae and 47 pupae died of unknown causes. Dissection of dead pupae revealed no visible signs of parasitism and no entomopathogenic fungi were observed after incubation.

A. comariana densities in the three fields with flower strips were a mean of 6.7, 0.4 and 3.1 larvae/m row (Table 2). Across fields there was a highly significant correlation between the density of *A. comariana* and the proportion parasitized ($N = 9$, Pearson correlation coefficient = 0.98, $P < 0.001$). There was no significant effect of distance from the flower strip on *A. comariana* density ($F_{1,5} = 0.15$, $P = 0.717$) or the proportion parasitized by *C. aretas* ($F_{1,5} = 1.62$, $P = 0.264$). In all fields mortality was highest near the flower strips (Table 2), with a significant effect of distance ($F_{1,5} = 29.11$, $P = 0.003$). Estimated mortality at the flower strip (distance 0 m) was 34% and this decreased by 1.2% per metre distance from the strip (intercept ± SE: 0.34 ± 0.05, $t = 7.10$, $P = 0.011$, slope: -0.012 ± 0.002 , $t = -5.38$, $P = 0.003$). Likewise total *A. comariana* mortality, including parasitism, was highest near the strip, with a highly significant effect of distance ($F_{1,5} = 118.4$, $P < 0.001$) (intercept ± SE: 0.68 ± 0.14, $t = 4.83$, $P = 0.040$, slope: -0.008 ± 0.001 , $t = -6.71$, $P = 0.001$).

Discussion

Longevity and survival of *A. comariana*

The various floral diets were equivalent in value for *A. comariana* except for phacelia which was still superior to water. Longevity of *A. comariana* sexes as a function of diet was not significantly different, though the survival distribution analysis showed differences in three cases, as males survived longer on strawberry and buckwheat than females early in the survival periods, and longer than females on water throughout the survival period.

The fact that *A. comariana* lived longer on sucrose than on any floral diet could be an effect of nectar accessibility. Flowers have their highest production of nectar in the morning, whereas moths are mostly inactive during the day. Though night time temperatures are lower and RH higher the nectar in the flowers may have crystallized or become very viscous for the moths to feed upon by the time they become active (Traynier 1983). In the experiment with sucrose, this remained liquid and accessible.

The high longevity of *A. comariana* on pollen (not significantly different from that on sucrose), indicates that this species may utilize pollen. This has previously only been documented for Micropterigidae and the families Nympalidae (*Heliconius* spp.) (O'Brien et al. 2004, Wäckers et al. 2007) and Gelechiidae (*Phyllanthus* spp.) (Luo et al. 2011). Since pollen grains do not have to be broken in order for insects to extract nutrients from them, mechanical constraints should not limit pollen feeding (Jervis et al., 1993), but the use of bee pollen may overestimate the dietary value of pure pollen, as honey bees mix pollen with regurgitated nectar or honey for transport on their legs (Roulston and Cane 2000). The proportion of added nectar or honey has never been estimated directly (Roulston and Cane 2000), and it is possible that insects use sugars to aid the digestion of pollen (Roulston and Cane 2000).

Longevity of *A. comariana* females on the floral diets did not vary significantly, except for phacelia which was inferior and equivalent to water. The low value of phacelia to *A. comariana* corresponds to its low value for another lepidopteran, *P. operculella* in a study by Baggen et al (1999). Phacelia is sucrose rich, like buckwheat (Baker and Baker, 1983, Irvin et al. 2007, Tompkins et al. 2010), indicating that nectar composition alone does not explain its low value. However, the long corolla of phacelia, outward pointing hairs on the style and ovary, and scale-like appendages within corollae, may limit nectar access, as discussed for *C. aretas* below.

The longevity of *A. comariana* ranged from 9 and 40 d, depending on diet, values that correspond to longevities reported for related species. For example, *E. postvittana* had an average longevity of 14.5 d at 20°C (Gutierrez et al. 2010), and in cages, *Acleris gloverana* (Walsingham) and *A. variana* (Fernald) males lived for about 14 days and females 28 days (EPPO 1980). In contrast to EPPO (1980) we did not find a difference between sexes in longevity. The ability of male *A. comariana* to live longer when deprived of food may reflect a higher food requirement of females (Wäckers et al. 2007).

Lepidopterans typically produce viable eggs in the absence of adult feeding, but with age, adult diet can be an increasingly important source of egg carbon (O'Brien et al. 2004). As a consequence, a positive impact of feeding on lifetime fecundity can be due to a prolonged oviposition period or an increase in the daily fecundity when adult feeding increases the oviposition period (Wäckers et al. 2007). Further studies are needed on the ovipositional activity of *A. comariana*. If it is concentrated early in adult life as in *P. xylostella* (Winkler et al. 2009), an extension of the pests longevity may not strongly affect pest abundance, whereas if the parasitoid has a prolonged oviposition period an extension of longevity may lead to greater fecundity.

Longevity and survival of *C. aretas* females

The longevity of *C. aretas* was greatest on pure diets of pollen or sucrose. Pollen is known to significantly increase the longevity of other encyrtids, e.g. *Trichogramma* spp. (Zhang et al. 2004). As for *A. comariana*, the use of bee pollen may have led to overestimation of the dietary value of pure pollen. Among the floral diets, buckwheat was superior for *C. aretas*, with an early survival distribution equivalent to that found on pollen. Borage was also of high quality, whereas intermediate values were obtained with strawberry and dill, and longevity on phacelia was less than on water. Longevity of *C. aretas* on the two sucrose rich flowers, buckwheat and phacelia, was very different, indicating that sucrose/hexose ratio cannot explain parasitoid longevity (Tompkins et al. 2010), provided that insects can access the sugars of both flowers.

Flower morphology can be an important factor influencing parasitoids' access to nectar (Vattala et al. 2006, Wäckers 2004), as the short mouthparts of parasitoids restrict their feeding to exposed sugar sources, usually unspecialized flowers (Wäckers 2004), as found for *D. semiclausum* (Winkler et al. 2006,Wäckers 2000). However, differences in corolla aperture did not seem to explain all differences in dietary value observed in this study. Dill and buckwheat both have exposed nectaries (Cawoy et al. 2008), and average corolla apertures of 2.2 and 6.6 mm, whereas borage has a corolla aperture of 0.10 mm (Baggen et al. 1999), strawberry has a wide corolla and phacelia has a corolla aperture of 5.1 mm (Baggen et al., 1999). The long corolla of phacelia, outward pointing hairs on the style and ovary, and scale-like appendages within the corolla, may limit nectar access by *C. aretas* (Baggen et al. 1999; Irvin et al. 2007) or even create mechanical stress from attempting to access the nectar, which may explain the low value of this floral diet. Interestingly, phacelia did increase female longevity of a similar-sized (0.9-1.1 mm long) congener, *C. koehleri* (Baggen et al. 1999; Guerrieri and Noyes 2005).

Greater parasitoid longevity does not necessarily imply higher fecundity. Although *Copidosoma* spp. can be predominantly proovigenic (Jervis et al. 2001), increased longevity can indirectly lead to increased parasitism as the time available to encounter and parasitize hosts is increased (Thompson and Hagen 1999, Witting-Bissinger et al. 2008). The fecundity of another *Copidosoma* species, *C. koehleri* increased when fed honey, and provision of flowers increased longevity (Baggen and Gurr, 1998).

The longevity of *C. aretas* observed in this study was shorter than that reported for *C. koehleri*. In a laboratory trial at 25 °C, *C. koehleri* lived on average 3.5 days without food or water, ca 5 d with water or plants without flowers, 7 d with glucose, 10 d with coriander, 12 d with borage and 13 d with dill (Baggen and Gurr 1998). In another study, the average longevity of *C. koehleri* on floral diets was 7-17 d, and longest with buckwheat (Baggen et al., 1998). *C. floridanum* longevity at temperatures from 15 °C to 35 °C varied from 30 days to 3 days, respectively, in the presence of food

(20% levulose solution), but dropped to 8 days and 1 day, respectively, if only water was supplied (Stoner and Weeks 1974). Further studies are needed to clarify if other diets may provide greater longevity in *C. aretas*.

Among the flowers tested, it was not possible to single out a floral diet that was beneficial to the parasitoid without being also beneficial to the strawberry tortricid. However, buckwheat was superior for *C. aretas* while buckwheat, borage and strawberry were not significantly different in quality for *A. comariana*. Therefore, we selected buckwheat for flower strip assays. Furthermore, its seeds do not overwinter so it does not pose a potential weed problem the following year, a problem which Danish strawberry growers have reported with dill. Other advantages of buckwheat are that it germinates easily, has a short sowing-flowering time and seed is inexpensive and readily available (Bowie et al. 1995).

The effect of distance to flower strips on *A. comariana* density, parasitism and mortality

No effect of flower strip proximity on *A. comariana* density or rate of parasitism could be found. The incidence of parasitism by the ichneumonid was too low to draw any conclusions. Still, the high level of parasitism by *C. aretas* found in two of the fields, and the strong correlation between pest density and parasitism shows the potential of this parasitoid to impact *A. comariana* populations in a density-dependent manner. A positive effect of flower strip proximity was demonstrated by Tylianakis et al. (2004); aphid parasitism by *Aphidius rhopalosiphi* De Stefani-Perez was 36% next to a buckwheat floral strip and declined exponentially to 0 % at 14 m distance. Likewise, a distance effect on parasitism of *P. operculella* by *C. koehleri* in potato was found when assessed at distances from 1-20 m (Baggen and Gurr, 1998). In that study, both species were field released, whereas insect numbers were not augmented in our study, and reliance on naturally-occurring field populations may have reduced the chances of demonstrating an effect of flower strip proximity. It is possible that greater distances than those used in our study may be necessary to discover a gradient in *C. aretas* parasitism. Though *D. semiclausum* is about four times larger than *C. aretas*, Lavandero et al (2005) found that it could move 80 m. Detection of effects at this scale would be difficult with normal field sizes and would require a design where fields with and without flower strips are compared. Strawberry had completed flowering by mid-late June, before parasitism, but flowering weeds occurred in low density. If *C. aretas* were able to utilize weed flowers this could have masked any distance effect of the floral strip. Finally, although buckwheat was found to increase longevity of *C. aretas* under laboratory conditions, this does not mean that it will preferentially feed from buckwheat in the field. Witting et al. (2007) found that relative attraction of trichogrammatids and two microhymenopteran parasitoids was not significantly higher in flowering buckwheat plots compared to plots where flowers had been removed or to non-flowering crabgrass controls.

The fact total *A. comariana* mortality near flower strips was 34% and decreased by 1.2% per metre distance from the strip shows that buckwheat had a positive effect on other mortality factors at the scale studied. The unknown mortality may result from viral or fungal pathogens (Stairs, 1966), predation, or nonconsumptive predator effects, as stress responses induced by exposure to predator cues can increase prey vulnerability to other mortality factors, and mere exposure to predators can result in increased mortality (McCauley et al 2011). Syrphid larvae were observed to prey on *A.*

comariana larvae inside rolled strawberry leaves (Sigsgaard, pers. obs.) and syrphid adults are frequent visitors of buckwheat flowers (Cawoy et al., 2008). Other predators observed in strawberry include spiders, anthocorids, coccinellids and carabids, but their respective contributions to *A. comariana* mortality has not been quantified. A positive effect of flower strips with buckwheat on both predators and parasitoids was found in several other studies (Irvin et al. 2006, Nicholls et al. 2000, English-Loeb et al. 2003, Tylianakis et al. 2004, Eggenschwiler et al. 2010). Similar to the present results, a 3-y survey of *A. comariana* found low mortality from fungal pathogens; fungal infection was only observed in one year and only in very few specimens (Sigsgaard et al unpublished.) This low mortality is noteworthy, since generalist insect pathogenic fungi are commonly found in soil and on insects in cropped fields (Meyling et al., 2011).

In conclusion, buckwheat was the best food plant tested, but was not selective for the parasitoid. Although buckwheat has high dietary value to *C. aretas,* and levels of parasitism in two of the fields tested were high, a positive effect of buckwheat proximity on parasitism could not be demonstrated at the scale of the study. Higher mortality of larvae near flower strips due to unknown sources calls for further study. Literature indicates that buckwheat flower strips can augment a more complex suite of natural enemies including predators (Irvin et al. 2000, Nicholls et al. 2000, English-Loeb et al. 2003, Tylianakis et al. 2004, Eggenschwiler et al. 2010) and one cryptic mortality factor could be nonconsumptive predator effects (McCauley et al 2011). A selective food plant for *C. aretas* remains to be identified that might aid in conservation biological control of *A. comariana*. If a positive effect of buckwheat on *A. comariana* is confirmed, buckwheat, might be utilized together with a selective food plant in flower strips to augment a more complex suite of natural enemies of *A. comariana*.

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Table 1

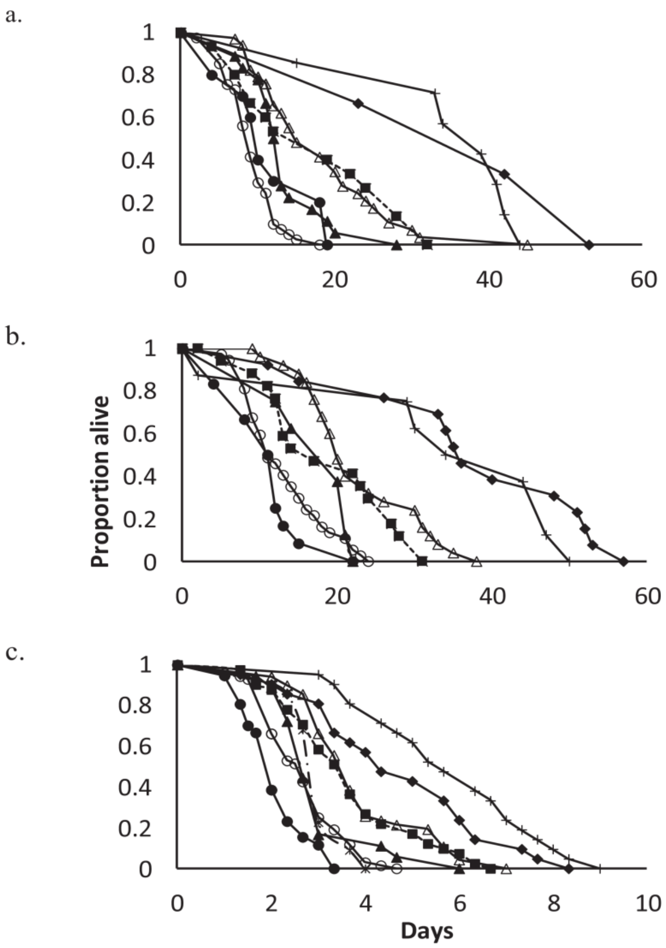
Mean longevity (± SE) of *Acleris comariana* and *Copidosoma aretas* on floral diets, pollen sucrose and water (n = number of individuals in each treatment). Within each species and sex, mean longevity in days is followed by a letter indicating significant differences according to a t-test of log-transformed population means. The last column provides a ranking based on pairwise comparisons of early survival (Wilcoxon). Where findings for late survival (LogRank) differ, this is mentioned in the text.

Species	Sex	Diet	n	Longevity	SE	Wilcoxon
Acleris comariana	F	Sucrose	7	35.43^{a}	3,73	a
		Pollen	3	39.33^{a}	8,76	a
		Buckwheat	34	16.71^{ab}	1,46	ab
		Borage	15	17.53^{ab}	2,51	b
		Strawberry	18	13.33^{b}	1,2	bc
		Phacelia	10	11.30^{c}	1,8	bc
		Water	41	9.07^{d}	0,49	c
	M	Sucrose	8	35.38^{ab}	5,58	a
		Pollen	13	37.77^{a}	4,01	a
		Buckwheat	28	21.29^{bc}	1,54	b
		Borage	18	17.89^{cd}	2,11	b
		Strawberry	8	17.75^{cd}	1,52	b
		Phacelia	12	11.00^{e}	1,4	c
		Water	37	13.03^{d}	0,84	c
Copidosoma aretas	F	Sucrose	63	5.81^{a}	0,31	a
		Pollen	63	4.73^{b}	0,35	ab
		Buckwheat	141	3.84^{b}	0,12	bc
		Borage	123	3.63^{bc}	0,15	cd
		Strawberry	54	3.00^{c}	0,18	d
		Dill	66	3.00^{c}	0,1	d
		Phacelia	96	1.91^{e}	0,09	e
		Water	204	2.66^{d}	0,07	d

Table 2. Density of *Acleris comariana* larvae, proportion of larvae parasitized by *Copidosoma aretas*, proportion dead from unknown causes before adult emergence, and total larval mortality in three strawberry fields (Farm 1, Farm 2, Farm 3) at three distances from a flower strip of buckwheat sown inside each field. Total proportion parasitized include parasitism by Ichneumonids.

Farm	Distance	Sampled stretches (1 m)	Larvae/ m row	Proportion parasitized by *C. aretas*	Total proportion parasitized	Proportion dead from unknown causes	Total mortality
1	1	20	4,95	0,65 ± 0,02	0,65 ± 0,02	0,26 ± 0,02	0,91 ± 0,01
	6	13	7,23	0,68 ± 0,04	0,68 ± 0,04	0,19 ± 0,03	0,87 ± 0,03
	11	13	7,77	0,68 ± 0,04	0,68 ± 0,04	0,15 ± 0,03	0,83 ± 0,03
2	1	60	0,45	0,04 ± 0,00	0,07 ± 0,00	0,37 ± 0,01	0,44 ± 0,01
	6	60	0,37	0,00 ± 0,00	0,00 ± 0,00	0,36 ± 0,01	0,36 ± 0,01
	11	60	0,34	0,00 ± 0,00	0,05 ± 0,00	0,30 ± 0,01	0,35 ± 0,01
3	1	31	3,55	0,32 ± 0,02	0,35 ± 0,02	0,32 ± 0,02	0,67 ± 0,02
	6	35	2,69	0,37 ± 0,01	0,40 ± 0,01	0,24 ± 0,01	0,65 ± 0,01
	11	32	3,00	0,41 ± 0,02	0,47 ± 0,02	0,14 ± 0,01	0,60 ± 0,02

Figure 1. Survival of a) *Acleris comariana* females, b) *Acleris comariana* males and c) *Copidosoma aretas* females on diets of buckwheat (*Fagopyrum esculentum* Moench) (Δ), borage (*Borago officinalis* L.) (■), phacelia (*Phacelia tanacetifolia* Bentham) (●), dill (*Anethum graveolens* L.) (--x--) and strawberry (*Fragaria x ananassa* Duchesne) (▲) as well as on pollen (♦), sucrose (+) and water control (○). Survival on dill was only assessed for *C. aretas*.



Appendix 3 Journal paper III: Shallot aphids, Myzus ascalonicus, in strawberry – biocontrol potential of three predators and three parasitoids

The paper has been resubmitted to Journal of Insect Science after revisions

Shallot aphids, *Myzus ascalonicus*, in strawberry – biocontrol potential of three predators and three parasitoids

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Keywords: *Adalia bipunctata, Chrysoperla carnea, Aphidoletes aphidimyza, Aphidius colemani, Aphidius ervi, Aphelinus abdominalis*

Abstract

The parasitization capacity of 3 parasitoids and the predation capacity of 3 predators towards the shallot aphid (*Myzus ascalonicus* Doncaster (Homoptera: Aphididae)) on strawberry was examined in laboratory experiments. In Petri dish assays both *Aphidius colemani* Viereck and *A. ervi* Haliday (Hymenoptera: Aphidiidae) readily stung shallot aphids with no significant difference in stinging frequency between the two species. *A. ervi* induced a significantly higher mortality (79.0±7.2%) in terms of stung aphids compared with *A. colemani* (55.3±4.1%). However, only a minor fraction (2.7±1.8% and 7.1±3.1%, respectively) of the killed aphids resulted in formation of mummies, presumably due to a physiological response to parasitism. The low percentage of mummification precludes the use of either *Aphidius* species in anything but inundative biocontrol. In similar set-ups *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) killed almost half (49.6±5.3%) of the exposed aphids through host feeding. In addition 23.2±7.3% of non-host-fed aphids developed into mummified aphids and 38.1±13.2% of non-host-fed aphids died from other parasitoid-induced causes. However, the host feeding rate was reduced to only 1.2±0.8% and no significant parasitization mortality was observed on strawberry plants, suggesting that host plants interfered with *A. abdominalis* activity. This parasitoid does not, therefore, seem to be suited to either inoculative or inundative biocontrol of shallot aphids in strawberry. The three predators studied were the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), the two-spotted lady beetle, *Adalia bipunctata* L. (Coleoptera: Coccinellidae) and the gall midge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Third instars of all 3 predators readily preyed upon the shallot aphid in Petri dish set-ups with significant differences in daily predation (34.62±3.45, 25.25±3.18 and 13.34±1.45, respectively). Further studies on *A. bipunctata* revealed that the larvae maintained their daily predation capacity (32.0±6.3) on strawberry plants. About 60% of already ovipositing *A. bipunctata* refrained from laying any eggs on the first day after transfer to set-ups with combinations of shallot or peach-potato aphids (*Myzus persicae* (Sulzer) (Homoptera: Aphididae)) and strawberry or sweet pepper leaves. The aphid species and the plant species did, however, not have a significant influence on the number of females laying eggs, the average number of eggs laid during the first day being 6.37±1.28 per female. Adult lady beetles had a significant preference for odor from controls without plants over odors from uninfested strawberry or pepper plants, but they showed no preference for either of the plant species, whether

infested with aphids or not. The predation capacity of *A. bipunctata* on shallot aphids holds promise for its use in inundative biocontrol and the results on egg laying cues suggests that inoculative biocontrol may be possible although further studies will be needed for a complete evaluation.

Introduction

Strawberry is the most important high-value berry crop in Denmark for fresh consumption (Danmarks Statistik 2005; Nielsen 2006). Danish strawberry is prone to infestations with a number of pest species, e.g. the strawberry tortrix moth (*Acleris comariana*), the strawberry blossom weevil (*Anthonomus rubi*), the strawberry mite (*Phytonemus pallidus*) and the two-spotted spider mite (*Tetranychus urticae*) (Lindhardt et al. 2003). In addition, new strawberry pest species known to cause serious problems in warmer climates (Karl 1983; Cross et al. 1994; Rabasse et al. 2001) are beginning to appear. One of these species is the shallot aphid (*Myzus ascalonicus* Doncaster (Homoptera: Aphididae)), which with the global climate changes in view can be expected to become an increasing problem in Denmark.

Aphids have not previously posed a serious problem in Danish strawberry grown in open fields or in tunnels. However, in 2008 shallot aphid infestations were observed in Denmark and Sweden in plastic-covered strawberry beds (Petersen et al. 2008). The shallot aphid, known to attack strawberry in Germany (Borchardt 1958; Martin Hommes, BBA, pers. comm.) and the UK (Cross et al. 1994; Anon. 2011), can inflict serious damage on this crop due to its induction of malformed leaves, stunted growth and shortened and distorted flower stalks (Anon. 2011). There are indications that shallot aphids can become especially problematic in strawberry if temperatures in February and March are above normal (Hurst 1969). Winters in Denmark are expected to become milder as a consequence of global climate changes; combined with elevated temperatures during production due to the ongoing shift in strawberry cultivation methods (from open field to plastic-covered beds or in tunnels) (Daugaard 2005) this is likely to increase the importance of the shallot aphid as a pest in Danish strawberry. To forestall the accompanying increase in the use of insecticides it is important to develop biological control methods against this pest.

Literature presents only few investigations on natural enemies of the shallot aphid (Wahab 1985; Wahab and Askew 1989); however, more information exists regarding natural enemies of the strawberry aphid *Chaetosiphon fragaefolii* (e.g. Easterbrook et al. 2006; Fitzgerald 2006). In addition, countless investigations have been made regarding the possibilities for biological control of other aphid species in various field and, especially, greenhouse crops. A number of aphidophagous natural enemies have well-documented beneficial effects and are commercially available. However, these natural enemies are not necessarily effective against the shallot aphid – this species may not be included in the host or prey range of the natural enemy, it may not occupy accessible spatial niches or it may display effective defense behavior (Enkegaard and Brødsgaard 2002). Consequently, this study screened a number of commercially available beneficials for their predation or parasitization capacity towards shallot aphids, and further studies were undertaken with the better suited candidate species.

Materials and methods

The experiments were conducted at light and temperature conditions mimicking those found in spring in Danish tunnel strawberry. For an overview of experiments, see Table 1.

Plants, insects

Onion plants (*Allium cepa* L., cv. Stutgarter Riesen), strawberry plants (*Fragaria ananassa* L., cv. Honeoye) and sweet pepper plants (*Capsicum annuum* L., cv. Arcano) were grown separately in insect-proof net-covered cages (68 × 75 × 82 cm) in a climate-controlled greenhouse compartment at 23±1°C, 70%±5% RH and 16:8 l:d at Research Centre Flakkebjerg, Aarhus University, Faculty of Science and Technology, Denmark.

Shallot aphids were received from the Julius Kuehn Institute (Federal Research Centre for Cultivated Plants, Braunschweig, Germany) and used to initiate a rearing. Shallot aphids were reared in cages at environmental conditions as described above. Peach-potato aphids (*Myzus persicae* (Sulzer) (Homoptera: Aphididae)) were obtained from a long-standing colony reared on sweet pepper and maintained at the Research Centre Flakkebjerg in similar cages and conditions.

Natural enemies (parasitoids [*Aphidius colemani* Viereck, *A. ervi* Haliday (Hymenoptera: Aphidiidae), *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae)]; predators [lacewings (*Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae)), lady beetles (*Adalia bipunctata* L. (Coleoptera: Coccinellidae)), gall midges (*Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae))]) were purchased from natural enemies producers (EWH BioProduction, Tappernøje, Denmark; Borregaard Bioplant, Aarhus, Denmark).

The parasitoids were supplied as mummies, which species-wise were placed in open Petri dishes (diameter: 9 cm) placed in a small cage (30.5 × 22 × 15 cm) in a climate cabinet (23±1°C, 70±1% RH, 16:8 l:d) until emergence. The parasitoids were allowed to mate in the cage for 1 day with access to sugar water after which females were sexed under stereo microscope and used for experimentation.

Gall midges were supplied as 3rd instars and used directly in the experiments. Lacewings and lady beetles were supplied as 2nd instars. For all experiments, except those mentioned below, larvae were reared individually in Petri dishes (diameter: 5.5 cm) to the 3rd instar on surplus flour moth eggs (*Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)) in a climate cabinet at similar conditions as above. One day prior to experimentation, predator larvae were starved for 24 h by keeping them individually in Petri dishes in a climate cabinet in the same conditions as above.

For the behavioral experiment and the experiment with egg laying of *A. bipunctata*, the rearing of the purchased 2nd instars continued until adulthood after which emerged adults were kept together in groups of 15-20 individuals of similar age. Both males and females were kept together to ensure mating. The lady beetles were fed on flour moth eggs with a supplemental feeding of grain aphids (*Sitobion avenae* (F.) (Homoptera: Aphididae)) every second day. For the egg-laying experiment adult lady beetles were collected one day prior to experimentation and placed individually in small Petri dishes with the same food. The following day females that had laid eggs during isolation were used for experimentation. For the behavioral experiments adult lady beetles were collected one day prior to experimentation and placed individually in small Petri dishes without food but with access to water. After a 24-h starvation period the lady beetles were used for experimentation.

Screening experiments

Parasitoids

A set-up used successfully in prior experiments (e.g. Enkegaard and Brødsgaard 2002) was originally intended for the screening experiments with parasitoids. In this Petri dish set-up aphids were exposed on detached leaves to a female parasitoid for a pre-defined period depending upon the parasitoid species; they were then left for approx. 1½ weeks after which the degree of parasitization was recorded. After several attempts this design had to be abandoned since the shallot aphid was unable to survive on detached strawberry leaves for the period needed for development of visible signs of parasitization. Instead the following experiments were undertaken:

Aphidius colemani, A. ervi

For recording frequency of parasitization, Petri dishes (diameter: 9 cm) with moistened filter paper and a detached strawberry leaf (approx. 4 × 7 cm) with 25 2nd instar shallot aphids were each supplied to a mated female parasitoid and observed under stereo microscope for 15 min in a climate-controlled greenhouse compartment at 18±1°C. The number of aphids stung was recorded. The number of replicates was 13 and 15 for *A. colemani* and *A. ervi*, respectively. For recording actual parasitization of stung aphids, similar Petri dishes with similarly sized aphid-infested strawberry leaves were each supplied to a mated female parasitoid and observed under stereo microscope in the same greenhouse compartment at 18±1°C. For each individual parasitoid (replicate) 10 aphid nymphs observed to be stung were immediately transferred to a small, 3-leafed potted onion plant (approx. 10 cm height), which was subsequently placed in a climate cabinet (23±1°C, 70±1% RH, 16:8 l:d) for 1½ weeks, after which the number of live, dead and mummified aphids, respectively, was recorded. The number of replicates was 13 *A. colemani* and 10 *A. ervi*, respectively. In addition, 5 onion plants each with 10 2nd instar shallot aphids unexposed to parasitoids were set up as controls.

Aphelinus abdominalis

Petri dishes (diameter: 9 cm) with similarly sized aphid-infested strawberry leaves as above were each supplied to a mated female parasitoid and placed for 24 h in a climate cabinet (18±1°C, 70±1% RH, 12:12 l:d), after which the number of dead and live aphids was recorded. For each replicate the live aphids were, as above, subsequently transferred to a small potted onion plant placed at similar conditions and recorded in a similar fashion after 1½ week. The number of replicates was 16 with 5 controls consisting of onion plants each with 25 2nd instar shallot aphids unexposed to parasitoids.

Predators

For the screening experiments a similar set-up as above was used. The Petri dishes were each supplied with a starved 3rd instar predator and placed in a climate cabinet (18±1°C, 70±1% RH, 12:12 l:d). After 3 h (*C. carnea*), 4 h (*A. bipunctata*) or 24 h (*A. aphidimyza*), respectively, the predator was removed and the number of consumed aphids recorded. The predation time for each predator species was defined, based on their maximum daily predation capacity. The number of replicates was 25, 25 and 23 per predator species, respectively. In addition, controls (n=10) without natural enemies were used for each predator species.

Further studies of the most suited predator and parasitoid species

Aphelinus abdominalis

Experiments were conducted to examine the parasitization/host feeding potential of *A. abdominalis* on whole strawberry plants. Small cages (23 × 23 × 23 cm) with either a small, 3- to 4-leafed potted strawberry plant or a small, 4- to 6-leafed potted sweet pepper plant with 60 2nd instar shallot aphids were each supplied with 1 female *A. abdominalis* (mated, max. 2 days old). After 24 h in a climate cabinet (18±1°C, 70±1% RH, 12:12 l:d) the parasitoids were removed and the number of dead aphids recorded. For each replicate the live aphids were subsequently transferred to a small potted onion plant (dimension as above) and placed in a climate cabinet (23±1°C, 70±1% RH, 16:8 l:d) for 1½ week after which the number of live, dead and mummified aphids, respectively, was recorded. The number of replicates was 11 (strawberry) and 20 (sweet pepper), respectively. In addition, plants with 60 2nd instar shallot aphids unexposed to parasitoids were made as controls for each plant species (n=10 for strawberry and n=13 for sweet pepper).

Adalia bipunctata

Predation on whole plants

Predation by *A. bipunctata* larvae was studied on whole plants. Similar cages as above with similarly sized aphid-infested strawberry or sweet pepper plants were each supplied with one 3rd instar lady beetle larva (starved for 24 h). After 24 h in a climate cabinet (18±1°C, 70±1% RH, 12:12 l:d) the larvae were removed and the number of consumed aphids recorded. The number of replicates was 12 and 14 for set-ups with strawberry plants and sweet pepper plants, respectively. In addition, controls without lady beetle larvae were made for each plant species (n=6 for strawberry and n=8 for sweet pepper).

Egg laying cues

Experiments were conducted to examine if ovipositing *A. bipunctata* would obtain sufficient cues from aphid infested strawberry to continue egg laying. Petri dishes (diameter: 9 cm) each with moistened filter paper and either a strawberry leaf or a sweet pepper leaf infested with either shallot aphids or peach-potato aphids (approx. 100 aphids per leaf) were each supplied with 1 adult female *A. bipunctata* 6-7 days old. After 24 h in a climate cabinet (18±1°C, 70±1% RH, 12:12 l:d) the females were removed and the number of eggs counted. The number of replicates was 15 for each treatment.

Behavior

The reaction of adult *A. bipunctata* to odor emitted from uninfested and aphid-infested leaves was examined in a still-air olfactometer set-up modified after Weeks et al. (2010). The set-up consisted of a rectangular plastic box (5 × 21 × 31 cm) with the edges sealed with parafilm and with two holes (4 cm diameter, covered with insect net) at the bottom, each hole positioned at opposite ends of the box on the longest central axis and 25 cm apart. A small glass container (3 l) was placed to contain test material under each hole. The top of the container was sealed against the box with isolation tape. A single adult lady beetle (less than 1 week old) (starved for 24 h) was introduced to the center of the set-up through a small hole (1 cm diameter) in the center of the box lid. The hole was plugged and the beetle then observed for 5 min during which the time spent in each of the two halves of the box was recorded. Individuals which remained inactive for more than 2 successive minutes were discarded from the analysis (only 2 individuals were discarded). Six experiments were conducted to examine the response of *A. bipunctata* to small 3-leafed (8-10 cm height) uninfested or aphid-infested sweet pepper or strawberry plants in the following treatment combinations: (1) uninfested strawberry plant versus empty container, (2) uninfested sweet pepper plant versus empty container, (3) uninfested strawberry plant versus uninfested sweet pepper plant, (4) aphid-infested strawberry plant versus empty container, (5) aphid-infested sweet pepper plant versus empty container, (6) aphid-infested strawberry plant versus aphid-infested sweet pepper plant. The infested material was produced by adding approx. 50 peach-potato aphids (mainly nymphs) to the plants 2 days prior to experimentation. The plants were placed in a climate cabinet (23±1°C, 70±1% RH, 16:8 l:d) until use. The test material was placed in the containers 5 min prior to initiation of the experiment. Three batches of 5 lady beetles were tested for each treatment. As is common practice in olfactometer studies (e.g. Souissi et al. 1998; Venzon et al. 1999), after testing each batch of insects the set-up was cleaned with alcohol (70%) and left to dry for 5 min. Subsequently odor sources were switched between the left-hand and right-hand side to minimize any spatial effect on choices. Each individual was tested only once. The experiments were conducted in a darkened room (23°C) with a light source positioned directly above the center of the set-up.

Data analysis

The recorded predation was corrected for the mortality in the corresponding controls (Abbott 1925). For comparison between the three predators, the corrected predation rates were extrapolated to daily consumption (assuming predation for 12 h of light). The number of stung, dead and mummified aphids in the screening experiments with *A. colemani* and *A. ervi* was analyzed in one-way analyses of variance with the species as fixed effects. Corrected number of dead aphids in the Petri dish screenings of *A. abdominalis, C. carnea, A. aphidimyza* and *A. bipunctata* was analyzed in one-way analyses of variance with the species as fixed effects. Because the variance depended on the species in question a separate variance was estimated for each species. Similar analysis was applied for the whole plant experiments with *A. abdominalis* and *A. bipunctata*. The egg laying of *A. bipunctata* was analyzed in one-way analyses of variance with the four combinations of aphids and plants as fixed effects. In order to examine the behavior the differences in time spent in the two halves of the set-up were analyzed in one-way analyses of variance with pairs of treatment as fixed effects. The comparison of the two different materials in each pair was examined by testing the hypothesis that the difference was zero (this analysis is similar to a paired t-test except that in this analysis the variance was assumed to be the same for all pairs). All analyses were performed using procedures glm, mixed and glimmix of SAS (SAS Institute Inc. 2008).

Results and discussion

Please refer to Table 1 for an overview of the experiments and to Table 2 for a summary of the results.

Parasitoids

Screening of parasitization capacity for Aphidius colemani *and* A. ervi

Both *Aphidius* species readily stung shallot aphids, which is in contrast to a previous study undertaken with a strain of shallot aphids (obtained from Campanula ("Campanula-strain")) with a strongly developed defensive behavior towards parasitization by both *Aphidius* species (Enkegaard and Brødsgaard 2002). The average number of stung aphids (± s.e.) was not significantly different ($F_{1,27}$ =0.48, P=0.785) between the 2 parasitoid species being 6.8±2.4 and 6.1±1.2 for *A. colemani* and *A. ervi*, respectively. Not surprisingly, a high proportion of aphids that had been stung by either species died within 1½ week. The average mortality (± s.e.) induced in aphids stung by *A. ervi* (79.0±7.2%) was significantly higher than the mortality induced in aphids stung by *A. colemani* (55.3±4.1%) ($F_{1,22}$=9.22, P=0.0063). However, only a minor fraction (7.1±3.1% and 2.7±1.8% for *A. colemani* and *A. ervi*, respectively) of the aphids killed after exposure to either of the parasitoids died as a result of a completed parasitization process leading to formation of mummies with no significant difference between these values ($F_{1,22}$=1.20, P=0.268).

Parasitization by the two parasitoid species leading to fully developed mummies has been reported in the literature to vary up from 40 to 80% at temperatures above 20°C, depending upon the offered aphid host species, host density and environmental conditions (e.g. Sequeira and Mackauer 1994; van Steenis 1995; Takada and Tada 2000; Zamani et al. 2007; Byeon et al. 2010). These rates, expressing mortality among all exposed aphids (i.e. not just stung aphids), are higher than the rates for mummification found here, indicating that the performance of *A. colemani* and *A. ervi* against shallot aphids on strawberry is far from optimal. The low degree of mummification suggests a physiological response to parasitism by *Aphidius* parasitoids in *M. ascalonicus* as demonstrated for clones of pea aphids (*Acyrthosiphon pisum*) to parasitization by *A. ervi* (Henter and Via 1995), although the physiological mechanism described by Henter and Via (1995) served as a true defense (i.e. survival) reaction, which was not the case here because the stung aphids actually died. It therefore seems that *M. ascalonicus* has the potential to form clones with resistance to parasitization based on physiological or behavioral mechanisms (Enkegaard and Brødsgaard 2002).

Even though the overall mortality rates induced in stung shallot aphids was quite high, the low degree of mummification precludes the use of either species in anything but inundative biocontrol in which no reproduction of significance can be expected. Given a choice, *A. ervi* should be preferred due to higher mortality induction.

Aphelinus abdominalis – *screening for parasitization capacity and parasitization on whole plants*

Contrary to the 2 *Aphidius* species, *A. abdominalis* uses its hosts for host feeding (Stary 1988; Haardt 1990) as evidenced in the present experiment with almost half (49.6±5.3%) of the aphids being killed within 24 h after exposure to the parasitoids. In addition, *A. abdominalis* inflicted additional mortality to the shallot aphids with 23.2±7.3% of the non-host-fed aphids developing into mummified aphids and 38.1±13.2% of the non-host-fed aphids dying from parasitoid-induced causes; this was presumably a result of parasitizations that did not develop to the mummified stage.

The results found for *A. abdominalis* are in contrast to the previous study mentioned above (Enkegaard and Brødsgaard 2002), in which the defensive behavior of the Campanula strain of the shallot aphid was obstructive also to parasitization by *A. abdominalis*. On the other hand, our findings agree with the study of Wahab (1985), in which dissections of shallot aphids 3 days after exposure to *A. abdominalis* at 18°C revealed that 40-70% of the aphids contained parasitoid eggs or larvae. The method used by Wahab (1985) allowed for neither determination of mortality due to host-feeding nor determination of the proportion of parasitized aphids that would eventually have mummified. However, in view of reports on successful aphid biocontrol with *A. abdominalis* (e.g. Hurni and Stadler 1993; Colombo and Fasce 1994; Blumel and Hausdorf 1996) it seems probable that the majority of the parasitized aphids in the study of Wahab (1985) would have mummified. The lower degree of mummification found in the present study is therefore likely a reflection of the presumed physiological mechanism mentioned above for the *Aphidius* species.

A. abdominalis and *A. ervi* inflicted the same overall mortality on shallot aphids (about 80%) although the mortality for the latter was based only on stung aphids. The mortality to be expected among a group of exposed shallot aphids would thus be higher for *A. abdominalis*, which – combined with its ability to reproduce on this aphid species – at first would seem to make it a better candidate for biological control with the potential to be used as an inoculative agent. However, the results from the experiment on realization of the potential of *A. abdominalis* on whole plants showed otherwise.

On whole plants the host feeding mortality inflicted by *A. abdominalis* on shallot aphids was significantly influenced by the host plant species ($P<0.0001$, $F_{1,29}=38.45$) with a corrected host feeding mortality (±s.e.) of 32.1±5.3% on sweet pepper and only 1.2±0.8% on strawberry. Compared with the relatively high host feeding mortality observed on strawberry leaves, the latter result suggests that qualities pertaining to whole strawberry plants were obstructive to *A. abdominalis* activity. Contrary to the results obtained in the screening experiment, no significant parasitization mortality was observed in the whole plant set-up with either of the host plants (strawberry: $P=0.73$, $F_{1,20}=0.12$; sweet pepper: $P=0.83$, $F_{1,32}=0.05$). Thus the obstructive factors pertained to both host-feeding and parasitization activities. It may be speculated that the trichomes of strawberry plants constituted an obstacle for parasitoid search, which primarily is based on walking since this species seldom flies (Biobest 2011). Another possibility is that volatile signals emitted from shallot aphid-infested strawberry plants were in some way discouraging to the parasitoids (Mölck et al. 1999; Mölck and Wyss 2003). On sweet pepper the occurrence of host feeding but the lack of parasitization was surprising because an obstructive influence from either plant structure (other than trichomes because sweet pepper is void of trichomes (Madadi et al. 2007)) or pest-plant volatiles would have been expected to affect both behavioral elements more or less equally.

Even though *A. abdominalis* may have some potential to control shallot aphids on sweet pepper, when used in an inundative fashion, the same potential is not likely on strawberry.

Predators

Screening of predation capacity

Third instar larvae of all 3 predators readily preyed upon the shallot aphid with a significant difference ($F_{2,70} = 19.34$, $P<0.0001$) in daily predation between predator species. The average daily predation rates (± s.e.) of larvae of *A. aphidimyza*, *A. bipunctata* and *C. carnea* were 13.34±1.45, 25.25±3.18 and 34.62±3.45, respectively. These results are within the range of predation rates reported on various aphid species for *A. bipunctata* (Timms et al. 2008; Jalali et al. 2009a), *C. carnea* (Athhan et al. 2004; Montoya-Alvarez et al. 2010) and *A. aphidimyza* (Morse and Croft 1987; Kulp et al. 1989; Harizanova and Ekbom 1997). Lacewing larvae were more voracious than larvae of both the lady beetle ($t_{70}=-2.00$, $P=0.0498$) and the gall midge ($t_{70}=-5.68$, $P<0.0001$), and lady beetle larvae were more voracious than gall midge larvae ($t_{70}=-3.40$, $P=0.0011$).

Predation by **Adalia bipunctata** *on whole plants*

Third instar *A. bipunctata* had significantly higher predation ($F_{1,24}$=14.17, P<0.001) on shallot aphids on sweet pepper (average daily corrected number eaten (± s.e.) 56.3±1.7) than on strawberry (32.0±6.3) with the latter predation rate being in accordance with that observed in the screening experiment. Contrary to the situation with *A. abdominalis*, the change in experimental dimensions did not seem to be obstructive to the activity of the lady beetle larvae. However, the difference in predation on the 2 host plant species showed that larvae were influenced by prey-plant characteristics (Timms et al. 2008; Alhmedi et al. 2010), perhaps due to the structure or texture of the plants (Banks 1957; Shah 1982; Carter et al. 1984).

Egg laying cues for **Adalia bipunctata**

A high proportion of the already ovipositing *A. bipunctata* refrained from laying any eggs during the 24 h experimental period (46.6 % (strawberry-shallot aphids), 53.3 % (strawberry-peach-potato aphids), 66.7 % (sweet pepper-shallot aphids) and 66.7% (sweet pepper-peach-potato aphids)). The number of females refraining from egg laying was, however, not affected by the species of the aphid and the species of the plant (χ^2=0.038, df=1, P=0.845). The results are in accordance with the study by Hemptinne et al. (2000) demonstrating female *A. bipunctata* in their oviposition site selection use other cues than those associated with aphids or plants.

The average number (±s.e.) of eggs laid per day by *A. bipunctata* was similar, irrespective of aphid and plant species ($F_{1,59}$=1.07, P=0.369) with the following values attained in each plant-aphid system: 8.67±3.08 eggs (strawberry-shallot aphids), 8.07±2.91 eggs (strawberry-peach-potato aphids), 5.93±2.59 eggs (sweet pepper-shallot aphids) and 2.8±1.24 eggs (sweet pepper-peach-potato aphids). Since the lady beetles in this experiment were only allowed a short egg laying period with the new prey, the average daily egg laying across all treatments (6.37±1.28) was, not surprisingly, lower than reported in other studies where egg production was observed for longer periods (16-20 eggs) (Hamalainen et al. 1975; Ware et al. 2008; Jalali et al. 2009b). Further studies will be needed to examine if the proportion of egg layers as well as the egg laying capacity of *A. bipunctata* would increase after longer exposure to shallot aphids on strawberry.

Behavior of **Adalia bipunctata**

The time spent by adult *A. bipunctata* over the different odor sources of uninfested and aphid-infested plant material is shown in Table 3. The lady beetles had a significant preference for clean air from the empty container to odors from uninfested strawberry (trial 1), and there was a tendency for similar preference for clean air over uninfested sweet pepper (trial 2). This apparent repellency of both plant species is in accordance with the findings of Timms et al. (2008), who found a significant negative olfactory response of adult *A. bipunctata* to uninfested Norway spruce compared to controls without plants. However, the results contradict previous observations that adult *A. bipunctata* are not attracted to volatiles from uninfested broad beans, rape or mustard (Francis et al. 2004) or to volatiles from uninfested broad bean or Nasturtium plants (Raymond et al. 2000). Avoidance or attraction of *A. bipunctata* to plant volatiles apparently depends upon the plant species in question. When *A. bipunctata* was given a choice between both uninfested plant species (trial 3), neither was preferred as was also the case when the lady beetles were given a choice between both infested plant species (trial 6). However, the presence of aphids made both plants species more attractive since the lady beetles no longer displayed a significant preference for clean air (comparison of trials 1 and 4 and of trials 2 and 5) – still, only trials 1 and 4 showed a tendency to being significantly different (t=-1.86, P=0.066) whereas this was not the case for trials 2 and 5 (t=-1.35, P=0.189). This result is in accordance with the findings of Raymond et al. (2000), who observed a tendency for *A. bipunctata* with previous feeding experience to show an olfactory preference for broad bean or Nasturtium plants infested with black bean aphids (*Aphis faba*) to uninfested plants.

Although *A. bipunctata* seems to find strawberry an inferior host plant compared with sweet pepper, its realized predation capacity on shallot aphids on strawberry indicates a potential in inundative biocontrol. In addition, the results on egg laying cues suggest that the lady beetle may be used in inoculative biocontrol strategies provided that adult lady beetles developing from released larvae will remain in the culture to initiate further generations. Even though *A. bipunctata* is apparently repelled by uninfested strawberry the extent of this reaction in the presence of aphids suggests that this may be the case. It might consequently be expected that adult *A. bipunctata* developing from released lady beetle larvae will remain in aphid infested strawberries. It would be valuable to investigate if short-term learning processes, as has been documented for the seven-spotted lady beetle, *Coccinella septempunctata* (Glinwood et al. 2011), in adult *A. bipunctata* developing from larvae released in a strawberry culture may increase their tendency to remain in the crop.

Conclusion

This study has demonstrated that the reproductive success of especially *A. colemani* and *A. ervi* but also of *A. abdominalis* is low on shallot aphids compared to the abilities of these parasitoids on other aphid species. The low reproductive success is not a result of behavioral defense reactions towards the parasitoids. Instead the shallot aphid seemingly responds physiologically to the internal presence of parasitoid eggs or larvae although this reaction does not serve as an effective defense mechanism as demonstrated in other aphid species (Henter and Via 1995) since the aphids themselves die. The very low reproductive success of the two *Aphidius* species precludes their use in biocontrol against shallot aphids in strawberry except for purely inundative releases. In addition, the reduced host feeding and reproductive capacity of *A. abdominalis* on whole plants with strawberry compared to the initial screening experiments likewise precludes the use of this species even as an inundative agent. Whether other parasitoid species than the three species examined here will be able to parasitize shallot aphids on whole strawberry plants remains to be seen. It likewise remains to be seen if other shallot aphid populations will exhibit the same resilience to parasitization as seen here.

All predator larvae readily preyed upon shallot aphids on strawberry with lacewing larvae being the most voracious. Although *A. bipunctata* seems promising as a biocontrol agent as judged from its predation capacity and its ability (at least for about half of the females) to lay eggs on shallot aphid infested strawberries further studies are needed to examine its departing tendencies in strawberry as are further studies on realized fertility, development and survival for individuals feeding continuously on shallot aphids. At the same time it would be interesting to further examine the two other predators, especially the green lacewing, to clarify the realization of their predation potential under more realistic conditions.

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Table 1. Overview of experiments.

Experiment	Natural enemy	Unit	Host plant	n	n (controls)
Parasitoid stinging	*A. colemani* *A. ervi*	Petri dish	Strawberry leaf	13 15	
Parasitisation among stung aphids	*A. colemani* *A. ervi*	Petri dish	Strawberry leaf	13 10	5
Parasitisation	*A. abdominalis*	Petri dish	Strawberry leaf	16	5
Parasitisation	*A. abdominalis*	Cage	Strawberry plant	11	10
			Sweet pepper plant	20	13
Predation	*C. carnea*	Petri dish	Strawberry leaf	25	10
Predation	*A. aphidimyza*	Petri dish	Strawberry leaf	23	10
Predation	*A. bipunctata*	Petri dish	Strawberry leaf	25	10
Predation	*A. bipunctata*	Cage	Strawberry plant	12	6
			Sweet pepper plant	14	8
Egg laying	*A. bipunctata*	Petri dish	Strawberry leaf	15	15 for 3 other aphid-plant combinations
Behaviour	*A. bipunctata*	Olfactometer	6 combinations	15	

Table 2. Summary of the average (± s.e.) values for the different biological characteristics examined for the 3 parasitoids (*Aphidius. Colemani, Aphidius ervi, Aphelinus abdominalis*) and 3 predators (*Aphidoletes aphidimyza, Chrysoperla carnea, Adalia bipunctata*) with shallot aphids as host/prey. NS: mortality was not significantly different from the controls.

	A. colemani	*A. ervi*
Number of stung aphids	6.8±2.4	6.1±1.2
Mortality induced in aphids stung	55.3±4.1%	79.0±7.2%
Completed parasitization	7.1±3.1%	2.7±1.8%

	set-up	*A. abdominalis*
Mortality due to host feeding after 24 h exposure	detached leaves	49.6±5.3%
Non-host-fed aphids developing to mummified aphids	detached leaves	23.2±7.3%
Non-host-fed aphids dying from parasitoid-induced causes	detached leaves	38.1±13.2%
Mortality due to host feeding after 24 h exposure	whole plants	sweet pepper: 32.1±5.3% strawberry: 1.2±0.8%
Mortality corrected for mortality in the controls	whole plants	sweet pepper: NS strawberry: NS

	A. aphidimyza	*A. bipunctata*	*C. carnea*
Daily predation rates	13.34±1.45	25.25±3.18	34.62±3.45

	prey-plant	*A. bipunctata*
Daily corrected number eaten, whole plants	shallot aphids-sweet pepper	56.3±1.7
	shallot aphids-strawberry	32.0±6.3
Egg laying in 24 h	shallot aphids-strawberry	8.67±3.08
	peach-potato aphids-strawberry	8.07±2.91
	shallot aphids-sweet pepper	5.93±2.59
	peach-potato aphids-sweet pepper	2.8±1.24

Table 3. Average time (± s.e.) (in sec) over the different odor sources in the six treatment combinations, as well as the test statistics for differences in preference between the two odor sources

	Odor source		Time spent over odor source			
Trial	A	B	A	B	t	P
1	uninfested strawberry	empty container	114.6 ± 22.73	185.4 ± 22.73	-2.23	0.028
2	uninfested sweet pepper	empty container	118.8 ± 15.31	181.2 ± 15.31	-1.97	0.052
3	uninfested strawberry	uninfested sweet pepper	138.2 ± 12.10	161.8 ± 12.10	-0.74	0.458
4	aphid-infested strawberry	empty container	156.3 ± 16.37	143.7 ± 16.37	0.40	0.690
5	aphid-infested sweet pepper	empty container	149.1 ± 14.33	150.9 ± 14.33	-0.06	0.953
6	aphid-infested strawberry	aphid-infested sweet pepper	163.9 ± 11.65	136.1 ± 11.65	0.88	0.384

Appendix 4 Strategipapir for skadedyrsbekæmpelse i jordbær - friland, tunnel eller væksthus

Indledning

Dette strategipapir er udarbejdet i forbindelse med gennemførslen af delprojekt IV i projektet: *"Biologisk bekæmpelse af viklere og bladlus i jordbær"*. GartneriRådgivningen har gennem projektforløbet vurderet eventuelle forandringer i allerede eksisterende skadedyrs betydning og omfang hos en række producenter på Fyn og Sjælland. Dette arbejde samt besvarelser fra spørgeskemaer ligger til grund for hvilke skadedyr, der er medtaget i dette strategipapir.

Formål

Strategipapiret har til formål at sætte fokus på videreudvikling og implementering af biologisk bekæmpelse i jordbær, hvad enten de dyrkes på friland eller under beskyttede forhold som i tunnel eller væksthus. Strategien for den biologiske bekæmpelse varierer alt efter, hvilken dyrkningsmetode, der anvendes.

Strategipapiret begynder med en baggrund og en generel omtale af nuværende og fremtidige muligheder for udnyttelse af funktionel biodiversitet. Dernæst følger en gennemgang af en række skadedyr i jordbær med omtale af den nuværende praksis for skadedyrsbekæmpelse i Danmark. For hvert skadedyr nævnes desuden en række eksisterende og potentielle muligheder for videre implementering af denne bekæmpelsesform i Danmark.

Strategipapiret omhandler følgende skadedyr: Viklere, hindbærsnudebiller, væksthussnudebiller, dværgmider, væksthusspindemider, trips, bladlus, tæger, snegle og mellus. Mindre hyppigt forekommende skadedyr som bladhvepse, uglelarver, gåsebiller, stankelbenslarver og glimmerbøsser vil ikke blive behandlet i dette strategipapir. Større skadedyr som rådyr, harer, mus og fugle vil heller ikke blive omtalt yderligere. Strategipapiret omhandler heller ikke skadevoldende nematoder eller sygdomsfremkaldende mikroorganismer.

Baggrund for strategipapir

Størstedelen af den danske jordbærproduktion sker stadig på traditionel vis i jorden på friland, men i fremtiden vil vi højst sandsynligt få flere danske jordbær dyrket i substrat i tunnel eller væksthus. Forandringen sker, fordi det er blevet vanskeligt at finde nye frilandsarealer tæt på producenten, hvor der ikke tidligere har været dyrket jordbær. Dårligt sædskifte kan føre til øgede problemer med sygdomme og skadedyr, hvilket gør det vanskeligt at dyrke jordbær, hvor de tidligere har været. Dyrkning i spagnum eller kokos forhindrer nogle af de kendte sygdomme for jordbær, men samtidig bør man være opmærksom på risikoen for opformering af skadedyr, når produktionen nu kan ligge det samme sted år efter år. Forebyggelse og god hygiejne er derfor vigtige faktorer i de nye systemer.

Ved implementering af biologisk bekæmpelse er det vigtigt at være opmærksom på en række kulturtekniske forhold for at opnå tilfredsstillende resultater. En af de vigtigste faktorer i forbindelse med anvendelse af biologisk bekæmpelse er klimaet. Alle nyttedyr har behov for specifikke forhold for optimal og succesfuld etablering. Det er især klimaforhold som temperatur og relativ luftfugtighed, som er vigtige. De fleste nyttedyr foretrækker en relativ luftfugtighed på 70 % eller derover. Dette kan dog godt være uønsket eller svært at opnå i en dyrkningssituation.

Brug af insekticider og nogle svampemidler kan medføre høj dødelighed for mange arter af nyttedyr, især hvis disse udsættes lige før eller lige efter en sprøjtning. Nogle insekticider har en meget lang nedbrydningstid, hvilket i nogle tilfælde kan betyde, at der skal gå mindst 8 uger, inden der kan udsættes nyttedyr efter en behandling.

Det bedste resultat af biologisk bekæmpelse opnås ved udsætning af nytteorganismer (snyltehvepse, prædatorer, insektpatogene svampe, nematoder), mens trykket af skadedyr endnu er på et lavt niveau, altså langt inden symptomer på angreb bliver tydelige. For at få en ide om skadedyrstrykket og for at kunne vurdere om introducerede nyttedyr stiger i antal, er det nødvendigt med løbende moniteringer af status i afgrøden.

Visse nyttedyr kan have svært ved at spredes mellem planter, inden de enkelte blade rører ved hinanden. Det er vigtigt at have i tankerne ved udsætning for at opnå en succesfuld behandling.

Fremme af naturlig regulering – funktionel biodiversitet

Fremme af naturlig regulering (engelsk ”conservation biological control”) beskriver tiltag i og omkring marken, hvor naturligt forekommende nytteorganismer fremmes, for eksempel ved såning af blomsterbræmmer eller ved anlæg af billevolde. Bevaring af eller oprettelse af økologiske infrastrukturer på en ejendom er også et væsentligt element. Økologiske infrastrukturer er strukturer eller arealer udenfor dyrkningen, som tjener som levesteder for nytteorganismer. Det kan være for eksempel hegn, diger, haver, grenbunker, gamle træer eller små pletter vilde planter. I IPM-produktion skal 5 % med mål om at nå 10 % af en ejendom bestå at økologiske infrastrukturer (IOBC-WPRS, 2009).

Nuværende praksis

Hele EU’s landbrug skal i 2014 overgå til IPM (Direktiv 2009/128/EU). Økologiske infrastrukturer nævnes som et af tiltagene i direktivet, men hvert medlemsland har frihed til at udvikle egne strategier. En række danske tiltag på IPM-området er derfor i gang. Indenfor funktionel biodiversitet kan nævnes demonstrationsforsøg med blomsterbræmmer, vildtstriber og billevolde i regi af rådgivningstjenesten. Blandt andet blomsterbræmmer kan der opnås støtte til at etablere som tilskud til natur- og miljøprojekter (Ministeriet for Fødevarer, Landbrug og Fiskeri, 2010).

Fremme af naturlig regulering indgår ofte som et element i bredere strategier for at øge landbrugs- og gartneriproduktioners naturindhold og sker ofte i forhold til en hel bedrift snarere end til en enkelt produktion. Som eksempel på tiltag, der fremmer naturlig regulering af multifunktionel karakter, kan nævnes plantning og vedligeholdelse af levende hegn, som også tjener til blandt andet læ omkring marker.

Levende hegn

Det er kendt og påvist, at levende hegn tjener som kilde til nyttedyr for omgivende marker (Navntoft et al., 2009, Sigsgaard et al, 2007). Det er nuværende praksis i ejendomme med jordbærproduktion, at hegn vedligeholdes og nye anlægges. Der savnes videre undersøgelser af betydningen af disse hegn for nytteorganismer i jordbær.

Andre økologiske infrastrukturer

Ejendomme med jordbær har også andre typer økologiske infrastrukturer end hegn, som for eksempel udyrkede pletter, stendiger, skove og vandhuller. Der er ingen tvivl om, at denne praksis fremmer nyttedyr og herved bidrager til at sænke pesticidforbruget i Danmark, men der er behov for videre undersøgelser, som kan bidrage til optimal anlæg og pasning rettet mod at fremme naturlig regulering.

Forestående (< 1 år)

Blomsterbræmmer

Blomsterbræmmer findes allerede i nogle økologiske æbleplantager og kan anlægges ret let og afhængig af valg af plante også for ret lave omkostninger. De er ikke specifikt anlagt med henblik på at fremme naturlig regulering, men ofte med multifunktionelle hensyn herunder hensyn til bestøvere, næringsstofbalance (bælgplanter) og æstetik. Der findes pt. kun ét studie af blomsterbræmmers værdi for naturlig regulering i jordbærproduktion (Sigsgaard et al., *in press*). I æble har blomsterbræmmer vist sig at fremme regulering af viklere i New Zealand (Stephens et al, 1998), mens et canadisk studie viste, at det har betydning, at en blomsterbræmme allerede anlægges ved etablering af afgrøden, da opbygning af nytteorganisme-populationerne tager tid, og det fulde udbytte i form at væsentligt nedsat pesticidpforbrug først blev opnået efter 4-5 år (Bostanian 2004).

Redekasser og insekthoteller
Insektædende fugle kan fremmes ved brug af redekasser. I jordbær skal det sikres at dette ikke fører til skader i frugt. Både redekasser og insekthoteller findes i handelen og kan installeres af interesserede avlere.

Fremtidsperspektiver, der kræver videre forskning (>1 år).
Optimal plantesammensætning af blomsterbræmmer og levende hegn
Undersøgelser til udvælgelse af selektive planter, der fremme nyttedyr men ikke skadedyr, er nødvendige. Blomsterbræmmer af boghvede i jordbær førte til et nedsat antal jordbærviklerlarver nær striben (Sigsgaard et al. *in press*).

Optimal placering af og afstand mellem økologiske infrastrukturer
Det er væsentligt at kende 'aktionsradius' af de nytteorganismer, man ønsker at fremme, da det vil give retningslinier for, hvor stor en mark nyttedyrene kan betjene fra en økologisk infrastruktur, og hvordan økologiske infrastrukturer kan placeres.

Viklere

På friland og i flerårige tunneller findes flere forskellige viklerarter i den danske jordbærproduktion. Jordbærvikleren (*Acleris comariana*) er den mest almindelige. Dens larver kan være et relativt stort problem om foråret og kan føre til betydelige økonomiske tab. Man kan også støde på arter som rød jordbærvikler (*Phiaris locunana*) og skyggevikler (*Cnephasia asseclana*), men da disse oftest forekommer i mindre antal, vil de ikke blive behandlet yderligere.

Ved enårige kulturer i tunnel eller væksthus er der normalt ikke de store problemer med viklerlarver, så længe man søger for at anvende rent plantemateriale ved begyndelsen af hver kultur samt sørger for at opretholde en god hygiejne.

Æg af jordbærvikler på blad, viklerlarve på blad og i blomst, jordbærviklerlarve i sidste larvestadie, puppe af jordbærvikler. (*Acleris comariana*) (Foto: Lene Sigsgaard og Nauja Lisa Jensen).

Biologi
Jordbærvikleren har normalt to generationer per år. Overvintring foregår hovedsageligt som æg, der lægges af anden generations viklere, men de kan dog også overvintre som larver. Æggene bliver lagt på under- eller oversiden af blade. Æggene klækkes over en relativ lang periode i løbet af foråret, hvilket betyder, at der både kan findes små og store larver på samme tid. Inden forpupning gennemgår larverne fem larvestadier. Det er i larvestadierne, at jordbærviklerne gør skade på blade og blomster. Larverne forpupper sig midt i juni, og jordbærviklerens første generation ses

efterfølgende flyvende i tusmørket i juni-juli. Anden generations viklerlarver ses fra slutningen af juli til september, og de voksne viklere flyver i september-oktober for at lægge deres æg (Jensen og Sigsgaard, 2011).

Nuværende praksis

Eksisterende monitering og varsling

Der er ingen kendte skadetærskler, men hvis man vælger at bekæmpe viklerlarverne, er det bedst at behandle, mens larverne endnu kun er få mm lange (de tidligste larvestadier). Det er nemlig nemmere at opnå en rimelig effekt, hvis man rammer dem, inden de spinder sig ind mellem blade eller blomster. Det kan dog være svært at ramme larverne, mens de endnu er helt små, da de her skjuler sig mellem de endnu uudfoldede småblade.

Kemisk bekæmpelse

På friland og i midlertidigt overdækkede tunneler er pyrethroiderne Fastac 50, Karate 2,5 WG og Cyperb 100 godkendt til behandling af jordbærviklere. Når man ikke at få behandlet viklerlarverne inden blomstring, skal man være opmærksom på behandlingsfrister samt bifaremærkning ved behandling med pyrethroider. Der er ingen kemiske midler godkendt til bekæmpelse af jordbærviklere dyrket i væksthus eller under anden permanent overdækning.

Biologisk bekæmpelse

På friland og i tunnel har man om foråret mulighed for at behandle larverne med den insektpatogene bakterie, *Bacillus thuringiensis*. Midlet har især effekt på de yngste larvestadier. Effekten er dog noget klimaafhængig, så det er ikke altid den ønskede effekt opnås. Der er indtil nu ikke ret gode erfaringer med *B. thuringiensis* på friland om foråret, da det ofte er for køligt til optimal effekt. Derimod kan en aftopning efter høst efterfulgt af en sensommersprøjtning være en mulighed for at reducere antallet af overvintrende æg til den kommende sæson. Sprøjt så snart larverne ses. Larverne vil standse fødeoptagelsen i løbet af få timer og dø i løbet af nogle dage. Sprøjt i tørvejr, da regn og kunstig vanding vil skylle midlet af. Vent med sprøjtning til sidst på dagen, da sprøjtning i stærkt solskin bør undgås. Behandlingen kan gentages 3-14 dage senere, hvis flere larver viser sig (Jensen og Sigsgaard, 2011).

Fremtidsperspektiver, der kræver videre forskning (>1 år)

Varsling og monitering

Det er nødvendigt med brugbare skadetærskler for jordbærviklerlarver under danske forhold. Det er ligeledes vigtigt at få beskrevet, hvordan monitering af de enkelte skadegørere skal foregå (Axelsen et al., 2012).

Biologisk bekæmpelse

Udvikling af ny biologisk bekæmpelse ud fra resultater fra dette projekt. I projektet er der fokuseret på to grupper af naturlige fjender, parasitoider og entomopatogene svampe. Den naturlige forekomst af entomopatogene svampe var meget lav. Projektet har dokumenteret høj forekomst af æg-larve parasitoiden, *Copidosoma aretas*. Arten egner sig ikke til laboratorieopdræt. Forsøg med blomsterbræmmer til fremme af funktionel biologisk bekæmpelse af jordbærvikler havde ingen effekt på parasitering, men øget larve-mortalitet indikerer aktivitet af andre nytteorganismer. Lav naturlig forekomst af entomopatogene svampe udelukker ikke, at de kan anvendes til biologisk bekæmpelse.

Hindbærsnudebille

Hindbærsnudebillen (*Anthonomus rubi*) er et relativt stort problem under blomstringen hos en række jordbærproducenter på friland, hvor den kan føre til betydelige økonomiske tab. Der er ingen eksakt skadetærskel, men man vil ofte vælge at bekæmpe hindbærsnudebillen selv ved ret få biller i marken. I erhvervet anbefaler man at plante jordbær så langt væk som muligt fra tidligere angrebne jordbærarealer. Der er indtil nu kun set få tilfælde med angreb af hindbærsnudebillen i væksthus, men den kan komme ind gennem vinduer, så man bør være opmærksom, hvis der er nærtliggende arealer med problemer.

Hindbærsnudebillen (*Anthonomus rubi*) og dens skader efter æglægning (Foto: Nauja Lisa Jensen).

Biologi

Hindbærsnudebillen overvintrer som voksen i plantematerialer tæt ved jordoverfladen. Når foråret kommer, og temperaturen stiger op mod 13-15 °C, bliver billerne aktive, først for at æde, senere for at lægge deres æg. Hunnerne kan lægge omkring 200 æg enkeltvis i lukkede blomsterknopper. Derefter bides blomsterstilkene halvt over for at stoppe udviklingen af knopperne. Æglægningen starter hovedsageligt i maj-juni, når temperaturen kommer over 18 °C. Hindbærsnudebillen ses derfor oftest på relativt varme, solrige dage. Efter 5-7 dage klækkes æggene til små 2-3,5 mm lange hvide larver, som ernærer sig af knopperne. Efter 18-28 dage er larverne udviklede og forpupper sig i knopperne. Senere vandrer de nye hindbærsnudebiller væk fra knopperne og æder af bladene uden at forvolde større skade, hvorefter de går til overvintring. Normalt forventes det, at hindbærsnudebillen overvintrer i læhegn, men norske undersøgelser har påvist, at den også kan overvintre i marken. Mere viden om billernes økologi kan bidrage til at målrette bekæmpelsen.

Nuværende praksis

Vær opmærksom på at undgå handlinger, der fører til opformering og overvintring af hindbærsnudebillerne i marken. Der er for eksempel diskussion om, hvorvidt meget halm mellem jordbærrækkerne vil øge billens overvintringsmuligheder i marken, som i givet fald vil føre til et øget problem det efterfølgende år.

Eksisterende monitering og varsling

Der er ingen kendt skadetærskel for hindbærsnudebillen, men selv få hindbærsnudebiller vurderes at kunne gøre relativt megen skade. I danske forsøg med hindbærsnudebillen har ubehandlede parceller i flere tilfælde medført skader på over 40 % (Forsøg nr. 05705, 1-2).

Kemisk bekæmpelse

På friland kan hindbærsnudebillen bekæmpes med et af de godkendte pyrethroider: Fastac 50, Karate 2,5 WG eller Cyperb 100. Man skal dog være opmærksom på behandlingsfrister samt bifaremærkning ved behandling med pyrethroider under blomstring. Biscaya OD 240 fik i april 2012 en godkendelse til mindre anvendelse i jordbær på friland og i væksthus til bekæmpelse af hindbærsnudebillen i Danmark. Aktivstoffet thiacloprid er ikke farlig over for bier og mere skånsom over for nyttedyr end pyrethroider.

Biologisk bekæmpelse

Der findes endnu ikke nogen tilladte biologiske bekæmpelsesmidler mod hindbærsnudebillen i Danmark.

Nært forestående (< 1 år)

Varsling og monitering

I England er der udviklet feromonfælder til monitering af hindbærsnudebillen. Fælderne er tidligere blevet afprøvet af GartneriRådgivningen og er ikke så effektive, at de er taget i anvendelse i Danmark.

Biologisk bekæmpelse

Der findes pt. ikke nogen godkendt biologisk bekæmpelse for hindbærsnudebillen.

Fremtidsperspektiver, der kræver videre forskning (>1 år)

Varsling og monitering

Københavns Universitet, PLEN (L. Sigsgaard) er partner i et nyt CORE Organic projekt ”Softpest Multitrap” som videreudvikler fælder og fangstmetoder til varsling og potentielt masseudfangning af hindbærsnudebillen og håret engtæge i jordbær, samt hindbærsnudebillen og hindbærbillen i hindbær. Projektet har deltagere fra Norge, Sverige, England, Schweiz og Letland. Projektet har fået tilskud fra ”Grønt Udviklings- og Demonstrationsprogram, (GUDP) under FødevareErhverv”. Undersøgelser påbegyndes i 2012 (se Organic eprints, søg: Softpest multitrap).

Biologisk bekæmpelse

Laboratorietest af insektpatogene svampe til bekæmpelse af voksne hindbærsnudebiller (KU, PLEN) har vist lovende resultater. Det betyder, at en mikrobiologisk bekæmpelse af billerne kunne være en mulighed. Dette forudsætter dog videre undersøgelser under mere realistiske forhold.

Manglen på biologisk bekæmpelse gør, at producenter må ty til sprøjtning med pyrethroider. Dette reducerer muligheden for anvendelsen af biologisk bekæmpelse af en række andre skadedyr, da pyrethroider har en negativ effekt på en stor del af nyttedyrene.

Væksthussnudebillen/øresnudebille

Meget tyder på, at væksthussnudebillen (*Otiorhynchus sulcatus*) vil medføre stigende problemer i fremtiden, hvor dens larver kan forårsage alvorlige problemer for jordbærproducenter på både friland, i tunnel og væksthus.

Det er på nuværende tidspunkt meget svært at komme væksthussnudebillen til livs på friland. Specielt på plastdækkede højbede kan dens larver skabe alvorlige problemer. I tunnel- og væksthusproduktion kan der også forekomme store angreb, men her er der større mulighed for biologisk bekæmpelse. Det kræver dog, at behandlingen bliver foretaget i tide.

Væksthussnudebillen og dens larver (*Otiorhynchus sulcatus*) samt bladklip fra den (Foto: Nauja Lisa Jensen/Bodil Damgaard Petersen).

Biologi

I traditionel frilandsdyrkning vil der normalt kun forekomme en generation af væksthussnudebillen per år. Væksthussnudebillen overvintrer som larve i jorden i 5 til 10 cm dybde, men de voksne biller kan dog også overleve vinteren og fortsætte æglægningen det følgende forår. De vil dog normalt ikke være lige så produktive som de ny biller. Forpupningen af de overvintrende larver foregår i maj-juni måned og varer tre til fire uger. I juni-juli kommer de nye biller frem og begynder at æde af jordbærbladene. Allerede efter ca. en til to uger begynder æglægningen, som varer omkring en måneds tid. Æggene lægges i revner og små sprækker i jorden, og hver enkelt bille kan lægge flere hundrede æg. Klækningen finder sted 1-6 uger senere afhængig af temperaturen. De nyklækkede larver begynder straks at æde af jordbærplantens rødder, først de små tynde, senere tykkere rødder. De graver også boregange i rodstokken og hæmmer derved planterne, som ved svære angreb kan gå ud.

Højbedssystemer på friland med sort plast, samt systemer i tunnel og væksthus kan derimod føre til en forskydning i den normale cyklus, hvilket betyder, at væksthussnudebillen kan nå flere generationer per år. Disse generationer vil være sammenflydende, så der kan findes æg, larver, pupper og voksne, på samme tid. Dette gør bekæmpelsen særdeles vanskelig, og det kan være nødvendigt at behandle gentagne gange over en længere periode.

Nuværende praksis

Eksisterende monitering og varsling

Der er ingen kendte skadetærskler for væksthussnudebillen i jordbær. I første omgang er det vigtigt at se efter en eventuel aktivitet af voksne biller. Dette gøres ved gentagne ugentlige moniteringer. Kig efter "billetklip" i blade eller efter biller, der gemmer sig under strå, ved spagnumsække eller under plastikken om dagen. Hvis planterne begynder at hænge, og de har været angrebet af larver i en længere periode, kan det betyde, at man er for sent ude med en behandling.

Kemisk bekæmpelse

Biscaya OD 240 fik i april 2012 en godkendelse til mindre anvendelse i jordbær på friland og i væksthus til bekæmpelse af bidende og sugende insekter som bl.a. bladlus, hindbærsnudebiller og glimmerbøsser. En sprøjtning med Biscaya OD 240 kan bruges til at bekæmpe de voksne biller, men ikke dens larver. Ved sprøjtning af de voksne øresnudebiller er det vigtigt at være opmærksom på, at sprøjtningerne skal udføres efter solnedgang, da det er et nataktivt dyr.

Biologisk bekæmpelse

Ved dyrkning af jordbær i substrat er det muligt at behandle et angreb ved udvanding af nematoder, men på friland er erfaringerne ikke helt så gode. Der findes forskellige nematoder til bekæmpelse af øresnudebillens larver. Nematoderne udbringes til substratet vha. drypvandingssystemet. *Heterorhabdites spp.*, f.eks. *Heterorhabdites megidis*, virker ved jordtemperaturer på 12-30 °C. Er temperaturen mellem 8-12 °C bør man i stedet forsøge sig med *Steinernema spp.*, f.eks. *S. feltiae* eller *S. kraussei.*

Det er også muligt at iblande substratet en insektpatogen svamp, *Metarhizium anisopliae*, inden plantning – dette middel er nu godkendt til brug i danske jordbær. Svampen angriber larverne i substratet. Det er også muligt at anvende dette biologiske middel på friland, men her foreligger der endnu ikke ret mange erfaringer.

Forestående (< 1 år)

Varsling og monitering:

Generelt må det siges, at selv et lille antal biller vil kunne medføre store skader, så en god monitering er vigtigt med hensyn til dette skadedyr.

Fremtidsperspektiver, der kræver videre forskning (>1 år).

Biologisk bekæmpelse:

Et nyt EU projekt ”Inbiosoil” hvor Københavns Universitet, PLEN er partner (J. Eilenberg, L. Sigsgaard) som starter i 2012 vil fokusere på mikrobiologiske bekæmpelsesmidler mod jordboende skadedyr, herunder væksthussnudebillen.

Dværgmider

Forekomsten af dværgmider (*Phytonemus pallidus*) ser ud til at stige. En af årsagerne hertil er, at man ikke længere har de samme muligheder som tidligere for at behandle småplanterne, inden de videredistribueres til producenterne. Dette øger risikoen for, at dværgmiderne bliver indført i marken med småplanterne. Nye metoder med varmebehandling af småplanterne (CATT) kan forhåbentligt mindske dette problem i fremtiden.

Produktionssystemer med højere temperaturer vil desuden øge antallet af generationer per år, og dette kan medføre større problemer end tidligere. Angrebene kan forekomme på både friland, i tunnel og væksthus.

Dværgmider (*Phytonemus pallidus*) og bladskader efter dem. (Foto: Magnus Gammelgaard).

Biologi

Dværgmider overvintrer som voksne hunner i kronen af jordbærplanterne. Der optræder mindst 4-5 generationer, da udviklingstiden fra klækning til fuldvoksen dværgmide er relativ kort. Den er dog stærkt afhængig af temperaturen. Ved 23-28 °C og høj luftfugtighed varer udviklingen fra æg til voksen kun 10-11 dage. Æglægningen begynder om foråret ved temperaturer over 6-8 °C. Hver hun lægger 35-90 æg, hvoraf langt størstedelen udvikles til hunner. Det betyder, at en plantning hurtigt kan blive stærkt angrebet, hvis arealet først er blevet inficeret med dværgmider. Dværgmiderne gennemgår æg- og nymfepuppestadier, inden de er fuldvoksne og cyklussen gentages. Hunnens levetid er ca. 4 uger.

Nuværende praksis

Eksisterende monitering og varsling

Skader af dværgmider kendetegnes ved de karakteristiske forkrøblede og fortykkede hjerteblade, der ikke folder sig rigtigt ud. I en række sorter kan symptomer på dværgmider desuden kendes ved, at der kommer små, tornede udvækster på blomster- og bladstilke. Det er især karakteristisk i en sort som ‘Honeoye’. Ved alvorlige angreb reduceres udbyttet kraftigt.

Kemisk bekæmpelse

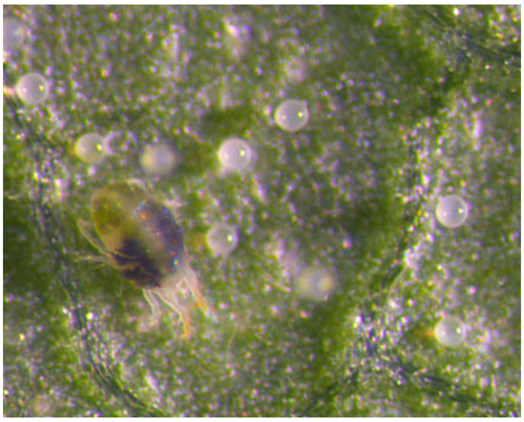
Har man et synligt angreb af dværgmider, særligt på plastdækkede bede, tilrådes det at bekæmpe dværgmiderne kemisk efter høst. I jordbær på friland, tunnel og væksthus kan dværgmiderne efter høst bekæmpes med Milbeknock. På friland opnås den bedste effekt ved at aftoppe jordbærrene og derefter bekæmpe med Milbeknock tilsat et sprede-klæbemiddel som f.eks. Silwet Gold. Man bør dog ikke aftoppe senere end i begyndelsen af august, da dette kan påvirke blomsterinitieringen markant. Danitron er også godkendt til bekæmpelse af dværgmider i jordbær på friland efter høst.

Biologisk bekæmpelse
Man kan også vælge at bekæmpe dværgmiderne med udsætning af tripsrovmider (*Amblyseius cucumeris, A. barkeri*). I nyplantninger kan man forebyggende udsætte 0,5 mio. tripsrovmider pr. ha, mens man i førsteårsmarker forebyggende kan udsætte 1 mio. tripsrovmider pr. ha. Rovmider kan med fordel udsættes ad 2 gange. Ved begrænset angreb på flad mark kan man vælge hurtigst muligt efter konstateret angreb at udsætte 2 mio. tripsrovmider pr. ha.

Rovmiderne kan i princippet godt udsættes før aftopning, idet de skulle kunne kravle tilbage i afgrøden efter aftopningen. Udsættelse af tripsrovmider efter 1. august er mere usikkert, da rovmiderne skal have tid til at opformere sig. Der må ikke have været bekæmpet med pyrethroider i 6 uger, når rovmiderne udsættes, og de bør udsættes på tørre planter, og ikke lige før et stort regnskyl.

Fremtidsperspektiver, der kræver videre forskning (>1 år).
Varsling og monitering:
Problemer med dværgmider vil kunne mindskes, hvis man gennem forskning fik udviklet en test af småplanter, inden de distribueres videre ud til producenterne.

Væksthusspindemider
Forekomsten af væksthusspindemider (*Tetranychus urticae*) har ikke tidligere været så stort et problem under traditionel jordbærproduktion på friland. I længere perioder med varmt og tørt vejr skal man dog være opmærksom på risiko for angreb. Tilsvarende skal man være opmærksom i produktionssystemer med højere temperaturer, da dette øger antallet af generationer per år og det vil føre til større risiko for problemer end tidligere. Væksthusspindemider kan resultere i betydelige angreb og dermed forringelse af kvalitet og udbytte. Derfor skal man være opmærksom på angreb af væksthusspindemider ved produktion på højbed, i tunnel og væksthus, mens frilandsproducenter hovedsagelig skal være opmærksomme i længere perioder med varmt og tørt vejr.



Væksthusspindemide (*Tetranychus urticae*) og dens æg (Foto: Magnus Gammelgaard).

Biologi
Hunnerne (røde) overvintrer på beskyttede steder i jord, under blade og planterester. I løbet af forsommeren søger de tilbage til jordbærplanterne, og æggene lægges på undersiden af bladene. Hunnerne lægger op til omkring 100 glasklare, runde æg. De klækkes under optimale forhold (temperaturer omkring 24 °C) efter 5-6 døgn, og de nyklækkede nymfer går straks i gang med at udsuge plantecellerne. Udviklingen fra æg til formeringsdygtige spindemider tager 2-3 uger i varmt

og tørt vejr. Der kan på friland forekomme 6-7 generationer om året. Fra september begynder de røde vinterformer at optræde igen.

Nuværende praksis

Eksisterende monitering og varsling

Der kan ikke angives nogen eksakt skadetærskel for væksthusspindemider, men det er tilrådeligt at foretage en bekæmpelse, hvis de nemt findes i toppen af planten på de nyere blade. Ved aftopning på friland efter høst vil mange af spindemiderne og deres æg gå til, når de afslåede blade tørrer ind. Derefter skal man holde øje med de nye blade, der vokser frem, hvor man efterfølgende må tage stilling til, om en bekæmpelse er aktuel. I væksthus skal man være ekstra opmærksom, da risikoen for en hurtig opformering her er større end på friland.

Kemisk bekæmpelse

På friland og i midlertidigt overdækkede tunneler er Nissorun 10 WP, Danitron 5 SC, Floramite 240 SC og Milbeknock godkendt til behandling af spindemider. Man skal dog være opmærksom på, at hvert enkelt middel ikke nødvendigvis er egnet til alle spindemidernes stadier. Man skal også være opmærksom på behandlingsfrister samt antallet af tilladte anvendelser per år. Ved produktion af jordbær i væksthus eller under anden permanent overdækning, må man anvende Floramite 240 og Milbeknock SC til bekæmpelse af spindemider.

Biologisk bekæmpelse

I tunneller og på plastdækkede bede er der gode erfaringer med at udsætte spinderovmider (*Phytoseiulus persimilis*). Der bør udsættes 25.000 spinderovmider per ha, gerne ad 2 gange, lidt afhængigt af antallet af spindemider. Resultatet af anvendelsen af rovmider er ofte overbevisende, idet de så at sige rydder helt op for spindemider, således at smittetrykket er lavt det følgende forår. Ved almindelig frilandsdyrkning på flad mark er der ikke helt så gode erfaringer med brugen af spinderovmider, da de kræver lidt højere temperatur for at fungere optimalt.

Udover manuel spredning af spinderovmiderne i jordbærrækkerne findes der metoder til spredning af rovmider mv. i marken, som for eksempel Airbug fra Koppert.

I jordbær i væksthus og tunneller kan væksthusspindemider også kontrolleres med galmyg (*Feltiella acarisuga*), da dens larver har en god appetit for alle stadier af spindemider. Den er aktiv ved temperaturer på 10-30 °C, men de optimale forhold er 20-27 °C. Dette er dog i den høje ende af, hvad der ønskes ved produktion af jordbær. Den kræver tilsvarende også en høj luftfugtighed (RH>80 %) og virker ikke ved en relativ luftfugtighed på under 50-60 %.

Forestående (< 1 år)

Biologisk bekæmpelse

Der forhandles for tiden 2 andre rovmider til bekæmpelse af spindemider i forskellige væksthuskulturer. Den ene art er *Amblyseius californicus*, som udover spindemider også kan leve af pollen og derved overleve i et stykke tid uden spindemider. I udlandet anvendes arten i jordbær i væksthus, tunneler og mark. Den anden art er *A. andersonii* som også menes at have nogen effekt på spindemider. Men den er under normale forhold ikke helt så effektiv som spinderovmiden. De kan dog klare sig i længere tid i kulturen uden føde og kan derfor muligvis anvendes i jordbær i kolde væksthuse og på friland tidligt på foråret, når der ikke længere er risiko for frost.

Trips

Trips kan forekomme i danske jordbær, men det er ikke klarlagt, hvilke tripsarter, der er tale om. I Holland har man blandt andet fundet *Thrips fusicipennis*, nelliketripsen (*T. tabaci*) og den californiske blomstertrips/saintpauliatrips (*Frankliniella occidentalis*). I Sverige har man fundet *F. tenuicornis*. Trips træffes som oftest i de sent blomstrende sorter, under den såkaldte 60-dages produktion af jordbær og i væksthus. Ofte opdager man dem først, når de har suget på de grønne bær, hvilket resulterer i, at bærrene får en kedelig brun overflade. Trips kan forårsage alt lige fra en ubetydelig kvalitetsforringelse til en total ødelæggelse af bærrene. Det er specielt i varmt og tørt vejr, at tripsene opformeres, og at angreb dermed kan blive kraftige.

Frugtkødet rundt om nødderne er brunfarvet. Bærret ligner en lille netmelon. (Foto: Stig F. Nielsen)

Biologi

Trips overvintrer som puppe, men kan også overvintre som voksen på beskyttede steder. Det er dog ikke alle arter, der kan overvintre udendørs. De lægger æg i blade eller blomster. Æggene placeres i små lommer, som tripsene skærer i vævet med deres savtakkede læggebrod. Ægstadiet varer en uges tid og efterfølges af 2 larvestadier og 2 puppestadier. Det er larvestadierne samt de voksne trips, der medfører skader. I slutningen af 2. larvestadie lader de sig falde til jorden for at forpuppe. Dette sker i små sprækker i jorden. Puppestadierne indtager ikke føde. Nogle arter har flere generationer pr. år.

Nuværende praksis

Varsling og monitering

Fangst og monitering kan foregå via opsætning af blå og gule limplader. Kig også efter trips under bægerbladene på blomster og bær. Der er endnu ikke udviklet nogen skadetærskel for trips i Danmark.

Kemisk bekæmpelse

Biscaya OD 240 fik i april 2012 en godkendelse til mindre anvendelse i jordbær på friland og i væksthus til bekæmpelse af bidende og sugende insekter. Biscaya OD 240 har dog oftest kun en delvis effekt mod bekæmpelse af trips.

Biologisk bekæmpelse

Der findes en række nyttedyr, som anvendes til bekæmpelse af trips i forskellige væksthus-kulturer. Af disse kan de nedenstående arter bruges til tripsbekæmpelse i væksthusjordbær og muligvis også i jordbær dyrket i tunneler.

Tripsrovmiden (*Amblyseius cucumeris*) er endnu det mest anvendte nyttedyr til bekæmpelse af trips i jordbær. Der er mange eksempler på gode resultater, men det kræver, at de udsættes rettidigt i de rette mængder. De bør udsættes før blomstring, så man er på forkant med udviklingen. Det er dog vigtigt, at bladene er så store, at de rører ved hinanden, så tripsrovmiden kan bevæge sig rundt mellem planterne. *A. cucumeris* vil kunne benyttes på friland.

Rovmiden *A. swirskii* tager flere forskellige skadedyr, primært mellus, men også trips. De har en markant hurtigere opformeringsrate og kan genfindes i længere tid end den almindelige tripsrovmide (*A. cucumeris*). *A. swirskii* er velegnet i længerevarende kulturer med en lav population af skadedyr. Tilstedeværelse af pollen bedrer rovmidernes mulighed for at opretholde deres bestand. *A. swirskii* kræver en relativ luftfugtighed over 50 % samt temperaturer over 18 °C og vil således ikke kunne anvendes på friland.

Tripsrovtæger *Orius spp*. flytter sig hurtigt rundt i planten og spiser både larver og voksne trips. De er ofte effektive til tripsbekæmpelse i væksthus, men man skal dog være opmærksom på, at de kan være følsomme over for en række parametre som bl.a. sprøjtning og for lav temperatur.

Jordrovmider (*Hypoaspis miles*/*H. aculeifer*) kan i væksthus udbringes ovenpå vækstmediet til bekæmpelse af tripspupper. Hvis jordrovmiderne udbringes sidst på sommeren, kan de være med til at reducere antallet af overvintrende trips til året efter. Der er endnu ikke mange erfaringer med anvendelsen af jordrovmiden i væksthusjordbær eller på friland. De skal helst have en temperatur over 10 °C for at være aktive.

Forestående (< 1 år)

Varsling og monitering

I England findes der en vejledende skadetærskel, som siger, at et lavt antal trips kan tolereres, men er der 2-3 trips per blomst, må en behandling tilrådes.

Biologisk bekæmpelse

Macrocheles robustulus er en stor jordmide med en stor appetit, som bruges til kontrol af trips i udenlandske væksthuse. *M. robustulus* æder tripspupper ligesom *Hypoaspis*-jordrovmiderne og er hurtigere end *H. miles* til at opbygge en stor population. Dette betyder, at den mere effektivt og hurtigere kan være med til at begrænse et tripsangreb. Rovmiden kræver temperaturer over 15 °C for at være aktive.

Bladlus

Der findes mange forskellige bladlusarter i Danmark, men det er ikke klarlagt, hvilke bladlusarter der findes i den danske jordbærproduktion. Bladlus er normalt ikke et problem i jordbær på friland, men i varme forår kan de dog give problemer, specielt ved produktion i tunneler. Bladlus er et stigende problem ved produktion af jordbær i væksthus. Nogle af de arter, der kan være aktuelle er blandt andet: stor jordbærbladlus (*Acyrthosiphon malvae rogersii*), rosenbladlus (*Macrosiphum rosae*), agurkebladlus (*Aphis gossypii*) og stribet kartoffelbladlus *(Macrosiphum euphorbiae*). Med de forventede højere temperatur i Danmark som følge af globale klimaforandringer kan det forventes, at bladlus vil blive et stigende problem i frilandsdyrkningen af jordbær, herunder bl.a. løgbladlusen (*Myzus ascalonicus*).

Biologi

Biologien afhænger lidt af, hvilken art det drejer sig om. Men generelt overvintrer de fleste bladlusarter som æg. I milde vintre eller under beskyttede forhold kan de dog også overvintre som voksne bladlus. I foråret flyver de vingede hunner fra vinteropholdsstedet til værtsplanten, hvor de føder uvingede unger. Er vejret varmt og tørt, kan de i løbet af ganske kort tid føde en mængde levende unger ved jomfrufødsel og danne en lille koloni. Så længe der er plads og nok med mad, vil afkommet være uden vinger. I løbet af en til to uger vil bladlusungerne være voksne og i stand til selv at formere sig. Typisk føder en bladlus 40-80 unger i løbet af 3-4 uger. Bladlusene kommer derfor hurtig til at sidde tæt, og der opstår fødemangel. Derefter kan bladlusene føde vingede bladlus, der kan opsøge nye afgrødeplanter eller vinterværter.

Nuværende praksis

Varsling og monitering

Bladlus er normalt ikke et problem i jordbær på friland med mindre, at der er tale om et varmt og tørt forår. En generation udvikler sig i løbet af 10 dage ved 15-20 °C. Lave temperaturer sinker udviklingen. Stærk regn kan dræbe mange bladlus. Kraftig vind og meget høje temperaturer hæmmer bladlusene. Medmindre forholdene for opformering er gode eller smittetrykket er meget højt, kan man godt tillade nogle bladlus i arealet. Men vi har ikke nogen præcis skadetærskel. I væksthusproduktionen bør man være mere på vagt og tjekke status allerede fra begyndelsen af vækstperioden.

Kemisk bekæmpelse
På friland, i midlertidigt og permanent overdækkede tunneler samt i væksthus er Pirimor G godkendt til bekæmpelse af bladlus. Det må anvendes max 1 gang pr sæson på friland og max to i væksthus. Biscaya OD 240 fik i april 2012 en godkendelse til mindre anvendelse i jordbær på friland og i væksthus til bekæmpelse af bidende og sugende insekter som bl.a. bladlus. Desuden er følgende pyrethroider: Karate 2,5 WG og Cyperb 100 godkendt til bekæmpelse af bladlus i jordbær på friland og i midlertidigt overdækkede tunneler.

Biologisk bekæmpelse
Der findes en række bladlusfjender, som anvendes til biologisk bekæmpelse af bladlus i væksthusjordbær. Anvendelse i tunneler og friland savner undersøgelser. Valg af nyttedyr vil komme an på, hvilken bladlusart, der er tale om.

Aphidius colemani, *Aphidius ervi*, *Aphidius matricariae*, *Aphelinus abdominalis* og *Praon volucre* er alle eksempler på snyltehvepse, der anvendes til bekæmpelse af bladlus i væksthus. Voksne snyltehvepse lever af nektar fra blomster og honningdug, mens larverne lever som snyltere i bladlus. Hunnen anbringer æggene i offeret ved hjælp af sin læggebrod. Snyltehvepse vil normalt skulle udsættes forebyggende for at opnå en tilfredsstillende effekt. Snyltehvepse til brug mod bladlus i jordbær i hus sælges enkeltvis eller som blandinger af forskellige snyltehvepsearter.

Guldøjelarver (*Chrysoperla carnea*) er specifikke bladlusprædatorer, som bl.a. ernærer sig af bladlus. Under opvæksten æder larverne flere hundrede bladlus. De er meget mobile og kravler let fra plante til plante, hvis bladene rører ved hinanden. I væksthuskulturer kan guldøjelarver udsættes til bekæmpelse af bladlus. De voksne guldøjer formerer sig ikke i kulturen, så der er ikke nogen langtidseffekt af guldøjelarverne, ud over den periode larver udsættes i. Optimale forhold for guldøjelarver er 22-25 °C. Hvis temperaturen er under 10 °C, har de ingen aktivitet.
Bladlusgalmyg (*Aphidoletes aphidimyza*) flyver om aftenen og natten, hvor de tiltrækkes af bladlusenes honningdug. De lægger deres små, orange æg tæt ved bladlusene, og æggene klækkes i løbet af tre til fire dage. Larverne begynder straks at suge væsken ud af bladlusene. Galmyglarverne æder alle stadier. Under gunstige forhold (20-30 °C, samt over 16 timers dagslys per døgn) kan bladlusgalmyg opformeres efter udsætning, men de er meget følsomme over for såvel insekt-, mide- som svampemidler.

Den toplettede mariehøne *Adalia bipunctata* anvendes til bekæmpelse af koncentrerede angreb af bladlus. En voksen mariehøne lægger 20-50 æg per dag, hvorefter larverne klækkes efter 4-8 dage. Derefter går de straks i gang med at æde bladlus. Både larver og voksne er grådige rovdyr. Larverne lever normalt 20 dage, men i løbet af 4. larvestadie indtager de ikke føde.

<u>Fremtidsperspektiver, der kræver videre forskning (>1 år)</u>.
Biologisk bekæmpelse:
På friland kan klimaforandringer betyde større problemer med bladlus.

Tæger
På friland og i tunneller findes der flere forskellige arter af tæger i den danske jordbærproduktion. Jordbærtægen (*Plagiognathus arbustorum)* er en af de mest almindelige og dens nymfer kan resultere i problemer hos jordbærproducenter på friland og i flerårige tunnelkulturer, hvor den kan føre til betydelige økonomiske tab. Håret engtæge (*Lygus rugulipennis*) er en anden almindelig blomstertæge, hvis nymfer tilsvarende kan resultere i skader.

Ved enårige kulturer i tunnel eller væksthus er der normalt ikke de store problemer med de skadelige arter af tæger, så længe man sørger for at anvende rent plantemateriale ved begyndelsen af hver kultur samt opretholder en god hygiejne.

Nymfe af håret engtæge (*Lygus rugulipennis*) (Foto: Magnus Gammelgaard).

Biologi
Jordbærtægen lægger sine æg om efteråret på den nedre del af bladstilken. Efter overvintring klækkes æggene på omkring samme tidspunkt som begyndende blomstring. Tægenymfer lever på blomster og grønne bær, og det er på dette stadie, at tægerne laver skader på jordbær. Når jordbærhøsten begynder, er nymferne udvoksede og søger andre steder hen.

Håret engtæge overvinter i modsætning til jordbærtægen som voksen. Om vinteren gemmer de voksne tæger sig under barkflager i krat og læhegn. Om foråret flyver engtægerne ud fra deres vinterophold for at opsøge egnede værtplanter. Ofte falder dette sammen med jordbærrenes blomstringsperiode, tilsvarende jordbærtægen. Der forekommer 2 generationer på friland. Derfor er det især de sene jordbær, som netop denne tægeart giver størst skade i.

Nuværende praksis

Varsling og monitering
De kraftigste angreb ses ofte nærmest hegn eller andet læ, så omkring begyndende blomstring vil det være oplagt at begynde moniteringen på lokaliteter tæt på hegn. Kig efter tægenymferne i blomster og på grønne bær. Nymferne kan minde lidt om bladlus, men de er meget mere livlige.

Kemisk bekæmpelse
Der findes p.t. ingen godkendte sprøjtemidler til bekæmpelse af tæger og tægenymfer hverken til frilands- eller væksthusjordbær.

Biologisk bekæmpelse
Der findes på nuværende tidspunkt ikke noget biologisk middel mod skadelige tæger.

Forestående (< 1 år)

Varsling og monitering
I England er der udviklet feromonfælder til varsling for håret engtæge. Det kunne være interessant at afprøve disse under danske forhold. Anbefalingerne lyder på 1 fælde per ha med en ugentlig opgørelse for fangsten i fælderne.

Mekanisk bekæmpelse

I England har der været udført forsøg med mekanisk 'opsugning' af tægenymferne, hvilket har haft en positiv effekt ved meget høje angreb. Bemærk, at der ses meget voldsomme angreb i sene kulturer i England.

Fremtidsperspektiver, der kræver videre forskning (>1 år).

Varsling og monitering

Håret engtæge vil indgå i nye undersøgelser af muligheder for varsling og masseudfangning (sammen med hindbærsnudebillen) projektet "Softpest Multitrap" (se ovenfor under hindbærsnudebillen).

Biologisk bekæmpelse

Foreløbige engelske undersøgelser har vist, at guldøjer og *Orius*-rovtæger kan æde engtægens nymfer. Hvorvidt disse prædatorer vil kunne anvendes i praksis er uafklaret.

Snegle

Snegle kan give problemer og økonomiske tab i produktion af jordbær på friland. Der findes flere arter, bl.a. *Deroceras reticulatum, D. agreste* (agersnegle), *Arion hortensis* (nøgen havesnegl) og *Arion lusitanicus* (iberisk skovsnegl).

Biologi

I de fleste tilfælde overvintrer sneglene som æg, men i milde vintre, kan de sagtens overvintre som voksne snegle, hvis de søger skjul i levende hegn. Æggene klækkes efter 3-4 uger afhængig af jordtemteraturen, og i løbet af et par måneder er de nye individer klar til selv at parre sig og lægge æg. Snegle er hermafroditter og har under gunstige betingelser (fugtigt og mildt vejr) en stor opformeringshastighed. Under tørre perioder er de i stand til at gemme sig i fordybninger og huller i jorden i lange perioder for så igen at komme til syne, når fugtigheden er optimal. Agersneglene lægger 400-500 glasklare æg, som gemmes i fordybninger i jorden eller under jordknolde.

Nuværende praksis

Varsling og monitering

Sneglene holder til på fugtige steder og er især aktive om natten eller i fugtigt vejr. Sneglene vandrer ofte ind i marken fra hegn og rabatter, hvor der er fugtige forhold. Angreb ses derfor typisk i randen af marken. Svære og knoldede jorde eller marker med ukrudt og halm er bedst for sneglene, fordi der her er gode betingelser for at gemme sig og lægge æg.

Kemisk bekæmpelse

Snegle kan bekæmpes ved at lægge sneglekorn ud, før der lægges halm mellem rækkerne. Der kan vælges mellem Ferramol, SmartBayt Professionel eller Ferrox, der alle består af samme aktivstof. Når sneglene har ædt sneglekorn ophører de med at indtage føde. De vil derefter søge i skjul og dø efter kort tid. Normalt vil der ikke findes døde snegle på arealet, så effekten ses bedst ved, at angrebet ophører.

Biologisk bekæmpelse

Snegle uden hus, dvs. agersnegle mm., kan behandles ved udbringning af nematoder. Nematoderne er naturligt forekommende i jorden, men med en ekstra dosering opnås en bedre bekæmpelse af sneglene. De kan også have nogen effekt på snegle med hus, men da snegle med hus oftest befinder sig på planterne, bekæmpes de ikke i større omfang.

Mellus

Der findes omkring 10 forskellige mellusarter i Danmark. Det er dog ikke klarlagt, hvilke af disse arter, der findes i den danske jordbærproduktion, men jordbærmellus (*Aleyrodes lonicerae*) og væksthusmellus (*Trialeurodes vaporariorum*) er i hvert fald to af de vigtigste. Der er endnu ikke set betydende problemer med mellusangreb ved produktion af jordbær i Danmark. På friland kan de ses i et mindre antal, men det har endnu ikke givet problemer. I andre lande skaber mellus specielt problemer i væksthus- og tunnelproduktion, så derfor bør man i Danmark også være opmærksom på risikoen for angreb i sådanne systemer. Specielt hvis produktionen ligger i et område med højt smittetryk.

Mellus (*Aleyrodes lonicerae*) (Foto: Magnus Gammelgaard).

Biologi

Væksthusmellusen er den mest almindelige. Den kan have adskillige generationer på en vækstsæson. De voksne mellus lægger små, opretstående, slanke lyse æg på bladundersiden. Efter 3-4 døgn farves æggene helt sorte. Klækketiden tager omkring 7- 10 døgn (ved 20 °C), hvorefter der fremkommer nogle små, flade, lyse nymfer. De nyklækkede nymfer vandrer i løbet af det første døgn rundt for at finde en god plads på bladundersiden, hvorefter munddelene plantes dybt i plantevævet. Herefter forsvinder benene, og nymfen er ikke længere i stand til at flytte sig. Efter 3-4 hudskifter afsluttes udviklingen med et puppelignende stadie, hvor nymfen forvandles til voksent insekt. Ved en gennemsnitstemperatur på 20 °C forløber hele udviklingen på ca. en måned.

<u>Nuværende praksis</u>

Varsling og monitering

Nyudviklede voksne mellus søger til unge blade for her at parre sig og lægge æg, hvilket medfører, at man normalt altid vil finde voksne mellus i toppen, mens pupper og nymfer kan findes på de nederste blade, da de en stor del af deres udviklingstid er fastsiddende på samme sted.

Mellus kan hovedsageligt give problemer, hvis der er en afgrøde i et nærtliggende væksthus, hvor der allerede er et etableret angreb. I sådanne tilfælde er det vigtigt at holde afgrøden ren og undgå ukrudt. Man bør også være opmærksom på vinduer samt vindretning. Monitering kan ske ved opsætning af gule limplader.

Kemisk bekæmpelse

Der findes p.t. ingen godkendte midler til bekæmpelse af mellus på hverken friland, tunnel eller væksthus.

Biologisk bekæmpelse

Der findes en række nyttedyr, som anvendes til bekæmpelse af mellus i forskellige væksthuskulturer. Af disse kan de nedenstående arter bruges til mellusbekæmpelse i væksthusjordbær og muligvis også i jordbær dyrket i tunneler. Grundet nyttedyrenes høje temperaturkrav er det ikke forventeligt, at de vil kunne anvendes i frilandsjordbær.

Snyltehvepsen *Encarsia formosa* er et af de vigtigste nyttedyr mod mellus. Snyltehvepsen parasiterer mellusens larvestadier, men dræber også nymfer ved at bruge dem som næringskilde. *E. formosa* er aktiv ved 16-30 °C, med et optimum omkring 20-25 °C og en relativ luftfugtighed på 50-85 %. Encarsia-snyltehvepse er meget følsomme over for rester af kemi og svovl.

Rovmiden *Amblyseius swirskii* er udover trips også glad for mellus. *A. swirskii* er velegnet i længerevarende kulturer med en lav population af skadedyr. Tilstedeværelse af pollen bedrer rovmidernes mulighed for at opretholde deres bestand. *A. swirskii* kræver en relativ luftfugtighed over 50 % samt temperaturer over 18 °C.

<u>Forestående (< 1 år)</u>
De overstående nyttedyr er de mest anvendte, men der findes andre nyttedyr som anvendes til bekæmpelse af mellus i væksthus jordbærproduktion i andre lande, men de vil ikke blive behandlet her.

Konklusion

Der er gode muligheder for at anvende biologisk bekæmpelse i jordbærproduktionen i Danmark. Specielt i væksthus er der efterhånden en del muligheder. Nolge af disse muligheder kan også tilpasses til brug i tunneler. Erfaringerne bliver mindre, når det drejer sig som om biologisk bekæmpelse på friland. Men med ekstra fokus på IPM fremover vil der forhåbentligt også komme ekstra fokus her. Man skal dog være opmærksom på de individuelle krav, der er ved anvendelsen af specifikke nyttedyr.

Udvikling af moniteringsværktøjer samt danske skadetærsker for skadedyr indenfor de forskellige dyrkningssystemer er nødvendig for at styrke anvendelsen af biologisk bekæmpelse og dermed bidrage til nedsættelsen af pesticidforbruget. Udvikling af populationsdynamiske modeller vil yderligere kunne forbedre mulighederne for optimal rådgivning indenfor området.

Sidst men ikke mindst er det vigtigt at få dokumentation for allerede eksisterende fornemmelser af forskelligheder mellem de enkelte jordbærsorters modtagelighed over for skadedyrene.

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En hjemmeside om skade- og nyttedyr

www.plante-doktor.dk

Forskning i økologisk jordbrug

Organic eprints: www.orgprints.org

Tabel 1: Oversigt over nuværende og forestående (< 1 år) muligheder inden for monitering samt kemisk og biologisk bekæmpelse på friland, i tunnel og væksthus.

Skadedyr	Friland					Midlertidig tunnel					Permanent tunnel og væksthus				
	Nuværende			Forestående		Nuværende			Forestående		Nuværende			Forestående	
	Moni.	Kemi	Bio.	Moni.	Bio.	Moni.	Kemi	Bio.	Moni.	Bio.	Moni.	Kemi	Bio.	Moni.	Bio.
Viklere		x	x				x	x					x		
Hindbærsnudebille		x		x			x		x						x
Væksthussnudebille		(x)	(x)				(x)	(x)				(x)	x		
Dværgmide		x	x				x	x				x	x		
Væksthusspindemide		x	x				x	x				x	x		
Trips	x	(x)	x			x	(x)	x			x	(x)	x		
Bladlus		x	(x)				x	x				x	x		
Tæger				x					x					x	(x)
Mellus	x					x		(x)			x		x		
Snegle		x	(x)				x	(x)					x		

Biological control of tortricids and aphids in strawberries

Cropping practice and biological control can contribute to reduced pesticide use in strawberries. Organic strawberries are less attacked by strawberry tortricid and buckwheat flower strips can augment its natural enemies. Against shallot aphid the two-spot ladybird is promising.

Dyrkningpraksis og biologisk bekæmpelse kan bidrage til nedsat pesticidforbrug i jordbær. Økologiske jordbær angribes mindre af jordbærvikleren og blomsterbræmmer med boghvede kan fremme dens naturlige fjender. Mod løgbladlus er toplettet mariehøne lovende.

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