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Biological aspects for forecasting of the cabbage stem flea beetle, *Psylliodes chrysocephala* L.

PhD thesis

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Title: Biological aspects for forecasting of the cabbage stem flea beetle, *Psylliodes chrysocephala* L.

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PREFEACE

This industrial PhD thesis is carried out as collaboration between SEGES and University of Copenhagen. The thesis has been submitted to The Faculty of Science, University of Copenhagen within the PhD study programme of Science. The research was carried out under the supervision of Professor Peter Esbjerg, Department of Plant and Environmental Sciences and Department and Head of Department Jens Bligaard, Crop & Environment, SEGES.

The thesis consists of an introduction which describes the background, objective and relevant biological information, three introductory chapters which provide an overview of the research topics and are based on existing knowledge from the literature as well as results from the present thesis. As part of this thesis, three manuscripts have been prepared for publication, two for publication in scientific journals and one conference paper published in the IOBC-WPRS Bulletin, 2014. Additionally, three publications in Danish on the cabbage stem flea beetle, aimed at the Agricultural Advisory Service have been prepared.

During the project I have presented experimental results from the project as a poster and one oral presentation at two International conferences, the 2011 and 2013 IOBC ICOC group meetings in the working group “Integrated control in oilseed crops”. The experimental work was carried out at University of Copenhagen, the experimental farm in Taastrup north of Copenhagen and the laboratory at Frederiksberg Campus, Copenhagen.

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I have participated in annual excursions/advisory meetings on plant production arranged by local advisory centres and thank all organisers and participants for having me along and telling about my work. Special thanks to Finn Olsen at Sønderjysk Landboforening for his interest, updates and an invitation to a winter oilseed rape field walk, Tine Lund, Kolding Herreds Landboforening and Benny L. Petersen, Gefion for field registrations.

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1. SUMMARY (ENGLISH & DANISH)

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae), is a serious pest in winter oilseed rape (WOSR) *Brassica napus* L. with variation in abundance and damage between years. The adult beetles invade fields at the time of crop emergence and cause a mostly minor damage by feeding on the leaves of young plants. The main damage is caused by the larvae mining the petioles and later stems from the autumn to following spring.

Forecasting is widely based on monitoring the activity density of the adult beetles with yellow water traps in the main period of field invasion. Uncertainties are attached to this forecasting as it is based on monitoring of a non-damaging stage. Further, there is not always a direct correlation between trap catches and subsequent larval density since temperature influences the number of eggs laid as well as the number of eggs hatching in the autumn.

The focus of this project is the problematic aspect of the existing monitoring of the activity density as the only basis of forecasting and the variation in abundance between years. The main focus has been on the influence of temperature on five parameters of reproduction (the preoviposition and oviposition period, the total and daily egg-laying capacity and female longevity), egg development and larval survival at low temperature. Tests were carried out in the laboratory. Reproduction was studied at five constant temperatures 4, 8, 12, 16 and 20˚. Larval survival was studied at -5 and -10˚C and the influence of cold acclimation and larval stage tested. As part of the project, an assessment of the initial plant injury was carried out and evaluated as an alternative monitoring method to estimate CSFB abundance in the field. This was tested in a field cage experiment with densities of 1, 2, 4 and 8 pairs of beetles at a plant density of 24.

The assessment of the initial plant injury showed a low level of infestation in terms of both plant injury and larval density per plant, and this monitoring not to be reliable indicator of beetle density. There was not a significant correlation between beetle density and the initial plant injury, assessed as number of damaged plants or “feeding holes”. There was a significant but very small effect of beetle density on number of damaged leaves and a significant effect on larval density per plant.

Temperature had an effect on the preoviposition period, the total and daily egg-laying capacity and female longevity, development and hatching rate of eggs as well as larval survival at low temperature. The temperature of maximum egg-laying capacity was found to be 16˚C. At 16˚C, the mean preoviposition period was 19 days, the estimated total egg-laying capacity and daily egg-laying rate 696 and 5 eggs, respectively and the estimated 50% survival time 186 days. The development time of eggs ranged from 12 days at 20˚C to 163 days at 4˚C and the hatching rate of eggs was roughly 70% at all temperatures except at 4˚C. At 4˚, approximately 50% of the eggs

developed into larvae. The results gave a requirement of 185 degrees-days (DD) above a developmental threshold of 5.1˚C to complete egg development.

Temperature, time of exposure, cold acclimation and larval stage had an effect on larval survival. The larvae were capable of surviving at -5˚C relatively long, whereas exposure to -10˚C was much more harmful. Cold acclimation increased cold tolerance of the larvae. Estimated time until 50% (LT_{50}) of acclimated and non-acclimated larvae had died at -5˚C was 9.6 and 7.4 days, respectively. Estimated LT_{50} of acclimated and non-acclimated larvae at -10˚C was 70.5 and 32.6 hours, respectively. Second instar larvae were more cold resistant when exposed to -5˚C, whereas first and second instar larvae were equally resistant when exposed to -10˚C. Weather data from 1990 to 2013 at two locations in Denmark showed that a sufficient number of continuous days with temperatures causing a high larval mortality rarely occur.

Based on the existing monitoring with yellow water traps, the time of field invasion can be estimated. With the new results and monitoring of temperature, it is possible to estimate the start and intensity of egg-laying, larval appearance in the field and larval mortality during winter. This can contribute to an improved basis of forecasting and decision support in pest management.

Rapsjordloppen, *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae), er et alvorligt skadedyr i vinterraps *Brassica napus* L. med stor variation i forekomst og angreb mellem år. De voksne biller kommer ind i marken omkring fremspiring af rapsplanterne og forårsager en oftest ubetydelig skade ved at æde af planterne. Den hovedsagelige skade forårsages af larverne, der minerer bladstilke og senere stængler fra efteråret til efterfølgende forår.

Varsling er udbredt baseret på monitering over tid af de voksne biller med gule fangbakker i hovedflyvningsperioden. Denne varsling er usikker, da den ikke bygger på monitering af det skadevoldende insektstadium og der er ikke altid en direkte sammenhæng mellem fangster og antallet af larver per plante senere i efteråret. Høje fældefangster betyder ikke nødvendigvis en høj larvedensitet, da temperaturen efter indflyvning i marken både påvirker mængden af æg, der lægges samt antallet af æg, der klækker i efteråret.

Det overordnede fokus for dette projekt er problematikken omkring monitering med gule fangbakker og en varsel baseret udelukkende på denne samt variationen i forekomst mellem år. Hovedfokus har været på temperaturforholds indvirkning på fem vigtige reproduktionsparametre (præovipositions- og ovipositionsperioden, det totale antal æg, den daglige æglægningsrate og hunnernes levetid), æg udvikling og larvernes overlevelse ved lave temperaturer. Forsøg blev udført i laboratoriet. Reproduktion blev undersøgt ved fem konstante temperaturer 4, 8, 12, 16 og 20˚C. Larvernes overlevelsesevne blev undersøgt ved -5 og -10˚C og betydningen af kulde akklimatisering og larvestadium undersøgt. Som en del af projektet, blev opgørelser af den tidlige bladskade foretaget og vurderet som en alternativ moniteringsmetode til at bestemme forekomsten af rapsjordlopper i marken. Dette blev undersøgt i burforsøg i marken med forskellige densiteter af biller, 1, 2, 4 og 8 par ved en plantedensitet på 24 planter.

Opgørelse af den tidlige planteskade viste en lav angrebsgrad, hvad angår både bladgnav og antallet af larver per plante, og monitering af bladgnav uegnet som indikator for antallet af biller. Der var ikke en signifikant sammenhæng mellem antallet af biller og den tidlige planteskade, opgjort som antallet af planter med bladgnav eller antallet af bladgnav. Der var en signifikant men meget lille effekt af antallet af biller på antallet af blade med bladgnav og en signifikant effekt på antallet af larver per plante.

Temperaturen havde en effekt på præovipositions-perioden, den totale og daglige æglægningskapacitet og hunnernes levetid, udviklingstiden og klækningsraten for æg samt larvernes overlevelsesevne ved lave temperaturer. Den maksimale æglægningskapacitet blev fundet ved 16˚C. Ved denne temperatur var præovipositions-perioden gennemsnitlig 19 dage, den estimerede totale æglægningskapacitet og daglige æglægningsrate henholdsvis 696 og 5 og den estimerede levetid for 50% af hunnerne 186 dage. Udviklingstiden for æg gik fra 12 dage ved 20˚ til 163 dage ved 4˚, og klækningsprocenten for æg lå omkring 70 % ved alle temperaturer på nær 4˚C, hvor kun ca. halvdelen af æggene udviklede sig til larver. En beregning ud fra resultaterne gav et varmesumskrav på 185 daggrader (DD) over 5,1˚C for udviklingen fra æg til larve. Både

temperatur, eksponeringstid, kulde akklimatisering og larvestadium påvirkede larvernes overlevelsesevne. Larverne var i stand til at overleve længere tid ved -5˚, hvorimod eksponering for -10˚C var betydelig mere skadelig. Kulde akklimatisering forøgede larvernes kulde tolerance og betød en højere overlevelse for akklimatiserede larver. Tiden indtil 50% (LT_{50}) af henholdsvis akklimatiserede og ikke-akklimatiserede larver forventes døde ved -5˚C blev estimeret til 9.6 og 7.4 dage. LT_{50} for henholdsvis akklimatiserede og ikke-akklimatiserede larver ved -10˚C blev estimeret til 70.5 og 32.6 timer. Første larvestadium var mere følsomt overfor eksponering for -5˚C, hvorimod første og andet larvestadium var lige følsomme overfor -10˚C. Vejrdata fra to områder i Danmark fra 1990 til 2013 viste, at et tilstrækkeligt antal sammenhængende dage, med temperaturer til at forårsage høj larvedødelighed, sjældent forekommer.

Med udgangspunkt i den eksisterende moniteringspraksis med gule fangbakker kan indflyvningstidspunktet i marken bestemmes. Med den nye viden om rapsjordloppens æglægning og kulde tolerance og ud fra et kendskab til temperaturen, er det muligt at bestemme starttidspunktet og intensiteten for æglægning, tidspunktet for larvernes fremkomst i marken og vinterdødeligheden for larver. Dette kan bidrage til et forbedret grundlag for varsling og beslutning omkring skadedyrshåndtering.

2. LIST OF PUBLICATIONS

This PhD consists of three manuscripts, one accepted and one prepared for publication in scientific journals and one published conference paper. Communications in Danish have been prepared for the Danish Agricultural Advisory Service. Prior to the manuscripts, four introductory chapters provide an introduction to the background, key aspects, project objectives and relevant biological information (chapter 3) and an overview of the research topics (chapter 4, 5 & 6). The presentation of the research topics is based on existing knowledge from the literature and results from this project. The experiments are described and results presented in details in manuscript I – III.

Manuscript I. Mathiasen, H., Esbjerg, P. & Bligaard, J. (2014): Early plant injury as an indicator of infestation level of the cabbage stem flea beetle? *IOBC/WPRS Bulletin; 2014.*

Manuscript II. Mathiasen, H., Esbjerg, P. & Bligaard, J. (2014): Effect of temperature on reproduction and embryonic development of the cabbage stem flea beetle, *Psylliodes chrysocephala* L.

Accepted for publication in *Journal of Applied Entomology*

Manuscript III. Mathiasen, H., Esbjerg, P. & Bligaard, J. Survival of cabbage stem flea beetle larvae, *Psylliodes chrysocephala* L., exposed to low temperatures

To be submitted to *Entomologia Experimentalis et Applicata*

Communication letters to be uploaded to SEGES, www.Landbrugsinfo.dk

Mathiasen H. (2015): Ny viden om rapsjordloppens æglægningsforløb kan forbedre varsling

Mathiasen H. (2015): Hvordan klarer rapsjordloppens larver lave vinter temperaturer

Mathiasen H. (2015): Monitering af rapsjordloppen; kan opgørelse af den tidlige bladskade sige noget om antallet af biller i marken

3. INTRODUCTION

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae), has a wide distribution in Europe, Northern Africa, the Middle East, Asia and North America (Bonnemaison 1965; Nadein 2010). The CSFB is a specialist herbivore of overwintering brassica crops and occurs as a pest in central and northern European countries in winter oilseed rape (WOSR), *Brassica rapa* L. (Bromand 1990; Alford et al. 2003). In Denmark, the CSFB occurred in former times as a pest in turnip grown for seed production (Thomsen and Bovien 1933). With a more or less complete shift in production from spring to winter oilseed rape in 1990, the CSFB increased in abundance and gained status as a pest. Attacks occurred first in southern Jutland, spread and became widespread from 2001 (Nielsen 1994). Before 1990 only spring oilseed rape was grown and the CSFB do not move into these fields even if they are located next to a WOSR field (Bonnemaison and Jourdheuil 1954).

The initial, usually minor, damage is caused by the adults feeding on leaves of the emerging crop. The subsequent and main damage is caused by larval offspring mining the leaf petioles and stem of plants from the autumn until following spring. The crop damage increases winter mortality of plants and reduces or impairs growth in spring (Winfield 1992).

The widespread management strategy consists of an initial monitoring of the leaf damage from the time of crop emergence. This monitoring is targeted control of adult beetles. If numerous, the beetles can cause lethal damage to plants by their feeding on young leaves and the threshold of leaf damage is related to plant tolerance. Due to the importance of larval damage, monitoring of the activity density of adult beetles with yellow water traps is carried out during the period of field migration (Pouzet and Ballanger 1984; Hossfeld 1993; Nielsen 1994). A problematic aspect of key importance is that forecasting of the CSFB is based on monitoring of a non-damaging insect stage, and that time between monitoring and the main damage is several months. In this period, important biological events of reproduction, development and winter survival take place which cannot be ignored in forecasting and risk assessment of the potential damage.

Chemical control relies on synthetic pyrethroid insecticides. Treatment against adults is carried out when the threshold of leaf damage is reached. Treatment against larvae is recommended when the given threshold of water trap catches is reached and in autumn (Purvis 1986). The pyrethroids are often applied prophylactically or unnecessarily (Ellis et al. 2009; Williams 2010). Another pest of oilseed rape, the pollen beetle, *Meligethes aeneus*, has developed widespread resistance to pyrethroids and recently resistance has been found in CSFB in Northern Germany (Zimmer et al. 2014).

These aspects put a pressure on the need for a better basis for decision support in pest management.

3.1. PROJECT AIM AND KEY ASPECTS

Overall, this project aims to define and study key aspects of the CSFB life cycle in the context of forecasting and pest management in WOSR production (figure 1). The problematic aspect of the existing monitoring with yellow water traps as the only basis of forecasting has been the starting point. The main focus has been on temperature effects on parameters of reproduction and winter mortality. Knowledge on these can improve the existing forecasting in terms of risk assessment and pest management and add to the understanding of the variation in abundance between years.

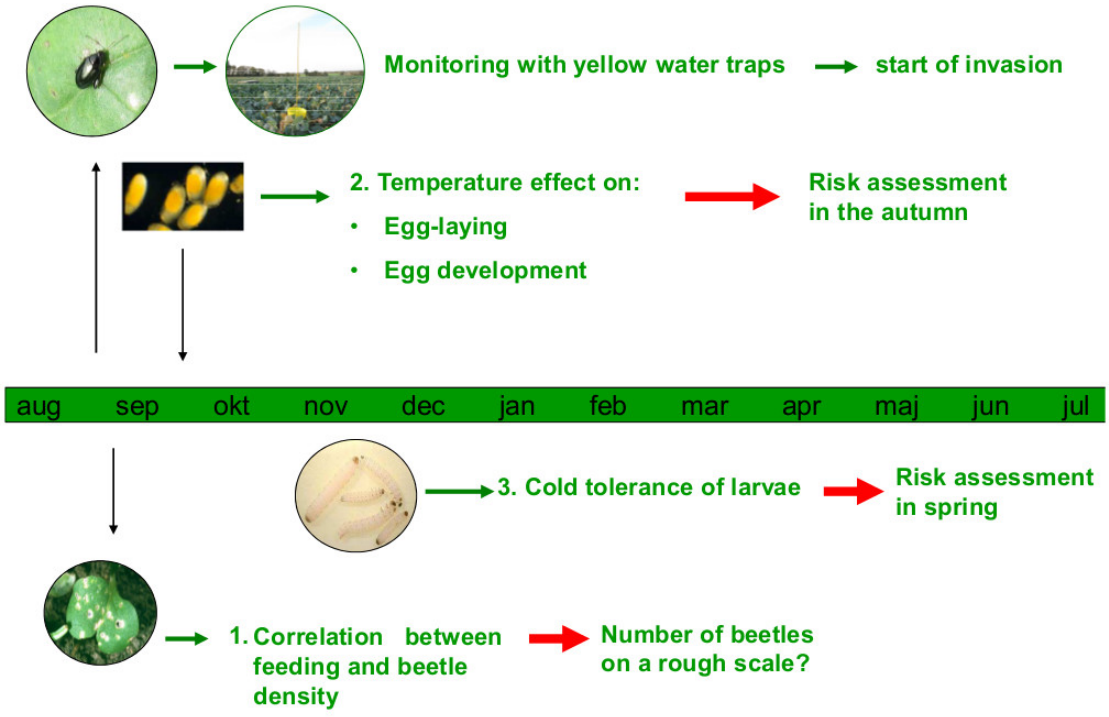

Figure 1: The key aspects of the life cycle of *Psylliodes chrysocephala* in relation to forecasting. The main aspects of focus in this project are numbered, the lacking information and its use presented. The approximate time of appearances of the insect stages are estimates taken from the literature.

The monitoring of the activity density of adult beetles with yellow water traps implies uncertainties because it monitors a non-damaging stage. The start of field invasion can be estimated from the monitoring with yellow water traps (figure 1). Yet, there is not always a direct correlation between trap catches and subsequent larval density per plant (Hossfeld 1993; Johnen and Meier 2000a). **Therefore, monitoring of the early plant injury from adults feeding on plants was tested as an alternative method to assess beetle density on a rough scale (Figure 1 (1) and manuscript I).**

The main damaging stage of the CSFB is the larvae and ideally, forecasting should be based on a prediction of larval density and time of appearance in the autumn. The larval density in the autumn depends on the egg-laying intensity and egg viability. The time of larval appearance in the field depends on the onset of egg-laying and egg development time. These aspects are ignored in the existing forecasting. **Therefore, temperature effect on parameters of reproduction and embryonic development time were studied (Figure 1 (2) and manuscript II).**

The larvae live throughout winter in leaf petioles and stems. Due to their geographical distribution, the larvae of the CSFB are exposed to a physical environment of a wide range of temperatures from the autumn to following spring with sometimes sub-zero winter temperatures. Larval population declines after hard winters have been reported (Meuche 1944; Buhl 1959). However, information on the cold hardiness of CSFB larvae is missing. **Therefore, survival of young larvae exposed to low temperatures was studied (Figure 1 (3) and manuscript III).**

3.2. BACKGROUND

3.2.1. THE CABBAGE STEM FLEA BEETLE THROUGHOUT THE YEAR

The CSFBs are univoltine and stay in the oilseed rape field most of their life cycle. They have one to two main periods of displacement which take place before and after their summer diapause in midsummer. The displacement before summer diapause is to avoid unfavourably high temperatures. The beetles move from the fields into cooler and shadier places of higher relative humidity in field margins and hedgerows. Alternatively, some stay in the field if the temperature is not too high, stay in the stubbles after harvest or disperse into field margins if disturbed (Meuche 1940). The next period of displacement is after summer diapause when the CSFB migrate to new fields of emergent WOSR plants.

Their flight ability can be characterized as fairly good. They are able to fly longer distances and have been caught up to a height of 18 meter (Ebbe-Nyman 1952). Their flight is influenced by wind and almost no flight is observed in strong wind (Ebbe-Nyman 1952; Bonnemaison and Jourdheuil 1954). Direction of flight is to some degree influenced by wind direction though this was shown to be most apparent at high flight frequency. No correlation between relative humidity and flight was found (Ebbe-Nyman 1952).

After field invasion, their behaviour gradually changes from movement by flight to jumping. This change is not influenced by temperature but takes place in line with sexual maturation and hardly any flight can be observed when all females are sexually mature (Ebbe-Nyman 1952; Bonnemaison and Jourdheuil 1954). After dispersion into the fields, the beetles restrict their main activity to after sunset (Bonnemaison and Jourdheuil 1954).

The time of field invasion can vary considerably between years, mainly influenced by temperature, but generally coincides well with the emergence of WOSR in late August/early September (Alford 1979; Derron and Goy 1991; Hossfeld 1993; Johnen and Meier 2000). After field invasion the beetles feed on young plants leaving small holes on the leaves. Oviposition starts from September after a period of feeding and sexual maturation (Bonnemaison and Jourdheuil 1954; Alford 1979; Vig 2003) and eggs are laid singly or in batches in the soil close to plants (Meuche 1944; Ebbe-Nyman 1952). Egg-laying can continue until the following spring, though interrupted at temperatures below 2˚C (Bonnemaison and Jourdheuil 1954; Schulz 1985). Three larval instars exist and are present from October to the following spring. Newly hatched larvae mine into petioles of plants and live within plants throughout winter (Alford 1979; Hossfeld 1993; Johnen and Meier 2000). In early spring the later instars, mainly third instars, often move into stems and growing points of plants and cause severe damage to plants by reducing or impairing growth. Later in spring (May/June), the fully grown larvae leave the plant to pupate in the soil, although pupation can occur already from late autumn. New adults emerge in early summer (June/July) and feed for a short period until they enter their summer diapause in midsummer (Bonnemaison and Jourdheuil 1954; Alford 1979; Saringer 1984; Thioulouse 1987; Vig 2003). Activity is resumed in

August/September (Ebbe-Nyman 1952; Bonnemaison and Jourdheuil 1954; Pouzet and Ballanger 1984; Derron and Goy 1991).

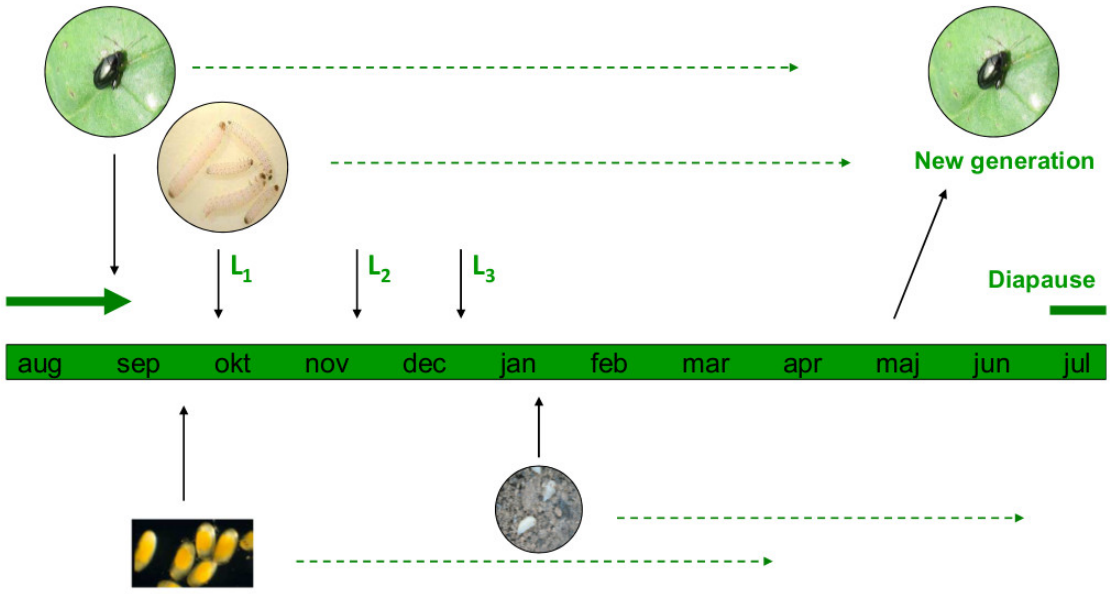

Figure 2: The main lines of the phenology of *Psylliodes chrysocephala* L.. The approximate times of appearance and presence of the insect stages are estimates taken from the literature.

3.3.2 POPULATION DYNAMICS

A wide range of direct and indirect (intrinsic and extrinsic) factors contribute to the population dynamics of insects, i.e. weather, predation, parasitoids, inter- and intraspecific competition. The factors may further be categorized as density-independent or density-dependent and the latter are considered having the major role in the dynamics of populations (Berryman 1996; Cornell et al. 1998). Often more interacting factors have a significant effect on a population and they can vary in relative importance from time to time, making predictions on population dynamic difficult (Davies 1988; Price et al. 2011).

The population dynamic of the CSFB is described as above all to be influenced by three main factors; the weather conditions from time of sowing, winter temperatures and to varying degrees natural enemies (Meuche 1944; Buhl 1959; Bonnemaison 1965; Hossfeld 1993; Johnen and Meier 2000; Nilsson 2002; Ulber and Wedemeyer 2004). The weather conditions from the time of sowing govern the start and intensity of egg-laying and a mild autumn will increase the time period of favourable conditions for egg-laying and larval development. Winter temperatures govern larval development time and mortality during winter. Natural enemies cause varying degrees of mortality of the CSFB (Ulber and Wedemeyer 2004; Williams 2010; Ulber et al. 2010).

3.3.3 NATURAL ENEMIES

Tersilochus microgaster (Scépligeti)(Hymenoptera:Ichneumonidae) has been identified as a larval parasitoid and *Trechus quadristriatus* (Schrank) (Coleoptera: Carabidae) as an egg predator (Warner et al. 2003; Ulber and Wedemeyer 2004). The role of these natural enemies in suppressing the CSFB population requires a spatial and temporal association with the vulnerable stage of the pest.

T. microgaster is a specialist endoparasitoid of CSFB larvae with synchrony in immigration into oil seed rape crops from March to May and appearance of CSFB larvae within plants. Varying levels of parasitism have been reported in different countries from 7.7 to 44.4% (Ulber and Wedemeyer 2004; Barari et al. 2005; Ferguson et al. 2006). In a three year study from 2001 to 2003 in Germany, the level of larval parasitism by *T. microgaster* ranged from 24.6 to 44.4% (Ulber and Wedemeyer 2004). Parasitism of CSFB larvae by *T. microgaster* has not been assessed in Denmark.

T. quadristriatus is a generalist and opportunistic predator primarily known as a predator of aphids in cereal crops (Sunderland 2002). This species, actively seeking food by random search, has shown a spatial association with CSFB larvae during October in the field and tests on feeding capacity in the laboratory confirmed this species to consume CSFB eggs (Warner et al. 2003). *T. quadristriatus* is common in Denmark (Hansen 1968), though its occurrence and association with the CSFB in oilseed rape fields has not been assessed.

Laboratory bio assays have shown the CSFB to be susceptible to isolates of the generalist insect-pathogenic fungus *Metarrhizium anisopliae* to varying degrees depending on isolate and spore dosage. However, natural infections have not been reported (Butt et al. 1992; Butt et al. 1994).

3.3.4 THE VARIATION IN ABUNDANCE

The variation in abundance and damage of the CSFB between years is occasionally described as a periodic pattern of seven years (Erichsen 1993; Nilsson 2002). The driving factors are believed to be natural enemies, weather conditions after field invasion and winter temperatures (Meuche 1944; Buhl 1959; Bonnemaison 1965; Hossfeld 1993; Johnen and Meier 2000; Nilsson 2002; Ulber and Wedemeyer 2004). Of these factors, only population oscillations of natural enemies can behave in a cyclic or density dependent manor and especially oscillations of specialist natural enemies are coupled with that of the prey (Price et al. 2011). Consequently, regulation by specialist natural enemies is often regarded as a principal driving factor of insect populations declines in general (Davies 1988; Cornell et al. 1998).

4. MONITORING ABUNDANCE

The abundance of the CSFB is mainly carried out by monitoring the adult beetles during the field invasion period with yellow water traps or the larval density per plant in the autumn or spring (Hossfeld 1993; Walters et al. 2001; Nilsson 2002). The monitoring of adults and larval density in autumn is used in forecasting the risk of damage (Purvis 1986; Hossfeld 1993). Monitoring larval density in spring is used as a prognosis of abundance and potential risk the following cropping season (Nilsson 1990). However, in some years the larval density in the spring may provide a fairly accurate prognosis of the population size the following season. In other years, it may not due to a high level of parasitism by *T. microgaster*.

In Denmark, monitoring with yellow water traps is carried in a large number of fields each year. In Sweden, an additional monitoring of larval density per plant in the spring is carried out. Peaks of high abundance are coinciding between countries with peaks in i.e. 1992, 2000 and 2008 (appendix 1 & 2). After a number of years with low abundance since 2009, it is assumed that the population is increasing. This is also confirmed by the latest monitoring in 2014.

4.1 MONITORING IN PEST MANAGEMENT

The objective of monitoring in pest management is to assess and provide an estimate of a pest density. Sampling can be direct by assessing the number of insects or indirect by assessing insect induced host injury. **Direct sampling** can provide absolute or relative estimates of population size (Pedigo 1994). **Absolute estimates** represent number of insects per unit area, i.e. plant, square meter or row or in the case of the CSFB, larval density per plant in spring or autumn. A representative sampling can then be extrapolated to the area of interest and provides a reasonably accurate estimation of the CSFB abundance. An advantage of sampling CSFB larval density per plant, if done in the autumn, is that it monitors the damaging stage. Despite the advantages, monitoring larval density per plant is rarely applied due to high labour requirement and cost of plant dissection. **Relative estimates** do not relate to unit area but to the sampling method i.e. observations or trapping per unit of time. These sampling methods provide count per unit effort of i.e. sweep netting, observations or in the case of the CSFB, trapping with yellow water traps (Pedigo 1994). Relative estimates are less accurate as they represent pest population abundance in time and space and therefore also depend on environmental conditions governing insect activity and behaviour (Davies 1988). Further, a threshold based on relative estimates works only in forecasting when monitoring is of the damaging stage as is not the case with monitoring of the CSFB with yellow water trap.

Still, forecasting of the CSFB is widely based on monitoring the activity density of adult beetles with yellow water traps from the time of crop emergence. The effectiveness and embedded uncertainties relate to the temporal and spatial field distribution of the CSFB and whether or not

there is a direct correlation between the relative number of adult beetles (trap catches) and subsequent larval density.

The CSFB are non-uniformly distributed in the field after dispersion (Thioulouse 1987; Warner et al. 2003; Ferguson et al. 2006). Sometimes, but not in general, this uneven distribution can be explained simply as a result of uneven germination of host plants, proximity to WOSR fields the previous season or aestivation places or as a result of oriented invasion flight (Pouzet and Ballanger 1984; Thioulouse 1987). To provide a sufficiently accurate estimate of abundance, 25 traps or sampling points per hectare was required in one study by Thioulouse (1987).

The control threshold originates from an evaluation of the correlation between trap catches and subsequent larval density per plant (Hossfeld 1993). However, a direct correlation does not always exist and the threshold should be interpreted as a negative or positive prognosis of potential damage from catches below or above the threshold, respectively (Hossfeld 1993; Johnen and Meier 2000). This prognosis is based on trap catches from a few traps per field. Consequently, the placement of traps becomes critical, as also exemplified by strongly differing trap catches within the same field from the Danish monitoring scheme (unpublished data, SEGES). Therefore, there is a potential risk of pest densities above the threshold not being discovered due to chance.

An indirect assessment of CSFB induced damage can be of leaf scarring by larvae (Walters et al. 2001) or feeding damage by adult beetles (manuscript I). As neither represents the main damage, the intention is to produce a damage index reflecting the pest density. Based on monitoring data, a threshold of 50% leaves scarred was estimated from a correlation between leaf scars and larval density per plant in the autumn (Walters et al. 2001).

In a field cage experiment, the initial feeding damage by adult beetles as damage incidence (number of damaged plants, damaged leaves or “feeding holes”) was assessed to test for a correlation between the induced damage and beetle and subsequent larval density (manuscript I). Sampling of the initial plant injury constitutes monitoring of the injury induced by a non-damaging stage, the adults. However, if feeding among adults is assumed to be similar under equal environmental conditions, then plant injury within a unit area, i.e. number of damaged plants, can be extrapolated to the managed area and beetle density estimated on a rough but absolute scale. This first attempt to evaluate monitoring of the initial leaf injury as an indicator of beetle density did not show this as a reliable method (manuscript I). There was not a significant correlation between the beetle densities tested and the plant injury expressed as number of damaged plants or number of feeding holes. There was a significant but small effect of beetle density on the number of damaged leaves. The increase in damaged leaves was as small as 3.3% per beetle at increasing density of beetles. The lack of a clear correlation between plant injury and beetle densities might be due to a great variation in feeding among beetles, as was confirmed in a parallel feeding test at five constant temperatures. The feeding of 20 females was assessed over a period of two months. There was a temperature effect on feeding though a great variation in

feeding among females within the same temperature, especially at increasing temperatures (appendix 3). Insect induced plant injury could not either be related to larval density per plant, though there was a significant correlation between densities of beetle and larvae (manuscript I). The beetle densities assessed resulted in a low feeding level and a low number of larvae per plant. The highest density of eight females only gave rise to a mean of 1.42 larvae per plant which is below the action threshold from the UK of five larvae per plant (Walters et al. 2001). On average, each female produced 0.2 larvae per plant or 4.8 larvae in total. Therefore, the beetle densities did not represent from low to high infestation levels. The beetle densities would roughly have to be increased to a scale between 2 and 24 pairs to yield a mean larval density per plant of 0.4 to 4.8.

5. REPRODUCTION AND DEVELOPMENT

Insect reproduction is defined as the per capita number of offspring production over a given time period (Carey 1993). Reproduction of the CSFB is characterized by iteroparity with long-lived females reproducing multiple times with the potential of females to remain reproductively inactive at times of unfavourable conditions or inadequate resources and resuming egg-laying as these become favourable (Fritz et al. 1982; Schulz 1985; Price et al. 2011). In cases of a short or cold autumn, fewer eggs are laid in the autumn and egg-laying is continued in spring where most eggs are laid (Bonnemaison and Jourdheuil 1954; Schulz 1985). The continuous egg-laying of the CSFB does not require repeated mating (manuscript II). CSFB females are syn-ovigenic with eggs maturing with time after emergence of adults. As a result, fecundity and longevity of the CSFB, opposed to semelparous and pro-ovigenic species, are more dependent on food availability and quality as well as the abiotic environment, with direct effects on start, intensity and end of egg-laying (Price et al. 2011). The daily oviposition rate may decrease over time as increasing age affect the rate of many insect species negatively (Regniere et al., 2012). To fully cover the reproduction potential of the CSFB, an evaluation therefore needs to include more significant components such as the length of the preoviposition and oviposition period, per capita total oviposition and oviposition rate, longevity and egg viability.

5.1 TEMPERATURE EFFECT ON REPRODUCTION AND DEVELOPMENT

Insect reproduction and development are influenced by the interaction between intrinsic life history traits and extrinsic factors as temperature, food, moisture and photoperiod (Awmack and Leather 2002; Malaquias et al. 2010; Price et al. 2011; Marchioro and Foerster 2012). Temperature is usually the most important abiotic factor (Smerage 1992; Hentz et al. 1998; Sagarra et al. 2000) with direct effect on reproduction parameters as oviposition period, fecundity, longevity, egg development and viability (Campbell et al. 1974; Smerage 1992; Kim and Lee 2003; Son and Lewis 2005).

Insect reproduction and development of different insect stages occur within a given temperature range normally experienced in their environment. Subsequently, the timing of many life history events is regulated by temperature. Knowledge on the effect of temperature on parameters of reproduction and development is therefore essential for predicting among others timing and intensity of egg-laying, development rate and time of hatching, and further for understanding population dynamics and managing pest populations. In this project, the focus has been on quantifying temperature effect on five important reproduction parameters; the length of the preoviposition and oviposition period, the total egg-laying capacity, the daily oviposition rate and survival time of females to determine the temperature of maximum reproduction.

The reproduction of the CSFB occurs within 2-30˚C (Bonnemaison and Jourdheuil 1954). Despite a wide temperature range, the parameters of insect reproduction in general and of the CSFB reach a

maximum at an optimal temperature and decrease towards the lower and upper limits (Bonnemaison and Jourdheuil 1954; Beck 1983; manuscript II). Temperature effect has been documented on the length of the preoviposition period, total number of eggs laid, daily oviposition rate, female longevity (Bonnemaison and Jourdheuil 1954; manuscript II). This study showed decreasing length of the preoviposition period at increasing temperature though with no significant differences at consecutive temperatures, no significant differences in the length of the oviposition period and decreasing female longevity at increasing temperature in the range 4-20˚C, though with no significant differences in longevity at 8, 12 and 16˚C. The temperature for maximum egg-laying capacity in terms of total egg-laying was found to be 16˚C and in terms of egg-laying rate to be above 16˚, as the rate at 16 and 20˚did not differ significantly (table 1 & manuscript II). These results are partly in contrast to the earlier findings by Bonnemaison & Jourdheuil (1954). In their study, the highest total number of eggs laid was found at 8˚C and the highest egg-laying rate at 16˚C (Bonnemaison and Jourdheuil 1954). At 16˚C in this study, the mean length of the preoviposition period was 18.8 days, the estimated 50% survival time 186 days, estimated total number of eggs 695.6 eggs/female and estimated daily egg-laying rate 5.4 eggs/female/day (table 1 & manuscript II).

Table 1: Reproduction parameters of *Psylliodes chrysocephala* (L.) females at 4, 8, 12, 16 and 20˚C; observed mean preoviposition period in days, estimated median total number of eggs laid and daily oviposition rate and estimated 50% survival time in days (manuscript II). The preoviposition period was analysed by a non-parametric test (Kruskal-Wallis and comparisons made by Wilcoxon tests. Total number of eggs laid and daily oviposition rate was estimated by linear regression on square-root transformed observed values. Reported values were transformed back to the original scale. A Wilcoxon test was used to compare values at subsequent temperatures. Survival time was estimated and compared across all temperature groups by a Cox proportional hazard model. Significant differences are indicated by different letters.

Temperature ˚C	Preoviposition period (days)	Eggs/female	Eggs/female/day	50% survival time (days)
4	93.07 [a]	57.34 [a]	0.81 [a]	239 [a]
8	41.56 [ab]	179.66 [b]	1.73 [b]	153 [b]
12	25.93 [bc]	199.06 [b]	2.31 [b]	195 [b]
16	18.83 [cd]	695.62 [c]	5.43 [c]	186 [b]
20	14.62 [d]	371.28 [d]	6.82 [c]	78 [c]

The rate of insect development, 1/development time, as a function of temperature, is usually graphically represented by a curvilinear relationship. The relationship is more or less linear within the intermediate temperature range and development rate gradually decreases and mortality increases at lower and higher temperatures (Campbell et al. 1974). The intermediate temperature range is assumed to match the range normally experienced by insects in the field (Campbell et al. 1974; Lamb 1992). Therefore, a simple linear model is often used to describe the relationship between insect development rate and temperature and to estimate two important parameters,

the lower threshold for development, (T_0), and the thermal constant, K. Below T_0 development is zero or negligible and K is the number of day-degrees (DD) above the lower developmental threshold required by an insect or insect stage to complete development. Appearance of an insect stage of interest can be predicted from T_0 and K.

The development of the CSFB has been described by the simple linear model and temperature effects have been documented on egg development and viability of the CSFB (Bonnemaison and Jourdheuil 1954; Alford 1979; Johnen and Meier 2000). CSFB eggs develop within a wide temperature range (Bonnemaison and Jourdheuil 1954) and are capable of developing at quite low temperature i.e. 2˚C, if the embryo has been differentiated before a drop in temperature (Buehler 1986) . Within the temperature range 4-20˚C development time decreased with increasing temperature from 163 days at 4˚C to 12 days at 20˚C. Hatching was around 70% at 8-20˚C and 50% and significantly lower at 4˚C (manuscript II). By the linear model and the DD concept, earlier studies have reported a developmental threshold (T_0) and thermal constant (K) values of 3.2˚C and 240 DD (Alford 1979), 4˚C and 200 DD (Johnen and Meier 2000) and 7˚C and 160 DD (Bonnemaison and Jourdheuil 1954). The estimates from our study were similar with a T_0 of 5.1˚C and a K of 184.9 DD (manuscript II). The estimates give similar predictions of egg development time at 12-20˚C and larger variation at 8˚C, especially the one by Bonnemaison & Jourdheuil (1954) (table 2). Development time could only be predicted at 4˚C by the estimates by Alford, as the other estimates of T_0 were greater or equal to 4˚C.

Table 2: Development time (days) of *Psylliodes chrysocephala* eggs predicted from reported values of the developmental threshold (T_0) and thermal constant (K) in our study (manuscript II) and studies by others. All studies have calculated T_0 and K by a simple linear regression.

Temperature ˚C	4	8	12	16	20	Reference
Development time (days)	-	64	26.9	17	12.4	Manuscript II
	300	50	27.3	18.8	14.3	Alford, 1979
	-	50	25	16.7	12.5	Johnen & Meier, 2000
	-	160	32	17.8	12.3	Bonnemaison & Jourdheuil, 1954

The objective was to provide estimates of T_0 and K to predict larval appearance in the field sufficiently adequately for pest management. A simple linear model was used to describe temperature dependent development of CSFB eggs. The linear model describes insect development well in the intermediate temperature range but can have limitations beyond this range with potential errors in estimating the developmental threshold (Worner 1992; Skinner et al. 2004). More nonlinear models exist varying in complexity and derived estimates (Kontodimas et al. 2004; Tran et al. 2012). Nonlinear models can describe insect development more accurately

when temperature extremes are included and comparison of estimates from more models is often carried out to validate the estimates (Kontodimas et al. 2004). Yet, the simple linear model is widely used (i.e. Johansen 1997; Nahrung et al. 2004) and often fit experimental data well with minor difference in accuracy of estimates compared to nonlinear models (Kontodimas et al. 2004). Additionally, extrapolation to estimate the lower developmental threshold is often necessary when using both linear and nonlinear models (Lamb 1992).

6. WINTER TEMPERATURE EFFECT ON LARVAL MORTALITY

Insects are exposed to a decrease in temperature during winter and their survival depends on their adaptations to survive these unfavourable temperatures. Their survival depends on both severity of the cold and duration of exposure, life stage and acclimation state or pre-exposure conditions (Hiiesaar et al. 2009; Berkvens et al. 2010; Buergi and Mills 2010). Declining temperature, photoperiods and nutrient quality of food initiate processes of **cold hardiness**, the adaptation of insects to survive winter (Somme 1999; Lee 2010).

6.1 COLD HARDINESS

The diversity of insects, their variety of habitats and overwintering sites has resulted in different strategies of surviving winter with various underlying physiological mechanisms. Two main categories of cold hardiness exist, freeze tolerance and freeze intolerance (Bale 1996; Somme 1999). The concept of cold hardiness has been further broadened to also include chilling tolerance and intolerance, as freeze intolerant insects existing in less severe winter conditions may be at greater risk of dying from chill injury than from freezing (Bale 1996; Lee 2010). Freeze tolerant insects are the most cold hardy insects capable of surviving partial freezing of tissue and body fluids. Their lower lethal temperature represents their limit of survival. Most terrestrial arthropods are freeze intolerant (Somme 1999). They rely on a capacity to increase their cold tolerance during winter by the mechanism of super cooling. **Super cooling** provides insects the ability to avoid freezing even below the freezing point of their body fluids and they are capable of surviving varying time periods in a super cooled state. The super cooling point (SCP) of a species is the temperature at which their body fluids freeze and death occurs and the super cooling capacity is the difference between the melting point of the haemolymph/body fluids and the SCP (Lee 2010). The SCP generally represents the lower temperature limit of survival though this can also depend on the chilling tolerance of a species. Some species suffer mortality from chilling injury above their SCP and may further be characterized as chilling susceptible. The SCP of these species is a poor indicator of their cold tolerance. The SCP of chilling tolerant species is a reliable indicator of their cold hardiness.

The depressing of the melting point of body fluids by super cooling is driven by increased concentrations of small, stable and highly soluble cryoprotective compounds and thermal hysteresis proteins (THP) in the haemolymph. Increased concentrations of these compounds have been found in the winter. Different kinds of compounds have cryoprotective ability but most often cryoprotectants are referred to as glycerol and other alcohols and sugars. Cryoprotectants enhance cold and freezing tolerance. Thermal hysteresis proteins (THP) or antifreeze proteins act by adhering to the surface of seedling ice crystals and thereby preventing growth and freezing. Ice nucleating bacteria and fungi, present on plants, are eaten by insects. The degree of super cooling capacity is further influenced by the content of these ice nucleating agents (INA) in the insect gut.

The coping strategies of insects to reduce or remove INAs are to stop feeding, to clear the gut and to mask the INAs in the gut by biochemical processes (Lee 2010).

Cold hardiness of insects can be evaluated by a determination of more indices, the **SCP**, the lower lethal temperature **(Ltemp)** and lethal time **(Ltime)**. The SCP is studied by exposing insects to decreasing temperatures at a relevant cooling rate. When the body fluids freeze, heat of crystallization is released and can be detected by thermocouples attached to insects. Due to the importance of pre-freeze mortality with the broadened and more ecologically relevant classification of cold hardiness, the SCP cannot always fully describe cold hardiness of insects. Cold hardiness should be assessed by a number of indices in combination rather than separately to fully describe mortality in response to low temperature (van Lenteren et al. 2006). Determination of LTime and LTemp can provide estimates of the time and temperature at which a given proportion (i.e. 50%) of the population would be expected to die. Importantly, these estimates can be compared with survival under fluctuating field temperatures, as fluctuating temperatures can increase survival (Colinet et al. 2011). LTime is studied by exposing insects to constant low temperatures for different times of duration (manuscript lll). LTemp is studied by exposing insects to different constant low temperatures for a fixed exposure time (i.e. one minute) (Koch et al. 2004). It is important to use an ecologically relevant cooling rate and duration of exposure to estimate the cold tolerance of insects in the field (Worland 2005; Lee 2010; Terblanche et al. 2011). In this project, the Ltime until 50% and 90% (LT_{50} and LT_{90}) of acclimated or non-acclimated larvae had died was estimated at -5 and -10˚C. The time of exposure was determined to ensure a mortality ranging from 0 to 100% and a cooling rate of 0.5˚C/min was applied. As acclimated and non-acclimated larvae proved to be first and second instars, respectively, a second test was carried out to test stage-specific mortality at -5 and -10˚C (manuscript lll).

Winter temperatures in the field have shown to affect the CSFB abundance with declines in larval density or trap catches of adults after cold winters (Meuche 1944; Kaufmann 1944; Bonnemaison and Jourdheuil 1954; Buhl 1959). Meuche (1944) assessed mortality of the three larval instars exposed to -11 and -16˚C for a few hours under dry conditions, in humid sand and in water. Mortality was affected by larval stage, temperature and the level of humidity with dry conditions and second instars favouring higher survival (Meuche 1944). Prior to the experiment on cold hardiness in this project, a pilot study with field collected larvae was initially carried out. The learning from this was that the range of exposure times at -5˚C needed to be expanded to reach a mortality ranging from 0 to 100%. Further, to test the effect of acclimation and larval stage, the age and pre-conditions needed to be standardized. An effect of temperature, exposure time, cold acclimation and larval instar on mortality was found (manuscript lll). The CSFB larvae are capable of surviving exposure to -5˚C for relatively long periods, whereas exposure to -10˚C is more lethal as mortality occurred much faster. Further, cold acclimation increases cold hardiness of the CSFB as survival of cold acclimated larvae was higher at both -5 and -10˚C as evident from the estimates

of LT_{50} and LT_{90} (table 3). In accordance with Meuche (1944), the survival of 2nd instar larvae was higher, though only when exposed to -5˚C (manuscript lll).

Table 3: Estimated times of 50% (LT_{50}) and 90% (LT_{90}) mortality of acclimated (AC) and non-acclimated (non-AC) larvae of *Psylliodes chrysocephala* (L.) exposed to -5˚C for 1, 2, 4, 8, 12, 16 and 20 days and to -10˚C for 6, 12, 36, 48, 72, 96, 120 and 144 hours (manuscript lll). Each temperature was analysed separately and values are estimated with parameters from logistic modelling of larval mortality as a function of temperature and acclimation.

Temperature ˚C	LT_{50} AC	LT_{50} non-AC	LT_{90} AC	LT_{90} non-AC
-5	9.58 days	7.38 days	15.14 days	11.03 days
-10	70.51 hours	32.60 hours	132.23 hours	66.84 hours

Approximately, 9 and 15 continuous days at a mean temperature of -5˚C or 3 and 5 continuous days at a mean of -10˚C are required to cause 50 and 90% mortality of acclimated larvae, respectively. A review of daily mean temperatures from 1990 to 2013 from two weather stations in Denmark depicts only one winter (2011-2012) in one location with sufficient continuous days at -5 or -10˚C to cause 50 and 90% larval mortality (appendix 4 & 5). If days at -3˚C is considered as harmful in a context of days at -5˚C, a larval mortality of 50% or more can occur in more years (appendix 4 & 5). Importantly, the temperature data reflects means of daily fluctuating temperatures and temporary increases in temperature allow insects to recover with both temperature and duration affecting the degree of recovery (Colinet et al. 2011). The question is how much temperature needs to increase and for how long to have a positive effect on mortality. Therefore, a comparison of the results and temperature data might not be fully applicable without knowledge on the effect of fluctuating temperature on mortality.

7. CONCLUSION & FUTURE PERSPECTIVES

This project was initiated due to the problematic aspects of monitoring the activity density of the CSFB and of forecasting being based on this monitoring alone. This project has focussed mainly on temperature effect on parameters of reproduction, egg development and cold tolerance. The results can serve to improve the existing forecasting as they can be used to predict times in the fall of an early start and high intensity of egg-laying, of larval appearance and times in winter of high larval mortality.

As part of the project, an assessment of the initial plant injury was tested as an alternative monitoring method but was not found to be a useful and reliable indicator of the beetle density. Firstly, beetle densities ranging from low to high were not achieved. Secondly, the results did not show a direct correlation between the initial plant injury and beetle density. A further evaluation needs to increase the number of beetle densities tested and to repeat the experiment more years to include variable weather conditions.

This project has documented and quantified temperature effects on important parameters of reproduction and egg development useful in forecasting the risk of larval damage by the CSFB. The temperature effect emphasizes the importance of weather conditions in the fall in relation to the potential infestation level and risk of damage. In forecasting the risk of larval damage, the start of egg-laying and daily egg-laying rate is of greatest relevance. The earliest start of egg-laying and times of maximum egg-laying rate were identified as occurring at temperatures of 16 and 20˚C. The maximum total egg-laying was found to take place at 16˚C. The temperature of maximum egg development and hatching rate was found at 20˚C.

It is evident from this project that young CSFB larvae are able to tolerate exposure to moderate sub-zero temperatures and that cold acclimation affected survival positively. Cold acclimated larvae were able to survive exposure to -5 and -10˚C for 9.6 days and roughly 3 days, respectively before 50% of the larvae had died. The increase in susceptibility between -5 and -10˚C was substantial and including more temperatures from i.e. 0 to -10˚C and periods of exposure could elucidate the severity factor of more ecologically relevant temperatures and durations of exposure.

The present results and the results by Meuche (1944) indicate that the lower lethal temperature of young CSFB larvae is below -10 and even -16˚C if this temperature limit of survival is determined by a minimum exposure time of one minute (Koch et al. 2004; Carrillo et al. 2005) . However, the present results document lethal chilling injury from brief to extended periods of exposure to temperatures above this lower limit. A more comprehensive understanding of cold tolerance of the CSFB could be provided by studying and comparing the SCP and lower lethal temperature. This comparison could reveal whether the SCP of CSFB, as in freeze intolerant species, represents the lower limit of survival.

To understand the influence of temperature on insect reproduction, development and cold tolerance, other conditions than temperature are frequently kept constant and only levels of temperature varied. Under field settings, more conditions might change simultaneously and over time. Despite this, empirical results from temperature studies constitute a framework for understanding temperature effects, as temperature is a main factor influencing reproduction, development and cold tolerance. The components of reproduction, development and cold tolerance are often studied under a range of constant temperatures, though importantly fluctuating temperatures, as experienced in the fields, can affect these parameters differently. Increased egg-laying and cold tolerance as well as slower, equal and faster development in response to fluctuating compared to corresponding constant mean temperatures have been observed (Beck 1983; Renault et al. 2004; Davis et al. 2006; Colinet et al. 2006; Colinet et al. 2011; Vangansbeke et al. 2013). Therefore, it is relevant to study the effect of fluctuating temperatures, both daily and between days. Future studies should also consider humidity as reduced egg-laying and development rate of the CSFB at low humidity has also been documented (Bonnemaison and Jourdheuil 1954).

The present results verify the importance of temperature in the autumn in relation to the egg-laying capacity and thereby abundance. There is a risk of a high level of larval infestation and increase in population size in mild autumns with continuing temperatures around 16˚C. In cold autumns with unfavourable conditions, fewer eggs are laid in the autumn and egg-laying is resumed and the majority of eggs are laid in the spring. These eggs do not have a great influence on plant injury, as the plants will not be damaged particularly by the younger larvae at this time. On the other hand, these eggs will influence the population with the greatest population increase in warm springs. In relation to the risk of damage, monitoring of temperatures in the autumn is relevant. In relation to prognosis of the future population size, additionally monitoring of spring temperatures is relevant.

Based on temperature data from two weather stations in Denmark from 1990 to 2013, the results do not seem directly to confirm a great effect of winter temperatures on the abundance of the CSFB. According to data and the results, low winter temperatures will rarely persist for a sufficient number of continuous days to cause a larval mortality of 50% or above. However, since the temperature data reflects daily fluctuating temperatures, the effect of fluctuating temperatures on mortality is required to estimate field mortality conclusively. However, with a broadened knowledge on cold tolerance of the CSFB, monitoring of winter temperatures and estimation of larval mortality might serve in forecasting the risk of larval damage in the spring and as prognosis of the abundance the following season.

The present results identify temperatures in the fall as an important and main factor affecting the abundance of the CSFB and winter temperatures as a contributing but not main factor. Varying levels of parasitism by the specialist parasitoid *T. microgaster* have been documented in Sweden, Germany and Poland (Williams 2010). Parasitism is most likely also a contributing factor in

population declines after years of high abundance, which is characteristic of the population dynamics of specialist natural enemies and their prey (Price et al. 2011). However, parasitism by *T. microgaster* has not been documented in Denmark. A documentation and evaluation could elucidate the role of parasitism in regulating the abundance of the CSFB and whether preservation of this specialist parasitoid is important in pest management.

With the widely used monitoring of the activity density of adult beetles, the start of field invasion can be estimated from the first trap catches. With the information on reproduction and egg development, times of high egg-laying and egg development rate can be estimated. If the threshold of trap catches is exceeded during field invasion, monitoring of weather can be used to predict the start and intensity of egg-laying and larval appearance. In line with the present results, a next step experimentally could be to further widen the knowledge on cold tolerance of the CSFB and to gain knowledge about the effect of fluctuating temperatures and humidity on reproduction and winter mortality. In a broader context, future studies should also include investigations on the possible role of natural enemies. A next step to improve forecasting and pest management decisions could be to construct a simple model based on the results and local temperature data to make real-time predictions on the level of reproduction in the autumn and larval survival rate during winter. Assessment of larval density per plant in the late autumn can be used to validate a model.

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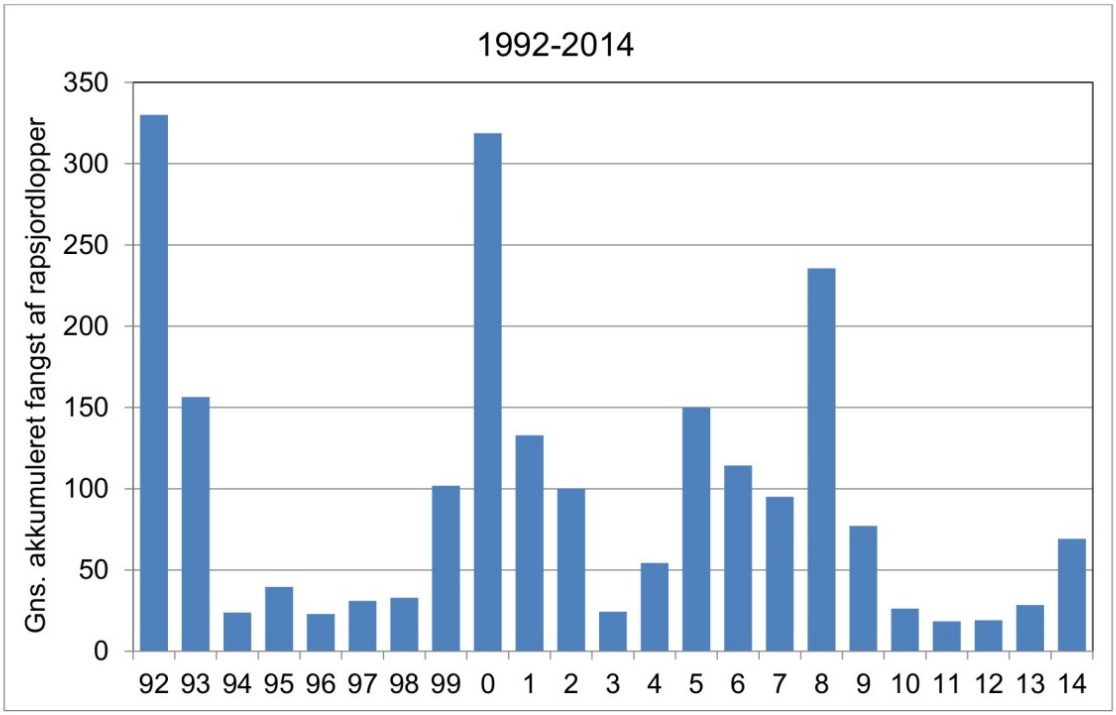
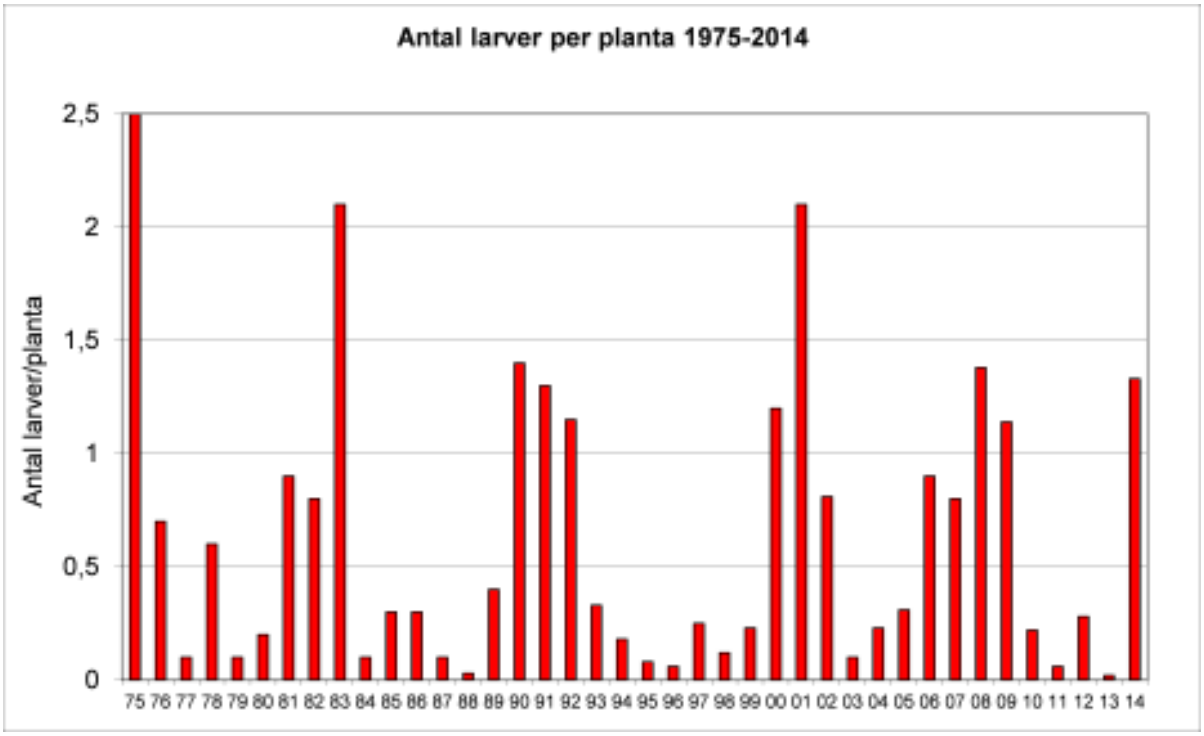
9. APPENDICES

9.1 1. MONITORING IN DENMARK FROM 1992-2014



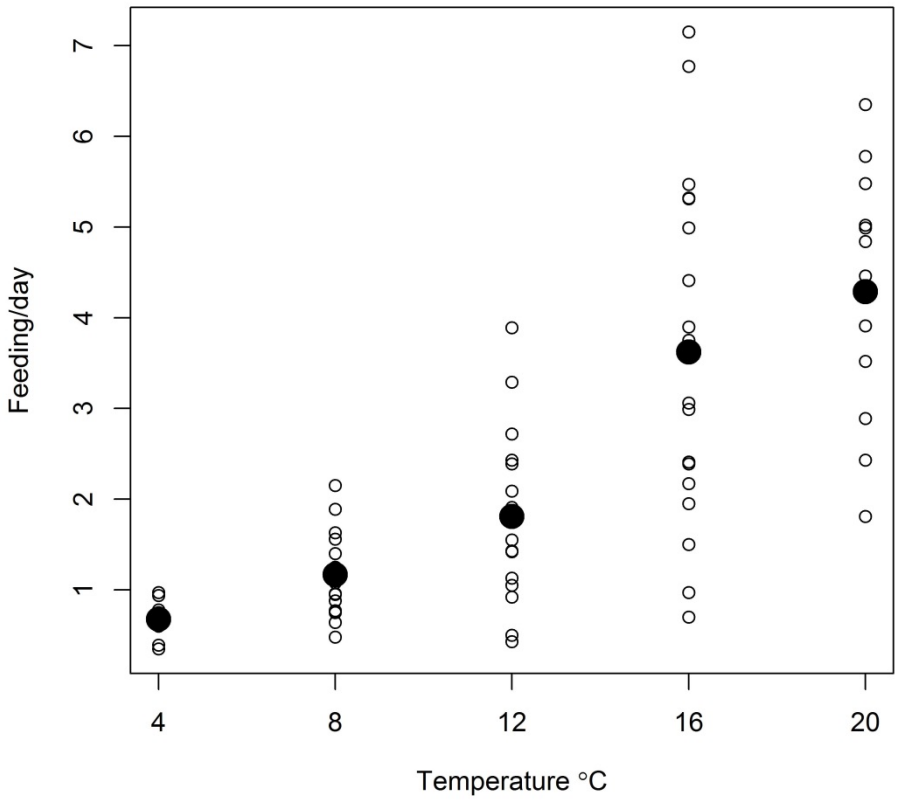
Appendix 1: Monitoring of adult cabbage stem flea beetles with yellow water traps during the main period of field invasion in autumn from 1992 to 2014 in Denmark. Monitoring is carried out with two traps per field in approximately 80 fields each year by local advisory centres. Traps are emptied and reported twice a week. The figure is provided by SEGES and shows the mean accumulated catches per field.

9.2 2. MONITORING IN SWEDEN FROM 1975-2014



Appendix 2: Monitoring of larval density/plant in the spring in Sweden from 1975 to 2014. Larval density was assessed in 25 plants in approximately 18 untreated fields each year in Skåne (Southern Sweden). The assessments were carried out by SLU, Alnarp 1974-1988 and Växtskyddscentralen, Jordbruksverket, Alnarp 1989-2014. http://www.jordbruksverket.se/amnesomraden/odling/vaxtskydd/vaxtskyddscentralerna/alnarp/vaxtskyddsbrevalnarp/nyhetsarkiv2014/nr5rapsjordjordloppapavagmotennytopp.5.37e9ac46144f41921cd751f.html

9.3 3. FEEDING TEST AT FIVE CONSTANT TEMPERATURES



Appendix 3: Observed mean feeding/day in mm^2 of 20 females at five constant temperatures 4, 8, 12, 16 and 20˚C with the mean of all females in bold. The values originate from assessment of feeding of beetle pairs over a period of two months from 13th of September 2012. Feeding "holes" were assessed visually into three categories increasing size. Mean size of each category was determined by measuring a number of feeding "holes" in mm^2. Temperature affected feeding ($p<0.001$, Kruskal-Wallis test). Significant differences were found between 4 and 8˚C ($p<0.001$, Wilcoxon test) and between 12 and 16˚C ($p=0.005$, Wilcoxon test).

9.4 4. WINTER TEMPERATURES AT FLAKKEBJERG, DK FROM 1990-2013

Locality: Flakkebjerg			
Winter	Days ≥ -3˚C	Days ≥ -5˚C	Days ≥-10˚C
1990–1991	3	1	0
1991-1992	4	0	0
1992-1993	3	2	0
1993-1994	6	0	0
1994-1995	3	1	0
1995-1996	8	7	0
1996-1997	9[x]	5	0
1997-1998	2	1	0
1998-1999	6	2	0
1999-2000	2	1	0
2000-2001	3	2	0
2001-2002	3	2	0
2002-2003	8	2	1
2003-2004	3	1	0
2004-2005	2	0	0
2005-2006	4	2	0
2006-2007	1	0	0
2007-2008	0	0	0
2008-2009	2	1	0
2009-2010	7	3	0
2010-2011	17[•]	6	1
2011-2012	11[x]	6	1
2012-2013	8	3	0

Appendix 4: Number of continuous days ≥ -3, -5 and -10˚C at the weather station Flakkebjerg on Zealand. [x] and [•] indicate potential years of 50% and 90% larval mortality, respectively according to the results from manuscript lll. Values on continuous days are extracted from graphs of daily mean temperature during each winter from December 1st to the end of February. Data is obtained from the Danish Meteorological Institute.

9.5 5. WINTER TEMPERATURES AT BILLUND, DK FROM 1990-2013

Location: Billund			
Winter	Days ≥-3˚C	Days ≥ -5˚C	Days ≥ -10˚C
1990-1991	3	2	0
1991-1992	3	1	0
1992-1993	4	3	0
1993-1994	8	1	0
1994-1995	5	3	0
1995-1996	9[x]	6	1
1996-1997	6	4	0
1997-1998	3	1	0
1998-1999	6	2	0
1999-2000	2	1	0
2000-2001	4	2	0
2001-2002	3	3	1
2002-2003	6	3	1
2003-2004	4	1	0
2004-2005	2	1	0
2005-2006	2	2	0
2006-2007	3	1	0
2007-2008	1	0	0
2008-2009	3	1	0
2009-2010	10[x]	3	0
2010-2011	18[•]	6	2[x]
2011-2012	12	10[x]	5[•]
2012-2013	8	3	0

Appendix 5: Number of continuous days ≥ -3, -5 and -10˚C at the weather station Billund in Jutland. [x] and [•] indicate potential years of 50% and 90% larval mortality, respectively according to the results from manuscript lll. Values on continuous days are extracted from graphs of daily mean temperature during each winter from December 1st to the end of February. Data is obtained from the Danish Meteorological Institute.

10. INCLUDED MANUSCRIPTS

Manuscript I.

Mathiasen, H., Esbjerg, P. & Bligaard, J. (2014):
Early plant injury as an indicator of infestation level of the cabbage stem flea beetle?

IOBC/WPRS Bulletin; 2014 Vol. 104, 31-37

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Early plant injury as an indicator of infestation level of the cabbage stem flea beetle?

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Abstract: Forecasting abundance of the cabbage stem flea beetle, *Psylliodes chrysocephala,* is widely based on monitoring of adult beetles with yellow water traps but there is not always a direct correlation between trap catches and larval density. The objective of this study was to investigate assessment of early plant injury as a monitoring method. This was done in a field cage experiment, by testing the relationship between beetle density and immediate and subsequent plant injury from adults feeding on plants and from larvae mining plant stems. Overall feeding of adults was low. A statistically significant relationship between beetle density and plant injury was not found when plant injury was expressed as damaged plants nor feeding holes. However the experiment revealed a small but significant increase in the number of damaged leaves: 3.3% per beetle at increasing density of beetles. The observed mean number of damaged leaves per cage of 24 plants was 11.6, 13.8, 13.2 and 19.0 at 2, 4, 8 and 16 beetles, respectively, four weeks after the beetles were released into the cages at plant growth stage BBCH 18. There was a statistically significant relationship between beetle density and larval density per plant. The larval density was low and the observed mean numbers of larvae per plant were 0.15, 0.38, 0.87 and 1.42 at 2, 4, 8 and 16 beetles. Overall the present study demonstrates a correlation between adult beetle density and subsequent number of larvae per plant. However, early plant injury was not a satisfactory indicator of infestation level. The possible causes of low levels of feeding and larval density per plant are discussed.

Key words: Monitoring, forecasting, Cabbage stem flea beetle, *Psylliodes chrysocephala*, plant injury, larval density

Introduction

The cabbage stem flea beetle, *Psylliodes chrysocephala*, is regarded as one of the most important pests in winter oilseed rape, causing damage at time of establishment in autumn and throughout winter. The initial damage is caused by the adults feeding on leaves of the emerging crop. Subsequent damage can be severe and is caused by larvae tunnelling and feeding within leaf petioles and stems, occurring from autumn until the following spring. Larval damage can increase the risk of plant death during winter and can lead to stunted growth in spring (Nilsson, 2002).

Forecasting is widely based on monitoring of the adult beetles with yellow water traps at the time of field invasion (Hossfeld, 1993; Nielsen, 1994; Johnen & Meier, 2000). This forecasting incurs some major limitations since monitoring occurs during a non-damaging stage of the pest and many biological events take place from the time of monitoring until larval damage is visible; i.e., oviposition, hatching and larval development. Embedded in the existing strategy is therefore the uncertainty of whether or not a correlation exists between trap catches and subsequent larval density. A simple correlation does not always exist (Hossfeld, 1993; Johnen & Meier, 2000).

After immigration into the crop, the adult beetles feed on leaves making characteristic damage "shot holes". A control threshold of 10% damaged leaf area until growth stage BBCH 14 occurs and is used in Germany and Denmark for targeted control of adult beetles (Nielsen G.C., The Knowledge Centre for Agriculture, pers. com.). This control threshold is aimed at protecting the young crop from damaging levels of feeding and does not relate plant injury to larval density or the major damage they cause.

Feeding is affected by temperature, but can be assumed to be more or less equal among beetles under the same conditions (Schulz, 1985). If this assumption holds, then monitoring of feeding can potentially be an indicator of beetle density on a rough scale. Assessment of plant injury can be categorised into incidence and severity of damage. Incidence is the number or percentage of either damaged plants or leaves or number of shot holes. Severity is the percentage of damaged leaf area. The latter is difficult to assess objectively. EPPO (2002) guidelines recommend 'assessment of damage incidence' (EPPO, 2002). Monitoring of damage incidence can be done on several levels; by binomial sampling where one plant or one leaf is assessed as 'with or without visual damage' and by monitoring the number of shot holes per plant. Which level of assessment will depend on the feeding behaviour of the cabbage stem flea beetle; whether they stay and feed on one plant or move around between plants and leaves.

A field cage experiment was carried out as a first step to developing an alternative monitoring method based on assessment of initial plant injury from adults feeding on plant. The relationship between beetle density and initial plant injury was tested at three levels: the number of plants with damage, the number of leaves with damage and the number of shot holes. The relationship between adult beetle density and subsequent larval density per plant was also tested as was the relationship between initial plant injury and subsequent larval density.

Material and methods

Experiments

A field cage experiment in which different numbers of adult beetles were released into twenty cages containing winter oilseed rape plants was carried out in 2012 in Taastrup, at the experimental farm of Copenhagen University, Faculty of Sciences, Denmark. The experiment aimed to represent low to high infestation levels and included four levels of pairs of beetles (1, 2, 4 and 8) with five replicates per treatment.

The crop was sown on August 20th 2012. A hybrid cultivar ('DK Expower'), seed-treated with Cruiser (active ingredients Thiamethoxam, Fludioxonil and metalaxyl-M) was used. Row width was 12 cm and plant density was aimed at 40 plants/m^2. Weeds were controlled with Command (active ingredient Clomazon) on August 23rd.

Cages were modified hotbeds (120 x 80 cm with height between 30-38 cm). Cages were placed in the field on September 7th at growth stage BBCH 12-13 in positions of even germination in the field, with a distance of at least 5 m between each cage. Plant density was assessed on September 11th and plants removed to reach a density of 24 plants per cage corresponding to 40 plants/m^2. The outer 10 cm inside the cages was kept free of plants giving a crop plant area of 100 x 60 cm.

The beetles used for the experiment were collected at harvest of winter oilseed rape at the end of July 2012 at time of their summer diapause. They were stored in incubators at 16 °C and a photoperiod of 12:12 h in big plastic containers with stalks of oilseed rape pods. The sex of beetles was determined based on tarsal morphology (Kaufmann, 1941). Pairs of beetles

(male & female) were then placed in small containers from mid-August and fed Chinese cabbage until release into the cages on September 11th at BBCH 12-13. The first egg-laying of beetles was observed before release on September 3rd.

Data on the mean daily temperature and precipitation was obtained from the nearest weather station (Danish Meteorological Institute, 2013) and was normal for the period of the experiment according historic records (1961-1999).

Plant injury assessment

Plant injury was assessed in cages once or twice weekly from September 14th - October 9th. The number of plants and leaves with damage and total number of feeding holes per cage was recorded.

Larval density assessment

The number of larvae per plant was assessed once by destructive sampling of twelve plants from each cage in early winter. Plants were stored at 5 °C until assessment. Each leaf stalk was carefully detached, dissected and the number of larvae counted.

Statistical analysis and models

The combined effect of beetle density and time on plant injury as the number of damaged plants, the number of damaged leaves and the number of feeding holes and on larval density were modelled by Poisson regression with a random effect of cage and analyzed by the GLMER procedure in R. Tests for significant effects and differences were assessed by model reduction and based on Likelihood ratio tests. Mean values of observed plant injury are presented and model estimates of the beetle variability from regression analysis. Observed mean values of larval density are presented with 95% confidence intervals estimated by parametric bootstrapping.

All p-values were evaluated at a 5% significance level. All analyses were carried out in R version 2.14.2 (www.r-project.org).

Results

Plant injury

As expected there was a significant effect of time on plant injury ($p < 0.001$) but there was not a significant effect of the number of beetles on plant injury expressed as the number of plants with damage ($p = 0.34$) or as the number of feeding holes ($p = 0.68$). There was a significant but small effect of the number of beetles on the number of leaves with damage ($p = 0.036$). Feeding was low and the difference in number of leaves with damage at the different beetle densities became more pronounced after 21 days (Table 1). The difference in feeding between 4 and 8 pairs of beetles was negligible. The model estimate showed an increase of mean leaves damaged per cage of 3.3% (± 1.6%) per beetle at increasing beetle density. There was no direct correlation between early plant injury and larvae density per plant.

Larval density

There was a significant effect of the number of beetles per cage on larval density per plant ($p = 0.023$). The majority of larvae found were first instars and larval density per plant was low. The standard deviation of the mean and the estimated 95% confidence intervals increased with increasing number of beetles (Table 2 and Figure 1).

Table 1. Mean number of damaged leaves (±standard deviation) per cage at different intervals (days) after adult cabbage stem flea beetles (pairs) were released into cages.

	Mean number (± sd) of damaged leaves (per cage = 24 plants)					
Beetles	**3 days**	**7 days**	**10 days**	**17 days**	**21 days**	**28 days**
2	1.00 (±1.41)	3.40 (±2.61)	3.20 (±1.92)	5.40 (±2.51)	6.60 (±2.51)	11.60 (±3.36)
4	0.40 (±0.89)	1.40 (±1.14)	1.20 (±1.10)	4.20 (±2.39)	9.80 (±4.97)	13.80 (±5.36)
8	1.20 (±1.79)	3.20 (±3.27)	5.60 (±3.78)	5.20 (±1.92)	9.20 (±4.66)	13.20 (±6.61)
16	1.60 (±1.95)	3.80 (±1.64)	4.20 (±2.39)	6.20 (±2.95)	13.00 (±4.42)	19.00 (±7.00)

Table 2. Mean number of cabbage stem flea beetle larvae per oilseed rape plant (± standard deviation) and estimated 95% CI. CI estimated from model by parametric bootstrapping.

Beetles	Larvae/plant (mean ± sd)	Estimated 95% CI
2	0.15 (± 0.66)	0.033-0.283
4	0.38 (± 0.99)	0.133-0.633
8	0.87 (± 1.59)	0.383-1.633
16	1.42 (± 1.90)	0.817-3.050

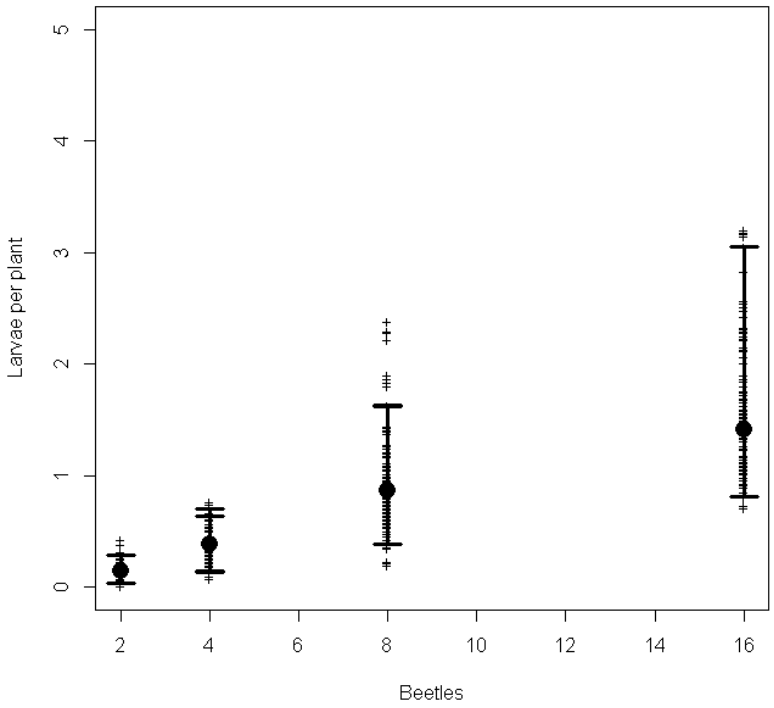

Figure 1. Observed mean number (•) of cabbage stem flea beetle larvae per oilseed rape plant and model estimates of 95% CI at 2, 4, 8 and 16 beetles per cage.

Discussion

In a study by Schulz (1985) a correlation between temperature, feeding and egg-laying was observed with increasing activity and a peak in activity approximately a month after the beetles had started feeding (Schulz, 1985); an observation indicating that feeding affects egg-laying and the potential larval population.

Insect activity in this experiment was low at all levels of beetle densities in terms of feeding, and egg-laying expressed by the subsequent low larval density. Even though plant injury as number of leaves with damage was significantly affected by beetle density, the feeding was low and the increase was only 3.3% more leaves with damage at an increase of one extra beetle per cage. This range of injury levels is not broad enough to distinguish a rough scale between beetle densities and is therefore not useful for practical monitoring and forecasting. The low larval density per plant could be due to low levels of egg-laying, high mortality of eggs and larvae or delayed egg-laying and larval development, so that eggs had not hatched before the time of assessment of larval density.

Weather conditions at time of release were normal according to Danish standards (Danish Meteorological Institute, 2013). Mean daily temperature was 12 °C, a temperature at which egg-laying is characterised as optimal (Bonnemaison and Jourdheuil, 1954; Buehler, 1986). It is often stated that beetles start egg-laying 10-12 days after crop invasion and that the egg-laying peak is around 4 weeks after crop invasion (Bonnemaison & Jourdheuil, 1954; Alford, 1979; Nielsen, 1994; Vig, 2002). Dependant on the onset of egg-laying, the first instar larvae could be present around 200-240 day-degrees (DD°) above 3.2-4 °C (Alford, 1979; Johnen & Meier, 2000). According to earlier findings, the first larvae in this experiment could then be present from around October 22nd based on the mean daily temperatures of the period. The sum of DD° did not permit hatching of eggs laid one month after the start of the experiment, a time corresponding to a peak of egg-laying. Larval development would not have reached the second instar stage. This corresponds well with the finding of this experiment of low larval density per plant and majority of the larvae found being mostly first instars.

Despite this it is noteworthy that the mean larval density at the high density of beetles was as low as 1.42 larvae per plant. Females at the highest density therefore, gave rise to an average total number of 4.26 larvae or less than 0.2 larvae per plant. Five larvae per plant is a former control threshold from Britain (Purvis, 1986). To reach this larval density per plant would then require 25 females per cage or approximately 1 female per plant. In this perspective the results reflect that a density of 16 beetles per cage of 24 plants did not correspond to a high infestation level.

The beetles used may have affected activity. Neither the developmental state of the beetles nor whether summer diapause had ended at time of release into the cages is known. The beetles were collected during their summer diapause and were kept at a constant mean daily temperature of 16 °C for approximately 42 days until the time of release. Earlier studies found diapause to be between 48-62 days under different conditions of temperature, relative humidity and food quality (Bonnemaison & Jourdheuil, 1954; Saringer, 1984). Furthermore, the start of crop invasion or resumed beetle activity has been shown to correspond to a decrease in temperature followed by an increase (Pouzet & Ballanger, 1984). In this experiment the beetles were kept at a constant temperature and then experienced a drop in mean daily temperature from the time of their release.

This first step in evaluating plant injury for improved monitoring did not show plant injury to be a useful and reliable indicator of beetle density. A direct correlation between adult beetle and subsequent larval densities was found, a prerequisite for a monitoring method

based on a non-damaging insect stage. If a direct correlation exists under specified weather conditions then monitoring of these conditions can be used to estimate damage risk. These results would benefit from further experiments using a broader range of beetle densities. Furthermore the use of beetles collected during crop invasion would ignore the uncertainties related to summer diapause and female reproductive status.

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ORIGINAL CONTRIBUTION

Effect of temperature on reproduction and embryonic development of the cabbage stem flea beetle, *Psylliodes chrysocephala* L., (Coleoptera: Chrysomelidae)

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Abstract

The cabbage stem flea beetle, *Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae), is a major pest of winter oilseed rape. Despite the importance of this pest, detailed information on reproduction to predict risk of crop damage is lacking. This study investigates the effect of temperature on parameters of reproduction, egg development and viability at five constant temperatures. Significant temperature effects were found on the pre-oviposition period, total number of eggs laid, daily oviposition rate, female longevity, egg-development rate and viability. The mean length of the pre-oviposition period ranged from 93.1 days at 4°C to 14.6 days at 20°C. Analysis of total number of eggs laid and daily oviposition rate during female lifespan estimated the highest total number of eggs laid (696 eggs/female) at 16°C and the highest oviposition rate (6.8 eggs/female and day) at 20°C. The daily oviposition rate at 20°C was not significantly higher than 5.4 eggs/female and day at 16°C. Female longevity was significantly longer at 4°C, shorter at 20°C and not significantly different between 8, 12 and 16°C. Estimated 50% survival time of females was 239, 153, 195, 186 and 78 days at 4, 8, 12, 16 and 20°C, respectively. A linear model of egg development at 8–20°C estimated the lower developmental threshold to be 5.1°C and the thermal constant for development 184.9 degree-days. The percentage of eggs hatching was significantly lower at 4°C than at all other temperatures tested. The estimated mean hatching percentages were 47.3%, 70.0%, 72.4%, 66.2% and 67.9% at 4, 8, 12, 16 and 20°C, respectively. These results can be used to predict the start and intensity of egg-laying in the autumn and the appearance of larvae in the field from knowledge about time of field invasion and from monitoring the weather.

Introduction

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae), is a local and serious pest of winter oilseed rape (*Brassica napus* L.) in central and northern European countries (Bonnemaison and Jourdheuil 1954a; Bromand 1990; Alford et al. 2003). In Denmark, this insect has gained renewed pest status in line with an increasing area of winter oilseed rape production from the late eighties. Today, the pest is widespread throughout Denmark, with a large variation in abundance and damage between years (Nielsen 1994). The widespread existing management strategy relies on monitoring of adult beetles in the main field invasion period and a threshold-based control against larval damage in the late autumn with pyrethroid insecticides (Purvis 1986; Hossfeld 1993; Nilsson 2002).

The beetles invade fields at crop emergence in late August/early September (Alford 1979; Derron and Goy 1991; Hossfeld 1993; Johnen and Meier 2000). The initial, though often least important, damage is caused by adults feeding on young leaves. The main damage is caused by larval offspring mining petioles and stems of plants from October to following spring, at which time the fully grown larvae drop to the ground to pupate in the soil. The damage may lead to increased winter mortality of plants and reduced regrowth in spring (Bonnemaison and Jourdheuil 1954a; Winfield 1992; Nilsson 2002). To estimate the risk of larval damage, the activity density of adult beetles is monitored with yellow water traps from the time of field invasion (Pouzet and Ballanger 1984; Hossfeld 1993; Nielsen 1994). However, the relationship between the activity density and the level of larval damage is unclear because of insufficient detailed biological information. A first step in evaluating the risk of larval damage is to gain information about the egg-laying phase and lifespan of female CSFB.

In response to differing weather conditions the time of CSFB larval appearance varies between years from October to the following spring (Bonnemaison and Jourdheuil 1954a; Alford 1979; Hossfeld 1993; Johnen and Meier 2000). The greatest risk of larval damage is linked to early hatching of larvae in the autumn (Buhl 1959; Bonnemaison 1965). Therefore, a second step in evaluating the risk of larval damage is to better understand the development time and viability of eggs to predict the time of hatching and larval abundance.

The period of field invasion and reproduction of the CSFB is long with egg-laying from autumn to following spring. Egg-laying may vary greatly with peaks either in the autumn or the following spring (Bonnemaison and Jourdheuil 1954a; Schulz 1985). There is a period of sexual maturation after field invasion and before egg-laying starts in the autumn. The length of this pre-oviposition period is 10–15 days depending on temperature, food availability and uptake (Ebbe-Nyman 1952; Bonnemaison and Jourdheuil 1954a; Alford 1979; Derron and Goy 1991; Vig 2003). Egg-laying occurs within the temperature range of 2–30°C, yet variable reports on the maximum egg-laying capacity have been presented in the literature (Bonnemaison and Jourdheuil 1954a; Buehler 1986; Derron and Goy 1991).

A major study on the CSFB was carried out by Bonnemaison and Jourdheuil (1954a). At a wide range of constant temperatures, they recorded the effect of increasing temperature on total number of eggs laid, oviposition period and longevity of the CSFB. Their results have remained unverified at other locations, where the CSFB is considered a pest. A decision support system for oilseed rape pests, ProPlant, has been developed by Johnen and Meier (2000) and is widely used. The system is based on data from the literature and 8 years of monitoring of field migration, egg-laying, egg and larval development (Williams 2010). The results of these monitoring studies have not been published.

Whether peaks of egg-laying take place in autumn or spring depends on weather conditions (Meuche 1944; Bonnemaison and Jourdheuil 1954b; Schulz 1985). The eggs laid in early autumn are most important in relation to the risk of crop damage (Buhl 1959). The time of hatching depends on temperature. Under favourable conditions, eggs laid in September or early October may develop into second or even third instars by October/November. The later instars are capable of mining into the growing point of plants and reducing their growth in the spring (Bonnemaison and Jourdheuil 1954a). The eggs laid in the spring are of minor importance in relation to crop damage because plants have reached a stage where they are able to tolerate mining by newly hatched larvae (Buhl 1959). They will, however, contribute to the total egg production which influences the population dynamics of the CSFB in the longer term and increases the potential risk of damage in following seasons.

This study aimed to evaluate reproduction, egg development and viability of the CSFB. This was carried out by testing the effect of five constant temperatures (4, 8, 12, 16 and 20°C) on components of reproduction; pre-oviposition and oviposition period, total number of eggs laid per female, daily oviposition rate, female longevity, and egg development and viability. The temperature for maximum fecundity was estimated taking into account total number of eggs laid, daily oviposition rate and female longevity. The development threshold and thermal requirement for egg development were estimated and the temperature for maximum egg survival was determined. The temperature range tested was chosen to approximately mimic conditions normally experienced from late summer and during autumn and early winter in Denmark.

Materials and Methods

Insects

The beetles were collected in Denmark from harvested winter oilseed rape seeds at the end of July 2012 and placed in plastic containers (16 × 22 × 32 cm) with winter oilseed rape stems and pods at 16°C and a photoperiod of 12 : 12 h (L : D).

Reproduction

The beetles were sexed and one male and female placed together at 16°C from mid-August in cylindrical plastic containers with a diameter of 6.5 cm and height of 7 cm. The lids were perforated to allow air circulation. They were fed leaves of Chinese cabbage until fresh oilseed rape leaves were available from the field. These leaves were stored at 5°C before use. On 13 September 2012, the containers were randomly divided into five groups and placed in incubators (Sanyo MIR-554 or MIR-254) at 4, 8, 12, 16 and 20°C (±0.5°C) and a photoperiod of 12 : 12 (L : D). Humidity was not controlled. Twenty replicates were used for each temperature and each replicate consisted of one mating pair. The beetles had not laid eggs before the start of the experiment. Dead males were removed and not replaced. The hatching of eggs from females with no male partner was assessed as a check of egg viability. Number of eggs and mortality were assessed every second day until 16 November 2012 and twice a week from this date until the end of the experiment. The beetles were fed leaves of oilseed rape at each assessment until 27 March 2013. From this date, they were fed leaves of Chinese cabbage. The experiment ran from 13 September 2012 until 4 October 2013. However, for practical reasons, the experiment at 20°C had to be terminated on 4 January 2013. At termination, there were three single females left at 4°C, one single female at 12 and 16°C and one couple and three single females at 20°C.

Egg development and viability

Eggs from a cohort of approximately 240 females and 145 males were collected daily from 13 September to 14 October 2012, transferred to Petri dishes with moist filter paper, and placed at 4, 8, 12, 16 and 20°C with a photoperiod of 12 : 12 h (L : D). Water was added to keep the filter paper moist. Hatching of eggs was recorded daily and the larvae removed. Egg viability was assessed as percentage of eggs hatched. Between 915 and 975 eggs were studied at each temperature.

Statistical models and analysis

Reproduction

The length of the pre-oviposition period was analysed with non-parametric methods as no transformation was found that stabilized variance adequately. More specifically, the overall effect of temperature on the pre-oviposition period was tested by a Kruskal–Wallis test, and pairwise comparisons were made with Wilcoxon tests. A Kaplan–Meier survival analysis was applied to test the effect of temperature on the length of the oviposition period. Data on total number of eggs laid and daily oviposition rate per female were square-root transformed to stabilize variance. One-way analysis of variance was carried out on transformed values, and an overall comparison of treatments was made by an F-test. It was furthermore tested with pairwise *t*-tests whether differences between consecutive temperatures (4 vs. 8, 8 vs. 12, etc.) were significant. Estimates and confidence intervals are reported after transformation back to the original scale. Raw data on female survival time are presented with a Kaplan–Meier plot (Andersen and Skovgaard 2010). A Cox proportional hazard model was fitted to compare longevity across all temperature groups, for pairwise comparisons and to estimate time of 50% survival (Andersen and Skovgaard 2010). Estimated values of 50% survival are presented with 95% confidence intervals. Nine beetles were alive when the experiment was terminated. The analyses of the oviposition period and longevity take this censoring into account.

The pre-oviposition period was calculated as the time from the start of the experiment until the first eggs were laid. The oviposition period was calculated as the time period between the first and final egg-laying. The daily oviposition rate was calculated as total number of eggs laid divided by the oviposition period in days.

Data were excluded from the analysis when the female was lost during the experiment or did not lay eggs. If a male was lost or died, data were included if the female had laid and continued to lay eggs. Altogether 15, 16, 17, 18 and 13 females were analysed at 4, 8, 12, 16 and 20°C, respectively.

Egg development and viability

The relation between temperature and egg development was tested by converting development days (time) to 1/development days (rate) and subjecting these to linear regression with heteroskedasticity, allowing for unequal variances between temperatures.

The linear relationship between development rates and temperature is modelled by:

$$Y = a \times T + b$$

Y is the rate of development (1/days), T is temperature, a is the slope and b is the intercept.

The minimum temperature of development, the developmental threshold T_0, was calculated by solving the equation for the development rate being equal to zero ($T_0 = -b/a$). The thermal constant or thermal requirement in day-degrees (DD), required to complete the egg stage, was obtained from the reciprocal of the slope of the regression line (1/a). Standard errors for T_0 and DD were computed by parametric bootstrap (Efron and Tibshirani 1993).

Percentage egg viability was analysed by mixed linear regression with temperature as fixed effect, day as random effect, and variance weights equal to the reciprocal of the total number of eggs (hatched and unhatched) corresponding to the observation.

All analyses were made in R version 2.14.2 (R Development Core Team 2014).

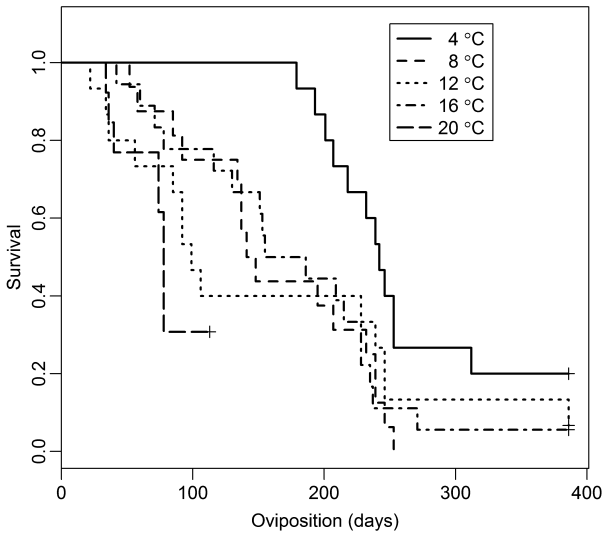

Fig. 1 Oviposition period of *P. chrysocephala* females in days at 4, 8, 12, 16 and 20°C.

Results

Reproduction

Overall, the period before egg-laying started decreased with increasing temperatures. At 4°C, the mean pre-oviposition period observed was 93.1 days and at 20°C 14.6 days (table 1). Temperature significantly affected the pre-oviposition period (P = 0.002, Kruskal–Wallis test). Pairwise comparisons showed no significant differences between consecutive temperatures, but significant differences between all other pairwise comparisons (table 1).

There was a large variation in the length of the oviposition period within all temperatures with periods ranging from one to above 300 days (fig. 1). Temperature did not significantly affect the length of the oviposition period (P = 0.13; Kaplan–Meier analysis).

In general, the frequency of egg-laying was regular at 12, 16 and 20°C as eggs had been laid at each time of assessment. The time in days between egg-laying increased at 8°C and even more so at 4°C. The observed total mean number of eggs laid per female was at a similar level at 4, 8 and 12°C, increased greatly at 16°C and then decreased at 20°C (fig. 2). The observed median total number of eggs laid was highest at 16°C (665 eggs/female), and lowest at 4°C (75 eggs/female). Temperature significantly affected the total number of eggs laid per female (P < 0.001, F-test) with significant differences between all temperatures except between 8 and 12°C (P = 0.78) (table 2).

The observed daily oviposition rate per female increased with increasing temperature (fig. 2). Temperature had a significant effect on daily oviposition rate (P < 0.001, F-test), although no significant difference was found between 8 and 12°C (P = 0.24) or between 16 and 20°C (P = 0.11) (table 2). Repeated mating was not needed for continuous egg production, as females could continue laying viable eggs for as long as 8 months, if the male was lost or died.

The observed female survival time decreased with increasing temperature with the largest observed difference between 4 and 20°C and little difference between 8, 12 and 16°C (fig. 3). Temperature significantly affected female survival time (likelihood ratio test $\chi^2 = 17.92$, d.f. = 4, P = 0.001). Pairwise comparisons showed significant differences in survival time at 4 and 20°C compared to all other temperatures and no significant differences between 8, 12 and 16°C (table 3).

Table 1 Observed mean pre-oviposition period in days of *P. chrysocephala* females at 4, 8, 12, 16 and 20°C

Temperature °C	Pre-oviposition period (mean ± SD) (days)	P (Wilcoxon test)
4	93.07 (±81.47) a	–
8	41.56 (±35.51) ab	0.06
12	25.93 (±13.03) bc	0.40
16	18.83 (±20.09) cd	0.05
20	14.62 (±9.63) d	0.97

Standard deviations in brackets. P-values from Wilcoxon test to compare pre-oviposition period at consecutive temperatures. Significant pairwise differences are indicated by different letters.

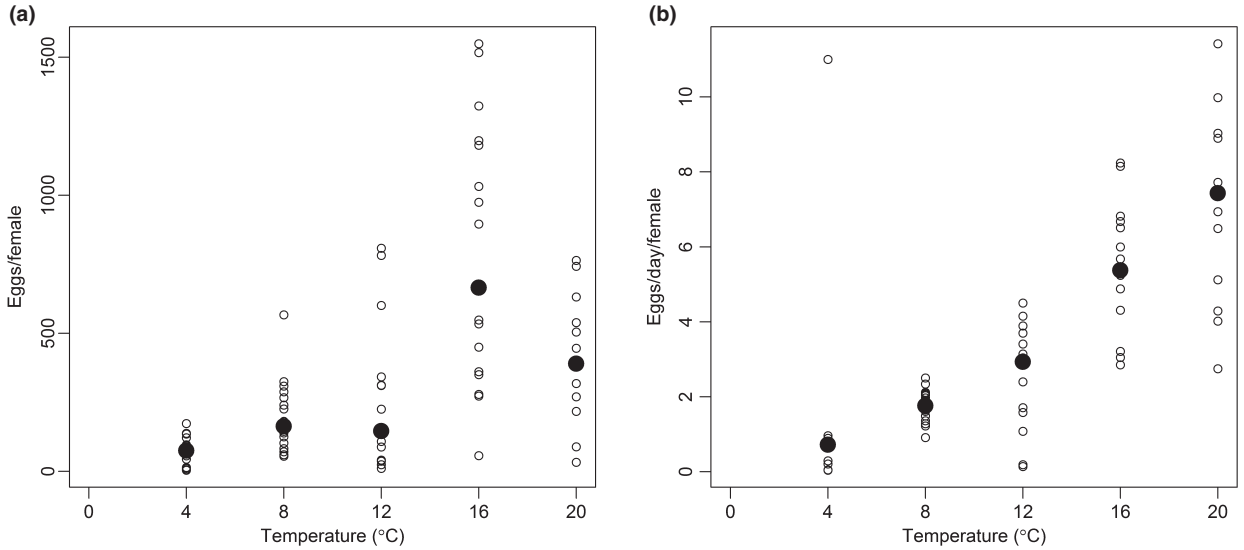

Fig. 2 Total egg-laying of *P. chrysocephala* females (a) and number of eggs laid per female and day (b) at 4, 8, 12, 16 and 20°C. Medians in bold.

Table 2 Estimated median number and 95% confidence intervals (CI) of total number of eggs laid and daily oviposition rate of *P. chrysocephala* females at 4, 8, 12, 16 and 20°C. Estimation by linear regression on square-root-transformed observed values. Reported values are transformed back to the original scale. P-values from Wilcoxon test to compare values at consecutive temperatures

Temperature °C	Eggs/female (estimated)	95% CI	P	Eggs/day/female (estimated)	95% CI	P
4	57.34	16.78–122.07	–	0.81	0.43–1.32	–
8	179.66	100.77–281.21	0.019	1.73	1.16–2.42	0.02
12	199.06	113.06–309.23	0.772	2.31	1.62–3.12	0.244
16	695.62	538.31–873.06	<0.001	5.43	4.43–6.54	<0.001
20	371.28	241.33–529.10	0.005	6.82	5.50–8.28	0.113

Egg development and viability

The mean observed development time for eggs ranged from 180.74 days at 4°C to 12.04 days at 20°C (fig. 4a). The linear regression model taking into account variance heterogeneity between temperatures described development rates within the temperature range 8–20°C well (fig. 4b). The parameter estimates from linear regression gave a development threshold of 5.1°C and thermal requirement of 184.9 degree-days (table 4).

The observed hatching percentages varied within the temperatures with only small differences in observed means between 8, 12, 16 and 20°C and a lower hatching percentage at 4°C (fig. 5). Temperature had a significant effect on the estimated hatching rate ($P < 0.001$) which was significantly lower at 4°C than at 8, 12, 16 and 20°C (table 5).

Discussion and Conclusion

Our results clearly document that temperature plays an important role in the reproduction of CSFB, as shown by its effect on the pre-oviposition period, total number of eggs laid, daily oviposition rate and female survival time. We found the temperature for maximum reproductive success in terms of daily oviposition rate and total number of eggs laid per female to be 20 and 16°C, respectively. We also showed that repeated mating is not required for a continuous and long period of egg-laying by the CSFB.

The results from this study show a great variation in eggs laid per female under constant temperatures, in agreement with both earlier field observations and laboratory studies (Meuche 1944; Bonnemaison and Jourdheuil 1954a; Schulz 1985). In this study, eggs

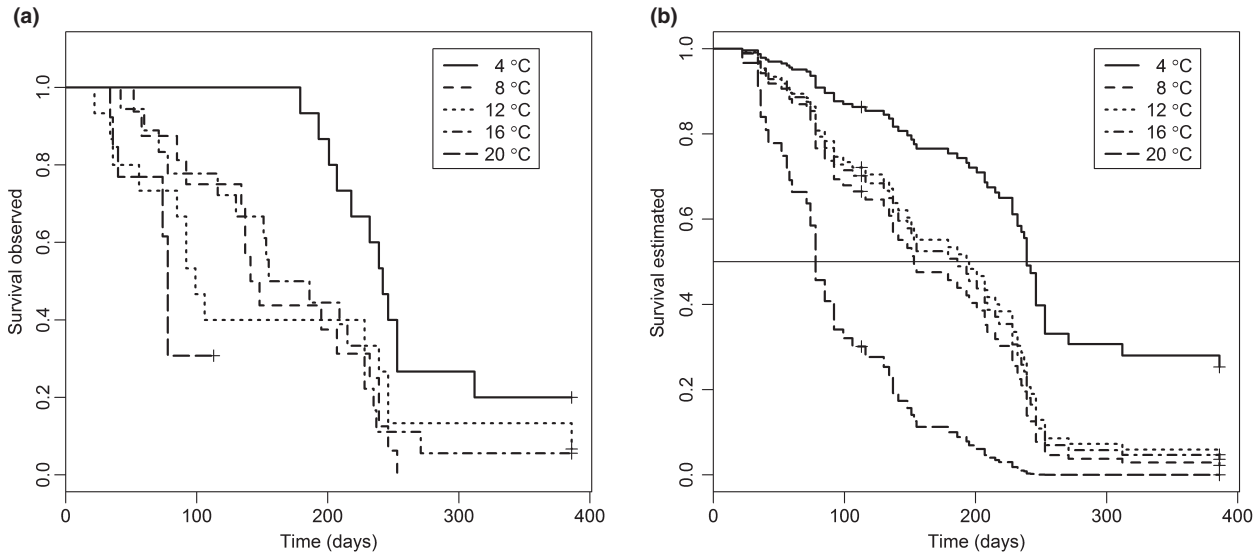

Fig. 3 Observed (a) and estimated (b) relative survival of *P. chrysocephala* females over time (days) at 4, 8, 12, 16 and 20°C. The horizontal line corresponds to 50% survival.

Table 3 Estimated 50% survival time of *P. chrysocephala* females and 95% confidence interval (CI) at 4, 8, 12, 16, 20°C. Estimation and pairwise comparisons across all temperature groups by a Cox proportional hazard model

Temperature °C	50% survival time (days)	95% CI
4	239 a	218–NA
8	153 b	116–232
12	195 b	137–239
16	186 b	137–237
20	78 c	60–215

Estimated values followed by different letters are significantly different. The upper limit of the 95% CI at 4°C could not be determined because of low mortality in this group.

were generally laid regularly every second day at 12–20°C, but with a longer period between egg-laying at 4 and 8°C.

Bonnemaison and Jourdheuil (1954a) reported a similar pattern of egg-laying with decreasing time intervals between egg-laying at increasing temperature. They observed egg-laying every day at 20°C, every second day at 16°C, every fourth to fifth day at 12°C, every eighth to tenth day at 8°C and every sixteenth to twenty-fourth day at 4°C. The pattern of egg-laying gradually changed for an increasing number of females at temperatures above 16°C. Above 16°C, egg-laying became less consistent among females, and sterility and reduced longevity occurred more frequently (Bonnemaison and Jourdheuil 1954a). In the present study, egg-laying was more frequent at 12°C than found by Bonnemaison and Jourdheuil (1954a). Also, in contrast to the results by Bonnemaison and Jourdheuil (1954a), a significantly reduced longevity and egg-laying in response to increasing temperature was first observed at 20°C in this study. However, no significant difference in the length of the oviposition period was found between any temperatures due to significant variation within all temperatures. The temperature of maximum egg-laying was 16°C in this study (estimated median of 695.6 eggs/female) in contrast to 8°C in the study by Bonnemaison and Jourdheuil (observed mean of 271.5 eggs/female) (1954a). This difference is due to a shorter longevity of 72.8 days at 16°C in the study by Bonnemaison and Jourdheuil (1954a) in contrast to the estimated 50% survival time of 186 days found in this study. In agreement with Bonnemaison and Jourdheuil (1954a), the temperature for maximum daily oviposition rate was 16°C.

Under controlled conditions within the temperature range 4–20°C, the CSFB is long-lived. A few individuals survived and continued to lay eggs for more than 1 year at 4, 8, 12 and 16°C. Such a long reproductive lifespan is not likely to occur under field conditions, where the CSFB is exposed to daily and seasonally fluctuating temperatures in addition to biotic and abiotic factors of mortality. Still, it is clear that the CSFB can have a long reproductive lifespan with peaks of egg-laying under field conditions in autumn or spring dependant on temperature (Bonnemaison and Jour-

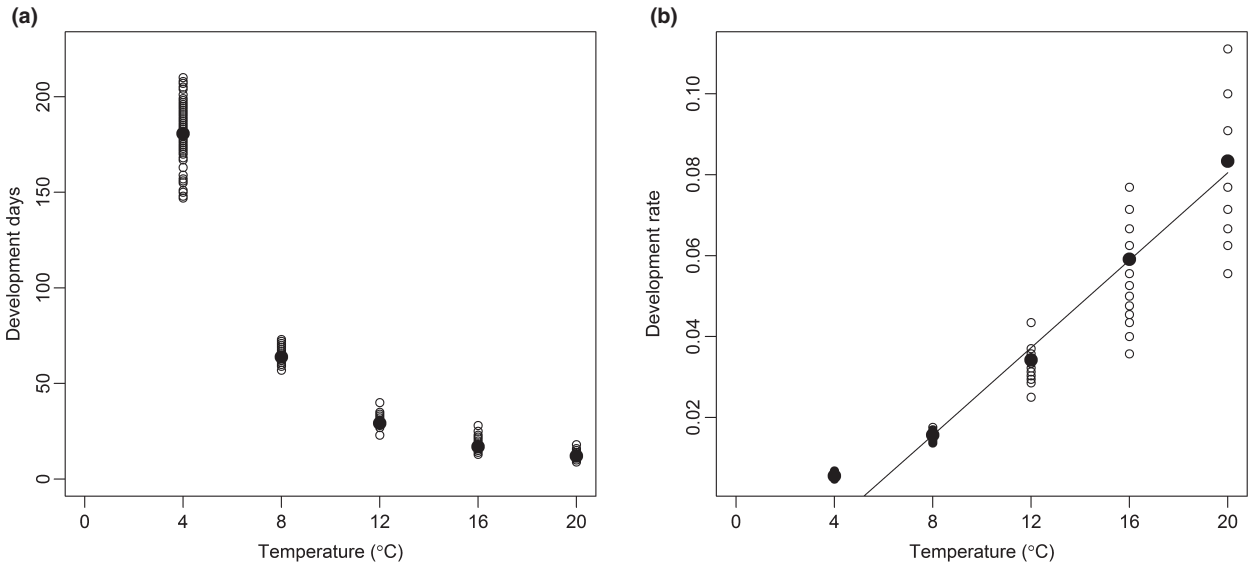

Fig. 4 Observed development time in days (a) and development rate (1/days) (b) of *P. chrysocephala* eggs at 4, 8, 12, 16 and 20°C with observed means in bold (●) and estimated regression line of development rate (see table 4).

dheuil 1954a; Schulz 1985). Further work should test the effect of daily fluctuating temperatures on reproduction of the CSFB as an increase in egg-laying has been observed for some insects and mites under fluctuating compared to the corresponding constant mean temperature (Davis et al. 2006; Mironidis and Savopoulou-Soultani 2008; Vangansbeke et al. 2013).

According to this study, the modelled egg development requires an accumulated sum of 185 DD above 5.1°C. This result is similar to earlier reported values of 240 DD above 3.2°C (Alford 1979), 200 DD above 4°C (Johnen and Meier 2000) and 160 DD above 7°C (Bonnemaison and Jourdheuil 1954a) suggesting no significant variation in development due to geographical location (Campbell et al. 1974). Development should further be tested under fluctuating temperatures as this regime compared to constant temperatures could have an impact on beetle development. Reports of faster, slower and equal development rates exist (Beck 1983). In a study by Alford (1979), egg develop-

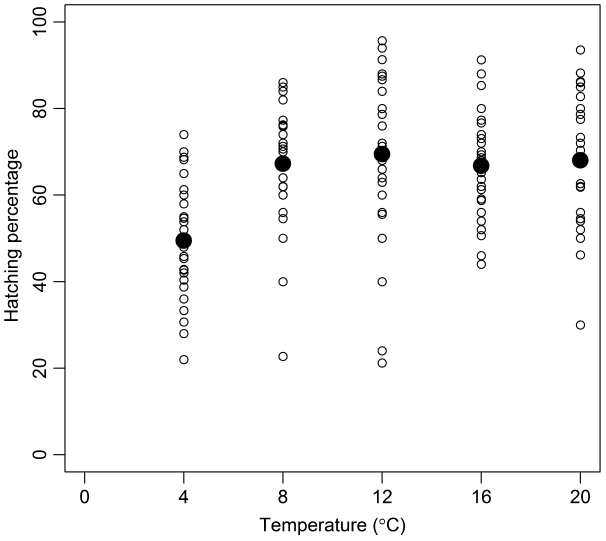

Fig. 5 Observed hatching rate of *P. chrysocephala* eggs at 4, 8, 12, 16 and 20°C. Means in bold.

Table 4 Parameter estimates, calculated developmental threshold and thermal requirement (DD) from the linear regression model of development rate of *P. chrysocephala* eggs

Constant (b)	Slope (a)	Development threshold T_0	Thermal requirement DD
−0.0276 (7.997e-05)	0.0054 (9.293e-06)	5.1117 (0.0065)	184.93 (0.32)

Standard errors in brackets.

Table 5 Estimated hatching rates of *P. chrysocephala* eggs at 4, 8, 12, 16 and 20°C with 95% confidence intervals (CI)

Temperature °C	Hatching %	95% CI
4	47.27 a	41.90–52.63
8	69.96 b	64.59–75.33
12	72.37 b	67.05–77.69
16	66.22 b	61.00–71.43
20	67.89 b	62.60–73.18

Significant differences are marked by different letters.

ment of the CSFB was found to be slightly faster under a low mean of fluctuating temperature compared to the corresponding constant mean. Fluctuating temperatures might therefore only significantly affect development rate of CSFB eggs at very low temperature, where development gradually slows and mortality increases. This has also been found for other species (Campbell et al. 1974; Worner 1992; Radmacher and Strohm 2011).

The linear model fitted data on egg development rate well in the range 8–20°C. However, egg development also took place at 4°C. At 4°C, the variation in development days was greater, indicating that this temperature is suboptimal for egg development. Still, an estimated threshold 1.1°C above 4°C, at which some development took place, can be considered contradictory. However, development below the developmental threshold has been demonstrated when very low temperatures are included, and a linear model is used to estimate the developmental threshold (Campbell et al. 1974; Liu et al. 2002; Sporleder et al. 2004). Consequently, the developmental threshold should be considered a theoretical value that is sufficiently adequate for pest management, which typically utilizes the straight part of the sigmoidal temperature development relationship (Campbell et al. 1974).

This study documents reproductive parameters of the CSFB in the northern range of its geographical distribution. It demonstrates the importance of temperature in relation to the infestation level, and it provides quantitative information on three important aspects of forecasting potential pest damage, the length of the pre-oviposition period, the daily oviposition rate and the development time of eggs at constant temperatures. If the threshold of trap catches is exceeded during field invasion, monitoring of weather can be used to predict the start and intensity of egg-laying and larval appearance.

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Survival of cabbage stem flea beetle larvae, *Psylliodes chrysocephala* L., exposed to low temperatures

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Abstract

The cabbage stem flea beetle, *Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae), is a major pest of winter oilseed rape. The larvae live throughout winter in leaf petioles and stems. Winter temperatures might play an important role in survival during winter and hence population dynamics, yet to what degree is unknown. This study investigates the effect of exposure time, cold acclimation and larval stage on survival at -5 and -10˚C. Exposure time at -5˚C was 1, 2, 4, 8, 12, 16 and 20 days and 6, 12, 24, 36, 48, 72, 96, 120 and 144 hours at -10˚C. Mortality increased with increasing exposure time and was significantly lower for cold-acclimated larvae. Estimated LT_{50} of non-acclimated and acclimated larvae exposed to -5˚C was 7.38 and 9.58 days, respectively, and estimated LT_{90} 11.03 and 15.14 days. Estimated LT_{50} for non-acclimated and acclimated larvae exposed to -10˚C was 32.60 hours and 70.51 hours, respectively, and estimated LT_{90} 66.84 and 132.23 hours. Significant differences in mortality due to larval stage were observed only at -5˚C. When exposed to -5˚C for eight days, mortality of first and second instar larvae was 81.22 and 51.26%, respectively. When exposed to -10˚C for two days, mortality of first and second instar larvae was 70.53 and 76.12%. Data on winter temperatures in Denmark from 1990-2013 showed that larvae were rarely exposed to a number of continuous days at -5 or -10˚C causing a larval mortality of 50 to 90%.

Keywords: Cold hardiness, stage-specific mortality, lethal times, acclimation

INTRODUCTION

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae), is a major pest of winter oilseed rape (WOSR), (*Brassica napus* L.), in central and northern European countries (Bonnemaison and Jourdheuil 1954b; Bromand 1990; Alford et al. 2003; Williams 2010). The egg-laying of the CSFB starts in autumn and continues until following spring at temperatures above 2˚C (Bonnemaison and Jourdheuil 1954a; Schulz 1985; Derron and Goy 1991). The larvae appear from autumn and overwinter as either first, second or third instars dependent on the start of egg-laying and temperature. In mild winters even adults overwinter (Alford 1979; Saringer 1984). The larvae live throughout the winter in leaf petioles and stems of winter oilseed rape and are exposed to a physical environment of a wide range of temperatures from the autumn to following spring, potentially with sub-zero temperatures during winter. The CSFB is adapted to temperate regions with moderately cold winter conditions and is able to withstand sub-zero temperatures during winter. However, earlier studies have reported lower trap catches or larval densities in response to particularly cold winters (Meuche 1944; Kaufmann 1944; Buhl 1959; Derron and Goy 1991). Therefore, winter conditions might be an important factor affecting population dynamics and the yearly occurrence of this species. Yet, detailed information on cold hardiness of the CSFB is lacking.

Cold hardiness can be described as the adaptations of insects to survive winter. These adaptations include various behavioral and physiological mechanisms often initiated by decreasing temperature, decreasing photoperiod and reduced nutrient quality (Sømme 1999). Overall, the physiological mechanisms behind cold hardiness can be divided into three main categories; freeze tolerance, freeze intolerance and chilling intolerance (Danks 1996; Bale 1996; Sømme 1999; Lee

2010). As many terrestrial insects from the temperate region, the CSFB might likely be defined as freeze intolerant. Freeze intolerant insects depend on super cooling; the ability to lower the temperature at which their body fluids freeze. The ability of super cooling is described by the super cooling point (SCP) which generally represents the lower lethal temperature of freeze intolerant species (Bale 1996; Sømme 1999; Addo-Bediako et al. 2000). Insects exhibit varying degrees of super cooling ability affected by among others the content of ice nucleating agents in the gut, the content of cryoprotectant substances and antifreeze proteins in the haemolymph (Sømme 1999; Lee 2010).

As insects often suffer mortality above the SCP and as SCP is often below the lowest temperature experienced by insects during cold periods, the ability of super cooling is not always a reliable indicator of cold hardiness (Bale 1996; Danks 1996; Renault et al. 2002). A more comprehensive understanding implies knowledge about the potential chilling injury above the SCP. Chilling injury plays a more important role in the ability of insects to survive winter because they exist in a physical environment where temperature rarely reaches the super cooling point but where longer periods of sub-zero temperature occur (Danks 1996).

The pre-exposure conditions before and during winter can put insects in an acclimated state and enhance their survival in cold periods (Carrillo et al. 2005; Overgaard et al. 2008; Hiiesaar et al. 2009; Berkvens et al. 2010; Soudi and Moharramipour 2011). Dependent on what triggers the onset and ending of an acclimated state and the speed of the process, insects can exhibit different degrees of survival during winter (Hiiesaar et al. 2009; Khani and Moharramipour 2010; Hiiesaar et al. 2011; Hiiesaar et al. 2012). Further, insects can exhibit different stage and even gender-specific

mortality in response to low temperature (Addo-Bediako et al. 2000; Koch et al. 2004; Carrillo et al. 2005; Jensen et al. 2007; Buergi and Mills 2010; Hiiesaar et al. 2011; Manrique et al. 2012).

This study aims to evaluate aspects of cold hardiness of the CSFB. More specifically, we investigated the lethal time until an expected larval mortality of 50 and 90% (LT_{50} and LT_{90}) of non-acclimated and acclimated CSFB larvae exposed to two constant sub-zero temperatures (experiment I) and the effect of larval stage on survival rate (experiment II). We compared the results on LT_{50} and LT_{90} with winter temperatures in Denmark from 1990-2013 and discussed a possible effect of these on population dynamics of the CSFB.

MATERIALS AND METHODS COLD

Experimental approach, insects and temperatures

The cold hardiness of CSFB larvae was studied as lethal time at -5 and -10˚C. Exposure to 0˚C was included as control and since the mean mortality at 0˚C was below 5%, no correction of mortality at -5 and -10˚C was carried out. In a pilot test prior to the experiment, field collected larvae were exposed to -5 and -10˚C for one to five days and mortality assessed to determine the range of exposure times. In the pilot test, mortality at -5˚C remained below 20% and ranged from around 5 to 100% at -10˚C. In the present experiments, exposure times at -5˚C were therefore extended up to twenty days and at -10˚C changed to shorter time intervals to ensure mortalities approximating from 0 to 100%. Newly hatched larvae from a cohort of approximately 800 beetles were used to standardize larval age and state of acclimation. Non-acclimated and acclimated larvae in experiment I proved to be second and first instars, respectively. Therefore, experiment II was

carried out to test the effect of age on mortality by exposing first and second instar larvae to -5˚C for eight days and -10˚C for two days.

The beetles used in the experiment were collected after harvest in 2013 in seeds of oilseed rape, kept in containers (diameter of 9.5 and height of 8 cm) at 16˚ and a photoperiod of 12:12 h (L:D) and supplied fresh leaves of Chinese cabbage, *Brassica rapa ssp. Pekinensis,* twice a week. At each time eggs were transferred to petri dishes with moist filter paper. The filter paper was kept moist by adding water regularly. Newly hatched larvae, not older than 24 hours, were transferred daily to Chinese cabbage leaves, kept at room temperature for a couple of hours until the larvae had mined into the leaves.

Data on daily mean temperatures during winter from 1990-2013 was obtained from the Danish Meteorological Institute for two weather stations, Flakkebjerg on Zealand and Billund in Jutland. The two weather stations are situated in areas of WOSR production and lower inland temperatures. The number of continuous days at a mean of -5 and -10˚C were quantified to identify potential years resulting in 50 and 90% larval mortality. The daily mean temperatures reflected fluctuating temperatures but only the daily means were considered.

Experiments

Mortality of acclimated and non-acclimated larvae from long time exposure to low temperatures – experiment I

To examine possible effects of acclimation on survival at low temperatures, leaves with larvae were placed at 12˚C and a photoperiod of 12:12 h (L:D) for one week. The leaves were then transferred to either 0 or 12˚C for one week, corresponding to acclimation and non-acclimation to

cold, respectively. The effect of long term exposure of larvae to constant low temperature was tested by assessing larval mortality at 0, -5 and -10˚C for various time intervals; 1, 2, 4, 8, 12, 16 and 20 days at 0 and -5˚C (±1˚C) and 6, 12, 24, 36, 48, 72, 96, 120 and 144 hours at -10˚C (±1˚C). Continuous replications, between two and seven for each treatment (temperature x exposure time), were made upon hatching of eggs, from 14th to 31st October 2013. Each replication consisted of one leaf with approximately twenty larvae. The leaves were transferred to low temperatures at a cooling rate of 0.5˚C/min.. The relative humidity was not controlled. Leaves were removed at the different time intervals and placed at room temperature allowing thawing of leaves for two to four hours. Subsequently, the leaves were carefully dissected, larvae counted and transferred to fresh leaves. Mortality was assessed as dead or alive by tactile stimulation with a small brush and was assessed twice; at time of recovery of the larvae, being two to six hours after removal from the incubators and again after 24 hours to ensure that a potential recovery was included. Mortality after 24 hours was included in the analysis.

Stage-specific larval mortality – experiment II

The possible effect of larval stage on survival at low temperature was tested by assessing mortality of larvae kept at 12˚C for 4-7 days representing first instar larvae and 14-21 days representing young second instars larvae. These larvae were transferred to -5 and -10˚C at a cooling rate of 0.5˚C/min. and exposed to these temperatures for eight days and two days, respectively. Mortality was assessed as described above in experiment I. Continuous replications (14-20) were carried out and each replication consisted of one leaf with approximately twenty larvae. In total, mortality of between 257-341 larvae was assessed.

Statistics

Larval mortality in both experiments was modelled by a logistic regression model with the glm function in R. Each temperature was analyzed separately with logit models with exposure time, acclimation temperature and larval stage as fixed variables. Differences were assessed by model reduction and log-likelihood ratio tests at a significance level of 5%. Lethal times, LT_{50} and LT_{90}, of non-acclimated and acclimated larvae were estimated from the model parameters and presented with standard error (SE). Significant differences in larval stage specific mortality were assessed by Wald tests at a 5% significance level and the mean mortality of larval stages presented as percentage mortality with standard deviation (SD).

All procedures were carried out in R version 2.14.2 (www.r-project.org).

RESULTS

Mortality of acclimated and non-acclimated larvae

Temperature, exposure time and acclimation had an effect on mortality of CSFB larvae ($p<0.001$). When exposed to -5˚C, the mean observed mortality of acclimated and non-acclimated larvae remained relatively low, from 0 to 13%, up to four days of exposure. Between four and eight days of exposure larval mortality started to increase and after eight days, the mortality of acclimated and non-acclimated larvae was 40% and 63%, respectively. As seen in figure 1, the mean mortality of non-acclimated larvae was slightly higher and most evident after eight and more days of exposure. 100% mortality of non-acclimated and acclimated larvae was reached after sixteen and twenty days of exposure, respectively (figure 1).

Mortality increased with time and the increase in mortality over time was significantly higher for non-acclimated than acclimated larvae ($p<0.001$). The estimated LT_{50} of non-acclimated and acclimated larvae was 7.38 (SE: 0.28) and 9.58 (SE: 0.34) days, respectively, and estimated LT_{90} was 11.03 (SE: 0.43) and 15.14 days (SE: 0.59).

When exposed to -10˚C, the larval mortality occurred much faster and the increase in mortality was more pronounced at shorter time intervals of hours (figure 2). Already after one day, mortality of acclimated and non-acclimated larvae was 20 and 36%, respectively. The mean observed mortality of non-acclimated larvae occurred slightly faster and 100% mortality was reached after 144 hours. The mean observed mortality of acclimated larvae did not reach 100% but 97% after 144 hours (figure 2).

The increase in mortality over time was significantly higher for non-acclimated larvae $p<0.001$. The estimated LT_{50} of non-acclimated and acclimated larvae was 32.60 hours (SE 2.05) and 70.51 hours (SE 3.42), respectively, and estimated LT_{90} was 66.82 (SE 3.79) and 132.23 (SE 7.30) hours.

Stage-specific larval mortality of non-acclimated larvae

The mean observed mortality of first and second instar larvae was 81.22 and 51.26%, respectively after exposure to -5˚C for eight days. The mortality of first instar larvae was significantly higher ($p<0.001$). After exposure to -10˚C for two days, the mean observed mortality of first and second instar larvae was 70.53 and 76.12%, respectively and not significantly different ($p=0.12$) (table 1).

Mean daily temperatures during winter at two weather stations in Denmark from 1990-2013

The daily mean temperature during winter in the period 1990-2013 ranged from well below 0˚C to above 10˚C and the lowest daily mean temperature was -14.2˚C. Days with a mean temperature of

-5˚C occurred frequently during winter, though not at all in some years and not always succeeding each other. The maximum number of continuous days at a mean of -5˚C was 7 and 10 days at Flakkebjerg and Billund, respectively. Days with a mean temperature of -10˚C occurred in a few years but more subsequent days at this temperature were rare, except in the winter 2011-2012 at Billund, where five continuous days of -10˚C occurred.

According to the weather data and the results from experiment l, low winter temperatures of -5˚C and -10˚C rarely persisted for time periods needed to cause a mortality of 50% of acclimated larvae. Approximately 9 and 15 days at a mean temperature of -5˚C or 3 and 5 days at a mean of -10˚C were required to cause 50 and 90% mortality of acclimated larvae, respectively. According to the data on daily mean temperature, a mortality of 50 and 90% would be reached in only one year (2011-2012) in Billund. At Flakkebjerg, sufficient continuous days of -5 or -10˚ to cause mortality above 50% did not occur in any year. If days at -3˚C is considered as harmful in a context of days at -5˚C, a mortality of 50% of acclimated larvae was possible in three years at Flakkebjerg and another three years at Billund.

Discussion & conclusion

Young larvae of the CSFB are able to tolerate exposure to sub-zero temperatures during winter as documented by the results from this study. Acclimated and non-acclimated larvae tolerated exposure to -5˚C for four days without significant mortality. Exposure to -10˚C was more harmful causing some mortality even after six hours of exposure. The time to reach 50% mortality of non-acclimated and acclimated larvae was 7.38 and 9.58 days at -5˚C and 32.60 and 70.51 hours (roughly 1.3 and 3 days) at -10˚C. The magnitude of cold tolerance varied for non-acclimated and acclimated larvae as mortality of non-acclimated larvae was significantly higher as has also been

found for other insect species (Overgaard et al. 2008; Hiiesaar et al. 2009). This effect tended to be even more pronounced at -10˚C suggesting that acclimation becomes even more important at the lower and more lethal temperatures. Acclimation was obtained after one week at 0˚C. At this temperature, the larvae were inactive in feeding. As both starvation and acclimation are recognized as important and interacting factors in cold tolerance (Sømme 1999; Block 2002), their effect may be difficult to distinguish (Worland 2005) also in this experiment. The acclimation period in our experiment was one week and extending the period of acclimation and decreasing the photoperiod could have positively affected larval survival (McDonald et al. 1997; Hiiesaar et al. 2009). Furthermore, different types of acclimation, such as developmental, gradual and rapid, exist and a combined acclimation of more types can result in cumulative effects on cold tolerance (Colinet and Hoffmann 2012). Therefore, Colinet & Hoffmann (2012) suggest that the mechanisms of the different acclimation types are independent, yet antagonistic interactions have also been found (Jensen et al. 2007). To fully comprehend the effect of acclimation on survival of CSFB larvae at low temperatures, combinations of different methods of acclimation should be tested, as natural acclimation as experienced in the field may expose insects to more types of acclimation, dependent on the yearly weather conditions.

Cold tolerance was also affected by larval stage, though only when exposed to -5˚C. The higher susceptibility of first instars is in accordance with results by Godan (cf. Buehler 1986) and Meuche (1944).

Meuche (1944) further documented a lower larval susceptibility to sub-zero temperatures under dry conditions. Desiccation or water balance is recognized as a contributing factor in the overwintering strategy of insects as the biochemical and physiological responses resemble those

of cold exposure (Block 1996; Sømme 1999; Zachariassen et al. 2008). The interaction between body water content and cold hardiness has been shown for other arthropods as increased production of cryoprotectants and super cooling capacity in response to reduced body water content (Block 1996). In our study, the larvae fed on Chinese cabbage leaves which have a higher water content compared to WOSR leaves. Therefore, the larvae might have possessed a dietary surplus of water compared to larvae in an oilseed rape field. This might have resulted in a higher experimental mortality.

This study has tested and documented some aspects of cold hardiness of young CSFB larvae. Other relevant aspects, yet to be studied, are the lower lethal temperature and/or their SCP to quantify the lower temperature limit of their survival and the effect of fluctuating temperatures on cold tolerance. Fluctuating temperatures during exposure to low temperature can enhance cold tolerance as temporary increases in temperature allow insects to physiologically recover. Both the temperature and duration of recovery can affect survival at low temperature positively (Renault et al. 2004; Colinet et al. 2006; Colinet et al. 2011). Therefore, our comparison of results with weather data on daily fluctuating temperatures with a mean of -5 and -10˚C might not be directly applicable without knowledge about the effect of fluctuating temperatures on mortality.

Despite the above mentioned consideration, significant overwintering mortality due to low temperatures seems to be rare according to the winter temperatures in Denmark from 1990 to 2013 and the present results. Winter temperatures are not likely to be a main factor driving the presumed population cycles with peaks approximately every seventh year (Erichsen 1993; Nilsson 2002).

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Figure 1: Observed (points) and estimated (lines) mortality in percent of acclimated (● -) and non-acclimated (× - -) larvae of *Psylliodes chrysocephala* after exposure to -5˚C for 1, 2, 4, 8, 12, 16 and 20 days. 50% and 90% larval mortality are indicated by the bold lines.

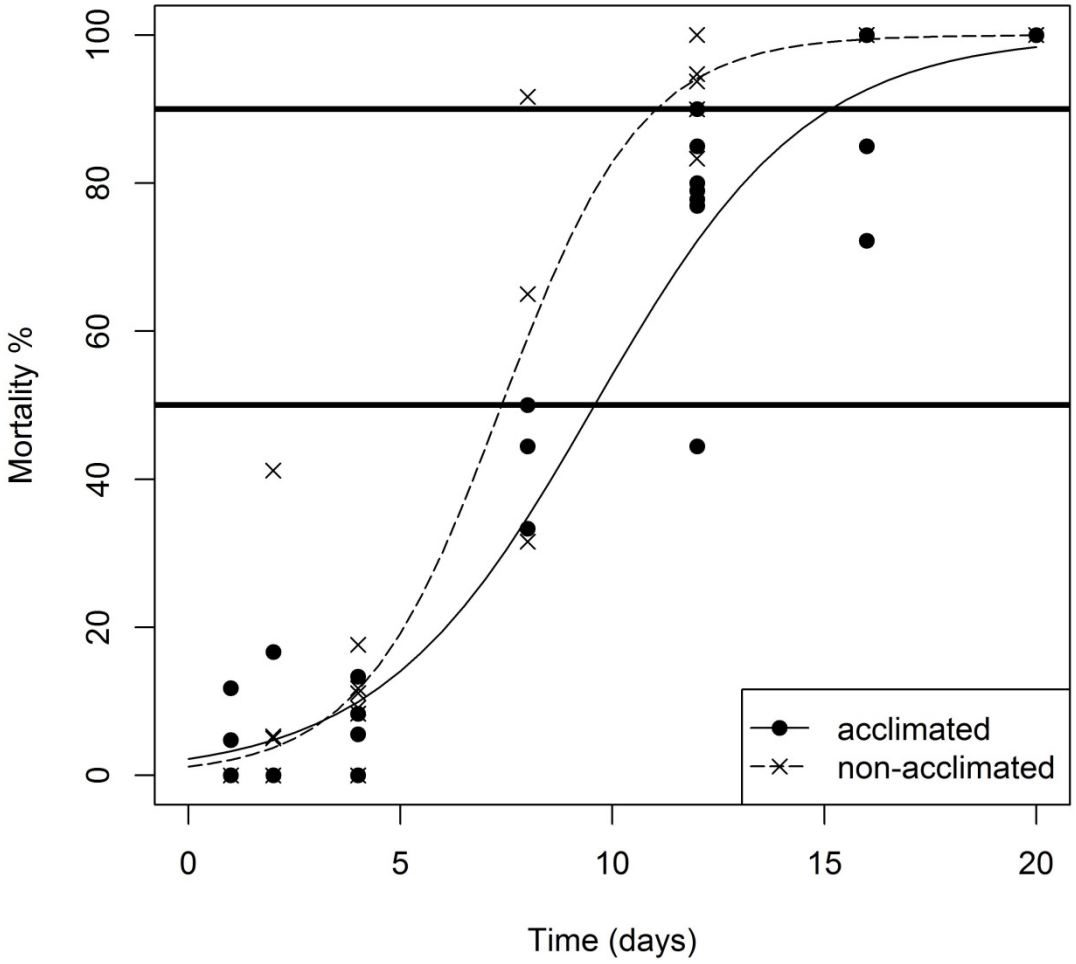
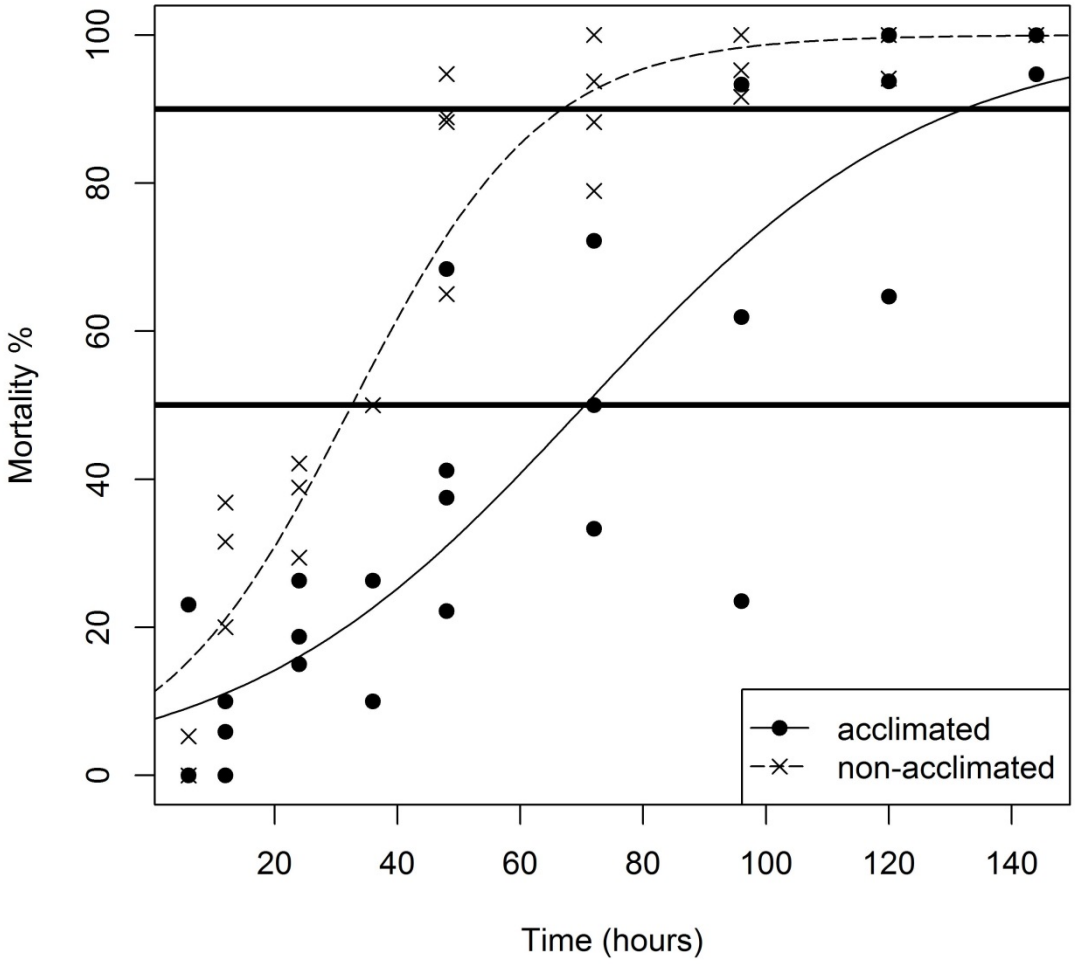


Figure 2: Observed (points) and estimated (lines) mortality in percent of acclimated (•-) and non-acclimated (×--) larvae of *Psylliodes chrysocephala* after exposure to -10˚C for 6, 12, 36, 48, 72, 96, 120 and 144 hours. 50% and 90% larval mortality are indicated by the bold lines.



Temperature	Larval stage	Mortality % (mean ± SD)	n	N (larvae)
-5˚C	L_1	81.22 ± 12.97 a	20	341
	L_2	51.26 ± 17.90 b	15	257
-10˚C	L_1	70.53 ± 19.49 a	16	278
	L_2	76.12 ± 11.36 a	14	269

Table 1: Mean mortality (%) and standard deviation (SD) of first and second instar larvae of *Psylliodes chrysocephala* exposed to -5˚C and -10˚C for eight and two days, respectively. Different letters indicate significant differences in mortality at -5 and -10˚C, respectively. The number of replications (n) and larvae assessed (N) are presented.

Communication letters to be uploaded to SEGES, Landbrugsinfo

Mathiasen H. (2015): Ny viden om rapsjordloppens æglægningsforløb kan forbedre varsling

Ny viden om rapsjordloppens æglægningsforløb kan forbedre varsling

Sammendrag

Forsøg viser, at temperaturen i efteråret har væsentlig indflydelse på hunnernes æglægningskapacitet, deres levetid og udviklingstiden og klækningsprocenten for æg. Temperaturregistreringer kan derfor bruges til at estimere tidspunktet for de første æg i marken, æglægningsintensiteten og tidspunktet for larvernes fremkomst, såfremt tidspunktet for billernes indflyvning i marken er fastlagt via gule fangbakker. Med denne nye viden er det muligt ud fra temperaturen i efteråret at forudsige en potentiel høj eller lav risiko for skade.

Æglægning

Et erhvervs-PhD projekt startet i 2011 har haft udgangspunkt i problematikken omkring monitering af raspjordloppen med gule fangbakker og fangsterne som det udelukkende grundlag for varslingen. Projektet har haft hovedfokus på temperaturforholds indvirkning på æglægning og larvedødeligheden gennem vinteren. Denne orientering handler om resultaterne fra et forsøg om rapsjordloppens æglægning, levetid og udviklingstiden for æg.

Rapsjordloppens æglægning og udviklingstiden for æg blev undersøgt ved konstante temperaturer (4, 8, 12, 16 og 20˚C). Æglægningskapacitet blev undersøgt ved at bestemme tidsrummet indtil æglægningen starter (den såkaldte præovipositions-periode), æglægningsperiodens længde, antallet af æg lagt pr. hun og deres levetid.

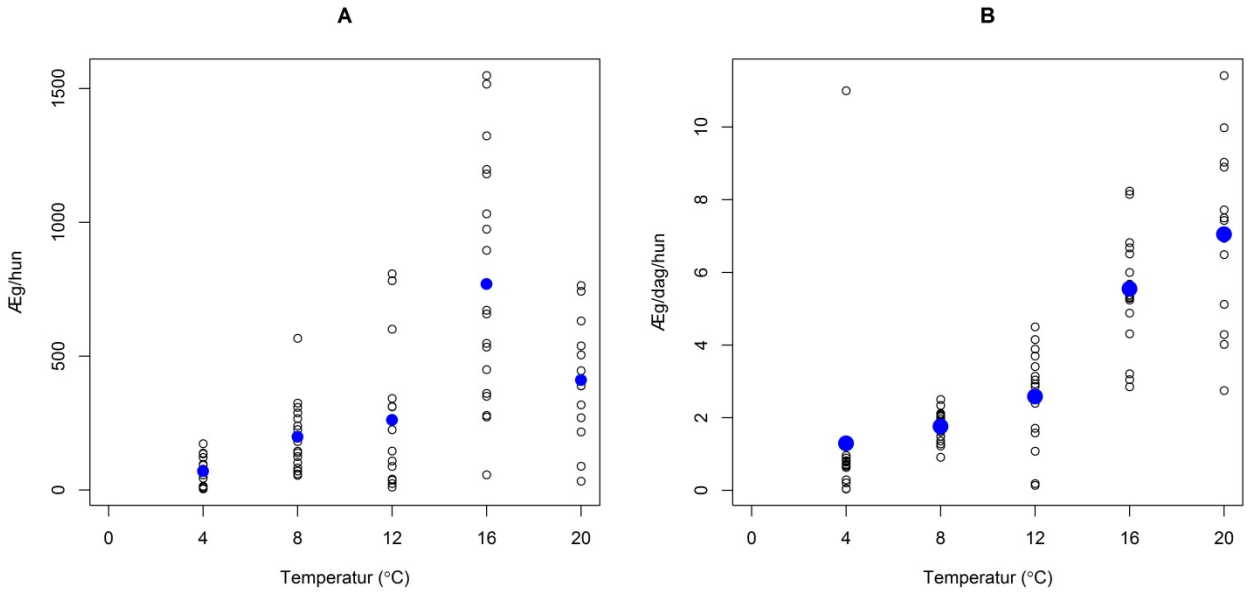
Af tabel 1 fremgår det, at tidsintervallet, indtil hunnerne begynder at lægge æg, falder med stigende temperatur. Der går i gennemsnit fra 15 dage ved 20˚C til 93 dage ved 4˚C, før en hun begynder at lægge æg. Forskellene i præovipositions-perioden mellem 20 og 16˚C og mellem 16 og 12˚C var henholdsvis 4 og 7 dage og ikke signifikant forskellige (tabel 1). Når æglægningen var gået i gang, lagde hunnerne generelt æg hver til hver anden dag ved 12, 16 og 20˚C, mens tidsintervallet steg ved lavere temperaturer. Længden af æglægningsperioden varierede kraftigt ved alle temperaturer og svingede fra 1 til over 300 dage. Til trods for en tendens til en kortere æglægningsperiode ved 20˚C, var der ingen statistisk forskel mellem temperaturer. Levetiden for hunnerne var ligeledes temperaturafhængig og faldt med stigende temperatur fra en gennemsnitlig levetid på 262 dage ved 4˚C til 79 dage ved 20˚C. Der var ikke forskel i levetiden ved 8, 12 og 16˚C.

Temperatur ˚C	Præoviposition perioden (dage)	Æglægningsperioden (dage)	Levetid (dage)
4	93.07 (± 81.47) [a]	126.1 (± 80.6) [a]	262.2 (± 71.3) [a]
8	41.56 (± 35.51) [ab]	113.3 (± 63.1) [a]	162.2 (± 69.1) [b]
12	25.93 (± 13.03) [bc]	121.3 (±107.3) [a]	156.9 (± 122.6) [b]
16	18.83 (± 20.09) [cd]	134.1 (± 67.7) [a]	175.1 (± 86.7) [b]
20	14.62 (± 9.63) [d]	60.77 (±33.5) [a]	78.6 (± 29.0) [c]

Tabel 1: Tidsrum indtil æglægning starter, æglægningsperioden og hunnernes levetid ved 4, 8, 12, 16 og 20˚C. Værdierne bygger på registreringer fra 15. september 2012 til 04. oktober 2013 for 20 billepar og er gennemsnit med standardafvigelsen i parentes. Værdier efterfulgt af forskellige bogstaver er signifikant forskellige.

Der var en stor variation i æglægningen pr. hun ved samme temperatur, og antallet af æg svingede fra 4 til 173 æg ved 4˚C, fra 55 til 309 ved 8˚C, fra 11 til 808 ved 12˚C, fra 57 til 1549 ved 16˚C og fra 33 til 764 ved 20˚C (figur 1). I en praktisk sammenhæng giver det mest mening at kigge på æglægningsintensiteten over et kortere tidsrum, da rapsjordloppen ikke oplever de undersøgte konstante temperaturer over en længere periode. Samtidig er det mest relevante i varslingsøjemed de æg, der bliver lagt tidligt i sæsonen. Disse æg kan nå at udvikle sig til larver allerede i efteråret og alt efter temperaturen til tredje larvestadium i foråret, hvor planterne genoptager væksten. De senere larvestadier, særligt tredje larvestadium, er i stand til at forårsage størst skade på dette tidspunkt. Figur 1 viser antallet af æg, som hunnerne lægger i alt (1A) og pr. dag (1B). Det højeste antal æg pr. dag, 7, blev lagt ved 20˚C, dog var antallet ikke signifikant højere end ved 16˚C. Ved 20˚C var det totale antal æg i alt 410 æg og lavere end ved 16˚C, hvor antallet var 770 æg. Alt i alt vil en temperatur på 16-20˚C efter rapsjordloppen indflyvning i marken betyde en tidligere start af æglægningen og den højeste intensitet.

Figur 1: Det totale antal æg for hunnerne (A) og antallet af æg pr. dag pr. hun ved 4, 8, 12, 16 og 20˚C og gennemsnit ved de forskellige temperaturer ●.



Ægudvikling og klækningsprocent

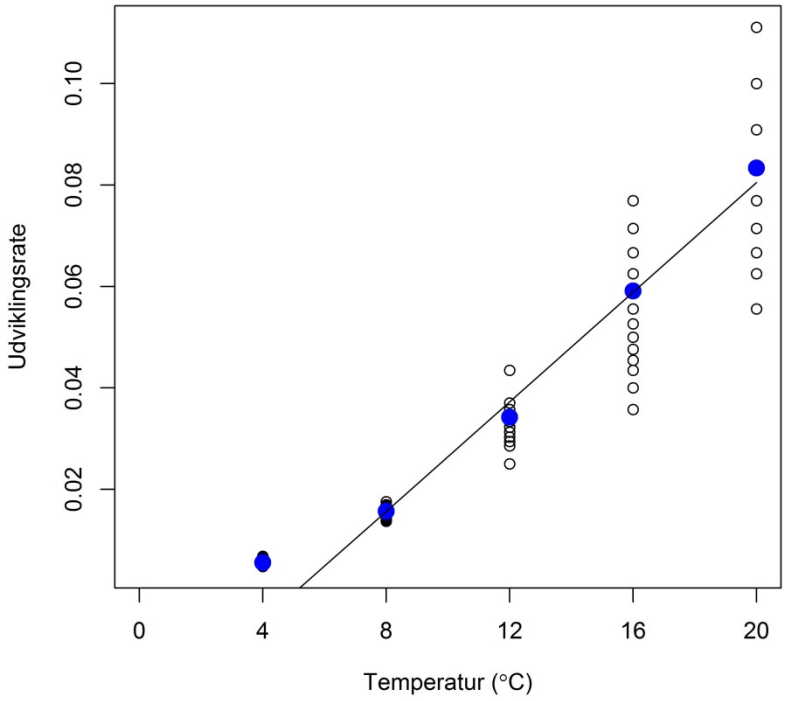
Udviklingstiden for æg var tydeligt påvirket af temperatur. Den gennemsnitlige udviklingstid for æg gik fra 12 dage ved 20˚C til 181 dage ved 4˚C (tabel 2). Klækningsprocenten var ligeledes temperaturafhængig, men kun ved 4˚C var der en signifikant lavere klækningsprocent. Ved de øvrige temperaturer lå klækningsprocenten mellem 67-69%.

Temperatur ˚C	Gennemsnitlig Udviklingstid (dage)	Klækningsprocent
4	180.74	49.46
8	63.89	67.24
12	29.25	69.44
16	16.97	66.75
20	12.04	68.02

Tabel 2: Gennemsnitlig udviklingstid i dage og klækningsprocenten for æg ved 4, 8, 12, 16 og 20˚.

Udviklingsraten for æg (1/udviklingstid) indenfor temperatur intervallet 8 til 20˚ opfører sig lineært (figur 2) og en extrapolering af kurven for dette område giver en beregnet minimumstemperatur for udvikling på 5,1˚C og et varmesumskrav til udvikling på 185 daggrader.

Figur 2: Observerede udviklingsrate (1/udviklingstid) for æg ved 4, 8, 12, 16 og 20˚. Blå punkter er observerede gennemsnit.



Disse værdier kan bruges til at beregne udviklingstiden for æg ved et kendskab til den daglige gennemsnitstemperatur i marken. En daglig gennemsnitstemperatur på f.eks. 12˚C bidrager med 6.9 (12-5.1) daggrader og et æg vil klække, når summen af daggrader rammer 185.

Resultaterne svarer nogenlunde til tidligere resultater, der har vist et beregnet udviklingsminimum på mellem 3,2 til 7˚C og et krav til antallet af daggrader på mellem 150 til 240.

Konklusion

Resultaterne fra dette forsøg viser, at temperaturen, efter rapsjordloppens indflyvning i marken, har stor indflydelse på den potentielle angrebsgrad. Resultaterne giver også kvantificerbar viden om tre vigtige faktorer i forhold til en forbedret varsling af risikoen for skade: Længden af præ-ovipositions-perioden, den daglige æglægningsrate og udviklingstiden for æg ved konstante temperaturer.

Der foreligger i forvejen kun få dokumenterede resultater for rapsjordloppens æglægning til sammenligning, men et fællestræk for tidligere og disse nye resultater er udover en effekt af temperatur, stor variation i de forskellige æglægningsparametre også ved samme temperatur. I modsætning til tidligere undersøgelser fra Frankrig, som beskriver 8˚C som den ”optimale” temperatur for æglægning, peger de nye forsøg på 16˚C som temperaturen for den højeste æglægningskapacitet, både hvad angår antallet af æg lagt i alt og pr. dag samt hunnernes levetid.

Denne forskel skyldes en kortere levetid ved 16˚C i de tidligere undersøgelser og dermed kortere æglægningsperiode og et lavere antal æg totalt. Ved 16˚C var præovipositions-perioden 19 dage, antal æg per hun i alt og per dag henholdsvis 696 og 5 æg, og levetiden 175 dage.

Døgngennemsnitstemperaturer i september og oktober kan svinge lokalt, fra år til år og dagligt. Der kan forekomme år, særligt i september, med relative høje gennemsnitstemperaturer omkring 12 til 16˚C over en længere periode. Sådanne perioder vil være grundlaget for en høj æglægningsintensitet tidligt. For landet som helhed er normalen for september og oktober for perioden 2001 til 2010 beregnet til henholdsvis 13.8˚C og 9.4˚C. Til eksempel blev september og oktober 2014 registreret som varme måneder med døgngennemsnitstemperatur for landet som helhed på 14.6˚C i september og 12.1˚C i oktober.

En forbedret varsling med de nye resultater

Ved monitering med gule fangbakker kan billernes indflyvning i marken følges og i tilfælde hvor tærsklen for fældefangster bliver overskredet, kan gennemsnitstemperaturen over tid bruges til at beregne starttidspunktet og intensiteten for æglægning samt tidspunkt for larvernes fremkomst. I varme sensomre og efterår og ved temperaturer på 16˚C og derover, er der gode betingelser for æglægning. Dette er grundlaget for et stort antal larver tidligt med efterfølgende høj risiko for en høj larvedensitet pr. plante i efteråret og planteskade det efterfølgende forår. En opgørelse af antallet af larver pr. plante i efteråret kan yderligere bekræfte en potentiel høj angrebsgrad.

Med denne nye viden er det muligt at forudsige en høj risiko for skade på et tidligere tidspunkt i efteråret fremfor først at opdage skaden i foråret, når denne er synlig. Samtidig vil en beslutning om en eventuel behandling kunne træffes på et forbedret grundlag, fremfor udelukkende på den noget usikre baggrund om fældefangster alene. Beslutningen kan endvidere støttes op af en opgørelse af antallet af larver pr. plante i efteråret.

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Mathiasen H. (2015): Hvordan klarer rapsjordloppens larver lave vinter temperaturer

Hvordan klarer rapsjordloppens larver lave vinter temperaturer?

Sammendrag

Både temperatur, eksponeringstid og akklimatisering til kulde påvirkede larvernes overlevelsesevne ved lave temperaturer. Larverne var i stand til at overleve længere tid ved -5˚, hvorimod eksponering for -10˚C var betydelig mere skadelig. Larverne havde en højere overlevelsesevne, hvis de var kulde akklimatiserede. Tiden indtil 50% (LT_{50}) af henholdsvis akklimatiserede og ikke-akklimatiserede larver døde ved -5˚C blev beregnet til 9.6 og 7.4 **dage**. LT_{50} for henholdsvis akklimatiserede og ikke-akklimatiserede larver ved -10˚C blev beregnet til 70.5 og 32.6 **timer** eller ca. 3 og 1.3 dage. Temperatur data fra to rapsdyrkende områder i Danmark fra 1990 til 2013 viste, at et tilstrækkeligt antal sammenhængende dage, med temperaturer til at forårsage høj larvedødelighed, sjældent forekommer.

Larvernes tolerance overfor kulde

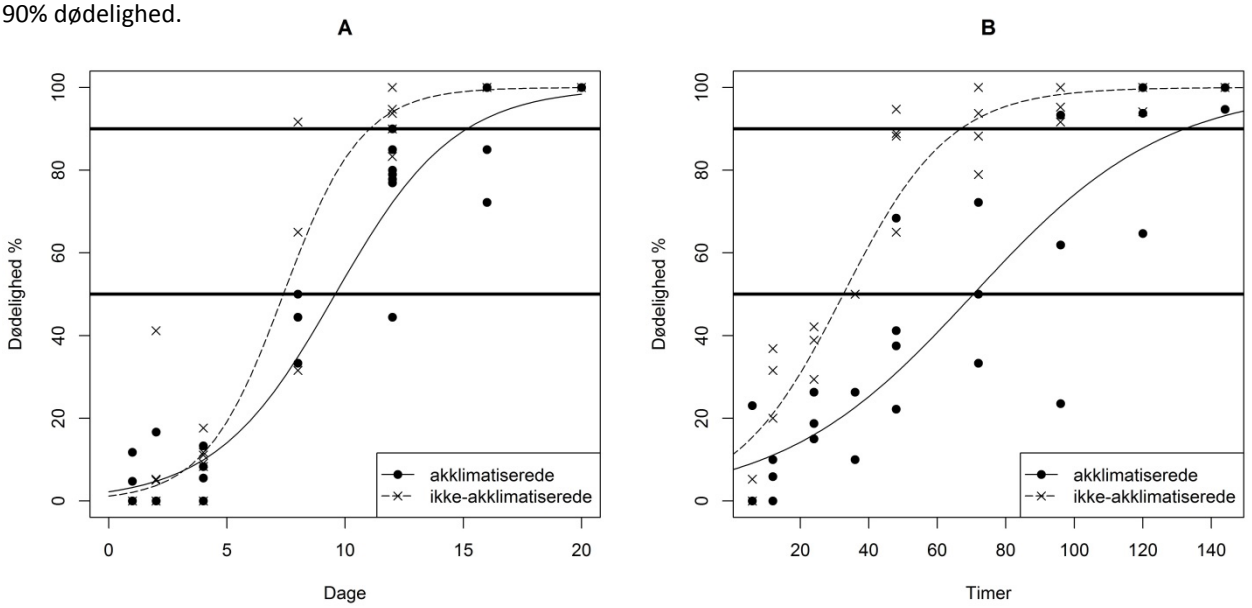
Et erhvervs-Ph.D. projekt startet i 2011 har haft fokus på variationen i forekomsten af rapsjordlopper mellem år og de mulige årsager til denne variation. Temperaturforholdene i efteråret og vinteren samt naturlige fjender er tidligere blevet beskrevet som hoved årsager. Projektet har haft fokus på temperaturforholds indvirkning på æglægning i efteråret og larvedødeligheden gennem vinteren. Temperaturforholdene efter billernes indflyvning i marken påvirker starten, det videre forløb og intensiteten af æglægningen med milde efterår som særligt gunstige for en opformering af bestanden. Vintertemperaturerne påvirker larvernes overlevelse gennem vinteren og dermed bestandens størrelse fremadrettet. Insekter er stand til at tolerere lave vintertemperaturer i forskellig grad. Deres evne til at overleve lave temperaturer kan bl.a. afhænge af, hvorvidt de er blevet akklimatiseret til kulde, deres alder og evt. stadium. Kendskab til rapsjordloppens kuldetolerance mangler stort set, men der er tidligere observeret et fald i bestanden efter hårde vintre. Denne orientering handler om resultaterne fra et forsøg om larvernes tolerance overfor lave vintertemperaturer.

I projektet blev to uger gamle larver eksponeret for -5 og -10˚C i forskellige tidsintervaller og derefter blev dødeligheden opgjort. Larverne havde forud for forsøget været opbevaret ved først 12˚C i en uge og derefter henholdsvis 0 og 12˚C i en uge svarende til de blev akklimatiserede og ikke akklimatiserede til kulde. Opgørelserne af dødelighed over tid blev brugt til at beregne tiden indtil 50% (LT_{50}) eller 90% (LT_{90}) af larverne forventes at dø ved de forskellige temperaturer. Vinter temperaturer fra to områder, Flakkebjerg og Billund, fra 1990-2013 blev brugt til at vurdere vigtigheden af vinterdødelighed af larver for bestandens størrelse.

På figuren ses stigningen i larvedødelighed over tid ved -5˚C (A) og -10˚C (B). Af figuren fremgår det, at larverne var bedre i stand til at tåle -5 end -10˚C. Dødeligheden ved -5˚ var forholdsvis lav i op til 4 dage, 40-63% efter 8 dage og nåede op på 100% efter 16-20 dage. Dødeligheden indtraf

tidligere ved -10˚C. Efter 24 timer lå dødeligheden på 20-43% og efter 144 timer (6 dage) på omkring 100%.

Figur 1 Larvedødelighed for rapsjordloppen: Gennemsnitlig observerede (punkter) og beregnede (linjer) dødelighedsforløb (%) for akklimatiserede (● -) og ikke akklimatiserede (× - -) larver. Larverne blev udsat for -5˚C i 1, 2, 4, 8, 12, 16 and 20 dage og -10˚C i 6, 12, 24, 36, 48, 72, 96, 120 and 144 timer. De vandrette linjer markerer 50% and 90% dødelighed.



Tilvænning eller akklimatisering til kulde forbedrede larvernes overlevelsesevne ved både -5 og -10˚C og særligt ved -10˚C (tabel 1). En larvedødelighed på 50 og 90% vil indtræffe efter omkring henholdsvis 9 og 15 sammenhængende dage ved -5˚C eller 3 og 5 dage ved -10˚.

Temperatur ˚C	LT_{50} AK	LT_{50} non-AK	LT_{90} AK	LT_{90} non-AK
-5	9.58 dage	7.38 dage	11.03 dage	15.14 dage
-10	70.51 timer	32.60 timer	132.23 timer	66.84 timer

Tabel 3: Den beregnede tid indtil 50 og 90% af larver var forventet døde for akklimatiserede (AK) og ikke akklimatiserede (non-AK) larver. Larverne blev udsat for -5˚C i 1, 2, 4, 8, 12, 16 and 20 dage og -10˚C i 6, 12, 24, 36, 48, 72, 96, 120 and 144 timer.

En gennemgang af vintertemperaturer fra 1990-2013 ved Flakkebjerg og Billund peger kun på en vinter (2011-2012 i Billund), hvor larvedødeligheden ville nå op på 50 og 90%. Hvis dage med -3˚C skulle vise sig tilsvarende skadelig i en sammenhæng med dage med -5˚, ville der være tre vintre ved Flakkebjerg og yderligere tre vintre ved Billund med høj larvedødelighed.

Konklusion

Rapsjordloppens larver er forholdsvis godt rustet til at klare den danske vinter de fleste år. Der kan dog forekomme vintre med høj larvedødelighed, særligt hvis -3˚C har samme effekt på dødeligheden som -5˚C. Forsøg med flere lave temperaturer kræves for at kunne dokumentere dette. Vi har undersøgt dødeligheden over tid. For at få et bredere kendskab til larvernes kulde tolerance, vil det også være relevant at undersøge den nedre temperatur grænse for larvernes overlevelse, da vinter temperaturerne i DK midlertidigt kan ligge en del lavere end -10˚C. Et bredt kendskab til larvernes kulde tolerance vil kunne bruges til at identificere år med høj dødelighed og nedgang i bestanden. I en praktisk sammenhæng vil dette kunne bruges i en prognose for det potentielle skadebillede den efterfølgende sæson.

Vinter temperaturer kan altså være en medvirkende men ikke en hoved faktor bag nedgange i bestanden. Vi har som en del af projektet vist, at temperaturforholdene i efteråret har stor betydning for æglægningsintensiteten og dermed bestandens størrelse. Tilbage er spørgsmålet om de naturlige fjenders betydning for nedgange i bestanden. Rapsjordloppens larver parasiteres af den specialiseret snyltehveps *Tersilochus microgaster* i foråret, men dette er ikke blevet undersøgt i DK.

Communication letters to be uploaded to SEGES, Landbrugsinfo

Mathiasen H. (2015): Monitering af rapsjordloppen; kan opgørelse af den tidlige bladskade sige noget om antallet af biller i marken

Monitering af rapsjordloppen; kan opgørelse af den tidlige bladskade sige noget om antallet af biller i marken?

Sammendrag

Burforsøg i marken med 2-16 biller per 24 planter i hvert bur har vist, at den tidlige bladskade ikke synes at være brugbar som en indirekte monitering af antallet af biller. Der blev ikke en vist en direkte sammenhæng mellem den tidlige bladskade opgjort som antal planter med skade eller antal bladgnav og billetætheden. Der var en lille effekt af billetætheden på antallet af blade med skade. Stigningen var dog så lille, at den må siges at være ubetydelig. Der var derimod en sammenhæng mellem billetætheden og antallet af larver/plante senere på året.

Monitering af rapsjordloppen

Erhvervs-Ph.D. projektet *Biological aspect for forecasting of the Cabbage stem flea bettle* kom i værk bl.a. pga. usikkerheden i moniteringen af rapsjordloppen med gule fangbakker og problematikken omkring at basere varslingen udelukkende på denne. Som en del af projektet blev en opgørelse af den tidlige bladskade testet og vurderet som en alternativ moniteringsmetode til at bestemme antallet af biller i marken.

Monitering af den tidlige bladskade blev vurderet ved at undersøge sammenhængen mellem antallet af biller og afgrødeskaden opgjort på tre niveauer; antal planter med skade, blade med skade og antal bladgnav. Sammenhængen mellem antallet af biller og larver pr. plante blev også undersøgt. Opgørelser blev foretaget i bure med 24 rapsplanter og forskellige antal biller (1, 2, 4 og 8 par), som skulle repræsentere en angrebsgrad fra lav til høj.

Der var ikke en direkte sammenhæng mellem antallet af biller og afgrødeskaden i form af antallet af planter med skade eller antal bladgnav. Der var en effekt men lille stigning (3.3%) i antallet af blade med skade for hver ekstra bille. Antallet af biller havde en effekt på antallet af larver pr. plante (tabel 1).

Antallet af biller	Larver/plante (gennemsnit ± standardafvigelsen)
2	0.15 ± 0.66
4	0.38 ± 0.99
8	0.87 ± 1.59
16	1.42 ± 1.90

Tabel 1: Gennemsnitlige antal larver pr. plante (±standardafvigelsen). Antallet af larver blev opgjort en gang i 12 ud 24 planter.

Konklusion

Både afgrødeskaden og antallet af larver pr. plante forblev på et lavt niveau. Det betød, at de undersøgte billetætheder ikke repræsenterede angrebsgrader op til et højt niveau. For bedre at kunne vurdere monitering af den tidlige bladskade, kræves der altså et større interval i antallet af biller, der bliver undersøgt. Det højeste antal biller (8 par) resulterede i et gennemsnit antal larver pr. plante på 1.42 og omregnet resulterede en hun i 0.2 larver pr. plante. Til sammenligning er tærsklen for behandling i England 2 larver pr. plante i efteråret. For at opnå en larvetæthed på omkring 5 pr. plante vil det, ifølge resultaterne kræve at det højeste antal biller ligger på 25 par (25 hunner), og det vil derfor give mening at anvende en skala fra 2 til 25 bille par i et tilsvarende forsøg.

Ud fra disse første resultater er monitering af den tidlige bladskade ikke et brugbart alternativ til monitering med gule fangbakker. Monitering med gule fangbakker er umiddelbart den mulighed, der findes og den er absolut bedre end ingenting. Denne metode kan med sikkerhed bruges til at bestemme tidspunktet for rapsjordloppens indflyvning i marken. Der er dog en væsentlig usikkerhed forbundet med at bestemme angrebsgraden af rapsjordlopper i marken ud fra fældefangster, da der ikke altid er en direkte sammenhæng mellem antallet af fældefangster i efteråret og antallet af larver pr. plante senere på året. Tærsklen er bestemt i tidligere tyske forsøg ud fra en vurdering af sammenhængen mellem fældefangster og det efterfølgende antal larver pr. plante. Da der ikke altid er en sammenhæng, skal tærsklen fortolkes som en negativ eller positiv prognose for potential skade ud fra fangster henholdsvis under eller over tærsklen. Der vil derfor altid være en potentiel risiko for skade trods en negativ prognose. Det sikreste ville til hver en tid være at opgøre antallet af larver pr. plante sent i efteråret. Det er dem, der forårsager skaden og det er deres antal, vi gerne vil kende. Denne metode kræver desværre enten megen tid eller udgifter til plantedissekering og er derfor ikke så anvendt i praksis.