A regional view of an impending invasion: western corn rootworm development in Northern Germany

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Summary

We examined the invasive maize pest western corn rootworm (*Diabrotica virgifera virgifera* LeConte; Chrysomelidae, Galerucinae; WCR) under Northern German temperature regimes. The experiments were conducted under quarantine conditions in climatic cabinets. Aim of the study was an improved knowledge of western corn rootworm biological key data under regional conditions which were necessary for an adequate integrated pest management strategy.

1. The influence of a delayed sowing date under recent temperature regime and a warmed temperature regime was studied:

- The temperature regime did not influence hatching rates.
- The time of mean hatch was accelerated from JD 195 under the temperature regime of today to JD 177 under the warmed regime.
- Sowing date did not affect development under the temperature regime of today.
- Under the warmed regime larval development was retarded at the late sown plants.

2. We evaluated the effects of varying day-night (4°C and 6°C difference) temperature regimes compared to the equivalent constant temperature regime on hatch, development and recovery of WCR larvae:

- Varying day-night temperatures resulted in earlier larval hatch and accelerated larval development.
- This effect was fortified by a temperature difference of 6°C compared to the 4°C-difference regime.
- Hatching rate and larval recovery were not affected by the different temperature regimes.

3. We compared two different European western corn rootworm field populations from Serbia and Hungary with the US non-diapausing laboratory population:

- There was a slight higher thermal requirement for hatching of the Hungarian and Serbian populations compared to the laboratory population.
- The Hungarian population showed highest thermal requirements for hatch.

There was a strong positive correlation between plant height and larval performance respectively, plant height and larval recovery in all experiments.

General Introduction

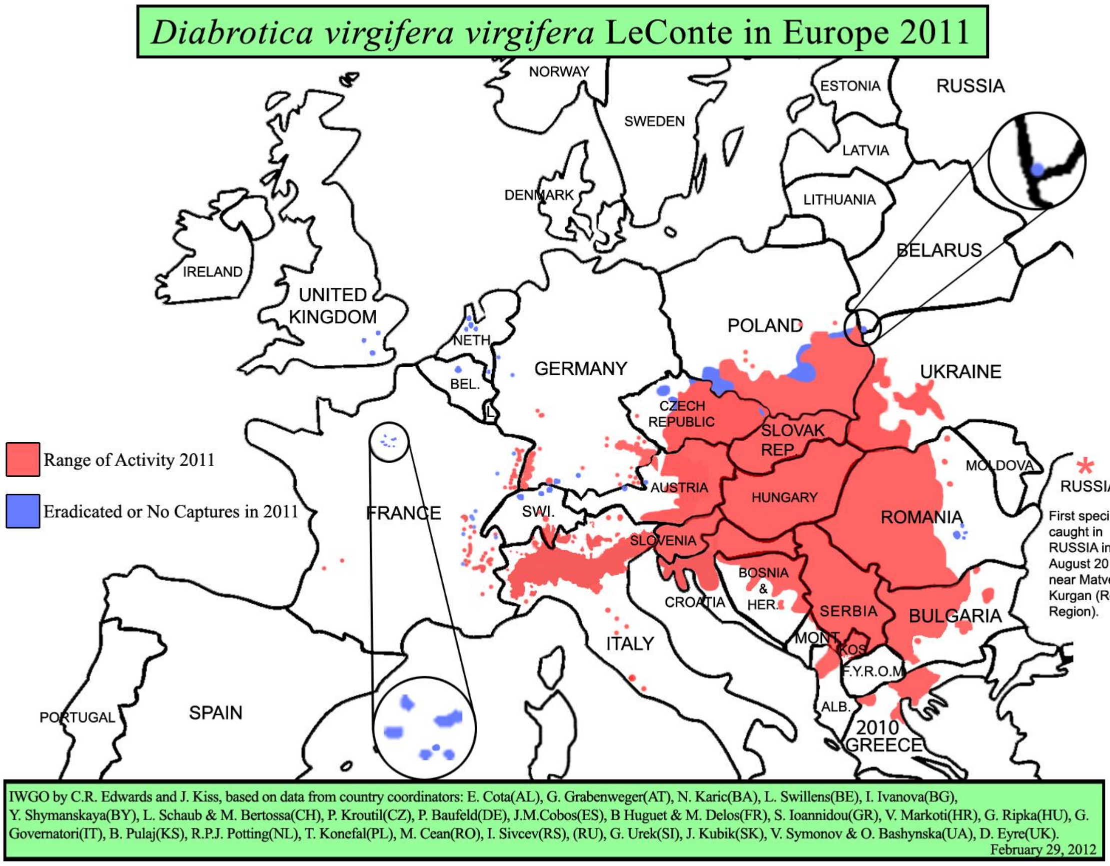
“Nothing endures but change.” – Heraclitus (535 BC - 475 BC)

It is a basic necessity for scientists and farmers to face new circumstances and adapt to new challenges like climate change and invasive species, which are considered to be gravely threads of biodiversity, ecosystem functions and agriculture (Pimentel, Zuniga, & Morrison 2005; Vidal, Kuhlmann, & Edwards 2005; Ziska *et al.* 2010).

It is challenging to make reliable predictions about climate change, because of technical and political uncertainties which caused different gas emission scenarios and the statistical bias of different models (Allen *et al.* 2000; Stainforth *et al.* 2005). Climate change has various direct and indirect effects on ecosystems and organisms, hence also on the relationship of organisms to each other (Dewar & Watt 1992; Buse & Good 1996; Cannon 1998; Stange & Ayres 2010) by elevated atmospheric CO_2-concentration (Johnson *et al.* 2011), changed precipitation and a general global warming by 0.6°C in the last 100 years (Walther *et al.* 2002). A close examination of climate change impact on species interaction like plant-pollinator or plant-pest synchrony is necessary (Tylianakis *et al.* 2008). A warming of the global mean surface by a temperature of 1.4-5.8 °C is assumed from the year 1990 till 2100 (Houghton *et al.* 2001) or 1.1-6.4°C till the end of 21st century (IPCC & I 2007). Climate change is supposed to lead to a higher productivity of temperate agriculture through longer growing seasons, but also to the worsening of many pests (Porter, Parry, & Carter 1991; Cannon 1998). Even if climate change is a global phenomenon, adaptations to its impacts have to proceed on a low spatial scale (Gregory *et al.* 2009; Rannow *et al.* 2010). Climatic conditions are the most important determinants of the distributions and abundance of poikilothermic species, therefore climate change leads to wide range shifts of several species (Walther *et al.* 2002; Parmesan & Yohe 2003; Thomas *et al.* 2004). Also the establishment and success of invasive species are likely to be affected by climate change (Mooney & Hobbs 2000; Ziska *et al.* 2010). An invasive species is defined as “a non-native species whose introduction does or is likely to cause economic or environmental harm or harm to human, animal, or plant health” (National Invasive Species Council 2006). Invasive crop pests cause high economic damage by yield loss and control costs: alone in the US economic losses by invasive pests sum up to almost $ 120 billion per year

(Pimentel *et al.* 2008). Most successful invaders have a high abundance and big native distribution range (Williamson & Fitter 1996).

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Galerucinae, Chrysomelidae; WCR) is a maize pest native to Central America (Melhus, Painter, & Smith 1954) which has spread over North America from the mid of 19th century till today (Gray *et al.* 2009). In the late seventies or early eighties of the 20th century it was accidentally introduced from North America into Serbia (Szalai *et al.* 2010), where it was detected in 1992 near the Belgrade airport (Gray *et al.* 2009). In 2010 this quarantine pest has already been reported from more than 20 European countries (EPPO 2011) (Figure 1). When the WCR has established in most maize growing regions in Europe, damage costs of more than 470 million Euros per year are predicted (Wesseler & Fall 2010). The distribution situation of the year 2011 is pictured in Figure 1.



Diabrotica virgifera virgifera LeConte in Europe 2011

IWGO by C.R. Edwards and J. Kiss, based on data from country coordinators: E. Cota(AL), G. Grabenweger(AT), N. Karic(BA), L. Swillens(BE), I. Ivanova(BG), Y. Shymanskaya(BY), L. Schaub & M. Bertossa(CH), P. Kroutil(CZ), P. Baufeld(DE), J.M.Cobos(ES), B Huguet & M. Delos(FR), S. Ioannidou(GR), V. Markoti(HR), G. Ripka(HU), G. Governatori(IT), B. Pulaj(KS), R.P.J. Potting(NL), T. Konefal(PL), M. Cean(RO), I. Sivcev(RS), (RU), G. Urek(SI), J. Kubik(SK), V. Symonov & O. Bashynska(UA), D. Eyre(UK).
February 29, 2012

Figure 1: western corn rootworm distribution in Europe 2011 monitored with pheromone traps (Edwards & Kiss 2012).

The first WCR was detected in 2007 in Germany (Gray *et al.* 2009), despite extensive containment and eradication measures WCR could establish in Southern Germany and still spread northward. WCR future spread and distribution is not assumed to be limited by the German climate (Baufeld, Enzian, & Motte 1996), but highly influenced by cropping practices. Western corn rootworm females lay their eggs in autumn mainly in maize fields. The eggs overwinter in the soil in diapause which ends in spring at a threshold temperature of 11°C (Wilde 1971). Larvae hatch and infest the host plant roots. Most damage is caused by the root feeding of the three larval stages, which upsets nutrient and water uptake and lowers plant stability (Kahler *et al.* 1985; Oleson *et al.* 2005; Riedell 1990; Spike & Tollefson 1989). The most effective management strategy of the WCR is crop rotation (Spencer *et al.* 2009), even though the larvae are also able to survive on some alternative host plants (Moeser & Vidal 2004). Figure 2 shows the areas in Germany with a high percentage of continuous maize in crop rotation, these areas have a high risk of permanent establishment and heavy infestations by the WCR (Schaafsma, Baufeld, & Ellis 1999).

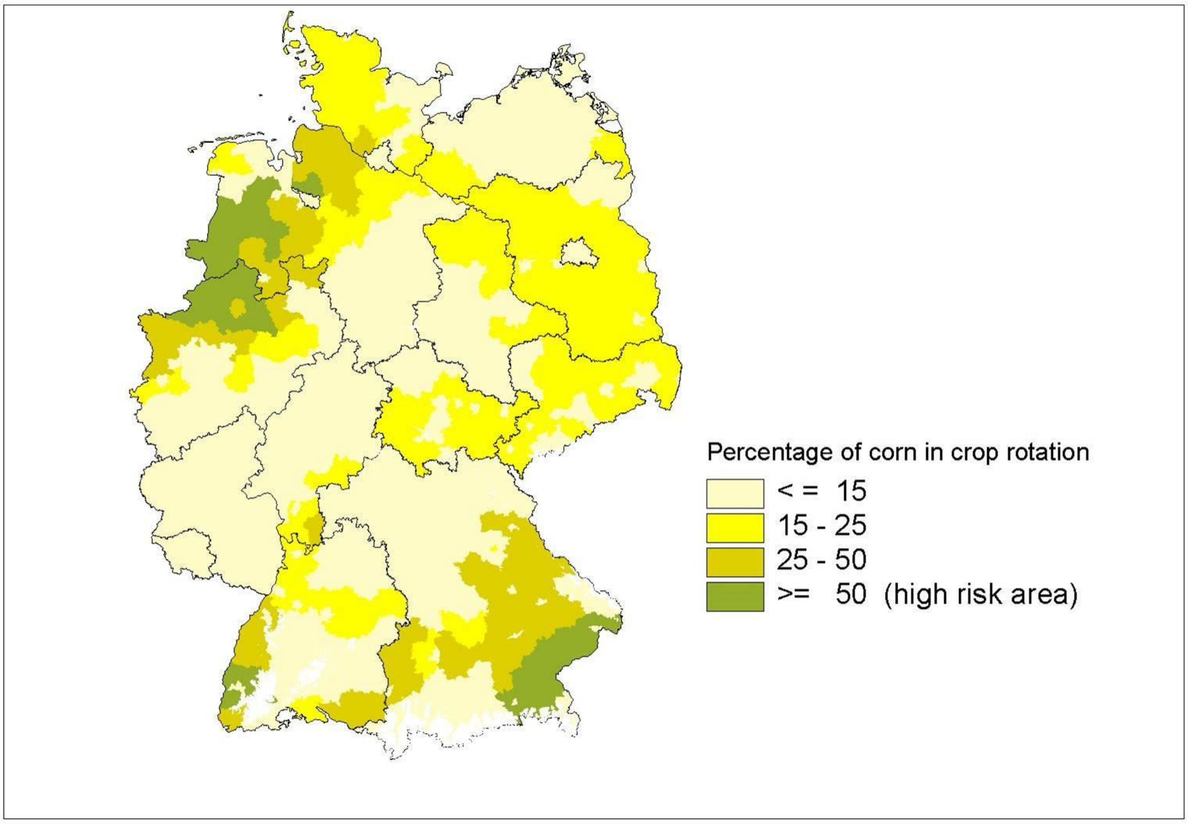


Figure 2: percentage of maize in the crop rotation in Germany (Schaafsma, Baufeld, & Ellis 1999)

WCR shows a high adaptability to pest management strategies, with one population even adapting to crop rotation when maize is planted after soybeans (Levine *et al.* 2002) or also showing locally evolved resistance to some insecticides (Meinke *et al.* 1998; Wright *et al.* 2000) and one cultivar of Bt maize (Gassmann *et al.* 2011). For this reason an integrated pest management (IPM) strategy against WCR is needed for prospective sustainable maize cropping in Europe (Gray 2010). Effective IPM strategies for agricultural pests need the detailed knowledge of key data from the ecology and biology of pest species, the host and their interaction (Awmack & Leather 2002; Spencer *et al.* 2009). Development of poikilotherms like insects and plants is highly temperature dependent (Beck 1983; Behrens *et al.* 1983; Liu, Zhang, & Zhu 1995; Bergant & Trdan 2006). There are species specific requirements for the amount of thermal energy (thermal constant) needed to accomplish different life stages (Damos & Savopoulou-Soultani 2012). Day-degrees and the thermal thresholds for development are reliable predictors for key data of poikilotherms like dormancy, insect egg hatch, budburst or plant flowering at constant temperatures. In most terrestric habitats varying day/night temperatures are pronounced and constant temperature regimes, typically set-up in laboratory tests, are thus unlikely to mimic the influences the insects are exposed to under field conditions (Howe 1967). The WCR itself has adapted well to his invaded areas. The threshold of development and needed thermal energy for development differs slightly between geographical separated populations (Wilde 1971; Wilde *et al.* 1972; Chiang 1973; Ruppel, Russell, & Jennings 1978; Fisher 1989; Levine, Oloumi-Sadeghi, & Ellis 1992; Davis, Brenes, & Allee 1996). Genetic differences between the US populations are low, but positive correlated with geographic distance (Kim & Sappington 2005). In consideration of the at least 5 independent WCR introductions from North America into Europe (Ciosi *et al.* 2008) it is very likely that European populations were introduced from different source populations with different thermal requirements and adaptations to management measures (Sappington, Siegfried, & Guillemaud 2006).

Objectives

In our study we analysed the western corn rootworm hatch, larval development and recovery under a Northern German temperature regime.

1. Does sowing date influence western corn rootworm development in Northern Germany under today's temperature regime or global warming?

In this experiment the influence of a moderate delay of the maize sowing date under simulated a) temperature regime of today and b) a changed temperature regime with elevated spring and summer temperatures by 2°C in Northern Germany was examined. Specifically addressing the questions:

- Does a delaying sowing date under the temperature regime of today influence WCR development and recovery by disrupting host-pest synchrony?
- Is this influence enhanced under the elevated temperature regime by the combined effects of an earlier start of development and a delayed sowing of maize?

2. Western corn rootworm development under constant and varying temperature regimes

We analysed the effect of varying day-night temperatures with 4°C difference, respectively 6°C difference compared with the adequate mean temperature:

- Do varying ambient temperatures influence WCR development and recovery in comparison to the adequate mean temperature?

3. Development of two different European western corn rootworm populations compared with the US non-diapausing laboratory population under a Northern German temperature regime

We tested for developmental differences between the US non-diapausing laboratory population and two European field populations from Serbia and Hungary:

- Are there differences between the populations with regard to their hatching patterns, development and recovery?
- Is the US-non diapausing laboratory population an adequate organism to study WCR development in Europe?

Does sowing date influence western corn rootworm (*Diabrotica virgifera virgifera* LeConte) development in Northern Germany under today's temperature regime or global warming?

Abstract

Adaptation to climate change and invasive species is an essential challenge for research. We tested the influence of two different maize sowing dates on JD 121 and JD 135 under two temperature regimes on the development and recovery of the invasive maize pest western corn rootworm in climate cabinets. We used a recent temperature regime from Northern Germany and the "climate change" regime which was increased by 2°C to simulate an earlier start of egg development in spring. The experiment started with the threshold temperature of western corn rootworm development at 11°C, which was reached at JD 121 at the first sowing date for the temperature regime of today and at JD 107 under the change regime. We assumed an effect on plant-pest synchrony by delayed sowing and a fortification of this effect under the increased temperature regime. Larval hatch did not differ between the temperature regimes with regard to the temperature sum necessary for hatch and the hatching rates. Due to the earlier start of egg development under the change regime the mean hatch occurred earlier (JD 177) compared to today's regime (JD 195). Under the recent temperature regime a delay of the sowing date did not affect larval development, but under the change regime larval development was retarded at the late sown plants. We assumed that early hatched larvae died by starvation on these plants. There was a strong positive correlation between plant height and larval performance and larval recovery in our experiment.

Introduction

The world around us changes. Scientists and farmers have to face new circumstances like climate change and invasive species (Ziska *et al.* 2010). On one hand a higher productivity of temperate agriculture by longer growing seasons is assumed, on the other hand the prediction of the worsening of many pests by climate change (Cannon 1998). Climatic conditions are the most important determinants of the distribution and abundance of poikilothermic species (Walther *et al.* 2002). In agricultural ecosystems, weather affects crop yield and quality as well as the dynamics of pests and their regulation by natural enemies. Today, there is little doubt that the climate changes, but it is challenging to make reliable predictions because of technical and political uncertainties which resulted in different gas emission scenarios and the statistical bias of different models (Stainforth *et al.* 2005). Even if climate change is a global phenomenon, adaptations to its impacts have to proceed on a small spatial scale (Gregory *et al.* 2009; Rannow *et al.* 2010).

But although if it is not known in detail how the climate will change, we can investigate how discrete factors would affect key data of the biology of organisms to give the possibility to model the influence when these factors changes. Effective integrated management strategies for agricultural pests are predicated on the detailed knowledge of key data from the pest species, the host and their interaction (Awmack & Leather 2002). An increase in temperature would lead to complex changes in species specific insect life-history traits and phenology (Deutsch *et al.* 2008; Musolin *et al.* 2010). While some studies predict distribution shifts for species like for *Ostrinia nubilalis* (Porter *et al.* 1991), an earlier pest activity by global warming such as for the cabbage root fly *Delia radicum* (Collier *et al.* 1991), or the faster development of the spittlebug (*Neophilaenus lineatus*) triggered by an earlier hatch (Whittaker & Tribe 1996), other studies deal with indirect climate effects by the interaction with the host-plant (Buse & Good 1996; Cannon 1998; Dewar & Watt 1992; Parmesan 2007; Stange & Ayres 2010). Under natural conditions synchrony of plant and pest development would be preserved when elevated temperatures proceed similarly for both. The expected temperature effect on larval survival of the winter moth (*Operophtera brumata*) (Dewar & Watt 1992) could not be verified in experiments, as there was no influence of elevated temperatures on the synchrony of the winter moth and budburst in oaks (Buse & Good 1996). In agricultural systems the synchrony of host plant and pest can be disrupted by human determination of the start of plant growing.

Subject of our study were the immature stages of western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Galerucinae, Chrysomelidae; WCR). WCR is an invasive quarantine maize pest native to Central America (Melhus *et al.* 1954). In the late seventies or early eighties of the 20th century it was accidentally introduced from North America to Serbia (Szalai *et al.* 2010), where it was detected in 1992 near the Belgrade airport (Gray et al. 2009). In 2010 this pest has already been reported from more than 20 European countries (EPPO 2011). During the last 20 years at least 5 independent introductions into Europe took place (Ciosi *et al.* 2008). In case of an establishment of this pest in most maize growing regions in Europe damage costs are expected to sum up to more than 470 million Euro per year (Wesseler & Fall 2010).

Western corn rootworm females lay their eggs in the maize field in autumn, rarely in other crops (Shaw *et al.* 1978). The eggs overwinter in the soil in diapause which ends in spring at a threshold temperature of 11°C (Wilde 1971). Larvae hatch and infest the host plant roots. Most damage is caused by the root feeding of larvae, which disrupts nutrient and water uptake and lowers plant stability (Kahler *et al.* 1985; Riedell 1990).

Even if WCR distribution is not assumed to be limited by German climate (Aragón & Lobo 2012; Baufeld *et al.* 1996), effects of climate change on WCR development, survival and damage potential are expected by more favourable conditions.

In this study we analysed the combined effects of an earlier start of development and a delayed sowing on the synchrony of WCR development and host phenology. In the past field studies in North America were conducted to examine the influence of varying planting dates or egg application dates on plant damage (Branson & Sutter 1986; Hibbard *et al.* 1999; Hibbard *et al.* 2008), however with contradicting results. While Branson and Sutter (1986) found higher damage ratings when the plants were infested with WCR after their emergence than on their planting date, Hibbard *et al.* (1999) detected heavier root damages when the plants were infested at the date of sowing instead of an infestation at the 2- or 5-leaf stage of maize. Hibbard *et al.* (1999) assumed this effect due to uncommon high experimental temperatures in their field. Bergman & Turpin (1984) and Musick *et al.* (1980) found that larval survival and larval development of WCR depend on the availability of maize roots at the hatching date of early hatched larvae. This resulted in changes of population dynamics and seasonal occurrence of life stages in the field occurred by a delay of the sowing date. In contrast, Fisher *et al.* (1990) did not find any influence of the sowing date on WCR immature stages or adult occurrence.

In this experiment the influence of a moderate delay of the maize sowing date under simulated a) today’s temperature regime and b) a changed temperature regime with elevated spring and summer temperatures by 2°C in Northern Germany was examined. We hypothesized that a late sowing date leads to a (i) retarded larval development and (ii) a diminished larval recovery of WCR and that these effects are enhanced under the change temperature regime.

Material and Methods

The experiment proceeded in section a) with a simulated temperature regime of today (1971-2000) and section b) with a global warming scenario with a by 2°C elevated temperature regime (change regime). Each section of the experiment started with egg application when the mean temperature of 11°C as a threshold for the development of WCR eggs (Wilde 1971) was reached. Therefore temperature simulation started a) at Julian Day (JD) 121 under the temperature regime of today and b) at JD 107 under the by 2°C changed temperature regime. Meteorological data were compiled by the DWD (Deutscher Wetterdienst), comprising the period from 1971 to 2000 at the location of Lingen. These data were used to calculate weekly adjusted mean temperatures in Northern Germany (Table 1). Lingen was chosen because of its location in a high risk area for establishment of high populations of WCR and thus a heavy damage potential (Schaafsma et al. 1991) because of the high frequencies of continuous maize. The experiment ended at a temperature sum of 370 day-degrees (D°) with a base temperature of 11°C after egg application. 13cm diameter pots were filled with Haplic Luvisol (sand/ silt/ clay-content: 3.0% / 55.2% / 41.8%; pH (H_2O) 7.6; C/N-ratio 15.8) from a field near Goettingen. The soil had been homogenized with a soil shredder (Unifix 300, Moeschle, Ortenberg, Germany). Eggs from the non-diapausing WCR laboratory population (USDA-ARS, North Central Agricultural Research Laboratory, Brookings, North Dakota, USA (Branson 1976)) were used in the experiment. The eggs were stored below 8°C until their use. They were washed with a sieve (250µm) from the soil and added to a sterile 0.15% agar solution. Near the brink of each pot 50 viable eggs were applied using a pipette (Multipipette Plus, Eppendorf, Hamburg, Germany) into the soil at a depth of 7cm. For each temperature regime 64 plants were sown (with 32 plants per sowing date). All maize seeds were pregerminated for 24h on sterile filter paper at 25°C and sown into 3cm depth into the middle of the pots at two different sowing dates, half at JD 121 the other half at JD 135. To monitor hatching rate and hatching date, 18 hatch tests for the temperature regime of today regime and 16 for the 2°C enhanced temperature regime were prepared. 1ml of the egg solution was added into Petri dishes with sterile moistened filter paper and the hatch tests were buried in pots with soil and reared under experimental conditions. The eggs were counted and daily checked for hatching when a temperature sum of 100 D° was reached. Relative humidity was adjusted to 60% ± 5% and the photoperiod was 14h:10h (light:darkness). The experiment was performed in climate cabinets (Mytron WB 750 KFL, Mytron Bio- und Solartechnik GmbH, Heiligenstadt, Germany) with by data logger (Voltcraft DL-120 TH, Conrad Electronic SE, Hirschau, Germany) controlled and adjusted weekly mean temperatures. At the end of the experiment plant height was measured and larvae were extracted from the soil using a high gradient Kempson device (Kempson et al. 1963) for 2 days at 60°C. Larvae were counted and head capsule width was measured using a binocular microscope with integrated object micrometer to determine larval instar (Hammack et al. 2003). Thereafter larvae were dried at 60°C for three days and dry weight was measured using a micro scale (MC5, Sartorius, Goettingen, Germany) with measuring accuracy of 0.001mg.

Data analysis

All statistical tests were performed using Statistica 10 (StatSoft®, Tulsa, USA).

All data were tested for the normal distribution with the Shapiro-Wilk-test and by visual observation of histograms. Levene's test was carried out to test the equality of variances. Required day-degrees (D°) (dependent variable) for hatching of the different temperature regimes (grouping variable) were tested for differences with Mann-Whitney U-test (MWU-test). The influence of the temperature regime (grouping variable) on hatching rate (dependent variable), respectively on Julian Day (JD) of hatch (dependent variable) was also calculated with the MWU-test.

To examine how the sowing date (grouping variable) influenced the WCR-larval development expressed as log transformed dry weight and headcapsule width (dependent variable) a Student's t-test for each temperature regime was performed. The effect of sowing date (grouping variable) on larval recovery (dependent variable) was tested with the MWU-test for both temperature regimes.

The effect of sowing date (grouping variable) on plant height at the extraction date (dependent variable) was tested with the MWU-test.

A simple regression was used to quantify the effect of plant height (predictor variable) on WCR development measured as log transformed dry weight (µg) and as headcapsule width (µm) (dependent variables). Effect of plant height (cm) as the predictor variable on recovery rate (%) as dependent variable was quantified with a simple regression.

Results

Temperature regime and western corn rootworm hatch

Temperature regimes did neither affect the required temperature sum (D°) for hatch (MWU-test: *P*=0.77, *U* = 135.00; *Z* = -0.29), nor the hatching rate (MWU-test: *P*=0.13, *U* = 99.00; *Z* = 1.54). 50% hatch occurred at a temperature sum of 290.2 D° under the today temperature regime, respectively at a temperature sum of 290.1 D° under the change regime. The Julian Day of 50% hatch accelerated from JD 195 under the temperature regime of today to JD 177 under the change regime (Table 2).

Sowing date and larval development

Under the temperature of today the sowing dates used in the experiment did not influence larval development measured as log transformed larval dry weight (Student's t-test: *P*=0.63, *t* (56) = -0.48, Figure 1A) or headcapsule width (Student's t-test: *P*=0.80, *t* (56) = 0.26).

Under the climate change temperature regime the late sowing date on JD 135 had a negative effect on larval development. The larvae of the early sown plants on JD 121 had a higher dry weight (Student's t-test: *P*=0.043, *t* (62) = 2.06, Figure 1B) and wider headcapsules (Student's t-test: *P*=0.006, *t* (62) = 2.88).

Sowing date and larval recovery rate

There were no differences between the recovery rates (%) of the two sowing dates under today's temperature regime (MWU-test: *P*=0.16, *U* = 407.00; *Z* = -1.40) and under the change regime (MWU-test: *P*=0.29, *U* = 432.00; *Z* = 1.07).

Sowing date and plant height

Under today's temperature regime sowing date did not affect plant height on the extraction date (MWU-test: *P*=0.152, *U* = 327.50; *Z* = -1.43; Figure 2A). On the change regime early sown plants were significantly higher than late sown plants (MWU-test: *P*=0.017, *U* = 334.50; *Z* = 2.38; Figure 2B).

Influence of plant height on larval development and recovery rate

Larval development measured as log larval dry weight and as headcapsule width was highly positively correlated with plant height on both temperature regimes. Regression equations are shown in Table 3.

Plant height was also positive correlated with recovery rate under the temperature regime of today ($P<0.01$; regression equation: $y = 48.2748 + 0.3751*x$; $R^2 = 0.19$) and the change regime ($P<0.01$; regression equation: $y = 40.7442 + 0.4053*x$; $R^2 = 0.26$).

Discussion

The different temperature regimes in our experiment did not affect larval hatch and development directly. Indeed, larval hatch occurred at JD 195 under today's regime compared to JD 177 at the change regime because of the earlier start of egg development, but there were no differences between the hatching rates detectable. The temperature sum necessary for 50% hatch was equal with 290.2 D° (today) and 295.1 D° (change) between the two temperature regimes: this was expected because the temperature sum is a very reliable predictor for certain developmental events under constant temperature regimes (Damos & Savopoulou-Soultani 2012). The temperature sum was also consistent to the 292 ± 69 D° Fisher (1989) observed in his laboratory experiment with constant temperatures and simulated field temperatures for 50% hatch.

At the temperature regime of today the sowing dates on JD 121 and 135 did not influence WCR larval development, but under the change regime larval development was retarded at the late sown plants. This influence of the sowing date at the change temperature regime was certainly an effect of the synchrony of larval hatch and plant phenology. At the extraction date there were no significant differences between plant height of early and late sown plants under today's temperature regime, because of the low simulated temperatures when the experiment started und the resulting small difference in the temperature sum between the two sowing dates (15.4 D° with a base of 11°C). Under the change regime early sown plants were slightly higher at the extraction date, compared with the plants planted at JD 135 (44.4 D° difference in temperature sum). The eggs received 50.4 D° at the change regime before the JD 135 plants were sown. The root biomass was not quantified in our experiment, but we assume that higher plants at the extraction date had a higher root biomass. Therefore a better food supply for the neonate larvae was provided as the plant heights at hatch and at the extraction date were highly correlated (data not shown). This assumption accords with the hypotheses by Branson *et al.* 1982 and Branson *et al.* 1983 that the availability of root biomass at the hatching date rather than the total root biomass, determines the survival of neonate larvae (Branson *et al.* 1982; Branson *et al.* 1983). Early hatched larvae on poor developed plants presumably died due to starvation because of the lack of sufficient root material (Bergman & Turpin 1984; Branson 1989; Hibbard *et al.* 1999; Musick *et al.* 1980) and the lack of preferred bigger newly grown nodal roots for older larvae (Hibbard *et al.* 2008).

Additionally well grown plants have a high root regrowth potential caused by a high photosynthetic biomass (Godfrey *et al.* 1993) which would lead to a high amount of suitable young roots for larval feeding. Food quality and quantity both are essential factors for herbivores (Awmack & Leather 2002). Not only the amount of ingested food, but also the quality such as nitrogen and root phytosterol content (Moeser & Vidal 2004) regulates WCR larval growth. Chiang (1973) reported that larvae preferred different root parts depending on their body size and age. Although we found that the larvae developed well an bigger plants, it can be expected that larval development on older plants would be retarded, due to the loss of nutritional value at aged plants (Chege *et al.* 2005) and the termination of root growth in plants at the generative growth stages (Robert *et al.* 2012).

We could not detect the negative effect of a late sowing on larval recovery other authors described for their field experiments (Bergman & Turpin 1984; Musick *et al*. 1980). However, these authors delayed the sowing up to early June for more than 6 weeks, not only two weeks, therefore our differences in plant growth were much lower. Well grown plants affected the recovery rate significantly positive. It is most likely that we would get similar results when we used these late planting dates, but such a late sowing would lead to considerable yield losses. On the other hand, the plant infestation rates by WCR larvae diminish at later sown plants (Chiang 1973). Another explanation that we found no significant differences in recovery between the treatments could be the low competition between the larvae because of the low infestation rates of only 50 eggs per plant (Branson *et al.* 1980). Whether plant damage tends to be positively or negatively affected by an early sowing has been and still is a topic in WCR research that results in conflictive discussions (Branson & Sutter 1986; Hibbard *et al.* 1999; Levine & Oloumi-Sadeghi 1991). Further investigation could be useful to find a balance between the disruption of plant-herbivore synchrony and yield losses under a changing climate with its predicted longer growing seasons (Cannon 1998). Overall with a later sowing of maize under a changing climate it can be presumed that more heat units for larval development accumulate before maize sowing, thus increasing larval mortality due to starvation.

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Annex

Tables

Table 1: weekly adjusted experimental mean temperature of today T_{today} (C°) calculated from historical (1971-2000) data from the DWD (Deutscher Wetterdienst) in Lingen and the by 2°C elevated temperature regime T_{change}

Julian Day	T_{today} (°C)	T_{change} (°C)
107 - 113		11
114 - 120		12
121 - 127	11.4	13.4
128 - 134	12.8	14.8
135 - 141	13.9	15.9
142 - 148	13.9	15.9
149 - 155	14.9	16.9
156 - 162	15.4	17.4
163 - 169	15.5	17.5
170 - 176	15.5	17.5
177 - 183	16.5	18.5
184 - 190	17.9	19.9
191 - 197	17.7	
198 - 204	17.2	
205 - 211	17.9	

Table 2: Julian day (JD) of 50% larval hatching under the temperature regime of Lingen calculated from 1971-2000 (today) and the by 2°C elevated temperature regime (change) (replicates = hatch tests: N_{totay} =18; N_{change} =16).

temperature regime	JD 50% hatch	N	JD min	JD max	SD
today	194.97	18	183.5	206.3	6.9
change	176.75	16	170.0	182.1	3.3

Table 3: Regression equations of plant height (cm) as predictor and log larval dry weight (µg) respectively headcapsule width (µm) as dependent variable at the temperature regime of today and the change regime

temperature regime	dependent Variable	y	*F*	*P*	*R²*
today	log larval dry weight	y=1.09 + 0.01*x	11.31	< 0.01	0.17
change	log larval dry weight	y=1.16 + 0.01*x	72.68	< 0.01	0.54
today	headcapsule width	y=175.96 + 1.85*x	16.70	< 0.01	0.23
change	headcapsule width	y=177.52 + 2.53*x	109.15	< 0.01	0.64

Figures

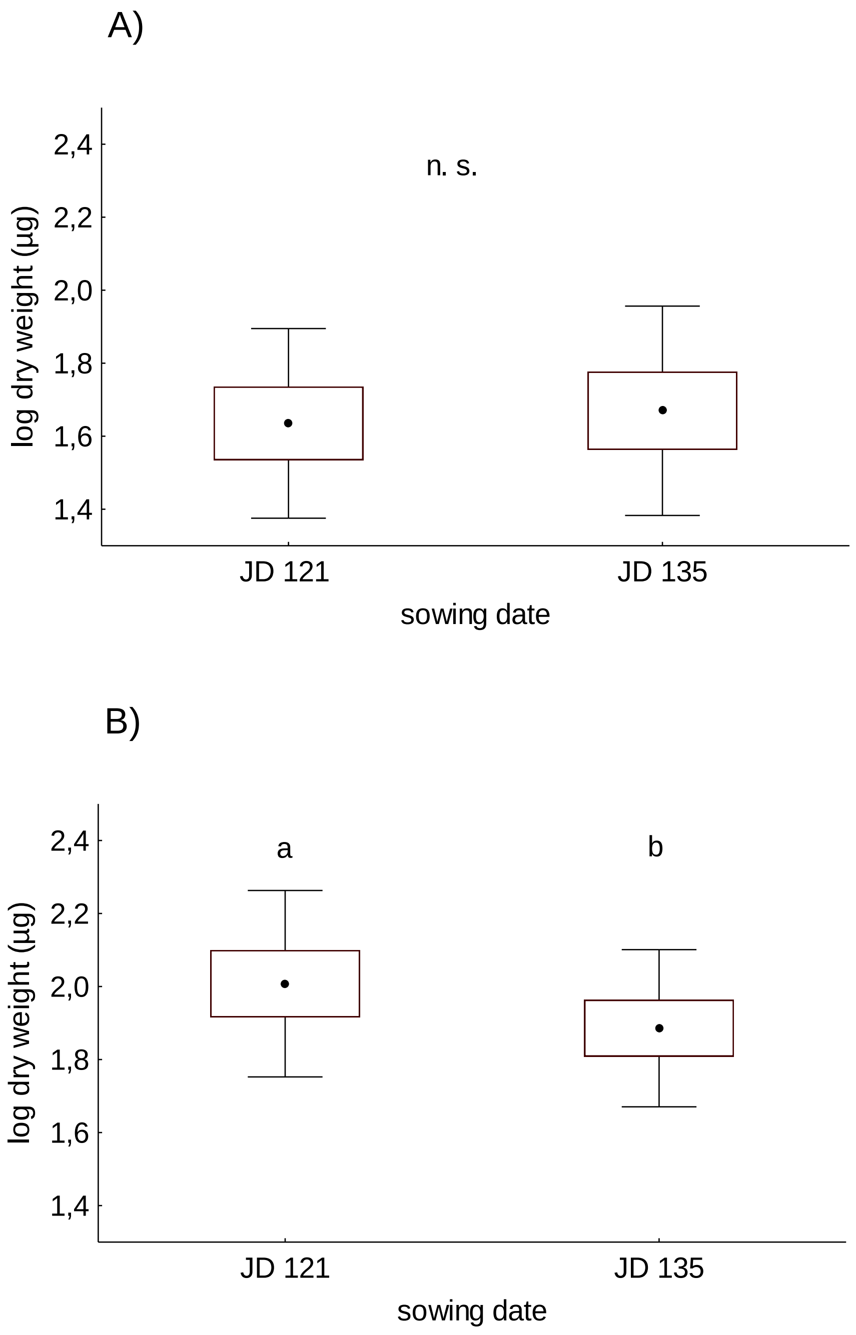

Figure 1: Influence of sowing date in Julian days (JD) on larval development measured as log larval dry weight (µg) at the temperature regime of today (A) and the change regime (B) (Student's t-test; dot = mean, box = mean ± 0.95 CI, whisker = mean ± SD; letters indicate significant statistical differences; n. s. = not significant: P > 0.05; replicates = plants: $N_{JD\ 121}$ =32; $N_{JD\ 135}$ =32 for each temperature regime).

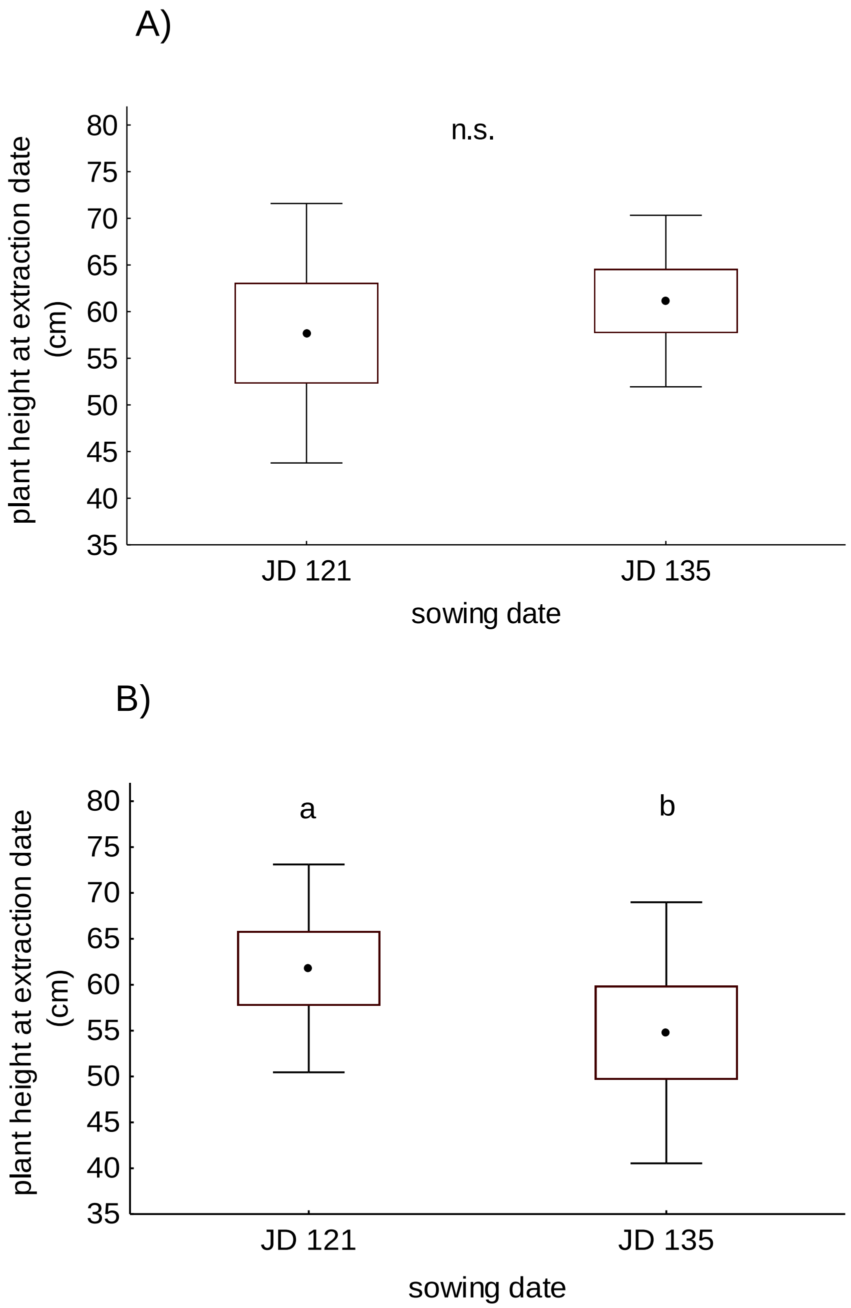

Figure 2: Influence of sowing date in Julian days (JD) on plant height (cm) at the temperature regime of today (A) and the change regime (B) (MWU-test; dot = mean, box = mean ± 0.95 CI, whisker = mean ± SD; letters indicate significant statistical differences; n. s. = not significant: P > 0.05; replicates = plants: $N_{JD\ 121}$ =32; $N_{JD\ 135}$ =32 for each temperature regime).

Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) development under constant and varying temperature regimes

Abstract

Forecasts of western corn rootworm (WCR) hatch and development in the field depend on models using experimentally determined day-degrees (D°). For constant temperature regimes this temperature sum is a reliable parameter to predict hatch and development of WCR larvae. In experiments in climate cabinets we evaluated the effects of varying day-night (4°C and 6°C difference) temperature regimes compared to equivalent the constant temperature regime on hatch, development and recovery of WCR larvae.

Varying day-night temperatures resulted in earlier larval hatch and accelerated larval development due to direct temperature effects like the rate summation (Kaufmann effect) and enhanced plant growth, with fastest development by the 6°C-difference regime. For western corn rootworm the temperature sum needed for hatch in the field are overestimated when they are determined by models based on constant experimental temperatures.

Hatching rates and recovery were not affected by temperature, but recovery was positively affected by plant height.

For herbivorous insects, such as WCR, plant growth effects induced by varying temperature regimes may result in increased variation in temperature based developmental parameters. These need to be taken into account to improve models on WCR hatch, development and optimal timing of control strategies especially for Northern German temperature conditions.

Introduction

Development of poikilotherms like insects and plants is highly temperature dependent (Beck 1983; Behrens *et al.* 1983; Liu, Zhang, & Zhu 1995; Bergant & Trdan 2006). There are species specific requirements for the amount of thermal energy (thermal constant) needed to accomplish different life stages (Damos & Savopoulou-Soultani 2012). This thermal energy is measured as sum of effective temperature, which is calculated as temperature range between lower threshold of development (no development below this temperature) and upper threshold of development (no development above this temperature) over a specific time (typically day: day-degrees (D°); Damos & Savopoulou-Soultani 2012).

Day-degrees and the thermal thresholds for development are reliable predictors for key data of poikilotherms like dormancy, insect egg hatch, budburst or plant flowering at constant temperatures. However, in most terrestric habitats varying day/night temperatures are pronounced and constant temperature regimes, typically set-up in laboratory tests, are thus unlikely to mimic the influences the insects are exposed to under field conditions. A review by (Howe 1967) has already pointed out these important shortcomings using constant temperature regimes. While for some species no detectable differences with regard to development at constant versus variable temperature regimes have been reported (Hagstrum & Leach 1973; Humpesch 1982) other species showed either retarded or accelerated development under varying temperatures (Hagstrum & Hagstrum 1970). These differences were dependent on species, life stage and temperature range (Kaufmann 1932; Hagstrum & Leach 1973). Understanding the impact of varying temperature regimes on biological key data is essential in improving predictions on the developmental time of pest species and will help to build upon forecasting models and ensure effective pest management strategies (Spencer *et al.* 2009).

Subject of our study were the immature stages of western corn rootworm (Diabrotica virgifera virgifera LeConte; WCR).

The WCR is an invasive quarantine maize pest in Europe which was accidentally introduced in the late seventies or early eighties of the 20th century from North America to Serbia (Szalai *et al.* 2010). It was detected in 1992 near Belgrade airport (Gray *et al.* 2009). In 2010 this pest has already been reported from more than 20 European countries (EPPO 2011). During the last 20 years at least 5 introductions into Europe took place (Ciosi *et al.* 2008). In case of an establishment of this pest in most maize growing regions in Europe damage costs are expected to sum up to more than 470 million Euro per year (Wesseler & Fall 2010).

Western corn rootworm females lay eggs in autumn in the maize field, rarely in other crops. The eggs overwinter in the soil in diapause which ends in spring at a threshold temperature of 11°C (Wilde 1971). Larvae hatch and infest the host plant roots. Most damage is caused by the root feeding of larvae, which upsets nutrient and water uptake and lowers plant stability (Kahler *et al.* 1985; Riedell 1990).

We study the effect of diurnal varying temperatures compared with constant temperatures on western corn rootworm immature stages under quarantine conditions.

Whereas several papers report on WCR larval development under constant temperatures (Kuhlman, Howe, & Luckmann 1970; Wilde 1971; Jackson & Elliott 1988; Schaafsma, Whitfield, & Ellis 1991), we are aware of only two paper yet on WCR egg development under varying temperatures (Levine, Oloumi-Sadeghi, & Ellis 1992) respectively under a simulated natural temperature regime (Fisher 1989). So far no data on the development of WCR larvae under Central European conditions or more specifically, under Northern German conditions have been published. For improving pest management strategies, such as optimal application time of insecticides knowledge on the hatching time of overwintering eggs and development pattern of the larvae are necessary.

In this study we investigated WCR hatching rate and hatching date, larval development, and recovery under constant and varying ambient Northern Germany temperature regimes under consideration of plant growth effects.

We hypothesize that varying ambient temperatures compared to a constant temperature will result in (i) an earlier WCR larval hatch, (ii) a higher hatching rate, (iii) an accelerated development rate and (iv) a higher larval recovery.

We conducted a second experiment to compare the effects of high, mediate and low constant development temperatures and their diurnal (12h/12h) by 8°C varying counterparts of the same mean on the needed thermal energy for WCR hatch. We expected that less thermal energy necessary under the varying temperatures.

Material and Methods

Experiment 1

Maize seeds (cultivar Susann, Saaten Union, Germany) were pregerminated for 24h on moistened filter paper at 25°C and were sown individually into pots (10cm diameter) with Haplic Luvisol (sand/ silt/ clay-content: 3.0% / 55.2% / 41.8%; pH (H_2O) 7.6; C/N-ratio 15.8) from a field near Goettingen. The soil was homogenized with a soil shredder (Unifix 300, Moeschle, Ortenberg, Germany). The plants were kept in the greenhouse (22°C ± 4°C). 245 one week old maize plants of equal size were selected for the experiment (BBCH 12 (Lancashire *et al.* 1991)).

Non-diapausing WCR eggs (USDA-ARS, North Central Agricultural Research Laboratory, Brookings, North Dakota, USA (Branson 1976)) were stored below 8°C until their use. Eggs were washed with a sieve (250µm) from the soil and added to a sterile 0.15% agar solution. Each plant was infested with 20 viable eggs using a pipette (Multipipette Plus, Eppendorf, Hamburg, Germany) into the soil at a depth of 5cm. To monitor hatching rate and hatching date, hatch tests were prepared. 2ml of egg solution was added into each of 24 Petri dishes with sterile moistened filter paper and 4 randomly chosen Petri dishes per cabinet were reared under experimental conditions and 4 Petri dishes were placed into an incubator with constant 25°C with 60% RH to observe hatching patterns (one Petri dish as one replicate) under experimental and optimal development conditions. The eggs were counted and daily checked for hatching.

49 infested plants per cabinet were randomly placed into 5 climate cabinets (Mytron WB 750 KFL, Mytron Bio- und Solartechnik GmbH, Heiligenstadt, Germany) with by data logger (Voltcraft DL-120 TH, Conrad Electronic SE, Hirschau, Germany) controlled equivalent adjusted weekly mean temperatures. Meteorological data compiled by the DWD (Deutscher Wetterdienst), comprising the period of 1971 to 2000 at the location of Lingen were used to calculate weekly adjusted mean temperatures in Northern Germany (Table 1). Lingen was chosen because of its location in a high risk area for an establishment of a high population of WCR and thus heavy damage potential (Schaafsma, Baufeld, & Ellis 1999) because of the high frequencies of continuous maize. In one cabinet the constant (with no daily variations) temperature regime of Lingen was established, two cabinets had a day-night (12h/12h) difference of 4°C and two cabinets a day-night (12h/12h) difference

of 6°C. The experiment started with the temperature-simulation at Julian Day (JD) 121 as sowing date and the first mean temperature above the base temperature of 11°C as threshold of development (Wilde 1971). Relative humidity was adjusted to 60% ± 5% and the photoperiod was 12h: 12h (light: darkness).

One week after the first hatch in the hatch tests and henceforward 7 plants per cabinet (one plant as one replicate) were randomly assessed per week. Plant height was measured and larvae were extracted from the soil using a high gradient Kempson device (Kempson, Lloyd, & Ghelardi 1963) for 2 days at 60°C. The larvae were counted and the head capsule width was measured using a binocular microscope with an integrated object micrometer to determine larval instar (Hammack *et al.* 2003). Thereafter larvae were dried at 60°C for three days and dry weight was measured using a micro scale (MC5, Sartorius, Goettingen, Germany) with measuring accuracy of 0.001mg.

Experiment 2

In the second experiment eggs were washed with a sieve (250µm) from soil and added to a sterile 0.15% agar solution. 1ml of egg solution was added into each of 96 Petri dishes with sterile moistened filter paper. 16 Petri dishes per cabinet were randomly chosen and the eggs were counted. The eggs were reared under 6 different experimental temperatures: high (20°C), mediate (17°C) and low (14°C) constant temperatures and the diurnal (12h/12h) by 8°C varying counterparts of the same mean (16/24°C, 13/21°C, 10/18°C). The hatch was observed thoroughly till larval hatch stopped.

Data analysis

All statistical tests were performed using Statistica 10 (StatSoft®, Tulsa, USA). All data were tested for the normal distribution with the Shapiro-Wilk-test and by visual observation of histograms. Levene's test was carried out to test the equality of variances.

Experiment 1

Required day-degrees (D°) (dependent variable) for hatching and hatching rates under different varying temperature regimes (independent variable) and the 25°C control regime were tested with a one-way analysis of variance (ANOVA) adjusted with Tukey-Kramer-test. Log-transformed larval dry weights (dependent variable) were used to test for differences in development under the three different regimes (independent variable) on all experimental days (ED), by means of a Kruskal-Wallis-ANOVA (K-W-ANOVA), adjusted with a multiple p-value comparison (two-sided). Temperature-dependent plant growth, grouped by ED, was tested with a one-way ANOVA and adjusted with a Tukey-Kramer-test (TK-test). To separate direct temperature effects (categorical predictor) and indirect effects by plant growth (continuous predictor) on larval weight an analysis of covariance (ANCOVA) for each experimental day was performed. A simple regression was used to quantify the effect of plant height (predictor variable) on WCR log transformed dry weight (µg) (dependent variable).

The impact of the different temperature regimes (independent variable) on larval recovery (%) as dependent variable was tested with the K-W-ANOVA, adjusted with a multiple p-value comparison (two-sided). Correlations between recovery (dependent variable) and plant height (predictor variable) were tested with a simple regression.

Experiment 2

The hatching rates (dependent variable) of constant temperature and corresponding varying temperature (temperature regime = grouping variable) were tested pair wise for differences with Mann-Whitney U-test (MWU-test) at five different temperature sums.

Final hatching rates (dependent variable) of all regimes after a temperature sum of 354D° were tested for differences with a K-W-ANOVA, adjusted with a multiple p-value comparison (two-sided).

Results

Experiment 1

Influence of temperature regimes on egg hatching

Different varying temperature regimes did not influence the hatching rates of eggs (one-way ANOVA: P=0.57, F (2, 17) = 0.59), and the hatching rates were not significantly different compared to the 25°C constant control regime (one-way ANOVA: P=0.22, F (3, 20) = 1.62).

The temperature sums larvae required for hatching significantly differed between the three treatments (one-way ANOVA: P<0.01, F (2, 17) = 30.85; Tukey-Kramer-test adjusted). Eggs reared at temperature regimes with varying day-night temperatures hatched at lower temperature sums (Figure 1). 50% of egg hatching would occur at JD 171 with regard to the 6°C difference regime, at JD 175 with regard to the 4°C difference regime and at JD 179 with regard to the constant temperature regime (Table 2).

Western corn rootworm larval recovery

Recovery was not affected by any temperature regime on the different ED (K-W-ANOVA, adjusted with a multiple p-value comparison (two-sided): ED 71 P=0.043, H (2, N=35) = 6.32, multiple p-value comparison shows no differences; ED 78 P=0.073, H (2, N=35) = 5.24; ED 85 P=0.059, H (2, N=35) = 5,67; ED 92 P=0.89, H (2, N=35) = 0.25)), but a simple regression showed slight positive correlations with plant height for experimental day 71, 78 and 85 (Table 3).

Influence of temperature regimes on larval development

Larval recovery was too low for statistical analyses at the first three extraction dates. The larval development shown as log larval dry weight (µg) of extractions from experimental day 71, 78, 85 and 92 (ED 71, ED 78, ED 85 and ED 92), respectively, is mapped in Figure 2. Developmental differences between the regimes changes between the extraction dates.

Larvae kept under varying day-night temperatures with a 4°C difference had a lower log larval dry weight (µg) on ED 71 than larvae from the 6°C difference regime. Larval development under the constant temperature regime did neither differ from the 4°C difference regime nor from the 6°C difference regime (K-W-ANOVA, adjusted with a multiple p-value comparison (two-sided): *P*=0.02, *H* (2, N=24) = 7.91).

One week later, on ED 78, larvae reared under constant temperatures had a lower weight gain than larvae reared under varying day-night temperatures. The larvae recovered from the 6°C difference regime had significant higher larval weights than 4°C regime larvae (K-W-ANOVA, adjusted with a multiple p-value comparison (two-sided): *P*<0.01, H (2, N=32) = 16.29).

On ED 85 the larval weight differences between the regimes were reduced, but were still significant between the constant temperature and the varying 6°C difference regime (K-W-ANOVA, adjusted with a multiple p-value comparison (two- sided): *P*<0.01, H (2, N=33) = 8.51).

On experimental day 92 no detectable differences in larval development between the regimes were detected anymore (K-W-ANOVA: *P*=0.37, *H* (2, N=31) = 1.99). Weight gain did not increase any more at the 6 °C difference regime compared to experimental day 85 (Figure 2).

Influence of temperature regimes on plant height

Plant growth was positively affected by varying temperatures (Figure 3). While at ED 71 no significant differences in plant height were detectable (one-way ANOVA: *P*=0.26, *F* (2, 21) = 1,43), plant height was positively affected by the varying temperature regimes on ED 78 (one-way ANOVA: *P*<0.01, *F* (2, 29) = 16.11; TK-test adjusted) and ED 85 (one-way ANOVA: *P*<0.01, *F* (2, 30) = 32.46; TK-test adjusted), plants height increased with increasing day-night differences. At ED 92 only under constant temperature grown plants were significantly smaller than those which were grown under varying temperatures (one-way ANOVA: *P*<0.01, *F* (2, 28) = 18.84; TK-test adjusted).

Correlation between plant growth and larval development

ANCOVA showed that a direct temperature effect on larval development only left at ED 71 and that plant height (covariate) significantly affected WCR-larvae log transformed dry weight (µg). Larval development was highly correlated with plant height (Table 4). Regression equations are shown in Table 5. On every experimental day larvae developed faster on higher plants than on smaller plants.

Experiment 2

Influence of temperature regimes on egg hatching

Over the whole experiment the hatching rate courses were higher at the diurnal varying temperature regime as the corresponding constant temperatures (Figure 4). The pair wise comparison of the varying and corresponding constant temperature showed that at varying temperatures a lower temperature sum is needed to reach a certain low level of hatching rate, even though this effect diminish for the high and mediate temperature regime when hatch was almost finished (MWU-test: exact results in Table 6). The final hatching rates between the four temperature regimes with mediate and high development temperature and even the low diurnal varying regime did not differ from each other. Only the constant 14°C regime lowers the final hatching rate (K-W-ANOVA, adjusted with a multiple p-value comparison (two-sided): P=0.000, H (5, N=96) = 50.53).

Discussion

In the first experiment western corn rootworm egg and larval development was notably enhanced at varying temperatures compared with development under constant temperatures. As we hypothesized especially hatch, as a fundamental key factor for pest management decisions, occurred considerably earlier at varying temperatures as expected under constant temperatures. In contrast to the assumption of Fisher (1989) the temperature sum necessary for hatch differed between the constant and the varying temperature regimes. Whereas 50% hatch was at Julian day 179 under constant temperature regime, at the 4°C-difference regime 50% hatch occurred at JD 175 and at the 6°C-difference regime at JD 171.

This finding contradicts studies which report retarded development (Hagstrum & Hagstrum 1970; Clarkson, Hobischak, & Anderson 2004; Siegel, Bas Kuenen, & Ledbetter 2010) or no influence (Hagstrum & Leach 1973; Humpesch 1982) of varying temperatures on insect development, but is accordant to prior studies (Hagstrum & Hagstrum 1970; Behrens *et al.* 1983; Roltsch, Mayse, & Clausen 1990; Blanckenhorn 1997; Bryant, Bale, & Thomas 1999) where insect development was enhanced at the varying temperature regimes. One explanation for this enhanced development rate is provided by the Kaufmann-effect, also known as rate summation. The “law of effective temperatures” which measures the species specific physiological time to complete a developmental stage (thermal constant) in degree-days (D°) presume a linear relationship between the temperature and the development rate (Damos & Savopoulou-Soultani 2012), but the relationship of temperature and the development rate is not linear over the whole temperature range between lower- and upper threshold of development, instead of that the development-temperature relationship can be pictured as a logistic curve (Kaufmann 1932). In the range of the logistic curve, where temperature and development rate have a linear relationship, the temperature sum to reach a developmental stage (like hatch) is identical under varying and adequate constant mean temperatures. When the development temperatures come near or fall below the lower threshold of development, a varying temperature accelerates the development rate and the development will be completed at a lower temperature sum, this effect is visible at the low temperature regime at Experiment 2. At the varying low temperature of 10 / 18°C a significant lower amount of accumulated day-degrees was necessary for hatch than the corresponding constant temperature (14°C) and all higher temperature. When the development temperature is near the upper threshold of development, a higher amount of day-degrees is necessary for the completion of the development under varying temperatures than predicted under constant temperatures.

Additionally, the fact that direct temperature effects in the first experiment were exclusively related to the time and temperature sum of hatch (Figure 1) which occurs under relatively low temperatures near the threshold of development and that hatch affected early larval development (ED 71), but diminished on later extraction dates with higher temperatures (Table 4) indicate the Kaufmann-effect as conclusive explanation. On the other hand approximation of development between the regimes on late extraction dates (ED 85 and 92) can be related to an early reached (ED 78) maximal weight of 6°C-difference regime larvae while larvae development of the other regimes decelerated (Figure 2). An additional explanation for the accelerated development under varying temperatures would be a potentially circadian feeding behaviour (Miyashita 1971; Behrens *et al.* 1983) of WCR-larvae as adaptation to naturally varying temperatures, gas proportions or humidity conditions. In this case higher day temperatures would enhance development supplementally (Neumann & Heimbach 1975). As it is challenging to observe the behaviour of soil dwelling insects directly (Brown & Gange 1990), there is yet no study available if larval behaviour of western corn rootworm changes during the day. Influence of biochemical processes on insect life which are dependent on higher temperatures like effectiveness of enzymes, proteins or hormones are discussed by some authors (Sharpe & DeMichele 1977; Cohet & David 1978; Beck 1983; Brakefield & Mazzotta 1995). The day-night temperature changes of 4°C and 6°C (respectively 8°C) used in this study can be regarded as moderate, as Petavy (Petavy *et al.* 2001) assumed daily fluctuations of 10°C and more for most terrestrial life.

Plant growth and chemical composition are as well highly temperature affected (Went 1953; Leather 2010). Varying temperatures extensively enhanced plant growth in this experiment. Plant growth was limited in the climate cabinets, due to the distance between the pots and the lamps which was 70cm and plant reached at ED 78 under the 6°C-temperature regime while the other plants approximated to this value during the experiment (Figure 3). The quality (Awmack & Leather 2002) and quantity of available adequate host biomass are essential factors for herbivores; hence here larval development (Table 5) and recovery (Table 3) were highly positive correlated with well grown plants.

The lack of preferred bigger newly grown nodal roots (Hibbard *et al.* 2008) or starvation due to the lack of sufficient root biomass (Musick *et al.* 1980) slowed larval development down on small plants compared to bigger plants of the same age (Bergman & Turpin 1984).

Our method is useful to observe early development under accurately controlled conditions. To optimise prediction of WCR hatch in the field further studies with various varying temperatures should be done for egg development and hatching patterns of different European WCR-strains. This is because they might differ from the US-non-diapausing laboratory strain, as US-strains differ from each other (Levine, Oloumi-Sadeghi, & Ellis 1992) and in Europe at least 5 different introductions from origin populations occurred (Ciosi et al. 2008).

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Annex

Tables

Experiment 1

Table 1: weekly adjusted experimental mean temperatures calculated from historical (1971-2000) data from the DWD (Deutscher Wetterdienst) in Lingen. Julian Day 121 is experimental day 1 and corresponds to the 1. May in the field

Julian Day	T (mean) (°C)
121 - 127	11.4
128 - 134	12.8
135 - 141	13.9
142 - 148	13.9
149 - 155	14.9
156 - 162	15.4
163 - 169	15.5
170 - 176	15.5
177 - 183	16.5
184 - 190	17.9
191 - 197	17.7
198 - 204	17.2
205 - 211	17.9
212 - 218	18.6

Table 2: Julian days for 50% hatch (JD 50%), first larval hatch (JD min), hatch of last larva (JD max) and standard deviation (SD) in the hatch tests (N_0 =4; N_4 =8; N_6 =8).

temperature difference	JD 50% hatch	JD min	JD max	SD
0	178.8	171	186	3.5
4	174.9	167	188	4.2
6	170.5	163	184	3.6

Table 3: Regression equations of plant height (cm) as predictor and larval recovery (%) as dependent variable at experimental days 71, 78, 85 and 92

Experimental day	recovery (%):plant height (cm)	*F*	*P*	R^2
71	y = 40.49 + 0.32 * x	5.94	0.02	0.16
78	y = 45.15 + 0.26 * x	9.42	< 0.01	0.23
85	y = 46.65 + 0.22 * x	6.94	0.01	0.18
92	y = 49.85 + 0.18 * x	1.48	n. s.	0.05

Table 4: Significance of plant height (cm) as covariate and day-night temperature difference on western corn rootworm log larval dry weight (µg) at experimental days 71, 78, 85 and 92 analysed with analysis of covariance (ANCOVA; n. s.: P > 0.05)

Experimental day	variable	*F*	df	*P*
71	plant height (cm)	17.12	1	< 0.01
	day-night temperature difference (°C)	5.84	2	0.01
78	plant height (cm)	10.28	1	< 0.01
	day-night temperature difference (°C)	2.95	2	n. s.
85	plant height (cm)	13.09	1	< 0.01
	day-night temperature difference (°C)	0.21	2	n. s.
92	plant height (cm)	12.52	1	< 0.01
	day-night temperature difference (°C)	0.81	2	n. s.

Table 5: Regression equations of plant height (cm) as predictor and log larval dry weight (µg) as dependent variable at experimental days 71, 78, 85 and 92

Experimental day	log (larval dry weight(µg)): plant height (cm)	*F*	*P*	R^2
71	y = 22.58 + 18.31 * x	14.02	< 0.01	0.39
78	y = 1.87 + 27.87 * x	45.33	< 0.01	0.60
85	y = 0.67 + 26.51 * x	33.10	< 0.01	0.52
92	y = 3.90 + 23.94 * x	15.72	< 0.01	0.35

Experiment 2

Table 6: The hatching rates (dependent variable) of constant temperature and corresponding varying temperature (temperature regime = grouping variable) for the high (20°C; 16 / 24°C), mediate (17°C; 13 / 21°C) and low (14°C; 10/18°C) temperature regimes, pair wise tested for differences with Mann-Whitney U-test at five different temperature sums (the asterisk marks significant differences; $N_{varying}$ = 16; $N_{constant}$ = 16).

temperature regime	temperature sum (D°)	*U*	*Z*	*P*	
20°C; 16 / 24°C	198	55.00	-2.75	< 0.01	*
	216	50.00	-2.92	< 0.01	*
	252	118.50	-0.34	0.73	
	330	127.00	0.02	0.98	
	354	127.00	0.02	0.98	
17°C; 13 / 21°C	198	8,00	-4.88	< 0.01	*
	216	0,00	-4.81	< 0.01	*
	252	55,00	-2.73	< 0.01	*
	330	102,00	-0.96	0.34	
	354	103,00	-0.92	0.36	
14°C; 10 / 18°C	198	0,00	-4.91	< 0.01	*
	216	0,00	-4.81	< 0.01	*
	252	2,00	-4.73	< 0.01	*
	330	2,00	-4.73	< 0.01	*
	354	2,00	-4.73	< 0.01	*

Figures

Experiment 1

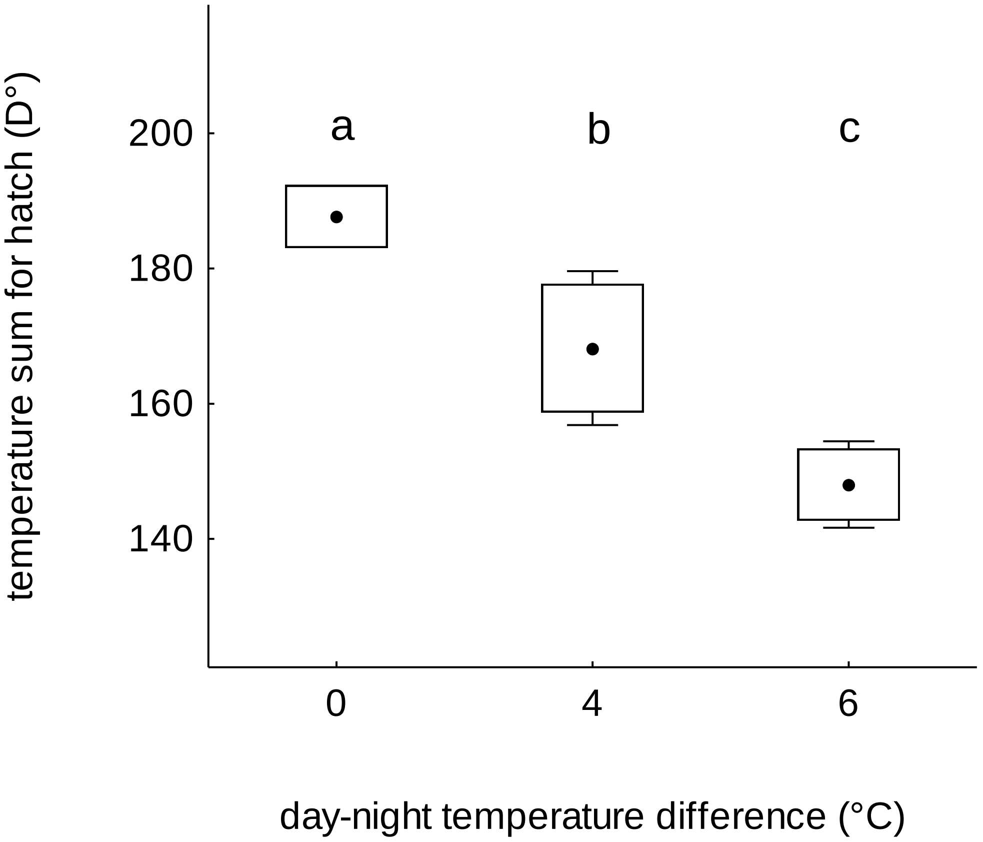

Figure 1: Temperature sum in day-degrees (D°) for a base temperature of 11°C required for larval hatch at temperature regimes with constant (0), varying day-night temperature with differences of 4°C (4) and 6°C (6) (ANOVA, Tukey-Kramer-test adjustment; dot = mean, box = mean ± 0.95 CI, whisker = mean ± SD; letters indicate significant statistical differences; hatch tests N_0 =4; N_4 =8; N_6 =8).

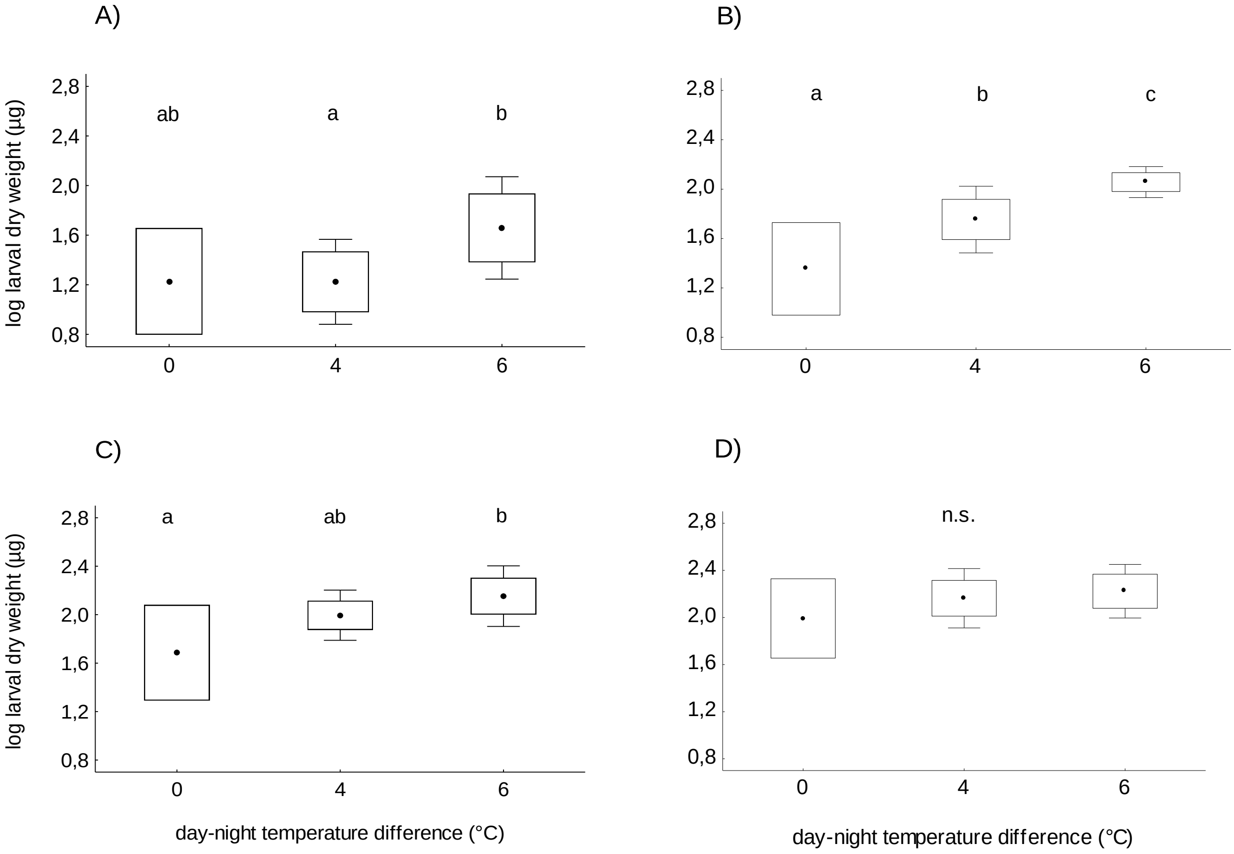

Figure 2: Compared larval development log larval dry weight (µg) at all experimental days (A = ED 71, B = ED 78, C = ED 85, D = ED 92) at temperature regimes with constant (0), varying day-night temperature with differences of 4°C (4) and 6°C (6) (Kruskal-Wallis-ANOVA; dot = mean, box = mean ± 0.95 CI, whisker = mean ± SD; letters indicate significant statistical differences; replicates = plants: N_0 =7; N_4 =14; N_6 =14 on every extraction date).

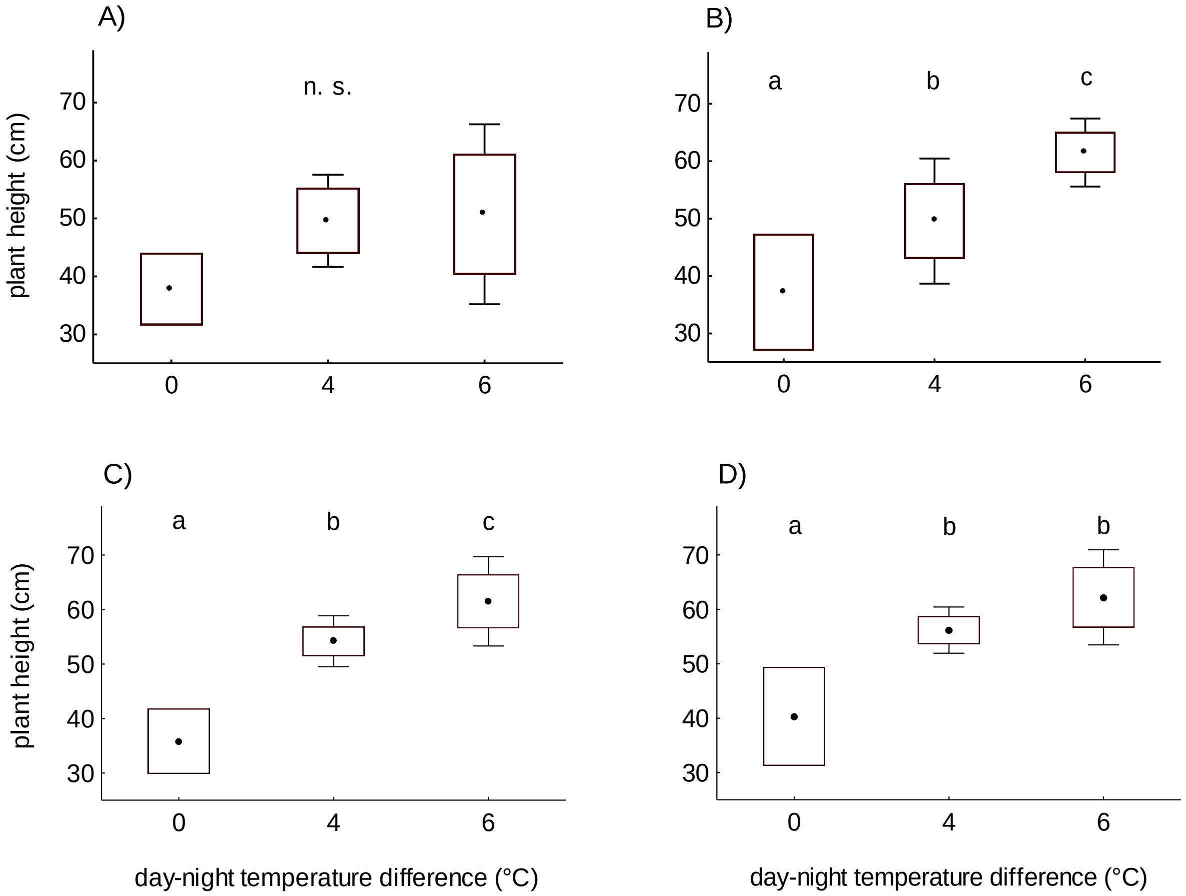

Figure 3: Plant height (cm) at all experimental days (A = ED 71, B = ED 78, C = ED 85, D = ED 92) at temperature regimes with constant (0), varying day-night temperature with differences of 4°C (4) and 6°C (6) (ANOVA, Tukey-Kramer-test adjustment; dot = mean, box = mean ± 0.95 CI, whisker = mean ± SD; letters indicate significant statistical differences; n. s. = not significant: P > 0.05; replicates = plants: N_0 =7; N_4 =14; N_6 =14 on every extraction date).

Experiment 2

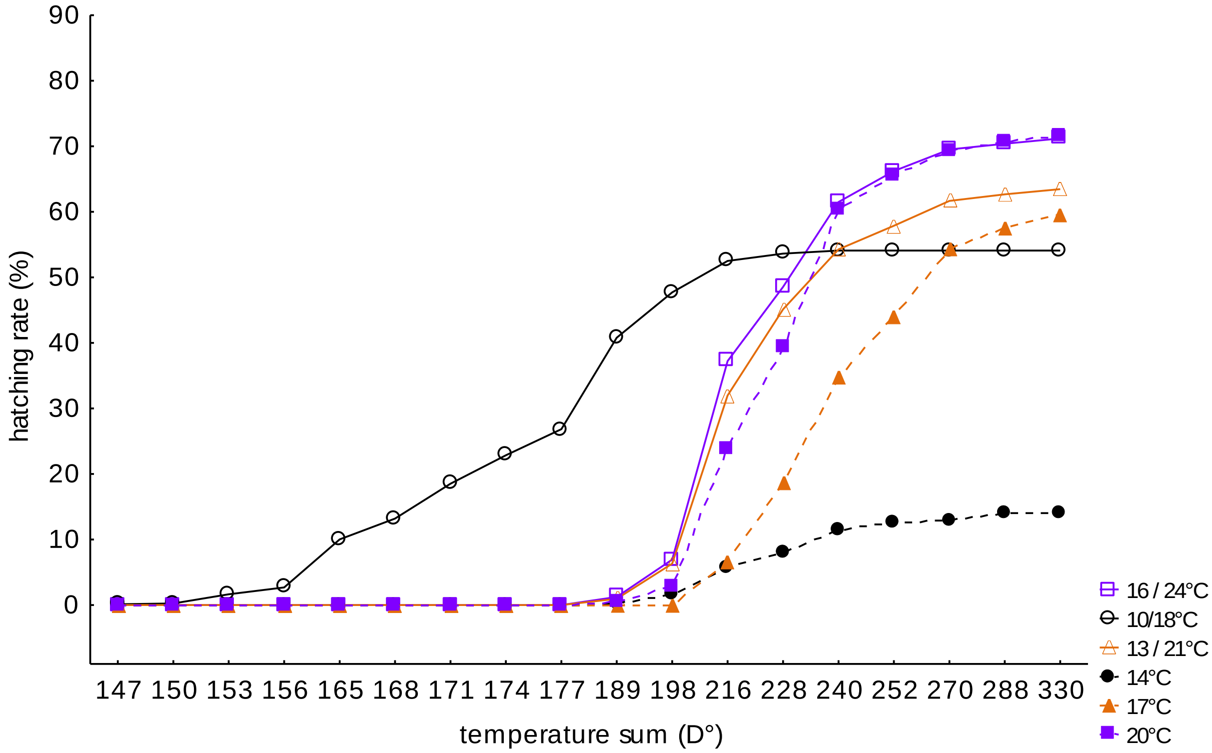

Figure 4: The courses of mean (from each 16 hatch tests) hatching rates of constant temperature (filled emblem) and corresponding varying temperature (empty emblem) for the high (20°C; 16 / 24°C), mediate (17°C; 13 / 21°C) and low (14°C; 10/18°C) temperature regimes.

Development of two different European western corn rootworm (*Diabrotica virgifera virgifera* LeConte) populations compared with the US non-diapausing laboratory population under a Northern German temperature regime

Abstract

Separated populations within one species typically differ with regard to their abilities and reactions on environmental impacts addicted to their special habitat. Insects reared in the laboratory additionally are mostly subjected to a loss of genetic diversity. In this study three populations of the western corn rootworm (WCR), an invasive maize pest in Europe, were tested for developmental differences in climate cabinets. We compared the US non-diapausing population, an established agent for WCR research, with two European field populations from Hungary and Serbia. Even if it is known that the laboratory population shows a loss of genetic diversity and differs genetically more from US field populations, than field populations with a high geographical range differs from each other, we could only detect a slight difference in thermal requirements for hatching between laboratory and the field populations. Hungarian population needed about 30D° (degree-days) more for hatch than the laboratory population did. Serbian population showed an intermediate thermal requirement for hatch. The differences between the two field populations indicate rapid adaptation processes of the introduced populations. We found a high impact of plant growth on larval development, with a higher larval dry weight on higher plants.

Introduction

Western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Galerucinae, Chrysomelidae; WCR) is a maize pest native to Central America (Melhus, Painter, & Smith 1954) and has spread over North America from the mid of 19th century till today (Gray *et al.* 2009). WCR shows a high adaptability to pest management like crop rotation (Levine *et al.* 2002) and also evolved resistance to some insecticides (Meinke *et al.* 1998; Wright *et al.* 2000). WCR populations in the USA vary in relation to temperature requirements. The threshold of development is reported as 11°C for South Dakota (James R. Fisher 1989), Michigan (Ruppel, Russell, & Jennings 1978) and New York population (Davis, Brenes, & Allee 1996), 11°C or 11.1°C for the Minnesota population (Wilde 1971), 12.7 for the Illinois population (Levine, Oloumi-Sadeghi, & Ellis 1992) and 12.8°C for the Kansas population (Chiang 1973). Wilde *et al.* 1972 showed that the populations from the North of the US from South Dakota and Minnesota need less thermal energy then the more southern populations from Iowa, Kansa, Missouri and Nebraska. Higher thermal requirements for hatch were reported from the Illinois population compared to the Minnesota and South Dakota populations (Levine, Oloumi-Sadeghi, & Ellis 1992). Genetic differences between the US populations are low, but positive correlated with geographic distance (Kim & Sappington 2005).

Since the late 70s or early 80s of the 20th century (Szalai *et al.* 2010) at least 5 independent introductions from North America into Europe occurred (Ciosi *et al.* 2008a). There is a high probability that different beetles from source populations with different abilities and adaptations could establish in Europe (Sappington, Siegfried, & Guillemaud 2006). In 2010 this pest has already been reported from more than 20 European countries (EPPO 2011). When the WCR has established in most maize growing regions in Europe damage costs from more than 470 million Euros per year are expected (Wesseler & Fall 2010). To model and predict WCR hatch for effective management, hatch, larval development and adult occurrence in the field in Europe should be determined experimentally under regionally climate conditions. A US non-diapausing laboratory population (Branson 1976) is commonly used by many researchers because it is easily available in high numbers, has a short generation time and can be used independently from season. Certainly the US non-diapausing population shows a loss of genetic diversity and differs genetically more from US field populations, than four field populations with a high geographical range differ from each other (Kim *et al.* 2007).

In this study we tested whether the US non-diapausing laboratory population is an adequate organism to study WCR development in Europe. Therefore in our experiment the US non-diapausing population was tested for differences to two European field populations from Serbia and Hungary with regard to (i) the required thermal energy for hatch, (ii) their hatching rate, (iii) larval development and (iv) recovery rate under a simulated Northern German temperature regime.

We hypothesize that there is a difference between the populations with regard to their temperature requirements.

Material and Methods

The maize seeds were pregerminated for 24h on moistened filter paper at 25°C (cultivar Susann, Saaten Union, Germany) and were sown individually in 3cm depth into pots (13cm diameter) with Haplic Luvisol (sand/ silt/ clay-content: 3.0% / 55.2% / 41.8%; pH (H_2O) 7.6; C/N-ratio 15.8) from a field near Goettingen. The soil was homogenized with a soil shredder (Unifix 300, Moeschle, Ortenberg, Germany). Eggs from three different WCR-populations were used in this experiment: the non-diapausing WCR laboratory population (USDA-ARS, North Central Agricultural Research Laboratory, Brookings, North Dakota, USA (Branson 1976)), one Serbian population collected from a field near Kula in the year 2009 and one Hungarian population from a field near Dalmand also collected in 2009. In the fields adults were caught, egg laying occurred in the laboratory. All eggs were stored below 8°C until their use.

Eggs were washed with a sieve (250µm) from soil and added to a sterile 0.15% agar solution. Each plant was infested with 50 viable eggs using a pipette (Multipipette Plus, Eppendorf, Hamburg, Germany) into the soil at a depth of 7cm. To monitor hatching rate and hatching date, hatch tests were prepared. 1ml of egg solution was added into Petri dishes with sterile moistened filter paper. 2 randomly chosen Petri dishes per cabinet from the field populations and one from the US population were reared under experimental conditions and 2 Petri dishes from each population were placed into an incubator with constant 25°C at 60% RH to observe hatching patterns. The eggs were counted and checked daily for hatching.

16 infested plants (6 with Hungarian, 6 with Serbian, 4 with US eggs) per cabinet were randomly placed into 3 climate cabinets (Mytron WB 750 KFL, Mytron Bio- und Solartechnik GmbH, Heiligenstadt, Germany) with by data logger (Voltcraft DL-120 TH, Conrad Electronic SE, Hirschau, Germany) controlled and adjusted weekly mean temperatures. Meteorological data compiled by the DWD (Deutscher Wetterdienst), comprising the period between 1971 to 2000 at the location of Lingen were used to calculate weekly adjusted mean temperatures in Northern Germany (Table 1). Lingen was chosen because of its location in a high risk area for an establishment of high WCR populations and thus heavy damage potential (Schaafsma, Whitfield, & Ellis 1991) because of high frequencies of continuous maize. Temperature regime of Lingen was established starting from Julian day 121 as the sowing date and the first mean temperature above the base temperature of 11°C as the threshold of WCR

development. Relative humidity was adjusted to 60% ± 5% and the photoperiod was 16h: 8h (light: darkness). The Experiment ended at Julian Date 212 at a temperature sum of 402 day-degrees (D°) for a base temperature of 11°C. Plant height was measured and larvae were extracted from soil using a high gradient Kempson device (Kempson, Lloyd, & Ghelardi 1963) for 2 days at 60°C. Larvae were counted and head capsule width was measured using a binocular microscope with integrated object micrometer to determine larval instar (Hammack *et al.* 2003). Thereafter larvae were dried at 60°C for three days and dry weight was measured using a micro scale (MC5, Sartorius, Goettingen, Germany) with measuring accuracy of 0.001mg.

Data analysis

All statistical tests were performed using Statistica 10 (StatSoft®, Tulsa, USA).

All data were tested for the normal distribution with the Shapiro-Wilk-test and by visual observation of histograms. Levene's test was carried out to test the equality of variances. Required day-degrees (D°) (dependent variable) for hatching of the three different populations (independent variable) were tested with a Kruskal-Wallis-ANOVA, adjusted with a multiple p-value comparison (two-sided). Hatching rate (dependent variable) of the three different populations (independent variable) was tested with a one-way ANOVA, adjusted with Tukey-Kramer-test to test for differences between the populations. The three populations (independent variable) were tested for differences in their log transformed dry weight (µg) respectively their headcapsule width (µm) (dependent) as an indicator for development with a one-way ANOVA adjusted with Tukey-Kramer-test. A simple regression was used to quantify the effect of plant height (predictor variable) on WCR development measured as log transformed dry weight (µg) and as headcapsule width (µm) (dependent variables). The effect of population (independent variable) on recovery rate (%) of larvae was tested with one-way ANOVA.

Effect of plant height (cm) as the predictor variable on recovery rate (%) as the dependent variable was quantified with a simple regression.

Results

Hatch

Required day-degrees (D°) differed between the three populations (Kruskal-Wallis-ANOVA, adjusted with a multiple p-value comparison (two-sided): $P<0.001$, H (2, N=649) = 127.90; Table 2). Highest amount of thermal energy for hatch is needed by the Hungarian field population with 199.9 D° (equivalent to Julian day 181) for mean hatch, Serbian population needed 184.7 D° (JD 178), fewest day-degrees were necessary for mean hatch of the US laboratory population (169.9 °D; JD 175). There were no differences (one-way ANOVA $P=0.09$, F (2, 12) = 2.93) of hatching rates (%) between the three populations, average hatching rate was 72.5%.

Development

Development measured as log larval dry weight (µg) (one-way ANOVA: $P=0.03$, F (2, 45) = 3.79, Figure 2) or as headcapsule width (µm) (one-way ANOVA: $P=0.02$, F (2, 45) = 4.25) did not differ because the adjustment with Tukey-Kramer test showed no differences between the populations. Development as log larval dry weight (µg) ($P<0.01$; regression equation: $y = 1.02 + 0.01*x$; $R^2 = 0.51$, Figure 2) and as headcapsule width (µm) ($P<0.01$; regression equation: $y = 198.78 + 1.91*x$; $R^2 = 0.39$) were highly positively correlated with plant height (cm) at the extraction date (Figure 3).

Recovery rate

Larval recovery (%) was not affected by WCR population (one-way ANOVA: $P=0.88$, F (2, 45) = 0.13), but was highly positive correlated with plant height (cm) at the extraction date ($P<0.01$; regression equation: $y = -9.16 + 0.49*x$; $R^2 = 0.40$).

Discussion

In our experiment thermal energy needed for egg hatch differed significantly between the three populations. Hungarian eggs needed about 30 D° more for mean hatch, than the US non-diapausing laboratory strain, while the Serbian field population showed intermediate thermal requirements (Table 2; Figure 1). These findings are consistent with a field study using artificial infestations, where larvae of the non-diapausing laboratory strain hatched 6 days earlier than neonates of the wild population (Branson *et al.* 1981). Hatching rates were not influenced by the origin of the populations. The hatch differences between the populations diminish during larval development (Figure 2). Eventually not all early hatched neonates could establish on the plants or Serbian and US-strain developed slightly slower than the Hungarian did. Differences between the Hungarian and Serbian population were not expected due to the assumption that both populations probable came from the same US source population and the same introduction event. Interestingly, both field strains are known to be resistant against the insecticidal compound aldrin (Ciosi *et al.* 2008b). Even though the Serbian and the Hungarian strain in our experiment showed significant differences in their thermal requirements for egg hatch. We assumed that the differences between the two field populations indicate a rapid adaptation process of the introduced population.

Some Authors described that insect reared over some generations in the laboratory on artificial diet performed worse than wild types did (Carpenter & Greany 1998; De Clercq & Degheele 1992), additionally laboratory rearing can cause changes in behaviour (Spates Jr. & Hightower 1967) and nutritional preferences (Schoonhoven 1967). Neither growth nor recovery rate did differ between the populations, thus the US non-diapausing population has not lost its viability compared to the tested field populations. The loss of genetic variability which was observed in laboratory colonies of for example *Anopheles albimanus* Wiedemann (Arias *et al.* 2005) or the New World sand fly *Lutzomyia longipalpis* Lutz and Neiva (Mukhopadhyay *et al.*) was shown for the US WCR non-diapausing population too (Kim *et al.* 2007). This genetic depletion and divergence had no consequence under moderate conditions like in our experiment for development, but could lead to misleading results when traits like insecticide resistance and behavioural studies are carried out or if responses to extreme conditions are examined. Experiments which deal with plant damage should be carried out carefully, because contradictious results were mentioned in literature.

While (Branson, Sutter, & Fisher 1981) showed a less damaging potential of the non-diapausing population, Hibbard *et al.* 1999 found higher damage levels caused by the non-diapausing population compared to a field population of WCR larvae. We did not test for differences in plant damage in our experiment. Plant growth itself had high impact on development of larvae (Figure 3) and recovery rate in our experiment. On well grown plants we had a higher recovery rate and larvae with a higher dry weight. It can not be clarified whether this was an effect of root quantity or a better food quality or both at high plants. Food quality and quantity both are essential factors for herbivores (Awmack & Leather 2002). The lack of preferred bigger newly grown nodal roots (Hibbard *et al.* 2008) or even starvation due to the lack of sufficient root biomass slowed larval development down on small plants compared to bigger plants of the same age.

When specific pest management measures for a region need to be verified, local populations should be used for the experiments. The use of the non-diapausing laboratory population offers benefits like its high availability, uniformity, inability to survive the winter in field (quarantine organism) and its usability over the whole year. For our comparative studies of the WCR traits hatching rate, development and recovery the US non-diapausing laboratory population is a very useful organism when the slight earlier hatch is considered in comparison to field populations. To optimise prediction of WCR hatch in the field further studies for egg development and hatching patterns of different European WCR-populations from different introduction events should be done under regional conditions.

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Annex

Tables

Table 1: weekly adjusted experimental mean temperatures calculated from historical (1971-2000) data from the DWD (Deutscher Wetterdienst) in Lingen. Julian Day 121 is experimental day 1 and corresponds to the 1. May in the field

Julian Day	T (mean) (°C)
121 - 127	11.4
128 - 134	12.8
135 - 141	13.9
142 - 148	13.9
149 - 155	14.9
156 - 162	15.4
163 - 169	15.5
170 - 176	15.5
177 - 183	16.5
184 - 190	17.9
191 - 197	17.7
198 - 204	17.2
205 - 211	17.9
212 - 218	18.6

Table 2: Day-degrees (D°) for mean hatch, first larval hatch (min D°), hatch of last larva (max D°) and standard deviation (SD) in the hatch tests ($N_{Hungary}$ =271; N_{Serbia} =281; N_{US} =97).

Population	mean D°	min D°	max D°	SD
US	169.6	136.6	297.4	25.9
Serbia	184.7	145.6	297.4	21.9
Hungary	199.9	154.6	304.1	25.8

Figures

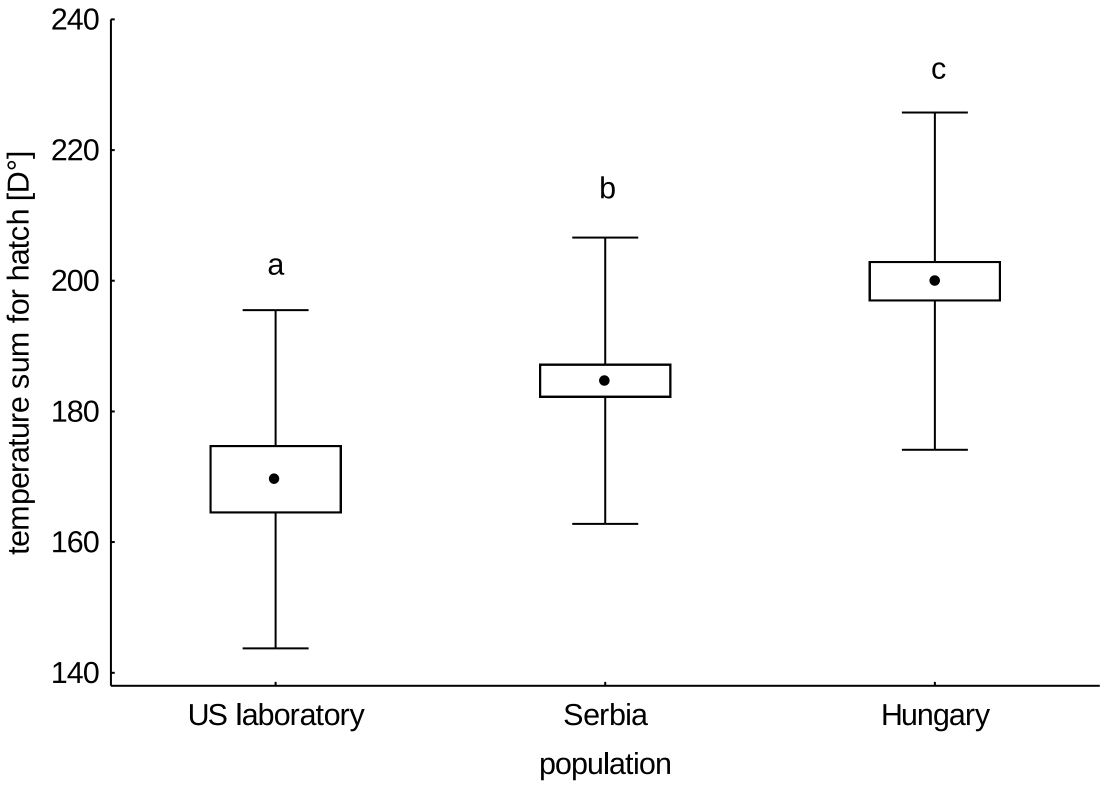

Figure 1: Temperature sum (D°) for a base temperature of 11°C required for hatch of the three different WCR populations (Kruskal-Wallis-ANOVA; dot = mean, box = mean ± 0.95 CI, whisker = mean ± SD; letters indicate significant statistical differences; replicates = plants $N_{Hungary}$ =271; N_{Serbia} =281; N_{US} =97).

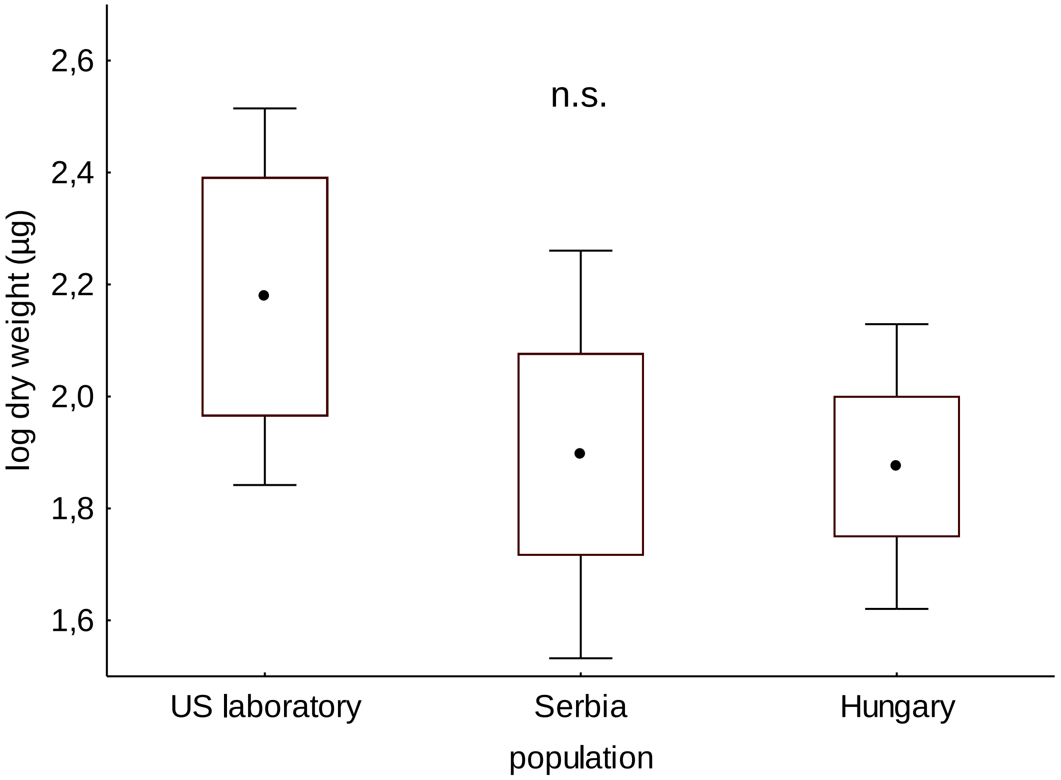

Figure 2: Compared larval development as log larval dry weight (µg) of the three different WCR populations (Kruskal-Wallis-ANOVA; dot = mean, box = mean ± 0.95 CI, whisker = mean ± SD; n. s. = no significant statistical differences; replicates = plants: $N_{Hungary}$ =18; N_{Serbia} =18; N_{US} =12).

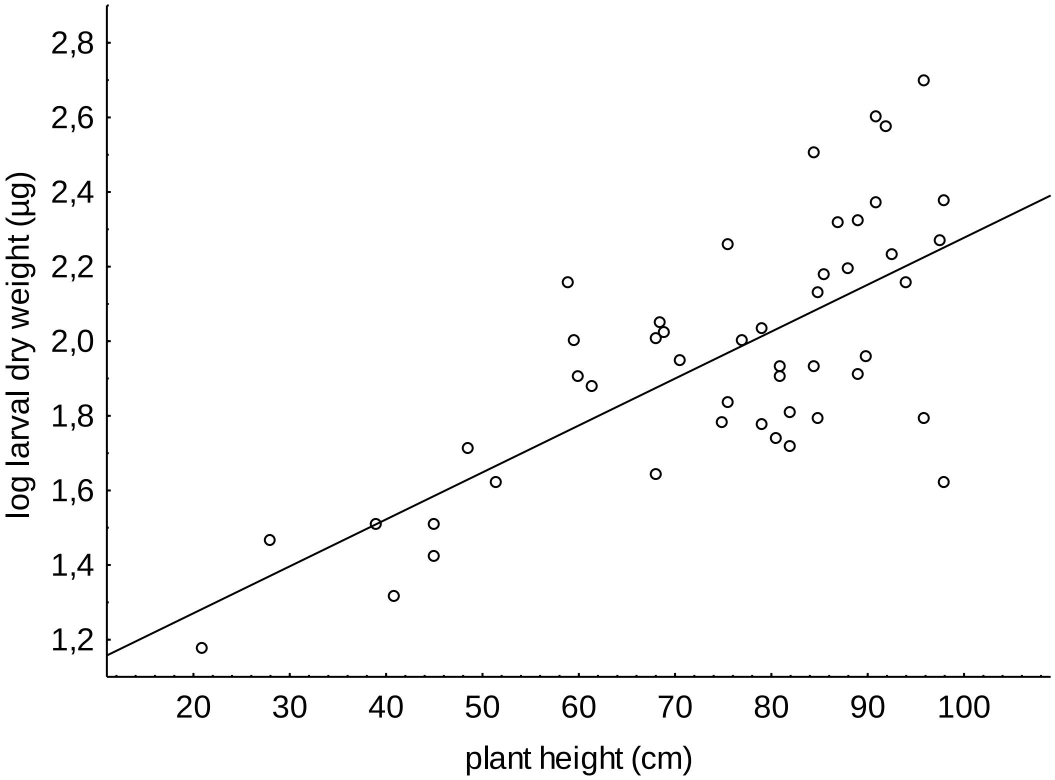

Figure 3: Correlation between log larval dry weight (µg) and plant height (cm) at extraction date.

General discussion

The western corn rootworm is well established in Europe and eradication of this maize pest unfeasible (Hummel & Deuker 2009). Extensive eradication and containment measures retarded WCR dispersion in Europe, but did not stop it. Even crop rotation is not sufficient to exclude WCR development, because of a low proportion of larvae which are able to develop on alternative host plants (Moeser & Vidal 2004; Chege, Clark, & Hibbard 2005) and a small proportion of females which lay eggs in non-maize fields. There is continuing extension of maize growing area for energy generation in Europe (Sims *et al.* 2006). Hence, there is a basic necessity of key data to establish regional integrated pest management strategies against WCR. Even though WCR larvae are soil insects, all the noted experimental temperatures were air temperatures. Elliott et al. stated that air temperatures are as well suitable to predict WCR development as soil temperature are (Elliott, Jackson, & Gustin 1990).

We studied the influence of an enhanced temperature on the synchrony of maize and pest in Northern Germany. Our experiment had the same limitation Musolin *et al.* mentioned in their work about the southern green stinkbug *Nezara viridula* (Musolin, Tougou, & Fujisaki 2010), we used fixed increase in temperature, while climate change predictions differ between the seasons with especially a warmer winter in northern hemisphere (Houghton *et al.* 2001). We looked on a scenario with increased spring and summer temperatures and a further begin of activity. Climate change will not only become manifest as global warming, but will also impact extreme events, carbon dioxide and precipitation (Harrington, Fleming, & Woiwod 2001). Jentsch *et al.* noted that climate change research has to deal with “events not trends.” (Jentsch, Kreyling, & Beierkuhnlein 2007), since extreme events have a very high influence on ecological processes. Nevertheless we found an influence of a moderate later sowing on larval development under a warmed temperature regime, assumedly due to a higher mortality of early hatched larvae on little developed plants. The possibility of the disruption of host-pest synchrony should be considered as a management strategy in infested second-year maize under a changing climate with its predicted longer growing seasons (Cannon 1998). Higher infestations levels will potentially lead to a higher degree of efficiency of this measure, because a higher mortality by intraspecific competition on small root systems (Bergman & Turpin 1984). Because of the contradicting findings of former studies (Branson & Sutter 1986; Hibbard *et al.* 1999, 2008), plant damage and resulting yield losses should be examined thoroughly. Precipitation and irrigation additionally influence WCR development and the regrowth potential of the plant.

We examined in the first experiment the effect of diurnal temperature alteration of two magnitudes (4°C and 6°C difference) on WCR development compared with the adequate constant mean temperature. In the second experiment we reared the eggs under 6 different experimental temperatures: high (20°C), mediate (17°C) and low (14°C) constant temperatures and the diurnal (12h/12h) by 8°C varying counterparts of the same mean (16/24°C, 13/21°C, 10/18°C). We found a high influence of alternating temperatures on hatching as the intrinsic factor for the timing of management measures. Western corn rootworm egg and larval development was significant enhanced at varying temperatures compared with constant temperatures. This phenomenon is known as Kaufmann effect or rate summation and the reason for this accelerated development is the logistic correlation of development rate and temperature for insects, the temperature sum deviate from predicted values if the temperature is below or above the area of linear dependence (Kaufmann 1932). This effect should be taken into account for the prediction of larval hatch. Even though it is clear that the differences of the hatching patterns were pure temperature effects the larval development could be influenced by behavioural changes like a circadian feeding behaviour (Miyashita 1971; Behrens *et al.* 1983) of WCR-larvae as adaptation to naturally varying temperatures, gas proportions or humidity conditions. Additionally plant growth and chemical composition of the host plant are highly temperature affected which could lead to a deviating host suitability (Went 1953; Leather 2010). In all of our experiments there was a strong positive correlation between plant height and larval performance respectively plant height and larval recovery. To exclude the effect of plant growth and observe direct temperature effects on larval development, larvae should be reared on an artificial diet in a further experiment.

We compared two different European western corn rootworm field populations from Serbia and Hungary with the US non-diapausing laboratory population. We found a slight higher thermal requirement for hatching of the Hungarian and the Serbian population compared to the laboratory population. We assumed that the differences between the two field populations indicate an adaptation process of the introduced population. US non-diapausing laboratory population showed not the loss of viability which is reported from other laboratory reared insect cultures (Carpenter & Greany 1998; De Clercq & Degheele 1992), even though a genetic depletion is evidenced in this population (Kim & Sappington 2005). This genetic depletion and divergence had no consequence under moderate conditions like in our experiment for development, but could lead to misleading results when studies were carried out addressing the responses to extreme conditions, the behaviour or resistance traits. It would be interesting to study differences between European populations from different introduction events. For the implementation of regional pest management strategies local populations should be used. The benefits of the non-diapausing laboratory population are very high and for our comparing studies of the WCR traits hatching rate, development and recovery the US non-diapausing laboratory population was a very useful organism. The earlier hatch has to be considered in comparison to field populations.

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Eidesstattliche Erklärung

Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbständig und ohne unerlaubte Hilfe angefertigt wurde.

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