PUBLIZIERBARER ENDBERICHT

A) Projektdaten

Kurztitel:	winsurv
Langtitel:	Predicting overwintering survival and establishment of exotic pest insects under future Austrian climatic conditions
Programm inkl. Jahr:	2.Call (2010)
Dauer:	01.07.2011 bis 30.06.2015
KoordinatorIn / ProjekteinreicherIn:	AGES
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Projekt- und KooperationspartnerIn (inkl. Bundesland):	ZAMG
Schlagwörter:	Winter, survival, model, *Tuta absoluta*, *Helicoverpa armigera*, *Trogoderma granarium*, temperature, prediction
Projektgesamtkosten:	346.778 €
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Projektübersicht

1 Kurzfassung

Die Modellierung der Überwinterungsmortalität von Insekten anhand „konsumierter" Wintertemperaturen würde ein wichtiges Werkzeug darstellen, um deren Populationsdynamik besser zu verstehen. Damit könnte auch eine mögliche Verschiebung von Arealgrenzen unter veränderten Temperaturbedingungen besser beurteilt werden. Leider gibt es derzeit kein solches allgemein akzeptiertes Modell. Bereits früher haben einzelne Autoren (z.B. Casagrande 1976, Nedvěd 1998) auf die enge Verwandtschaft zwischen Wachstumsmodellen und Mortalitätsmodellen für Insekten hingewiesen. Für dieses Projekt wurde entschieden, ein Überwinterungsmodell zu entwickeln, welches auf der stündlichen Summierung von Kälteeinheiten basiert. Diese Kälteeinheiten, welche auf empirische Weise gewonnen werden, sollten den stündlichen Beitrag zur Gesamtmortalität wiedergeben. Wie diese Kälteeinheiten jedoch genau zu definieren wären, war bei Projektbeginn noch nicht klar. Durch Vergleich von modellierten mit beobachteten Mortalitäten während der Überwinterung sollte dieses Modell geeicht werden: 3 wichtige landwirtschaftliche Schädlinge, nämlich die Tomatenminiermotte (*Tuta absoluta*; Tafel I), die Baumwollkapseleule (*Helicoverpa armigera*; Tafel II) und der Khaprakäfer (*Trogoderma granarium*; Tafel III) wurden für solche – mechanisch gut abgesicherten - Überwinterungsexperimente während dreier Jahre an 5 verschiedenen Standorten verwendet. Um den zukünftigen Überwinterungserfolg dieser Arten abzuschätzen, sollen die hier entwickelten mathematischen Überwinterungsmodelle mit prognostizierten Temperaturdaten angetrieben werden.

Zur Entwicklung der artspezifischen Überwinterungsmodelle wurde die maximale Überlebensdauer (LT_{100}) für die 3 Versuchstierarten bei konstant niedrigen Temperaturen (0°, -2°, -4°, -6° -8°, -10°C und noch tiefer) ermittelt. Deren Kehrwert mal 100 wurde als mittlere stündliche Mortalität [%] aufgefasst. Diese Berechnung ist identisch mit dem geforderten Gewichtungsprozess der einzelnen Temperaturen gemäß ihrem Beitrag zur Gesamtmortalität. Nichtsdestotrotz zeigte diese modellierte Mortalität im Freilandversuch Abweichungen von der beobachteten Mortalität. Daher wurde in einem Eichungsprozess versucht, diese beiden Werteserien in Übereinstimmung zu bringen. Als Randbedingung sollte gelten, dass beim Gefrierpunkt der Insekten immer 100% Mortalität auftreten. Die Eichung erfolgte durch Streckung oder Verkürzung der Kurve der mittleren stündlichen Mortalitäten in Richtung der X- und Y Achse. Dieser Schritt der Kalibrierung war auch deshalb notwendig, da das Überwinterungsmodell hauptsächlich die schädlichen Temperatureffekte erfasst (Influx), während die Erholung unter gemäßigt niedrigen Temperaturen (Efflux), eine erhöhte Mortalität bei starken Temperaturschwankungen oder eine erniedrigte Mortalität durch zusätzliche Adaption während der Überwinterung noch nicht berücksichtigt wird. Diese „Störfaktoren" werden durch die Kalibrierung kompensiert.

Zur Abschätzung des Überwinterungserfolgs bei historischen (bekannten) Temperaturverläufen wurden Zeitserien (1951 – 2010) mittlerer Tagestemperaturen und Ensemblewerte möglicher regionalisierter Klimaszenarien (A1B, rcp25 und rcp85) vorbereitet. Die durch eine Quantiltransformation dynamisch transformierten („downscaled") Temperatur-Zeit-Serien wurden zu den lokalen Temperatur-Zeit-Serien der lokalen Stationen in Beziehung gesetzt. Ein einfaches Bodenmodell wurde modifiziert, um ein Bodenklima zu simulieren, das für das Mikrohabitat von Insekten typisch ist, welche in oberen Bodenschichten bzw. der Bodenstreu überwintern. Auf Grundlage der aus den Laborexperimenten abgeleiteten Mortalitätsmodellen wurden Wintermortalitäten für Europa für alle drei Insektenarten berechnet und als Karten dargestellt. Dazu wurden beobachtete Temperaturzeitreihen (1961/1962 – 1998/1999) und Temperaturzeitreihen regionalisierter künftiger Klimaszenarien verwendet (A1B, rcp25 und rcp85, 2079/2080 – 2098/2099). Im Fall von *Helicoverpa armigera* und *Trogoderma granarium* wachsen die Gebiete, in denen während des Beobachtungszeitraumes keine Wintersterblichkeit vorkam, bis zum Ende des 21. Jahrhunderts stark an. Dann würden große Gebiete in Mitteleuropa zur Überwinterung beider Arten ohne jegliche klimabedingte Sterblichkeit geeignet sein. *Helicoverpa armigera* kann in Teilen Ostösterreichs schon jetzt ohne Probleme im Boden überwintern. In tropischen Sommern wie 2003 oder 2015 konnte sie sich in diesen Gebieten ausreichend vermehren und dann Peststatus erlangen. Für *Trogoderma granarium* wirkten sich im Überwinterungsexperiment Temperaturen um -16°C bereits sehr schädlich aus, wenn sie länger als einige Nächte lang andauerten. Bezüglich der sommerlichen Entwicklung müssten mittlere Monatstemperaturen von 20°C in 4 hintereinander liegenden Monaten überstiegen werden. Dies war selbst in den tropischen Sommern der Jahre 2003 und 2015 nicht der Fall. Im Fall von *Tuta absoluta* beschränkt sich die Vergrößerung der möglichen Überwinterungsgebiete auch gegen Ende des 21. Jahrhunderts auf Südwesteuropa und den Mittelmeerraum. Das restliche Europa bleibt auch dann für die Überwinterung dieser Art ungeeignet. Die Labordaten zeigten, dass *Tuta absoluta* höchstens 72 Tage überleben konnte - bei +4°C.

Zusammenfassend lässt sich sagen, dass im Rahmen dieses Projektes alle notwendigen Vorgangsweisen und Methoden vorbereitet wurden, die eine Beschreibung und Extrapolation der potentiellen Wintersterblichkeit von drei Insektenarten ermöglichen: Labor- und Freilandversuche mit Insekten im geeigneten Überwinterungsstadium, die Ableitung von Wintermortalitätsmodellen, die Einrichtung eines Bodentemperaturmodells, die Aufbereitung der künftigen Klimaszenarien und zuletzt die Modellierung der potentiellen Insektenmortalitäten. Das hier vorgestellte Überlebensmodell stellte sich als gutes Werkzeug zur Analyse spezifischer Überwinterungssituationen heraus. Nichtsdestotrotz ist dies erst der erste Schritt in der Analyse unterschiedlicher teils divergierender Vorgänge während der Überwinterung. Für eine Feinabstimmung des Modells sind noch weitere Informationen nötig bezüglich der Erholung während Phasen mäßig niedriger Temperatur, einer zusätzlichen Schädigung durch starke Tag-Nachtschwankung sowie durch mögliche zusätzliche Adaption während der Überwinterung. Derzeit werden diese „Störfaktoren“ für das Modell durch die Kalibrierung kompensiert.

Ein Folgeprojekt oder eine andere Arbeitsgruppe kann auf dieser Basis aufbauen und die hier verwendeten Methoden verfeinern und erweitern. Ein wichtiges quantitatives Ergebnis ist die Beschreibung der Wirkung der Klimavariabilität auf die potentielle Wintermortalität der drei Insektenarten. Das schließt sowohl die Variabilität von Jahr zu Jahr als auch die Wirkung von Temperaturtrends mit ein. Das Wintermortalitätsmodell, das in diesem Projekt entwickelt wurde, könnte in ein umfassenderes Modell, das die Populationsdynamik simuliert, integriert werden.

2 Executive Summary

Modelling of winter survival of insects would form a basic component for assessment of the ability of insects to spread into new habitats or change their distribution area in the course of climate change. Unfortunately no such generally accepted models exist. Already earlier some authors have realized the relationship between modelling winter mortality and modelling growth processes of insects. Therefore it was decided to develop an overwintering model which should be based on accumulation of "chill units" analogue to heat units in growth models. These chill units should be derived from laboratory experiments and weighted in a way that was not yet clear at the start of the project. By comparison of modelled vs. observed mortalities in overwintering experiments a calibration of the model was assumed to be necessary. Three important agricultural pests namely the tomato leafminer (*Tuta absoluta*, Plate I), the cotton bollworm (*Helicoverpa armigera*, Plate II) and the Khapra beetle (*Trogoderma granarium*, Plate III) would be used for these experiments at several locations in Austria during 3 years. For prediction of future overwintering success the mathematical models would have to be run with predicted temperatures obtained from temperature scenarios.

So as to develop overwintering models for 3 experimental insect species, laboratory experiments were carried out to obtain maximum survival times (LT_{100}) at constant low temperatures. Their reciprocal values (100/x) were interpreted as mean hourly mortalities [%] for each temperature. This calculation turned out to be identical with the weighting process necessary for chill units according their effect to mortality. Modelling of field mortalities is achieved by accumulation of hourly mortalities. However they showed some deviations to observed mortalities. Therefore a further step of calibration was necessary to make calculated mortalities congruent with mortalities observed during field experiments. This calibration was achieved by mathematical dilation or reduction of the calculated mean mortality-temperature curve along the x- and y-axes. As a result the recorded field mortalities could be described as a function of suffered temperatures. This step of calibration was necessary - presumably because our overwintering model summarizes only the influx of adverse temperature effects but does not take into account the recovery at moderate low temperatures (efflux) or increased mortality due to temperature fluctuations or extraordinary adaptation during overwintering. During the step of calibration these disturbing factors are compensated.

Data sets of observed daily mean temperature time series (1951 – 2010) and ensembles of possible future climate scenarios from regional climate model output data bases (A1B, rcp25 and rcp85) have been prepared. Via a quantile mapping method the dynamically downscaled temperature time series have been adjusted to the local station temperature time series. A simple soil model has been selected and adjusted to simulate the soil climate, which is typical for insects overwintering in a litter and soil environment. Winter mortality distributions were calculated for Europe on basis of the winter mortalities deduced from the lab experiments for all three insect species. As an input observed temperature time series (1961/1962 – 1998/1999) and temperature time series from regionalized future climate scenarios (A1B, rcp25 and rcp85, 2079/2080 – 2098/2099) were used. In case of *Helicoverpa armigera* and *Trogoderma granarium* the „zero mortality" areas (areas with no mortalities during the

observational period 1961/1962 – 1998/1999) strongly increase for all three scenarios at the expense of the other classes. Large areas in central Europe would become suitable for overwintering of both species. In case of *Tuta absoluta* the spatial gain through temperature increase of the climate scenarios remains always restricted to the Mediterranean and SW Europe. The temperature requirements of this insect species is such that no matter what amount the winter temperature increases, Europe remains largely an inaccessible continent for this insect. Interpretation of laboratory or overwintering experiments and other outdoor observations revealed that *Tuta absoluta* can survive for 72 days at a maximum, whereas *Helicoverpa armigera* can survive in parts of Austria without problems in the ground. In tropical summers like 2003 or 2015 *Helicoverpa armigera* was able to develop efficiently and to achieve pest status. For *Trogoderma granarium* winter temperatures beneath -16°C are deleterious if they last longer than a few nights. Mean monthly temperatures must be higher than 20°C in 4 consecutive months (Banks 1977). These values were not exceeded during the tropical summers of 2003 and 2015.

3 Hintergrund und Zielsetzung

Due to enlarging worldwide traffic and due to climatic change increasing numbers of new invading insects are recorded now in Austria and other countries during the last years. Among noxious species agricultural pests and vectors of human diseases are of special interest. Little is known about their ability to establish outdoor populations in their new habitat. Important limiting factors are thought to be severe winter conditions that would affect their overwintering success. A basic element for its estimation would be the development of a method allowing its calculation by the help of mortality data obtained from the laboratory at constant temperatures. Up to now no such generally accepted method exists. Some authors (e.g. Casagrande 1976) had realized a close relationship between winter survival models and growth models by accumulation of heat units. Nedvěd (1998) developed a model accumulating linear “chill units” [degreedays] below a threshold temperature. Kaliyan 2007 accumulates “mortality rates” in a cumulative lethality index model implying a logarithmic relationship between mortalities and temperatures. The type of relationship had been derived from Arrhenius’ equations by theoretical considerations. Beside these “chill unit accumulation models” other authors still improve the method of freezing points (super cooling points; SCP) of insects. If temperatures fall below the SCP of freeze-susceptible insects overwintering is completely prohibited (e.g. Andersen 2014, Berkvens 2009, Hart 2002, Hatherly 2005). Therefore we see the necessity to develop a model based fully on empirically derived chill units. An appropriate model would be an effective tool for prediction of overwintering success for all kinds of poikilothermic species. This would also facilitate the development of warning services for agricultural pests or for vectors of human and animal diseases.

Since the project submission in 2010 the essential assumptions concerning global and regional temperature have remained valid. Although cold season temperatures show a so called hiatus, which is a levelling off of the temperature increase, the warm season temperature time series have constantly been rising (IPCC 2013). So the problem of climate amelioration for various introduced exotic insects with potential hazard for the European agricultural plants is still relevant. As stated in the Austrian Assessment Report 2014 (APCC, 2014), the regional temperature time series of

Austria shows a higher short term variability than the global time series. The negative anomaly during 1870-1900 and the strong positive anomaly during the last three decades has led to a stronger temperature increase in Austria than on the global scale.

The extrinsic objective for this project was the prediction of overwintering of 3 important exotic agricultural pests - *Tuta absoluta, Helicoverpa armigera* and *Trogoderma granarium* – in a possible future climate. For its achievement an intrinsic objective had to be pursued by the development of an overwintering model based on hourly accumulation of chill units. Temperatures derived from appropriate future scenarios would be used to drive our 3 survival models. This would allow the prediction of overwintering probabilities for these species.

4 Projektinhalt und Ergebnis(se)

All activities were arranged in work packages (WP) and are structured by milestones.

According WP1 all experimental species *Tuta absoluta*, *Helicoverpa armigera* and *Trogoderma granarium* were reared in the laboratory in sufficient quantities. Whereas for *Tuta absoluta* a continuous plant breeding of tomatoes was necessary *Helicoverpa armigera* could be reared on a semisynthetic diet based on yeast and germinating beans. *Trogoderma granarium* was reared on malt or broken wheat grains without problems.

In WP2 appropriate overwintering stages and conditions enabling optimal cold adaptation for these stages were identified by comparisons of SCPs and by cold exposure experiments. In *Tuta absoluta* which has no diapause the SCPs of pupae were -16,8°C and that of adults -19°C. Contrary to these SCP measurements pupae turned out to be more cold resistant in exposure experiments. The least susceptible stages were found to be young pupae with hardened cuticle and black eyespots just in the centre of their compound eyes (Table Id). In preliminary exposure experiments acclimatisation was best after storage at 6°C for 12-14 days. For *Helicoverpa armigera* the overwintering stage is clearly defined by a facultative diapause during the pupal stage. Periodic measurements of SCPs in overwintering pupal populations showed values between -18 and -19°C without seasonal oscillation. For mortality experiments diapausing pupae were stored at 12°C for up to 4 months and were hardened 1 week at 2°C before starting the exposure. Preliminary experiments had shown that in such conditions cold hardiness remained constant for about 3 months. In contrast *Trogoderma granarium* shows a kind of quiescence under adverse rearing conditions like overcrowding and excess of faeces at 25°C. For overwintering experiments preferably less active bigger larvae in the 4th stage were used. Overwintering larvae in the field had a mean SCP of -21°C and showed no decrease of mean values of SCPs but a decrease of minimum values during progress of winter. For experiments quiescent larvae adapted 1 week at +15°C and 3 weeks at +6°C were used - they were similar cold resistant as field individuals from midwinter. Summing up this item it became clear that exposure experiments were more reliable than measurement of SCPs. Small differences in cold adaptation between overwintering insects and laboratory adapted insect stages were hardly to avoid. They seem to be compensated by proper calibration of the overwintering model (see WP5).

In WP3 cold adapted overwintering stages of the experimental species were exposed to constant low temperatures in order to reveal the influence of temperature and duration of exposure to survival. Maximum survival times (LT_{100}) were used as output of these experiments because their values did not fluctuate so much as LT_{50} values.

The results for *Tuta absoluta* are given in figure 1a and 1b. It is assumed that at temperatures below -10°C the corresponding maximum survival is represented by a slowly bending curve which reaches the x-axis at approximately -17°C (SCP). At a temperature of +6°C (developmental zero) measured values varied widely – probably death was caused by exhausted energy reserves rather than by chilling. Maximum survival time seems to form a logistic curve, which is characteristic if more than one factor causes overall mortality – i.e. chilling and exhausting energy reserves. It has to be stated that chilling for its part may include different types of cold injuries. Hatched adults without deformed wings were stated as surviving.

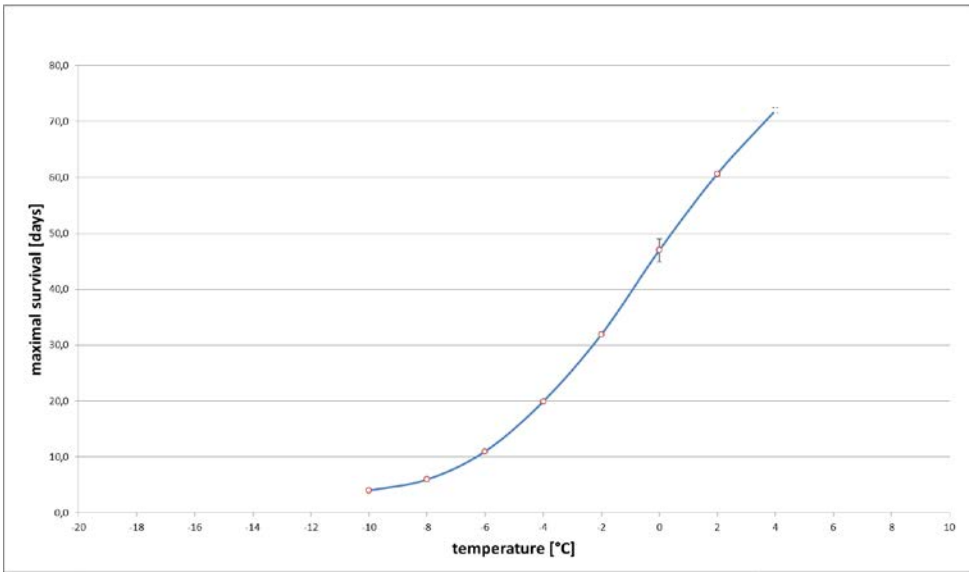


Figure 1a: Maximum survival [days] of *Tuta absoluta* pupae at constant low temperatures. Standard deviations (marked by whiskers) were mostly less than 0,5 and are hardly visible.

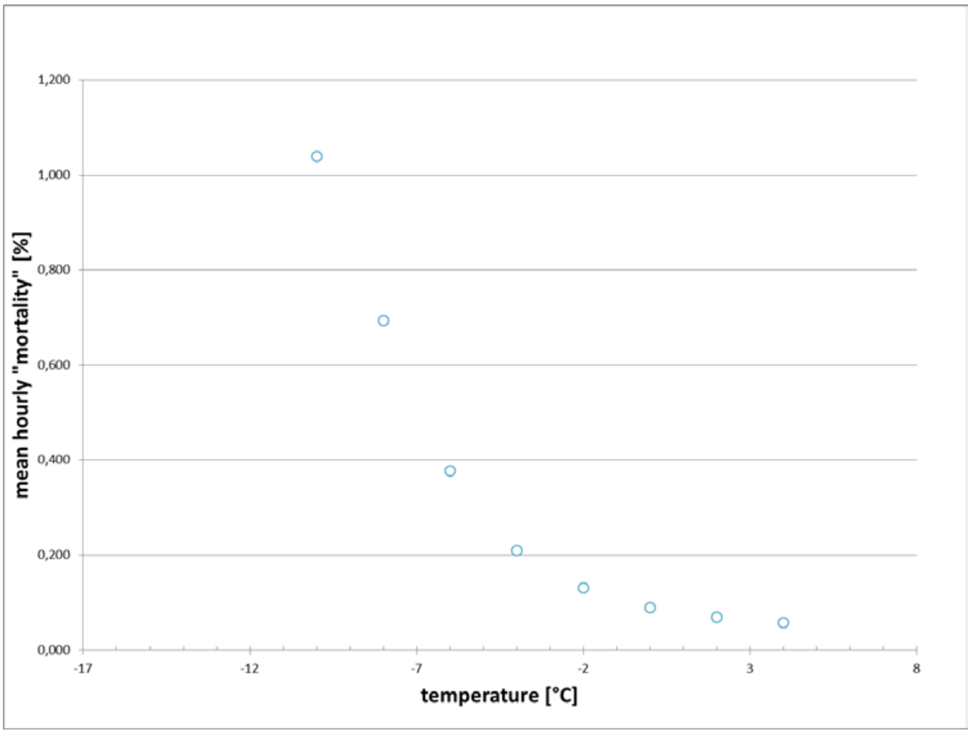


Figure 1b: reciprocal values of maximum survival x 100 (blue circles) are interpreted as mean hourly mortalities [%].

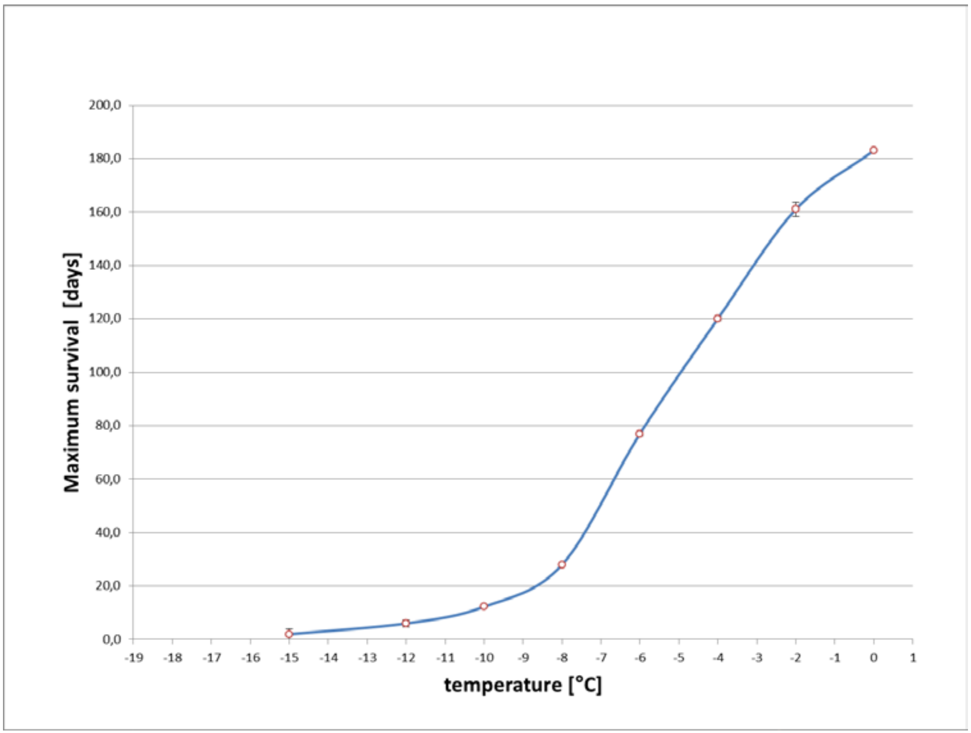


Figure 2a: Maximum survival [days] of *Helicoverpa armigera* pupae at constant low temperatures. Standard deviations (marked by whiskers) were mostly less than 0,5 and are hardly visible.

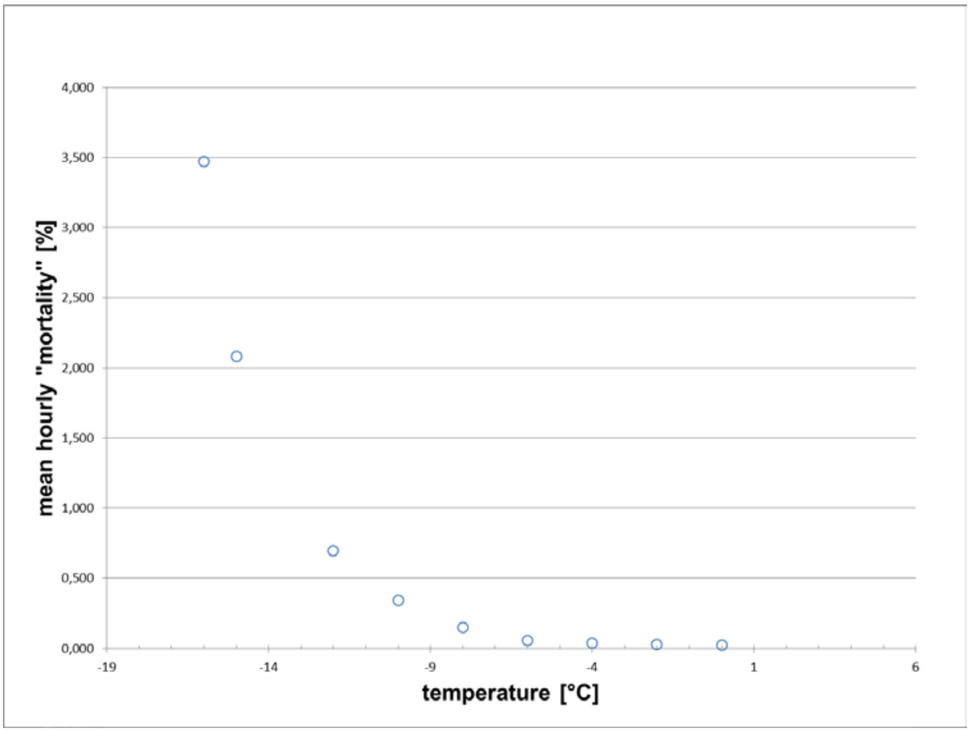


Figure 2b: Reciprocal values of maximum survival x 100 (blue circles) are interpreted as mean hourly mortalities [%].

The results for *Helicoverpa armigera* are given in figure 2a and 2b. It is assumed that at temperatures below -12°C the corresponding maximum survival is represented by a slowly bending curve which reaches the x-axis at approximately -19°C (SCP). The developmental zero temperature is reported 12°C. Maximum survival seems to form a logistic curve, which is characteristic if more than one factor causes overall mortality. Hatched adults without deformed wings were stated as surviving.

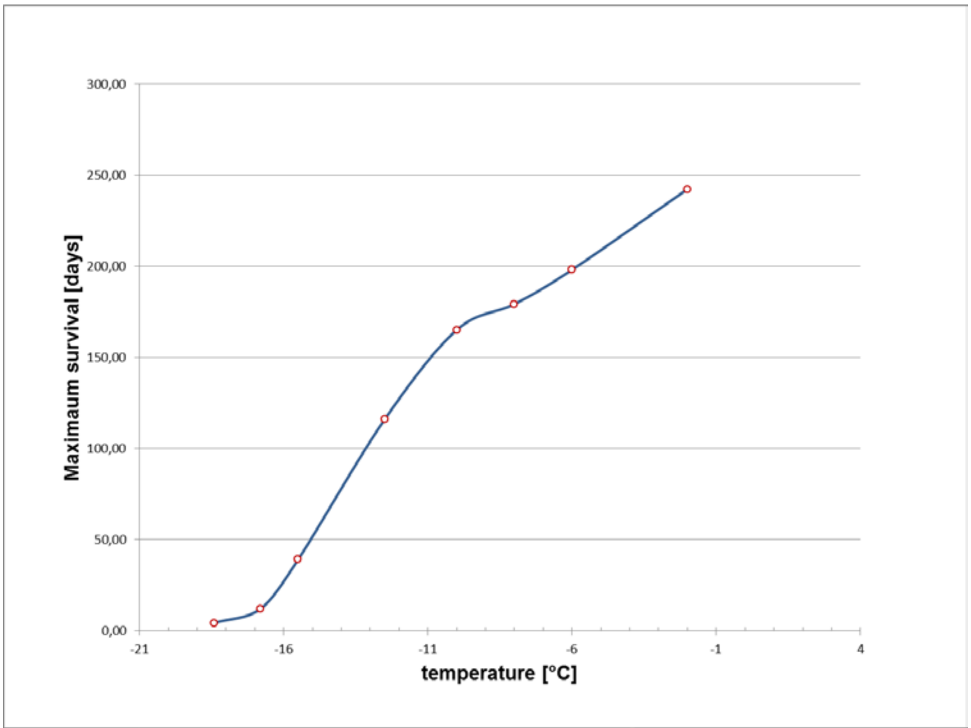


Figure 3a: Maximum survival [days] of *Trogoderma granarium* L_4 larvae at constant low temperatures. Standard deviations (marked by whiskers) were mostly less than 2 and are not visible therefore.

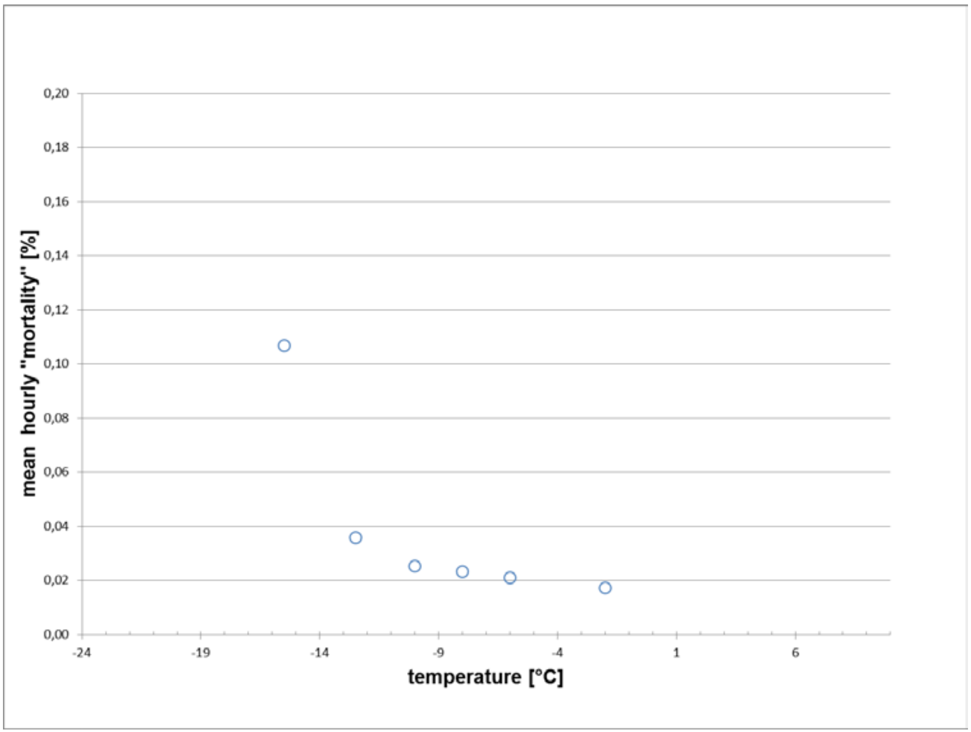


Figure 3b: Reciprocal values of maximum survival x 100 (blue circles) are interpreted as mean hourly mortalities [%].

The results for *Trogoderma granarium* can be obtained from figure 3a and 3b. It is assumed that at temperatures below -18°C the corresponding maximum survival is represented by a slowly bending curve which reaches the x-axis at approximately -21°C (SCP). The developmental zero temperature is reported 20°C. In contrast to *T. absoluta* and *H. armigera* maximum survival shows some deviations from a logistic curve. The reason for this is not known, but may be caused by physiological stages of experimental insects being not homogenous enough or by factors causing additional mortality. During experiments it was sometimes difficult to separate insect stages according their outer appearance not taking into account their physiological condition. Therefore it may be possible the test insect populations were not homogenous enough. It has to be noted that overwintering larvae are very cold resistant and can survive e.g. up to 155 days at -10°C. The decision if a larva had survived was sometimes difficult. To avoid any errors all larvae were reared until they gave rise to an adult or died at last. These two features lead to extraordinary long durations of single experiments.

WP4: Because the model based on linear chill units – as defined by Nedvěd - yielded no results it was necessary to modify the original plan. The efforts to compensate this failure lead to application of the concept of weighted chill units which would be a great improvement of the original concept. The relation of chill units to temperature should not be derived by theoretical considerations but by empirical methods. For this purpose 100 was divided by the particular LT_{100} (duration of exposure for maximum survival) for each temperature. This reciprocal value (rate of mortality - Kaliyan 2007) is denominated as mean hourly mortality (=chill unit) and is a measure of the hourly impact of each temperature to overall mortality. The division ($^{1}/_{\text{duration of exposure}}$) implies a linear relationship between duration of exposure and mortality – which is only partially the case. In contrast this relationship corresponds rather to a sigmoid curve (Casagrande 1976, Nedvěd 1998) – which means that the model would overestimate low mortality values and underestimate higher mortalities. In the planned publications this nonlinear relationship will be considered. Nevertheless this so far used calculation seemed to be accurate especially in the range of high mortalities near LT_{100} which is of special interest. The used method of calculating "chill units" is very similar to the method of computing "rates of development" in growth models. By plotting these "mean hourly mortalities" against temperatures it became evident that its relation to temperature was not linear (fig. 1b, 2b and 3b). Therefore linear models (like that proposed by Nedvěd) will not work here. For accumulation of hourly mortalities (chill units) it was necessary to find adequate mortality values also for temperatures not covered by experiments. For this reason data points (fig. 1b, 2b and 3b) had to be interpolated using appropriate nonlinear functions. This was achieved by using the trendline option of Excel™. This enabled the accumulation of hourly mortalities for various field temperatures. These interpolated curves of chill units are of similar type for all experimental species (fig 4a, 5a, 6a). Polynomial functions (6^{th} degree - provided by Excel™) for the "trendline" fitted best with an R^2 being very near to 1 - it will be indicated separately for each experimental insect (see results of WP5).

<u>WP5</u>: Modelled mortalities (by accumulation of weighted chill units) in field experiments showed some deviations to recorded mortalities. Therefore a further step of calibration was necessary to make calculated mortalities congruent with observed mortalities. This calibration was achieved by mathematical reduction or dilation of the calculated mean mortality - temperature curve along x- and y-axes (fig. 4a, 5a, 6a). As a result of this simulation modelled mortalities were equal or somewhat lower than recorded mortalities in the field. As it was intended to define conditions without survival field mortalities somewhat higher than predicted by the model did not matter. The calibrated mortalities are displayed in figure 4b for *Tuta absoluta*, in figure 5b for *Helicoverpa armigera* and in figure 6b for *Trogoderma granarium*. Horizontal reductions or dilations were carried out towards the ordinate (x=SCP). This ensured that 100% mortality at the freezing point was invariable - which is characteristic for the species in question. Also the vertical reductions had to ensure that the data point (SCP/100%) had to be invariant and the empiric mortalities would be proportionately scaled down. This step of calibration was inevitably necessary - presumably because our overwintering model summarizes only the influx of adverse temperature effects but does not take into account the recovery at moderate low temperatures (efflux) or increased mortality due to temperature fluctuations or further adaptation during overwintering. The process of calibration was achieved by varying the parameters for upscaling and downscaling along the x- or y axis (compare figures 4a, 5a and 6a) The results could be seen in the summarising charts for overwintering success (figures 4b, 5b and 6b). All mean hourly mortality curves were approximated by polynomials (6^{th} degree) which were valid in that certain range. For outlying temperature values corresponding mortalities were calculated by straight lines between the lowest empirical value and the SCP or between the highest empirical value and the developmental zero respectively. Mortality was calculated 100% for temperatures below the SCP and 0% for temperatures higher than the developmental zero.

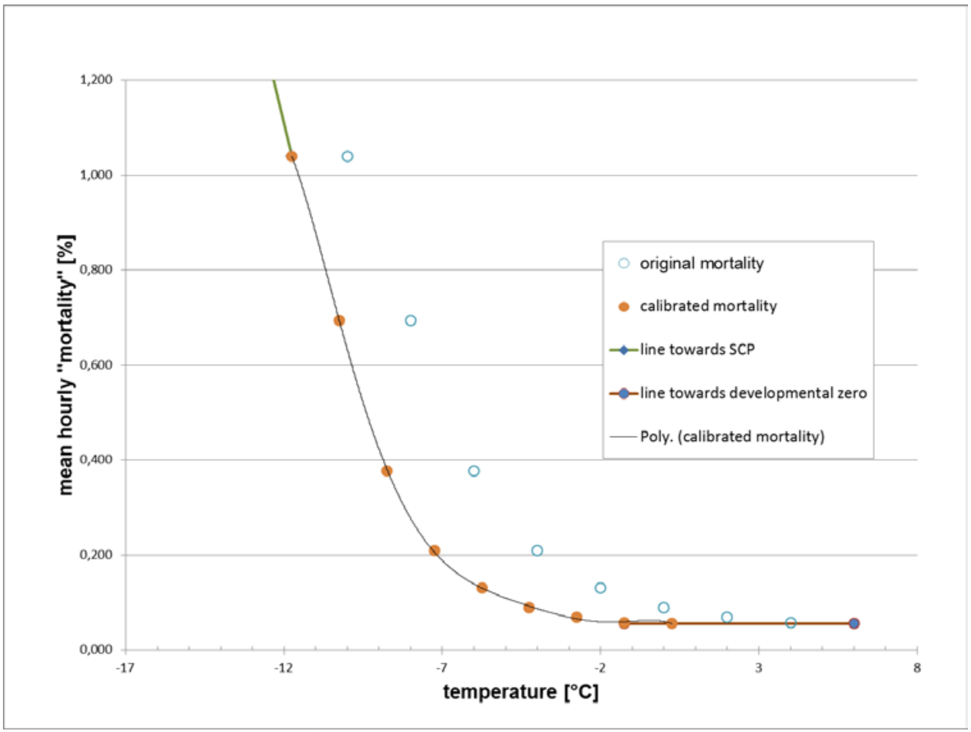


Figure 4a: Calibration of mean hourly mortalities for *Tuta absoluta* by horizontal reduction of the mortality values (compare fig 1b).

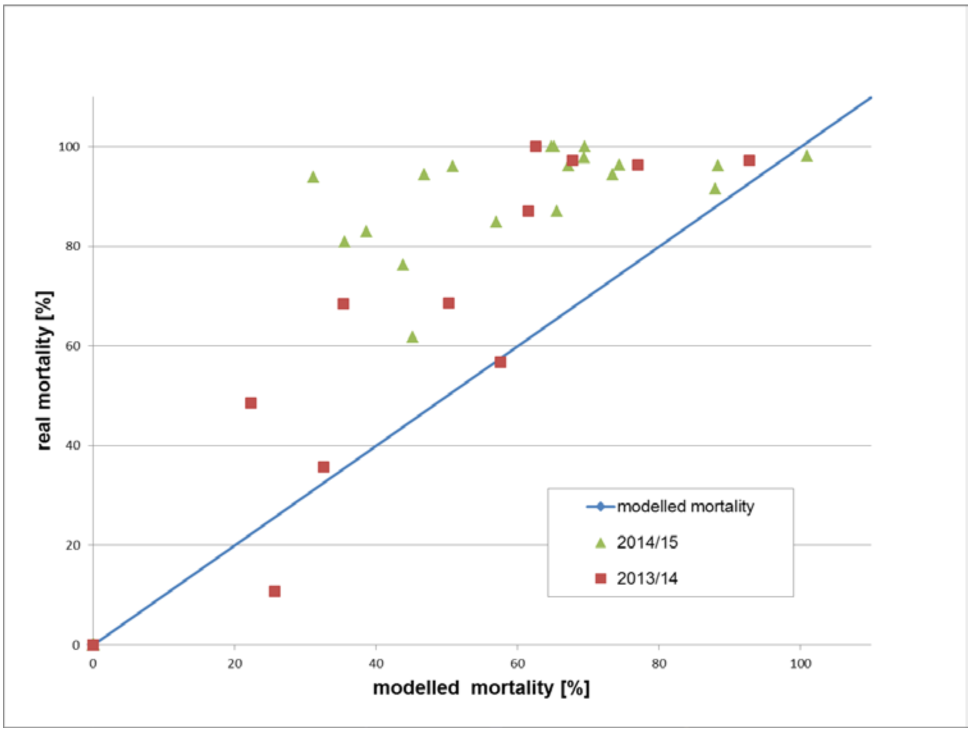


Figure 4b: Application of the calibrated "winsurv overwintering model" to overwintering data 2013/14 and 2014/15.

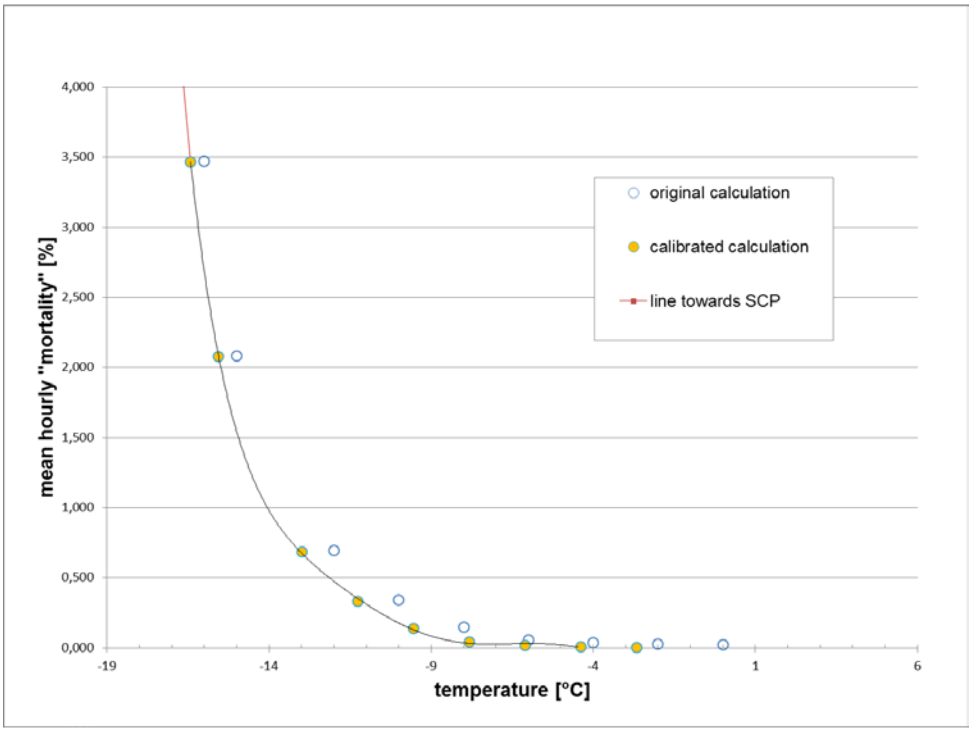


Figure 5a: Calibration of mean hourly mortalities for *Helicoverpa armigera* by horizontal and vertical reduction of the mortality values (compare with fig 2b).

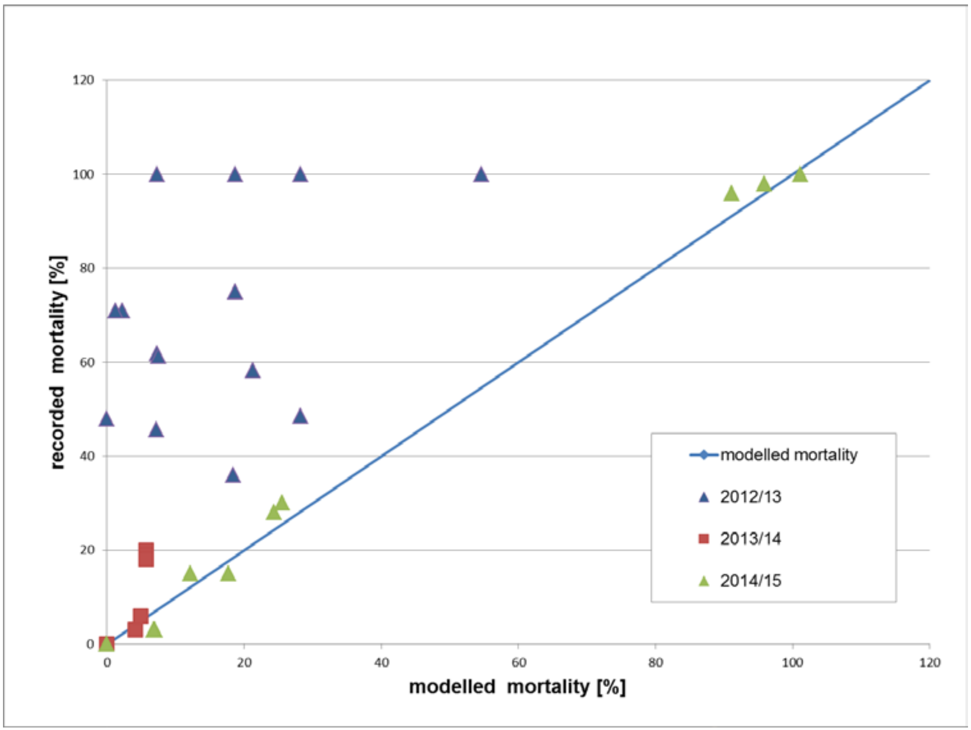


Figure 5b: Application of the calibrated “winsurv overwintering model” to overwintering data 2013/14 and 2014/15. Note that values for 2012/13 may be influenced by deleterious fungal growth.

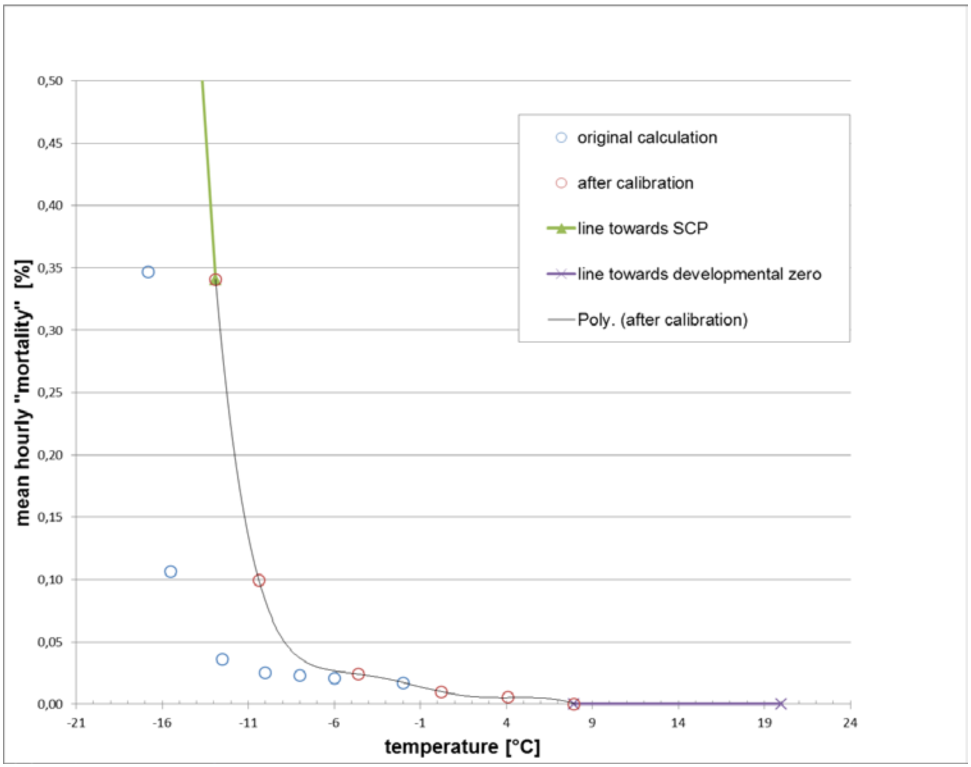


Figure 6a: Calibration of mean hourly mortalities for *Trogoderma granarium* by horizontal dilation and vertical reduction of the values (compare fig 3b).

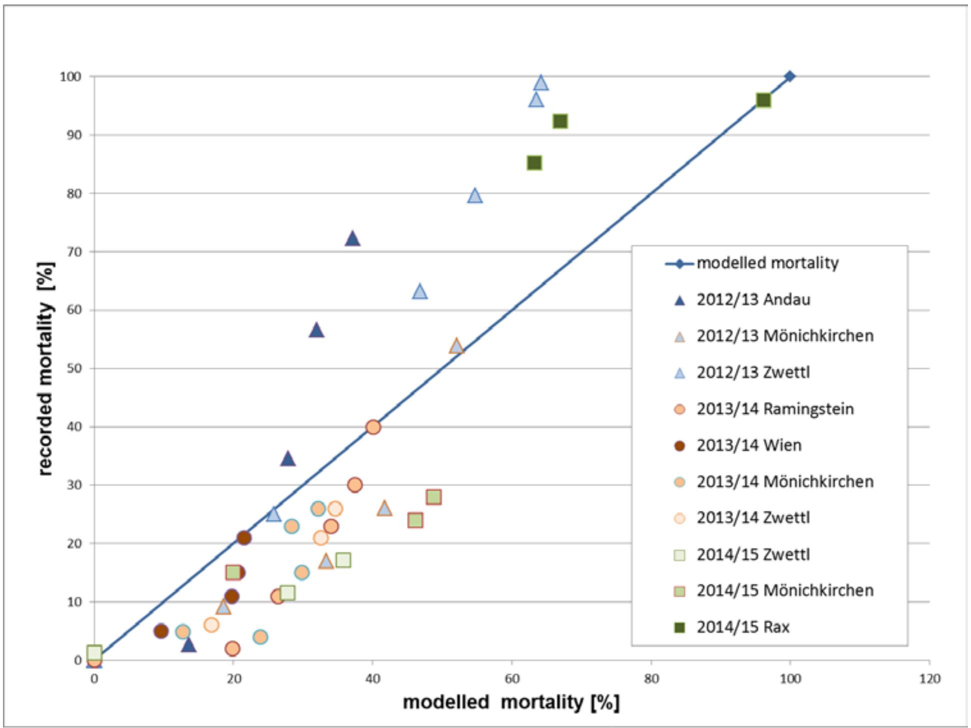


Figure 6b: Application of the calibrated “winsurv overwintering model” to overwintering data 2013/14 and 2014/15.

By variation of parameters for upscaling or downscaling also the data points in figures 4b, 5b and 6b moved. This procedure was repeated until most data points were situated at or above the blue line which marks optimal concordance of the model with the field data.

Tuta absoluta: the method of calibration worked satisfactory for *Tuta absoluta* (figure 4b) and all data points represent equal or higher mortality than predicted by the model (blue line in fig. 4b). From figure 4a it can be derived that maximum survival of 72 days occurs at +4°C. The equations describing mean hourly mortality (y) as functions of temperature (x) are: the endpoint (super cooling point) is situated at -17°C, the line connecting the SCP and the endpoint of the transformed polynomial at -11,75°C has the formula y= -0,27827257254 x -2,23063373313. The polynomial has the formula y = -0,00000713159x^6 - 0,00021325753x^5 - 0,00222291888x^4 - 0,01027837900x^3 - 0,01726840387x^2 - 0,00520535761x + 0,06450275151 (R^2 = 0,99995174408). The connecting line between the upper endpoint of the transformed polynomial at -1,25°C and the developmental zero at +6°C has the formula y= 0,06218905473

Helicoverpa armigera: the method of calibration worked satisfactory (fig. 5b) and all data points represent equal or higher mortality than predicted by the model (blue line in fig. 5b). Nevertheless many data points for the experimental year 2012/13 (blue markings) are located near to 100% mortality. This is not surprising as *Helicoverpa* pupae were affected very much by fungi in that experiment. The equations describing mean hourly mortality (y) as functions of temperature (x) are: the endpoint (super cooling point) is situated at -19°C, the line connecting the SCP and the endpoint of the transformed polynomial at -16,42°C has the formula y = -2,53014642550 x - 38,07278208441. The polynomial has the formula y = 0,00001607985x^6 + 0,00082126955x^5 + 0,01669529425x^4 + 0,17027328852x^3 + 0,91212544100x^2 + 2,41018583717x + 2,43404313690 (R^2 = 0,99991429948). The connecting line between the upper endpoint of the transformed polynomial at -4,38°C and the developmental zero at +12°C has the formula y = -0,00132518779 x + 0,02006892019.
Trogoderma granarium: the results for *Trogoderma granarium* (fig. 6b) are less conclusive than for other test species. The calibration worked better for higher mortalities than for low mortalities. The reason may be that *Trogoderma granarium* reacts sensitive to temperature fluctuations (Voelkel 1924) so that enhanced mortalities occurs at very low temperatures of ~-16°C. This can be concluded from overwintering experiments 2014/15 at the locations Mönichkirchen and Rax. In Mönichkirchen - 957 m altitude - with minimum temperatures of -10,4°C mortalities ranged at 28% whereas at the mountain Rax (close Mönichkirchen but 1547 m in altitude) -15,4°C appeared during 3 nights which resulted in a mortality of 96,5 %. The equations describing mean hourly mortality (y) as functions of temperature (x) are: the endpoint (super cooling point) is situated at -21°C, the line connecting the SCP and the endpoint of the transformed polynomial at -12,89°C has the formula y = - 0,20467947382 x - 2,29826895030. The polynomial has the formula y = 0,00000005870x^6 - 0,00000080289x^5 - 0,00000658501x^4 + 0,00006212766x^3 + 0,00030460169x^2 - 0,00300263283x + 0,01079788541 (R^2 = 1,00000000012). The connecting line between the upper endpoint of the transformed polynomial at 7,95°C and the developmental zero at +20°C has the formula y =0,00000020955 x + 0,00041247574.

The overwintering model summarizes the influx of adverse temperature effects but does not take into account the recovery at moderate low temperatures (efflux) or increased mortality due to intense temperature fluctuations or varying adaptation during overwintering – these adverse effects are compensated by calibration. Nevertheless it seems difficult therefore to validate the model by overwintering experiments on its own. An additional method is seen in a validation of the model by laboratory experiments: for that purpose a climatic chamber was programmed for variable slow cooling followed by variable slow heating over a period of 2 weeks (figure 7a). Observed mortalities were compared with modelled mortalities. The results can be seen from figure 8b. It shows a good correlation of modelled to observed mortalities. Few data points are too high – presumably because at the end of exposure unintended heat shocks happened. From that experiment it can be derived that accumulation of adverse temperature effects is described adequately by the “winsurv overwintering model”.

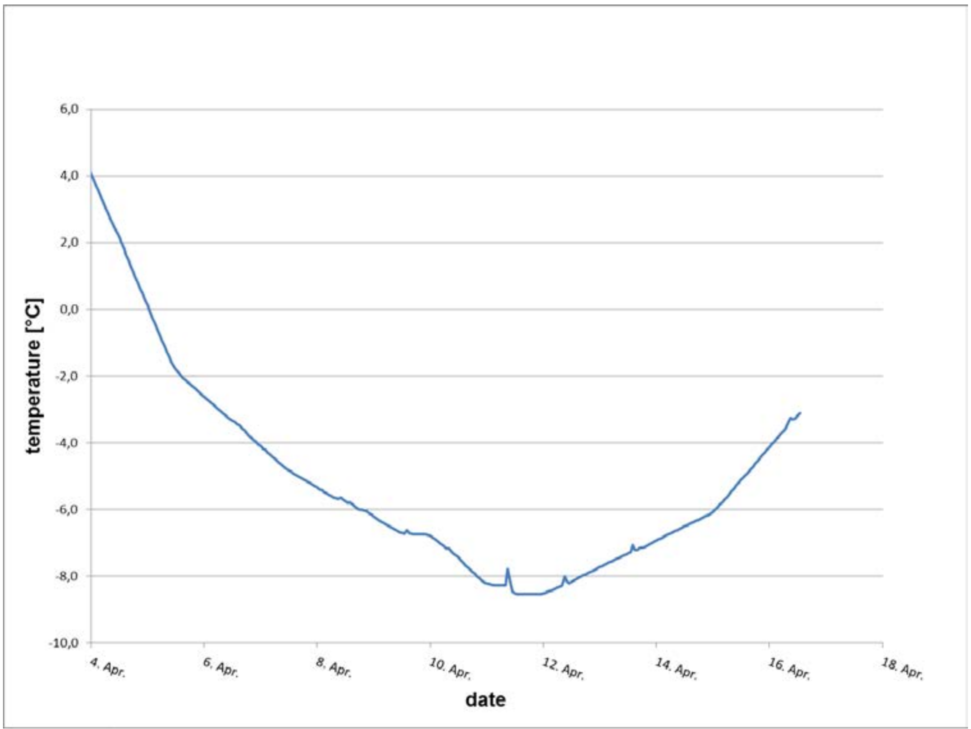


Figure 7a (left side): Temperature course in a laboratory experiment resulting from a period of slow cooling followed by slow warming.

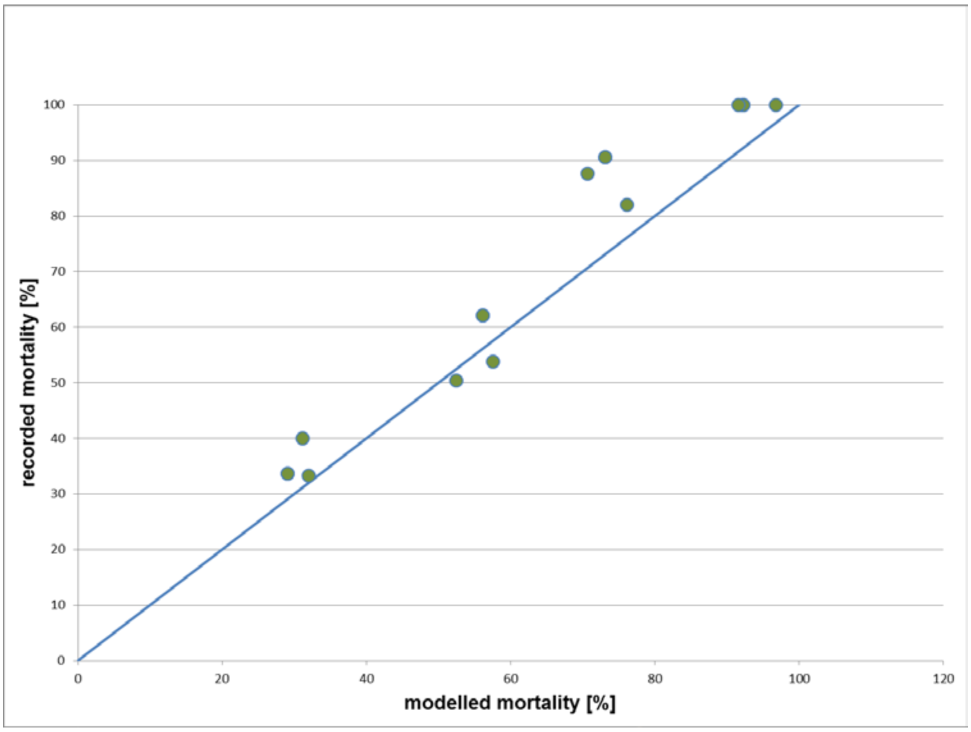


Figure 7b (right side): modelled mortality (of the uncalibrated model) marked by a blue line vs. recorded mortalities of acclimatized young pupae of *Tuta absoluta* marked by green dots.

WP6: **Present climate and future climate scenarios**

The evaluation of past climate conditions and future climate scenarios is based on the observational data from the monitoring stations and the results from the regional climate models. In the scope of the project, different types of climate models and climatic scenarios were used in the analysis. The results of the climate models are known to show systematic errors and the future climate projections are considered to have high uncertainties. Therefore, it is generally advisable to account for a large number of climate simulations in order to evaluate correctly the future climate

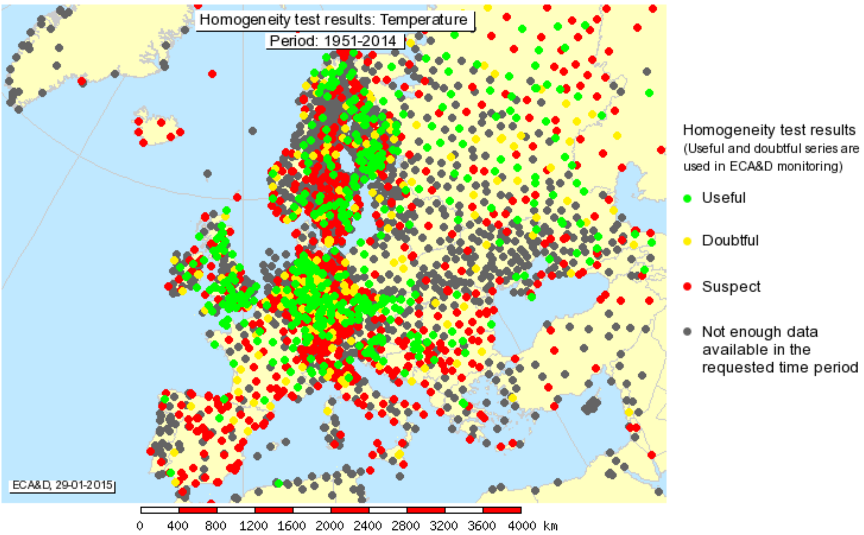


Figure 8: Homogeneity map for temperature time-series for the time period 1951–2014. (Source: http://eca.knmi.nl/utils/mapserver/homogeneity.php)

signal, its magnitude and possible spread of extremes. For that reason, we applied the model ensemble approach in order to evaluate possible future climate changes.

Observational climate data

The analysis of past climate conditions, which is also the reference for the evaluation of the future climate, was performed on the base of the observational data from the monitoring stations of the European network. The ECA&D – European Climate Assessment & Dataset (http://eca.knmi.nl/) provides the database of daily meteorological data. At the moment, the ECA dataset contains 40429 series of observations for 12 elements at 10257 meteorological stations throughout Europe and the Mediterranean, in total. The blended time-series were used in this study (blended series are series that are near-complete by infilling from nearby stations). Those monitoring stations were selected, which lay within the boundaries of the modelling domains for Europe. The time-series which contain mean daily temperature, maximum daily temperature and minimum daily temperature for the time period 1961–1999 without gaps were used in the evaluation. The number of time series used for the respective modelling domains are listed in Table 1. The quality of observational data is controlled by ECA&D and homogeneity maps are provided (Figure 8).

Climate model data

Two groups of future climate scenarios were considered in the project, from which a number of regional climate simulations were evaluated. The climate simulations were taken from three European climate modelling projects: ENSEMBLES, CLM Consortial Simulations and EURO-CORDEX (Table 2).

The future climate scenarios refer to projected emissions of greenhouse gases and other pollutants in the atmosphere throughout the 21th century, which are integrated as a forcing component in the global climate models (GCMs). The GCMs calculations, which take into account both natural and anthropogenic factors, provide an idea about the possible evolution of the future climate. The climate simulations based on two generations of emission scenarios have been considered in this study:

1. The Emissions Scenarios of the Special Report on Emissions Scenarios (SRES) – IPCC 4th Assessment Report (IPCC 2007)
2. The Representative Concentration Pathways (RCPs) – IPCC 5th Assessment Report (IPCC 2013)

Table 1: Overview of the model setup for different regional climate modeling projects for the European domain and the amount of ECA&D times series used for evaluation.

Project	**ENSEMBLES**	**Consortial Simulations**	**EURO-CORDEX**
Resolution	0.22° (25 km)	0.165° (18 km), 0.2°	0.11° (12 km), 0.125°
Longitude	34.42104°W–59.21399°E	10.6°W - 36.8°E	21.34759°W – 64.96444°E
Latitude	26.5861°N -71.6499°N	34.6°N - 69.8°N	35.33156°N – 72.58271°N
Xsize	170	238	424
Ysize	190	177	412
Periods	1951-2100	1960-2100	- Hindcast (ERA Interim): 1989 – 2008 - Control: 1951–2005 (1981–2010, 1951-1980) - Scenario: 2006–2100 (2041-2071, 2011-2040, 2071-2100)
Future scenarios	A1B	A1B, B1	RCP 2.6; 4.5; 8.5

Number of time series from ECA&D	426	394	428

Climate variables

From the available climate modelling dataset an ensemble of model simulations for daily mean, maximum and minimum air temperature was extracted only for simulations with a Gregorian calendar. Overview of used model runs is given in Table 2. Based on the daily data, the time-series of mean annual (Figure 9) and winter temperature (Figure 10) averaged over the European domain were calculated. All future climate scenarios show increase in temperature by the end of the 21st century. The highest increase is simulated for the scenario RCP8.5, however the spread between different model simulations is very large. Similar warming trend can be observed for daily mean, minimum and maximum temperature.

Table 2: Overview of the climate scenarios and number of model runs in regional climate modeling projects for European domain used for evaluation having a Gregorian calendar.

Scenarios	**historical**	**A1B**	**B1**	**rcp26**	**rcp45**	**rcp85**
ENSEMBLES	7	7	0	0	0	0
CLM Consortial Simulations	3	2	2	0	0	0
EURO - CORDEX	9	0	0	1	8	9
Sum	19	9	2	1	8	9

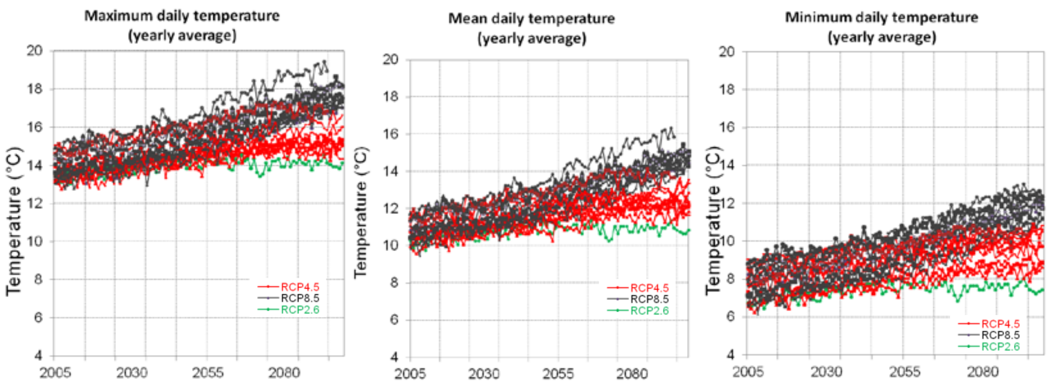


Figure 9: Timeseries of mean annual minimum (left), mean (center) and maximum (right) daily temperature for the European domain from EURO-CORDEX model results.

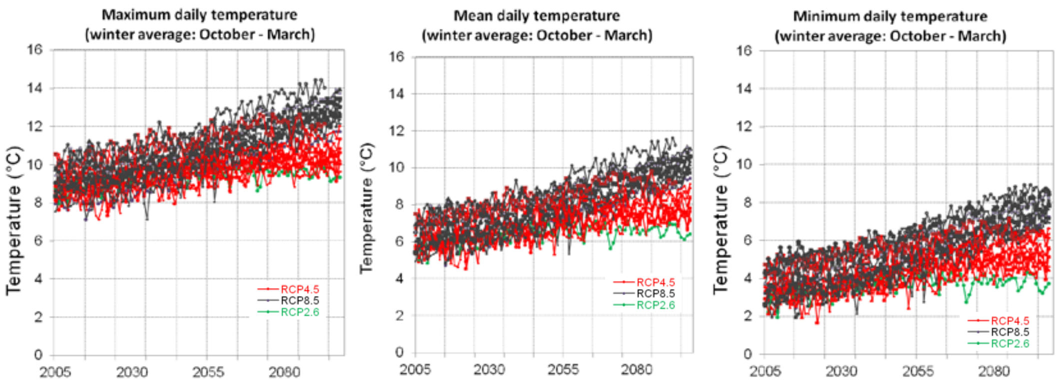


Figure 10: EURO-CORDEX model results of minimum (left), mean (center) and maximum (right) daily temperature for the winter period (October – March) averaged over the European domain.

Adjustment of the climate scenarios to local climate stations
Quantile Mapping – Method description
Climate models (CM) are known to exhibit a systematic bias, denoted as a difference between the model data and the observed value. Thus CM´s have to be post processed to reduce the bias and make the difference as small as possible. An often used post processing approach for bias correction is the so called quantile mapping (QM). QM attempts to find a transformation of the modelled cumulative distribution function (CDF), so that the new distribution of the model data equals the distribution of the observed variable. Different approaches are known to find the most suitable transformation of the model data such as **distribution derived transformations**, **parametric transformations** or **nonparametric transformations**.
In this project several transformations were tested using the qmap-package of R, a software environment for statistical computing and graphics (Gudmundsson et al., 2012; Gudmundsson, 2014):

- **fitQmapPTF (QM1):** fits a parametric transformation to the quantile-quantile relation of observed and modelled values.
- **fitQMapDIST (QM2):** fits a theoretical distribution to observed and modelled data and returns the parameters as well as the transfer function derived from this distribution – but not working at all.
- **fitQmapRQUANT (QM3):** estimates the values of the quantile-quantile relation of observed and modelled data for regularly spaced quantiles using local linear least square regression.
- **fitQmapQUANT (QM4):** estimates values of the empirical CDF of observed and modelled data for regularly spaced quantiles.
- **fitQmapSSPLIN (QM5):** fits a smoothing spline to the quantile-quantile plot of observed and modelled data.

These transformations were tested with 19 different CMs (compare Table 2) and observations from the ECA-D observation network for the period from 01.01.1961 to 31.12.1999 (see Table 1). To obtain the model values for the respective station, the gridbox with the smallest distance to the station was extracted.

Results – Transformation Test
Transformations with QM3 and QM4 were quite similar und showed the best fit of the model data to the observations. For further bias corrections QM4 (empirical quantile method) is the method of choice.

Results – Bias Correction with QM4 of historical data
Based on the results of the different transformation tests described in Section 0, method QM4 was used to perform the bias correction of the historical climate model data. Boxplots for the Root Means Square Error (RMSE) before (left; model minus observation) and after the bias correction (right, QM4 minus observation) are displayed in Figure 11. The mean difference between model and observation of -1.794 to 0.007 could be reduced with the bias correction (QM4) to values between -0.00124 and 0.00043. Also the root mean square error (RMSE) could be reduced by 0.2-0.5°C for all models.

Results – Bias Correction with QM4 of future scenarios

The parameters for the QM4 Quantile mapping method were identified for every scenario using the respective historical model data and observation data from the ECA-D Network within the time period from 01.01.1961 to 31.12.1999. Afterwards the bias correction was performed for the future scenarios using the previously identified parameters.
After bias correction, the historical model data for T_{mean} represent the observation data very well. Comparing the historical and future model data, the shift towards temperature increase is illustrated.

Validation

To validate the bias correction, the parameters identified for the QM4 method with the historical model data and observation data for every scenario within the time period from 01.01.1961 to 31.12.1999 were applied on the future model data within the time period of. The bias-corrected future model data was then compared to observation from the ECA-D Network within the same time period (01.01.2001 – 31.12.2010). The RMSE between models and observations was reduced by about 20 to 50% through the application of the QM4 method.

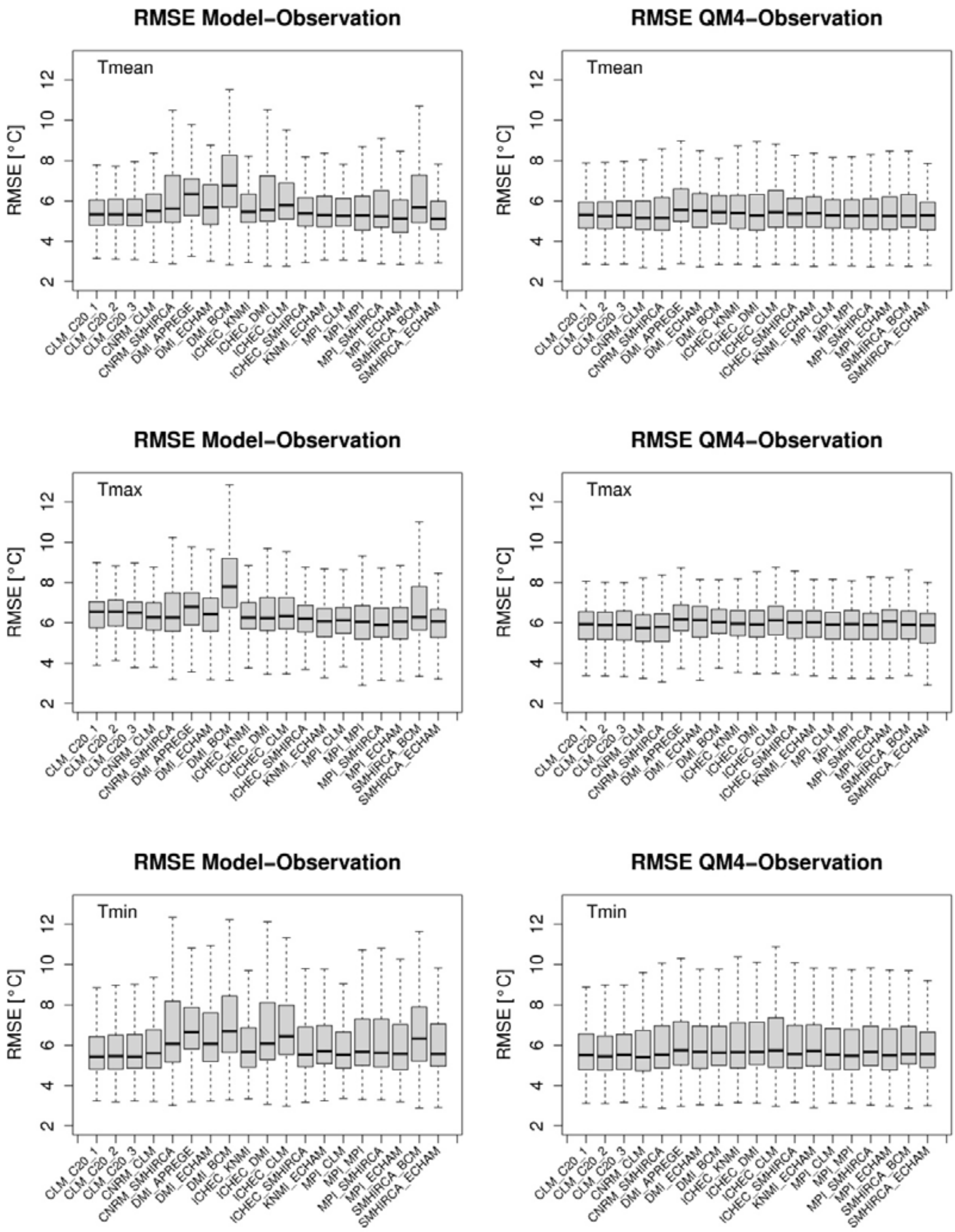


Figure 11: Boxplots of the RMSE for all different scenarios within the period from 01.01.1961 to 31.12.1999 between the historical model data and the observations (left) and the bias corrected model data with them QM4-method and observations (right).

WP7:

The final observational and scenario temperature data set

In order to get comparable results, only such station have been selected, which were available in all observational and scenario data sets (

Table 3). At each of the selected stations the mortalities for each winter were calculated with the AGES mortality models for each insect species as they were derived from the laboratory experiments

Table 3). The minimum, 10%, 25% 50% mean, 75% 90% and maximum mortalities were determined at each individual station for each data set (observations and scenarios).

Soil temperature data sets and soil model

One of the overwintering strategies of poikilothermal animals includes creeping into the soil at a certain developmental stage. Soil heat capacity, thermal conduction to the surface and thermal isolation protect the animals from the harsh winter temperatures in mid to high latitudes. Snow cover can act as additional isolating factor. Therefore, in order to model the winter mortality over Europe, soil temperatures are required, but generally unavailable. Considering all essential factors influencing the day to day and the seasonal variability, modelling of soil temperature can become a complex undertaking, because a number of factors influence it, which are difficult to get for large landscapes.

Table 3 Compilation of the temperature data sets used in this work and their metainformation.

	Observations	Scenarios		
		A1B	rcp45	rcp85
Time span	1961/1962 – 1998/1999	2079/2080 – 2098/2099		
Number of scenarios	1	9	7	9
Number of years	38	20	20	20
Number of winters = number of scenarios * number of winters	38	180	140	180

In order to be able to have some soil temperature information available over a rather large spatial range from the Mediterranean to Scandinavia, a simple soil temperature model was selected to simulate a standard winter soil temperature environment. If one is willing to accept a number of assumptions, there are some methods available, which allow modelling a reasonable soil temperature on basis of the 2 m daily mean air temperature. An average soil depth of 10 cm was assumed to be a typical overwintering depth for many insects. Soil temperature data were available from a small set of Austrian stations for model calibration.

Kang et al. (2000) developed a robust and easily parameterized model for estimating spatial soil temperature distribution in heterogeneous terrain. They combined principles of heat transfer physics with an empirical model proposed by Zheng et al. (1993), requiring standard 2 m air temperature, LAI and ground litter. The hybrid model of Kang et al. (2000) simulates the soil temperature behavior reasonably well that its results can be used as an approximation of soil temperature on the European continent.

Present and future insect mortality

Concept of mortality related mean winter temperature

In order to be able to relate the winter mortality with temperature, the mean winter temperature T_w was calculated as the average of the daily mean temperature, if the daily mean temperature was lower than the threshold temperature of the insect,0°C for *Helicoverpa armigera*, -2°C for *Trogoderma granarium* and +6°C for *Tuta absoluta:*

$$T_w = \frac{\sum_1^n T_t}{n} \quad with\ T_t \quad \begin{Bmatrix} 0 \ if \ T \le T_{th} \\ T \ if \ T > T_{th} \end{Bmatrix}$$

(Equation 1)

where T_{th} is the insect specific threshold temperature (**Fehler! Verweisquelle konnte nicht gefunden werden.** top three rows). Thus only daily mean temperatures were added up, which contributed to the mortality of the insect. In case of zero mortality no temperatures occurred below the threshold and the threshold temperature was taken as the winter average. In case of areas with zero mortality, the corresponding temperature distribution shows areas with the respective threshold temperatures.

Map representation of the modelled insect mortality

Winters are not alike and especially in the mid and high latitude continental areas the winter to winter variability can be fairly pronounced. A series of warm or cold winters can make a huge difference for the survival of bugs. Additionally one has to consider that the winter temperature variability is overlaid with non – linear winter mortality response of insects, which implies strong regional winter to winter shifts of the insect mortality. Here one is confronted with the problem to represent the results of the mortality modelling in such a way that it makes most of the information contained in the modelled mortality time series and avoids wrong conclusions. The results of the mortality modelling have been prepared in two different ways:

1. A simple average is inadequate to describe the winter climate conditions for any overwintering animals in mid to high latitudes. In this work it was decided to base the conclusions on percentiles (minimum, 10%, 25% 50%, mean, 75%, 90% and maximum) of a time span, which include 38 winters for the observational period (1961/1962 – 1998/1999) and 20 winters from the modelled future climate scenarios (2079/2080 – 2098/2099).
2. Such a series of percentile maps is still cumbersome to read and interpret. A simple classification scheme could convey the spatio-temporal variability of the insect mortality more concisely (
3. Table 4). The resulting maps should be called "potential mortality maps", because of the great number of assumptions and the lack of European outdoor observations and experiments.

Table 4: Definition of 5 potential mortality classes. At each valid station there is a time series with a certain number of winters available, at least 15 or 20 years. Then the winter with the minimum and the winter with the maximum mortality is determined. The table below lists the classification criteria into one of 5 non-overlapping classes, depending on the minimum and maximum mortality determined at a station.

Minimum mortality (%)	Maximum mortality (%)		
	= 0	> 0 & < 100	= 100
= 0	Class 1: The warmest conditions: over the total sequence of winters all winters were without any mortalities	Class 2: Next warmest conditions: over the total time period there occurred at least one winter with zero mortality, but never a winter with a total extermination	Class 3: Extreme case: still at least one winter over the total time period with zero mortality, but also at least one winter with total extermination
> 0 & < 100	X	x	Class 4: Cold case: all winters show non – zero mortalities and at least one winter occurred with total extermination.
= 100	x	x	Class 5: Coldest case: all winters with no exception exterminate all animals

Variability of potential mortality in space and time

The potential mortality maps give a consistent picture of the mortality distribution over Europe. The lines of equal mortality are running along NW-SE and the gradients perpendicular to the isolines from SW to NE with increasing mortality towards NE. A few ECA&D stations are at a higher elevation producing small areas of higher mortality within large areas of generally lower mortality. The species specific winter hardiness of the three insect species under investigation produces fairly different potential mortality distributions over Europe. Combined with the spatial temperature gradients the mortality distribution appears rather flat in case of *Trogoderma granarium*, whereas in case of *Tuta absolute* the lines of equal mortality appear compressed in a narrow band. Mild winters appear to guarantee protection through adequate winter warmth only at stations close to the sea, either Mediterranean or Atlantic. Stations on the continent, from the Balkans to Scandinavia, always have the potential to wipe out the total overwintering population. The non – linear relationship between temperature and potential mortality can be seen on the maps. The threshold temperature creates the 0% mortality zone, which can be extended over western and southern Europe. Then follows the transition zone from 0 to 100%. If the station density is high and the temperature gradient large, this transition zone can be rather narrow. In even colder climates a large region with > 100% mortality follows towards the NE. The year to year variability of temperature over Europe causes the transition zone to move along the SW-NE axis. One might call the area between the maximum and minimum mortality the transition zone space, where the transition zone is caused to move by the year to year variability of the winter temperature. Only the most western and southern sections can safely be used for overwintering and in the northeastern section of the map there is never an overwintering chance.

Climate scenarios

In order to demonstrate the effect of the expected temperature trend, the winters of the 20 year period from 2079/2080 – 2098/2099 are being compared with the presently observed climate (1961/1962 – 1998/1999). The figures of the sensitivity experiment can directly be compared with the results of the scenarios. The comparison between sensitivity experiments and climate scenario plots (**Fehler! Verweisquelle konnte nicht gefunden werden.**) reveals a good agreement between the temperature change and the corresponding change in mortality classes. The shift of the mortality classes towards NE due to the temperature increase from the sixties to the nineties corresponds to a temperature increase of 1 – 2°C of the sensitivity experiment, which agrees well with the observed temperature increase of 1 – 2°C between the two decades. All three scenario plots (A1B, rcp45 and rcp85) reveal a temperature increase, which is definitely stronger than just +1°C.

The insect specific winter temperature increase is about the same for all species in the various scenarios. The temperature increase is larger in the continental NE of Europe, which has something to do with the threshold temperature and the climate scenario. The weakest temperature increase is found in western and southern Europe, the strongest in NE continental Europe.
In case of *Helicoverpa armigera* and *Trogoderma granarium* the zero mortality areas strongly increase for all three scenarios at the expense of the other classes. Large areas in central Europe would become suitable for overwintering.

In case of *Tuta absoluta* the spatial gain through temperature increase of the scenarios remains always restricted to the Mediterranean and SW Europe. The temperature requirements of this insect species is such that no matter what amount the winter temperature increases, Europe remains largely an inaccessible continent for this insect, apart from many other problems.

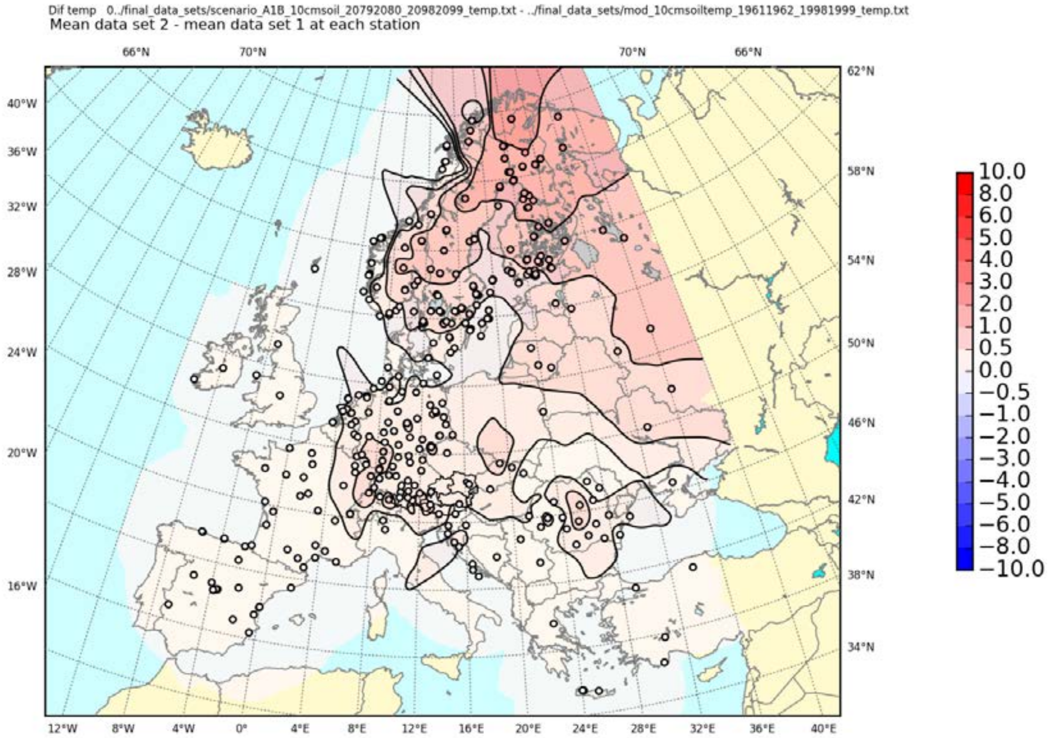


Figure 12 a: Insect specific winter temperature difference scenario A1B (2079 – 2099) – observations (1961 – 1999) *Helicoverpa armigera*

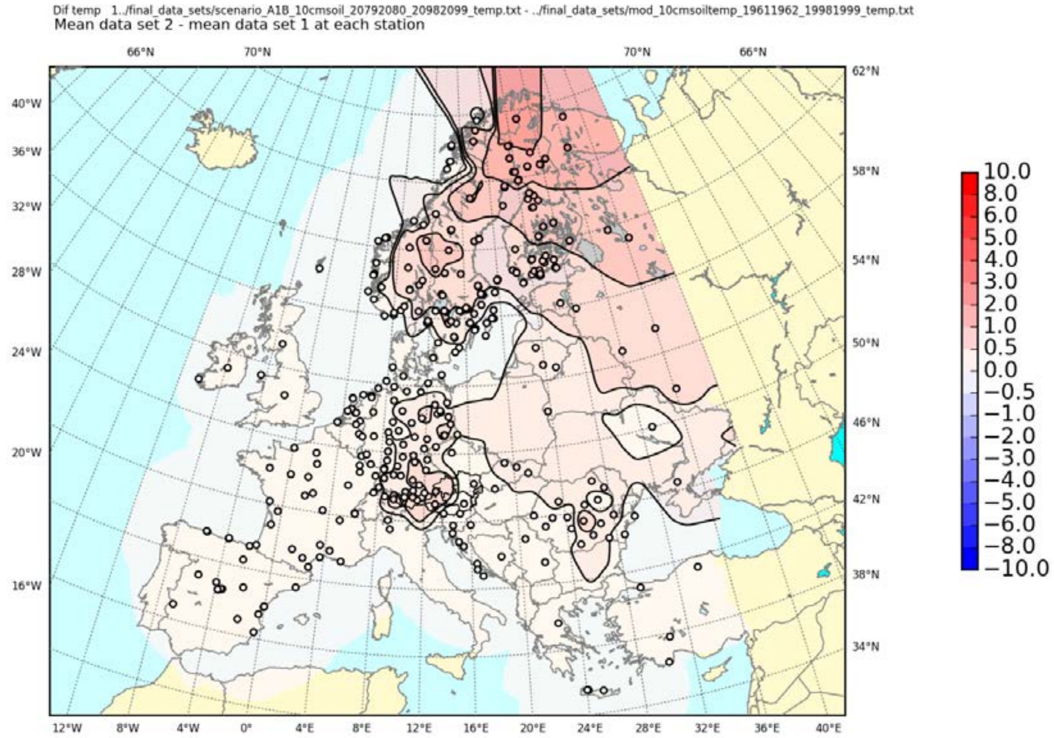


Figure 12 b: Insect specific winter temperature difference scenario A1B (2079 – 2099) – observations (1961 – 1999) *Trogoderma granarium*.

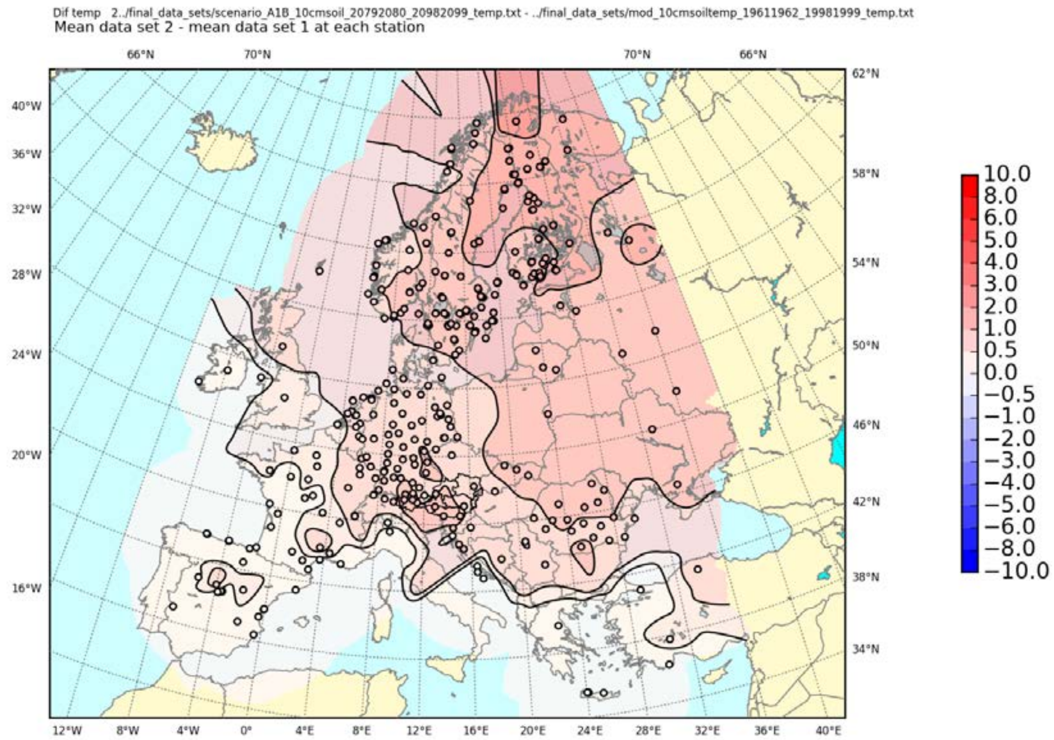


Figure 12 c: Insect specific winter temperature difference scenario A1B (2079 – 2099) – observations (1961 – 1999) *Tuta absoluta*.

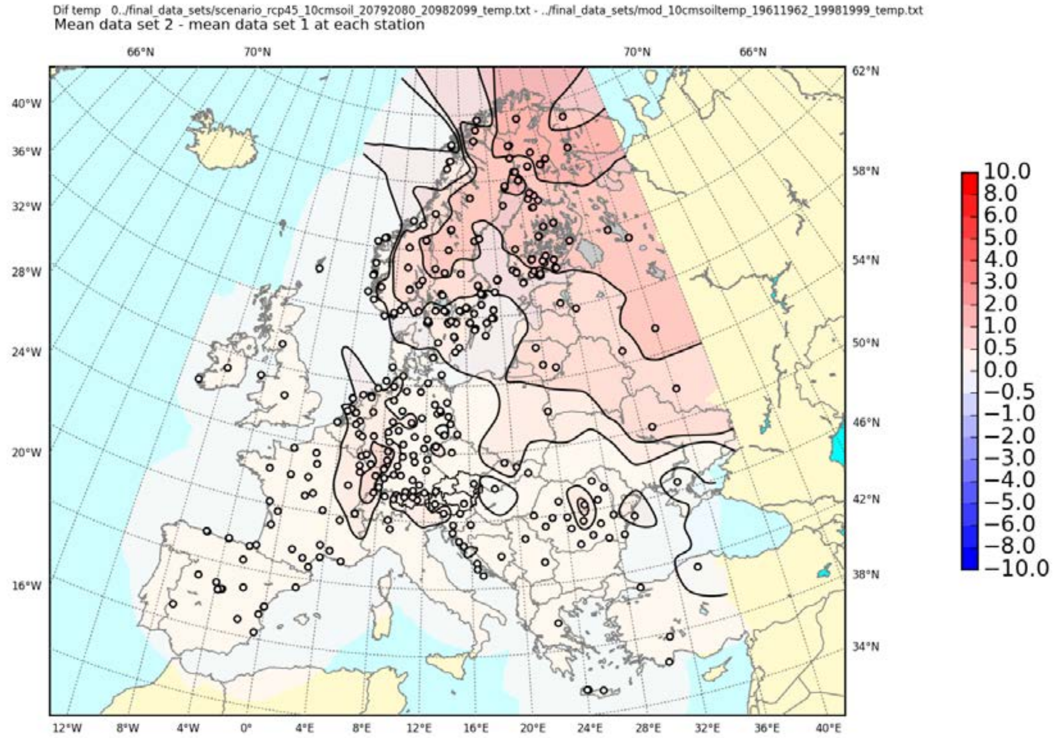


Figure 12 d: Insect specific winter temperature difference scenario rcp45 (2079 – 2099) – observations (1961 – 1999) *Helicoverpa armigera*.

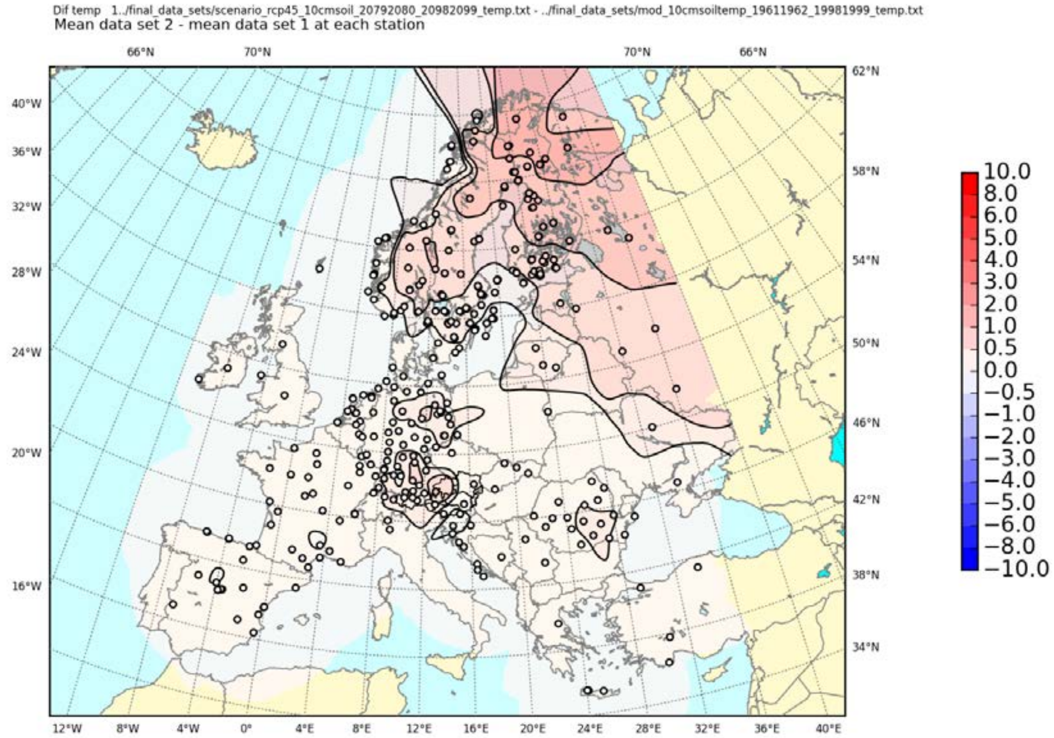


Figure 12 e: Insect specific winter temperature difference scenario rcp45 (2079 – 2099) – observations (1961 – 1999) *Trogoderma granarium*.

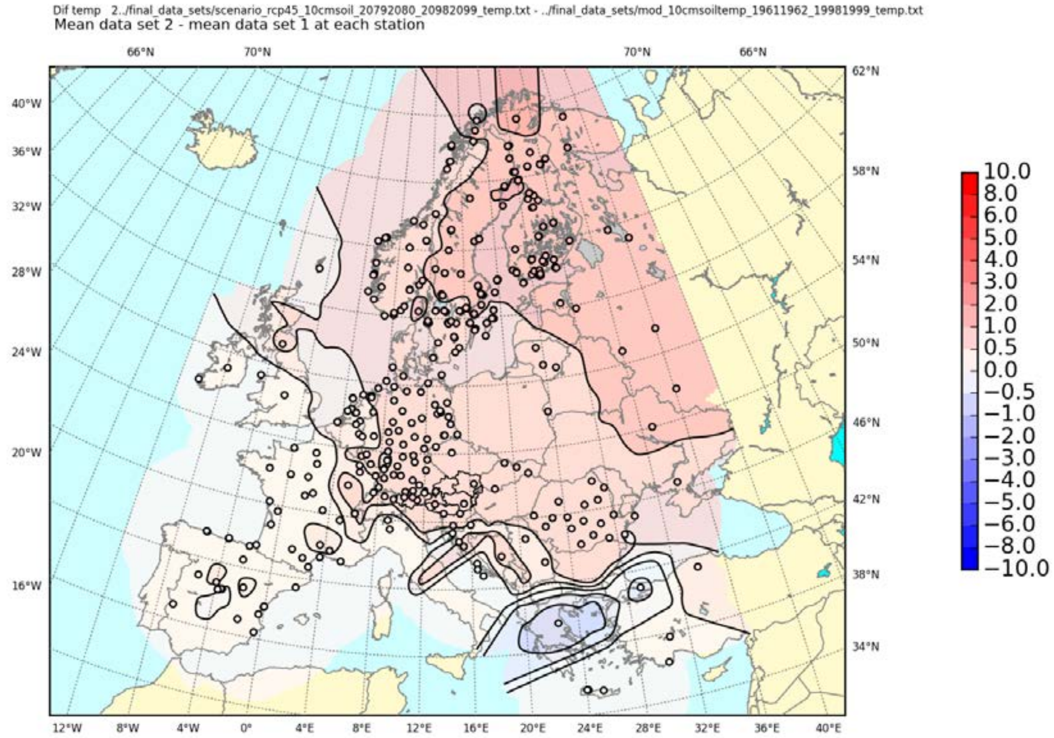


Figure 12 f: Insect specific winter temperature difference scenario rcp45 (2079 – 2099) – observations (1961 – 1999) *Tuta absoluta*.

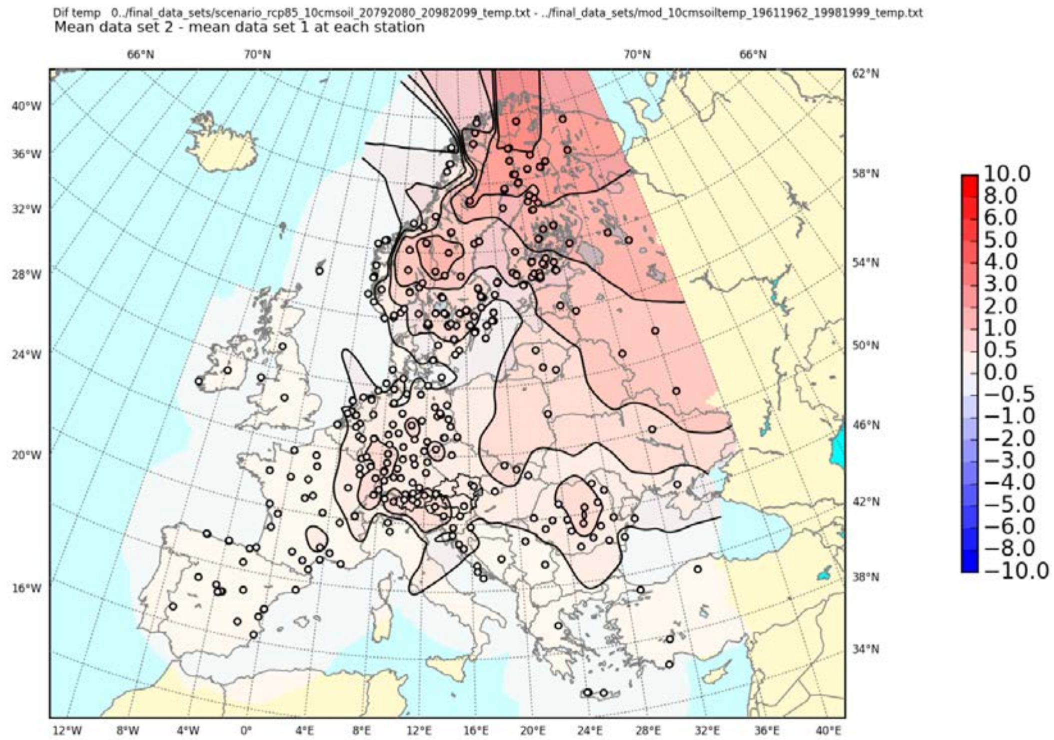


Figure 12 g: Insect specific winter temperature difference scenario rcp85 (2079 – 2099) – observations (1961 – 1999) *Helicoverpa armigera*.

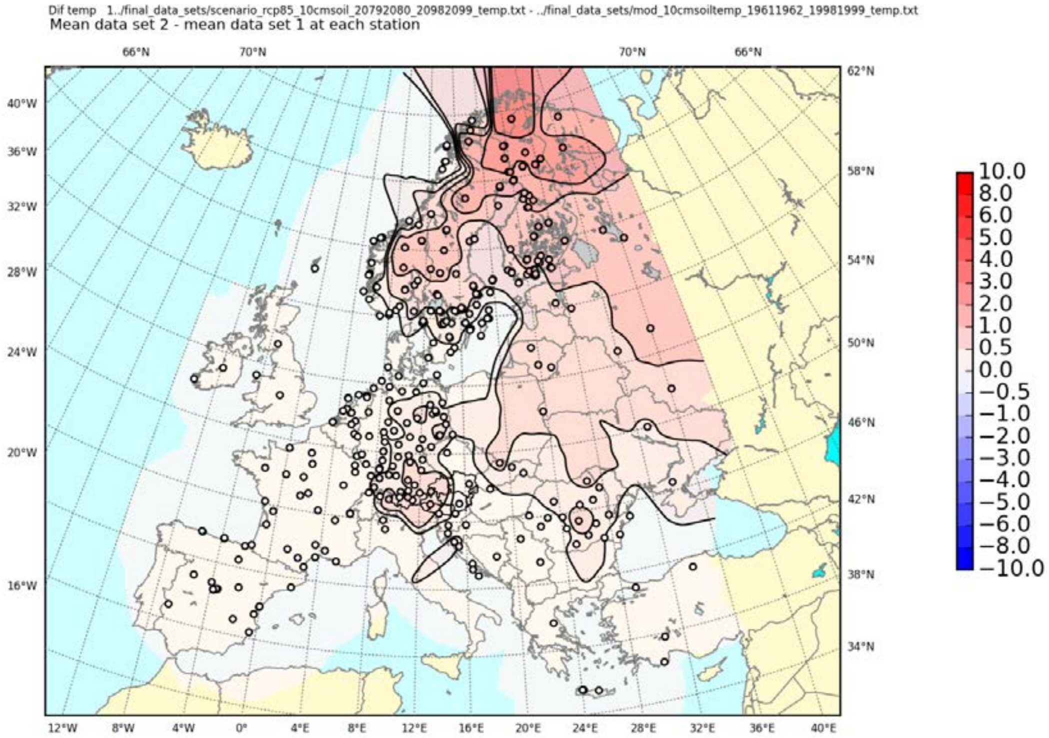


Figure 12 h: Insect specific winter temperature difference scenario rcp85 (2079 – 2099) – observations (1961 – 1999) *Trogoderma granarium*.

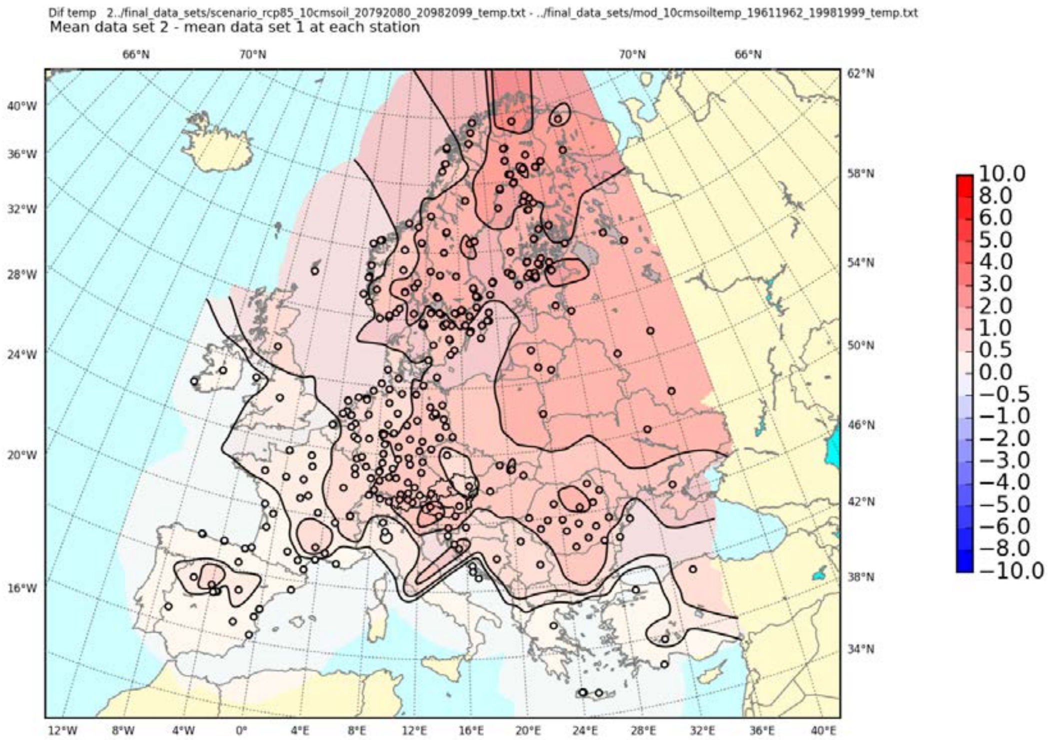


Figure 12 i: Insect specific winter temperature difference scenario rcp85 (2079 – 2099) – observations (1961 – 1999) *Tuta absoluta*.

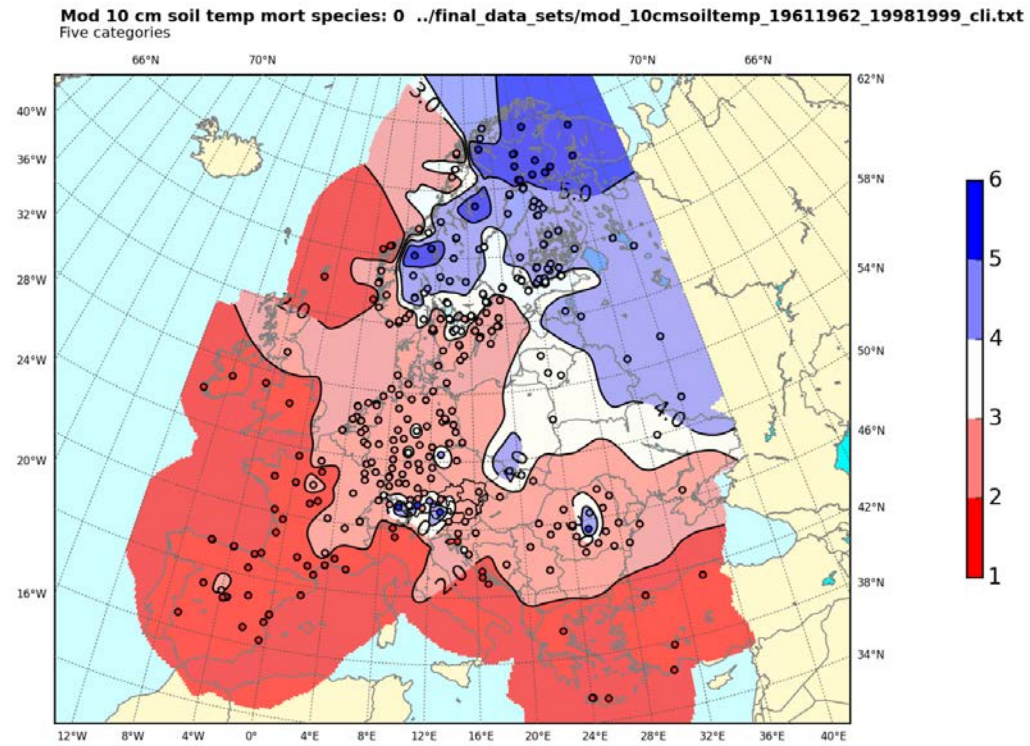


Figure 12 j: Mortality classes. Present climate 1961 – 1999, *Helicoverpa armigera*.

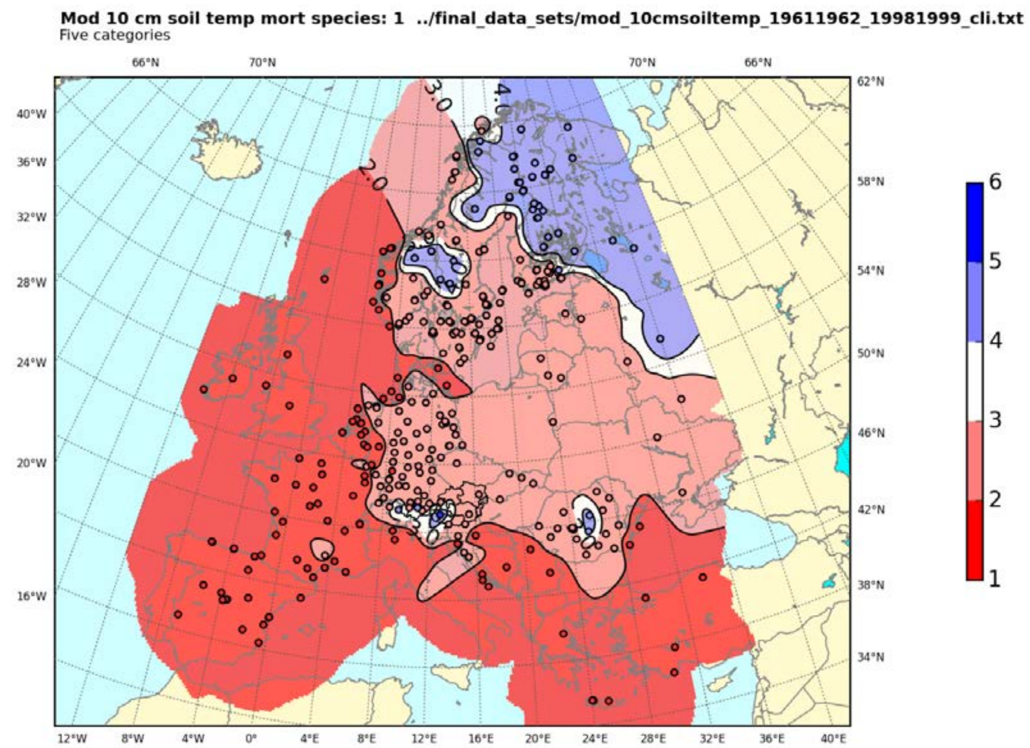


Figure 12 k: Mortality classes. Present climate 1961 – 1999, *Trogoderma granarium*.

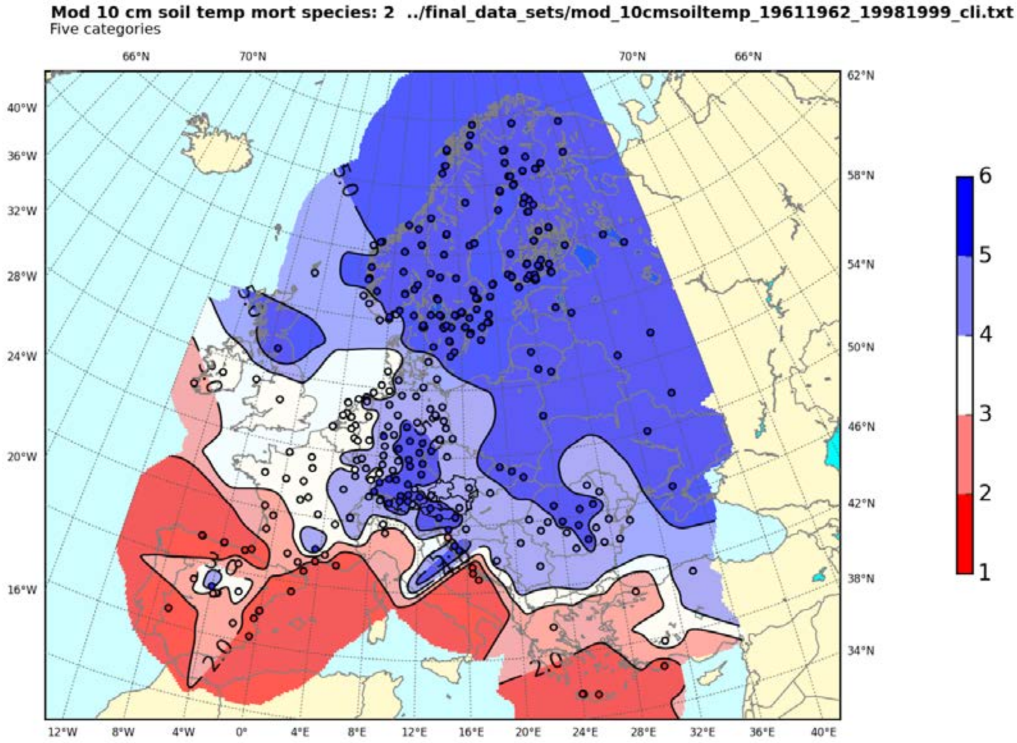


Figure 12 l: Mortality classes. Present climate 1961 – 1999, *Tuta absoluta*.

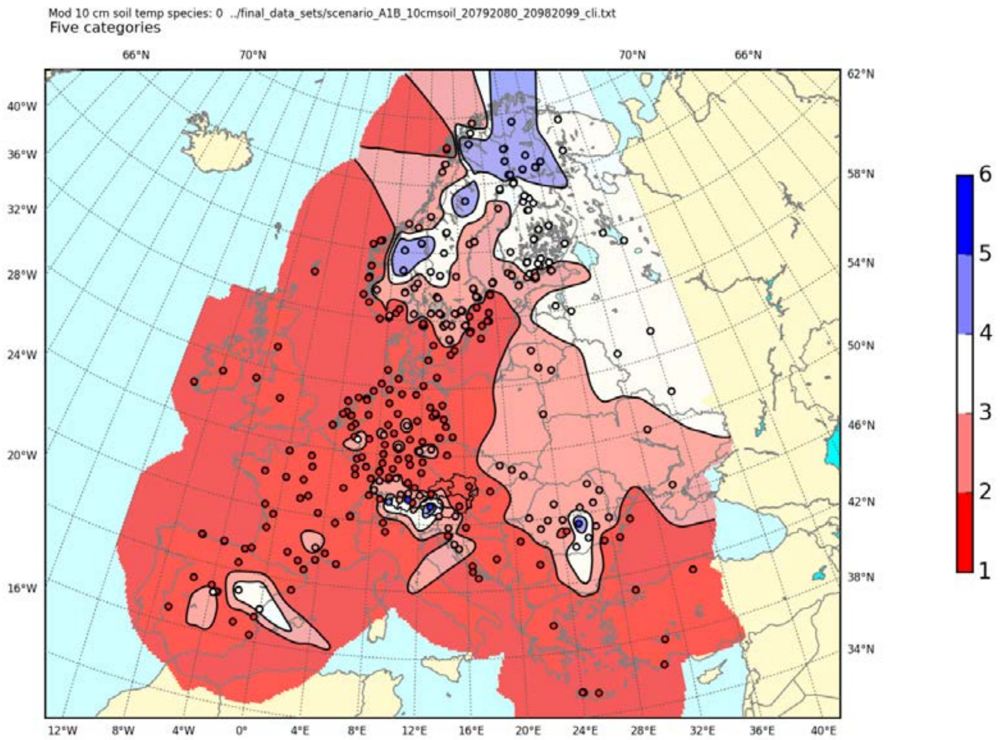


Figure 12 m: Mortality classes. Scenario A1B 2079 - 2099, *Helicoverpa armigera*.

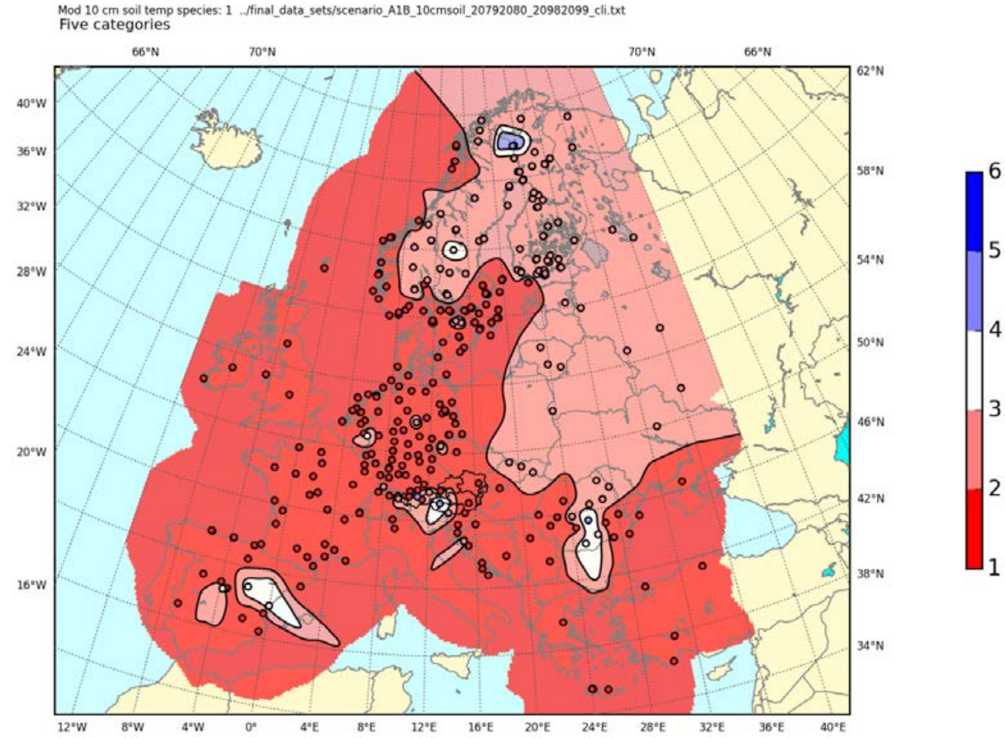


Figure 12 n: Mortality classes. Scenario A1B 2079 - 2099, *Trogoderma granarium*.

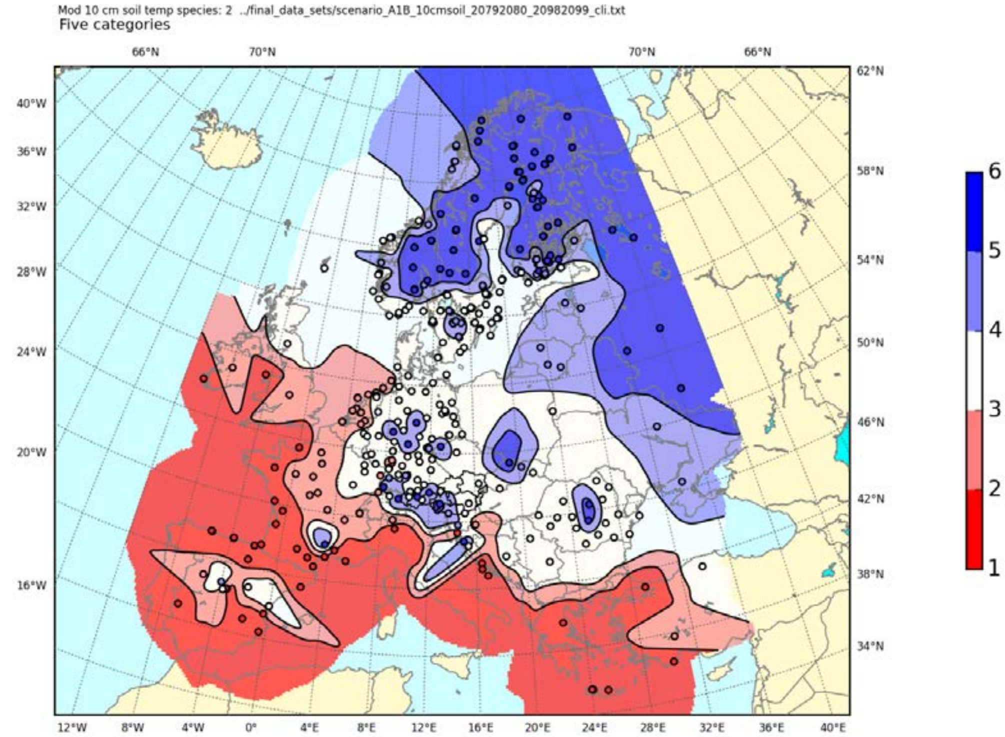


Figure 12 o: Mortality classes. Scenario A1B 2079 - 2099, *Tuta absoluta*.

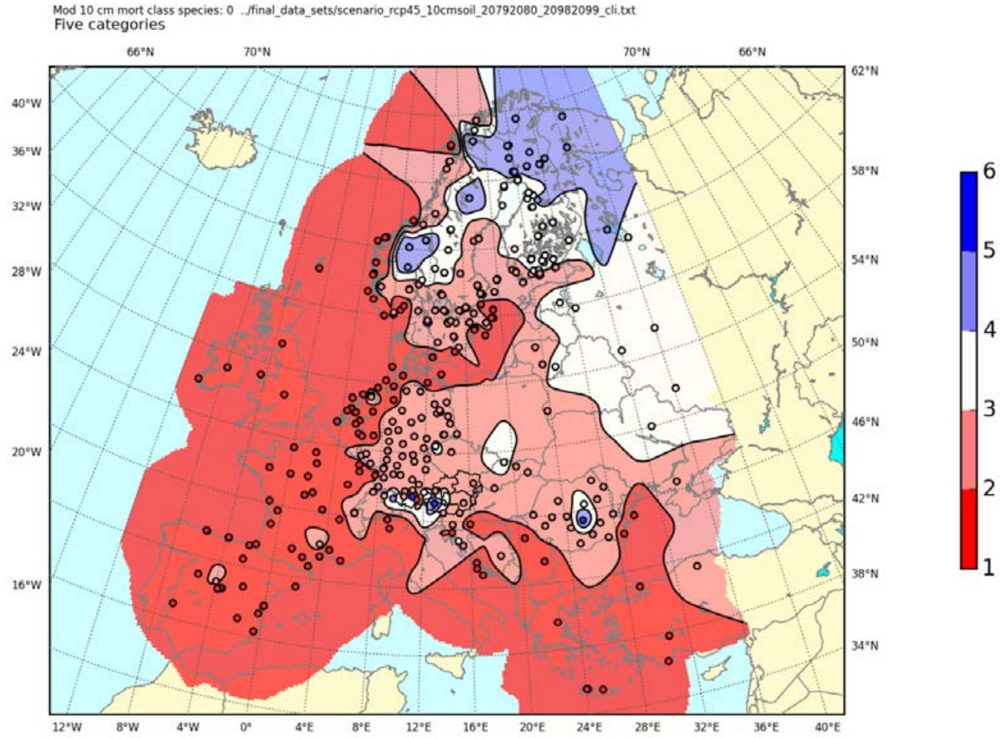


Figure 12 p: Mortality classes. Scenario rcp45 2079 - 2099, *Helicoverpa armigera*.

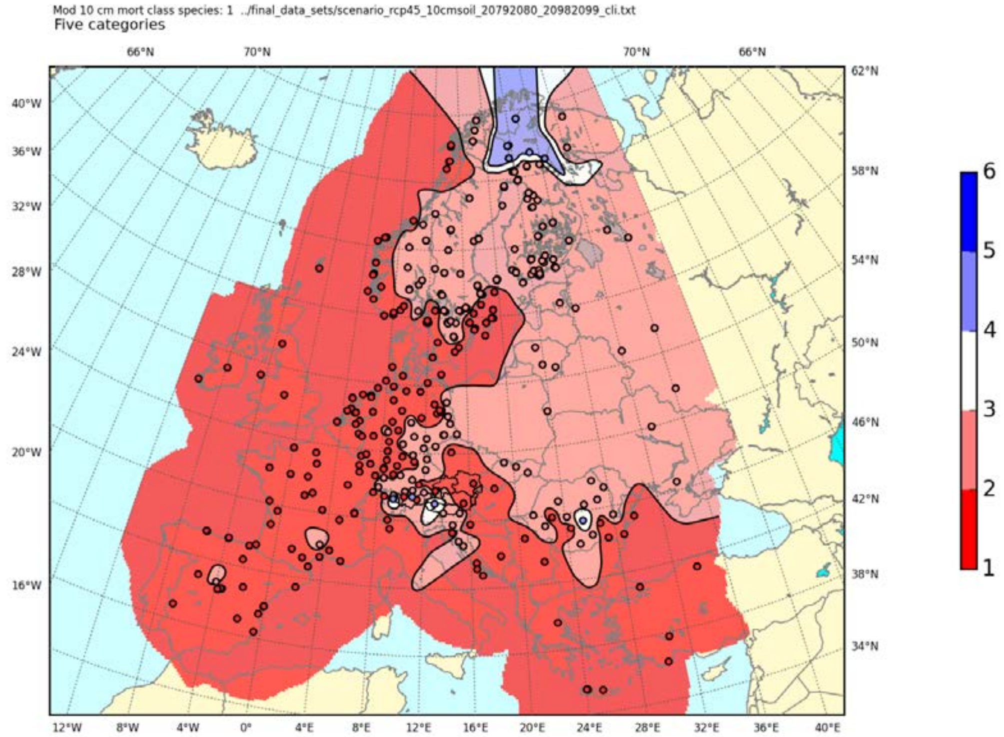


Figure 12 q: Mortality classes. Scenario rcp45 2079 - 2099, *Trogoderma granarium*.

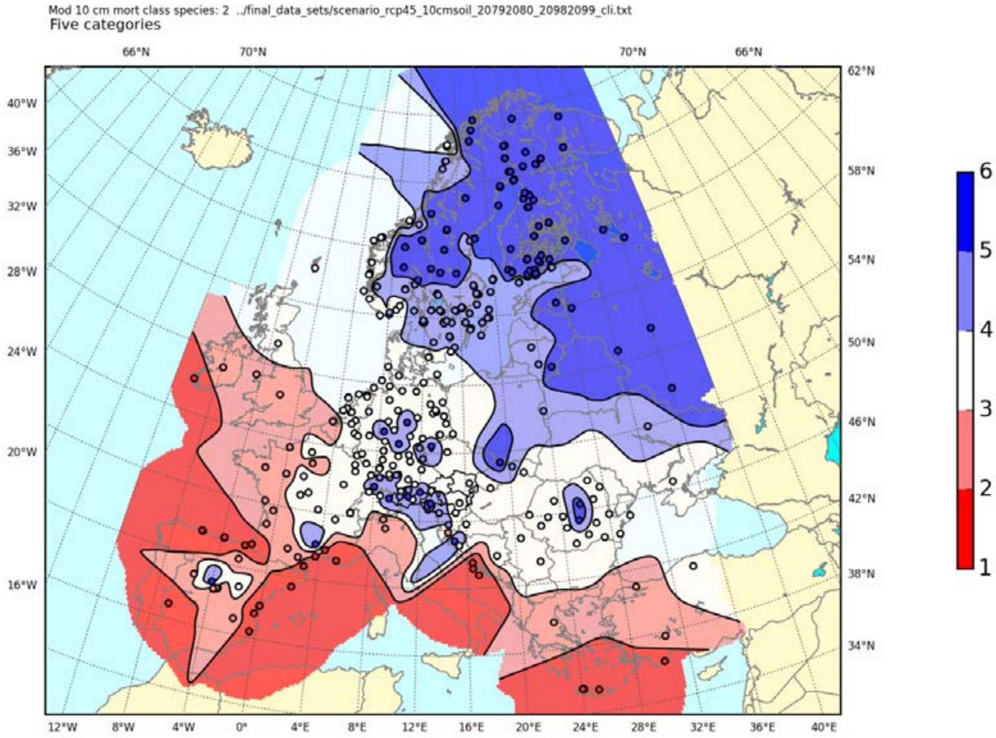


Figure 12 r: Mortality classes. Scenario rcp45 2079 - 2099, *Tuta absoluta*.

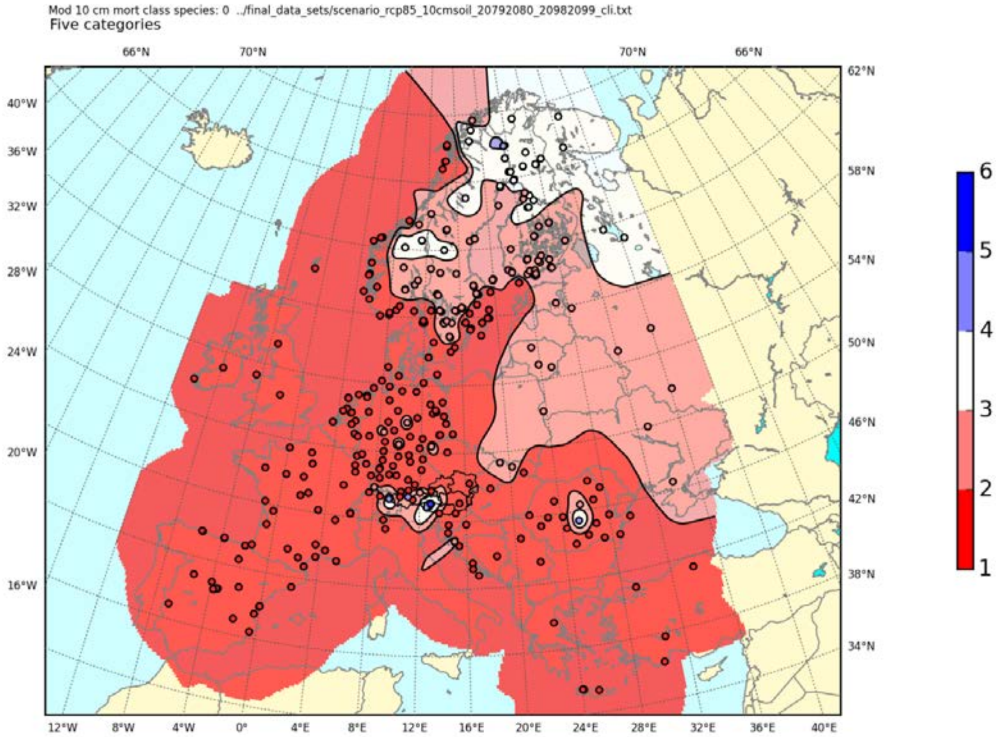


Figure 12 s: Mortality classes. Scenario rcp85 2079 - 2099, *Helicoverpa armigera*.

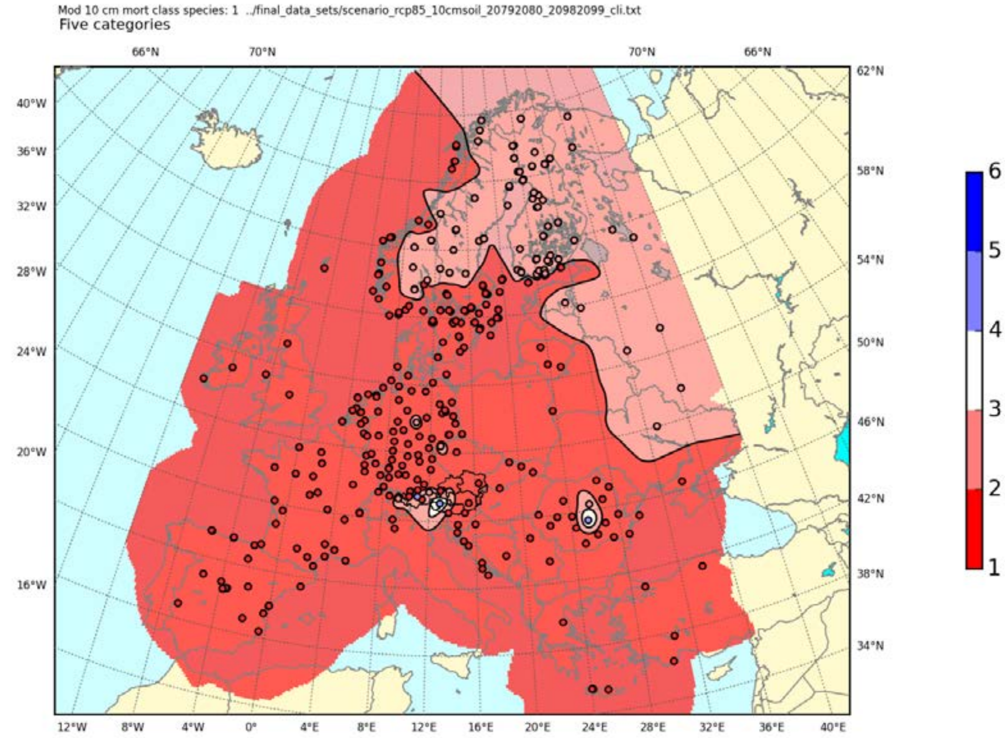


Figure 12 t: Mortality classes. Scenario rcp85 2079 - 2099, *Trogoderma granarium*.

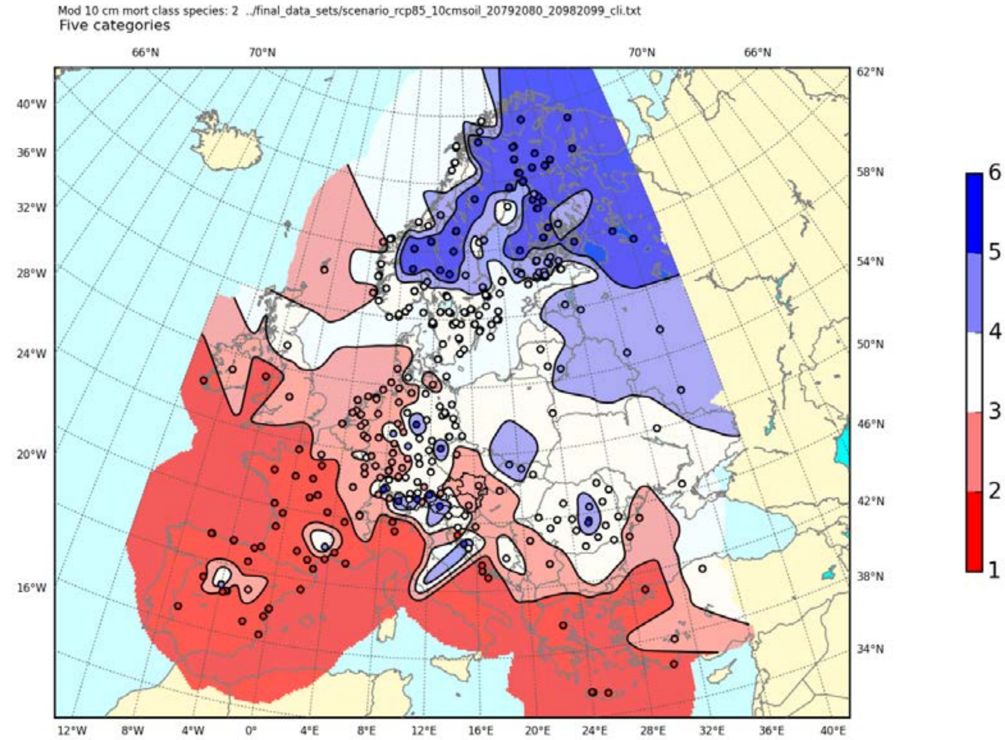


Figure 12 u: Mortality classes. Scenario rcp85 2079 - 2099, *Tuta absoluta*.

a)

b)

c)

d)

Tafel I / Plate I: Adult moths of tomato leafminers (*Tuta absoluta*; Lepidoptera; Gelchiidae) measure about 6 mm (a) and lay their eggs (b) which are hardly visible because of their diminutiveness of 0,3 mm on all parts of tomato plants. Larvae produce tunnels and galleries (c) within the leaf mesenchyme in which they live and where they form their pupa (d) within a cocoon.

a)

b)

c)

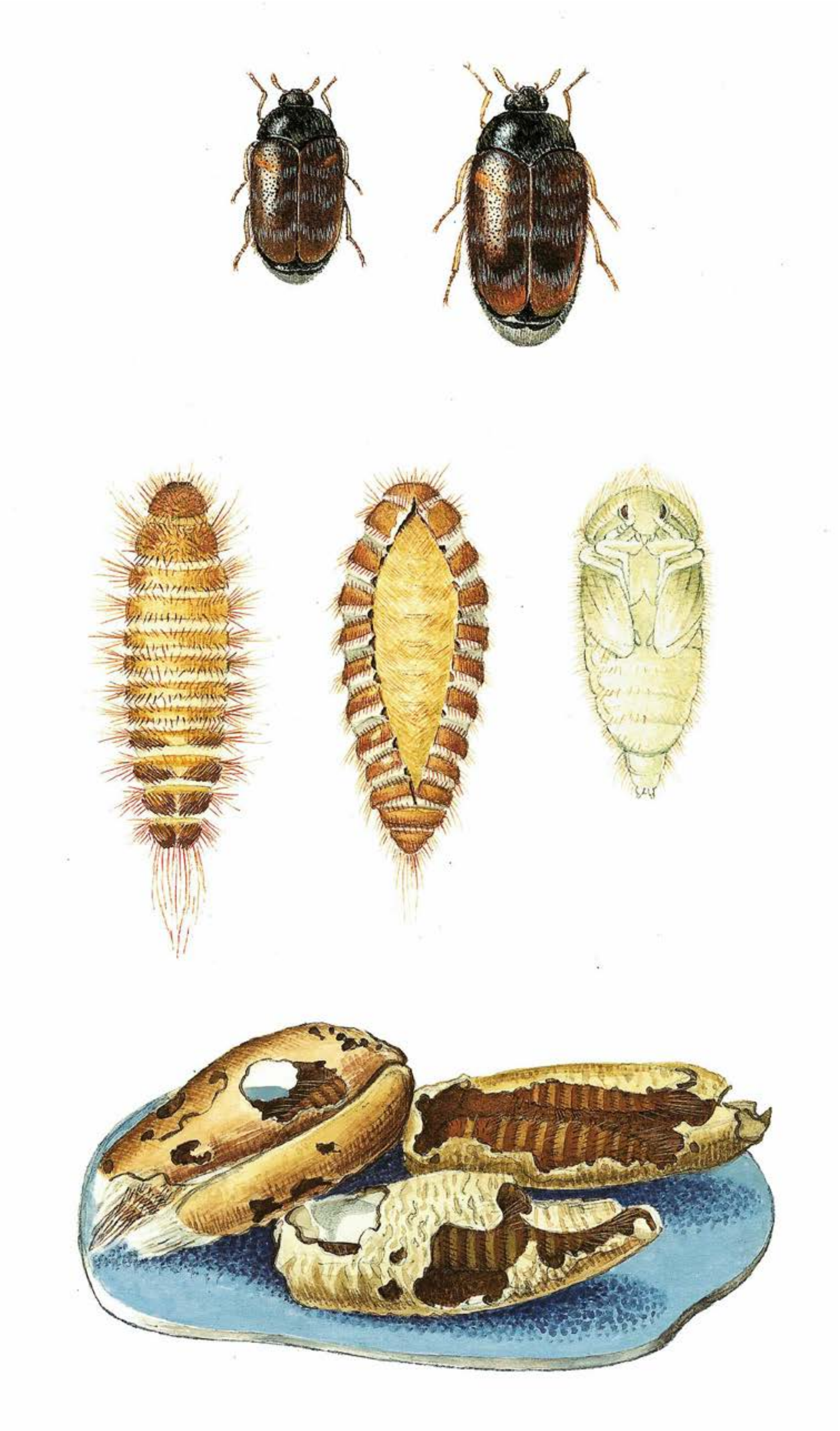
d)

e)

f)

g)

Tafel II / Plate II: Cotton bollworm is the name of caterpillars (c) of (*Helicoverpa armigera*; Lepidoptera; Noctuidae). Adult moths are of unspectacular appearance (a) and fly during and after dawn. They lay their eggs (b) singly in the vicinity of flowers or fruits of their host plants. Caterpillars feed mainly inside of buds, flowers or fruits. Pupation (d) occurs in a cocoon buried shallow in the ground. Beside fruit vegetables (e), (f), (g), also ornamentals and corncobs are perished by their activity.



Tafel III / Plate III: adults, larvae, pupae and damage of Khapra beetles (*Trogoderma granarium*). All stages are confined to stored products – mostly cereals. They originate from India, in European countries they would be forced to survive in human surrounding – like heated malt stores.

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5 Schlussfolgerungen und Empfehlungen

A temperature and time dependent model for winter survival was developed for *Tuta absoluta, Helicoverpa armigera* and *Trogoderma granarium* based on laboratory data. By calibration of the model it was possible to describe recorded field mortalities as functions of experienced temperatures and to calculate at least minimum values for mortality. Beside winter mortality also summer conditions are important for the establishment of an insect pest.

The application of hourly temperature values to the survival equations showed that Tuta absoluta can survive for 72 days at a maximum, whereas *Helicoverpa armigera* can survive in parts of Austria without problems in the ground. In tropical summers like 2003 or 2015 *Helicoverpa armigera* was able to develop efficiently and to achieve pest status. For *Trogoderma granarium* winter temperatures near -16°C are deleterious if they last longer than a few nights. Mean monthly summer temperatures must exceed 20°C for 4 consecutive months. These values were not exceeded during the tropical summers of 2003 and 2015.

Austria and the Alps are positioned in the area with the greatest effect of the winter temperature climate on the insect mortality. It is a sensitive zone and any winter temperature increase will produce an increased presence of some insects, native or introduced. According to the potential mortality maps, constructed on the mortality models derived from lab experiments, Austria lies today just at the border of the class “no winter mortalities” for *Helicoverpa armigera* and *Trogoderma granarium*. Any further winter temperature increase will move Austria from the area of the class “winter mortalities can happen, but no total extermination” into the area of the class “no winter mortalities”. At least according to potential winter mortality models, there exists an observational basis of all three entities, climate, soil, insect winter mortality, but it is extrapolated in space from the lab and a few outdoor experiments in Austria to Europe and the future. Although it is not possible to quantitatively asses the uncertainty of the results, this exercise can still be considered to be useful:

We have combined all the necessary procedures and models to describe the potential winter mortality. The chain of experiments with the insects, software tools and models does exist and has been worked through all steps, from lab and outdoor experiments, the deduction of mortality models, atmospheric data, soil model to the final result, mortalities in a future climate scenario. The basic structure has been created. Either a follow-up project or another working group can build on this basis, refine and extend the procedures. One important quantitative result is the description of the effect of the climate variability on the potential winter mortality. This includes the year to year variability and trends. The potential winter mortality model developed in this project could be implemented in a model, simulating the population dynamic processes with all the necessary additional components.

The winter survival model developed in this project turned out to be a useful tool for analysis of specific overwintering situations. Nevertheless it is only a first step in analysis of a complex of divergent processes during overwintering. During the step of calibration these disturbing factors are compensated. For further fine tuning of the model more information would be needed about recovery of overwintering stages in periods of moderate low temperatures, about possible adverse effects by fluctuating low temperatures and about adaptation processes during overwintering. It seems desirable to continue the process of modelling winter mortality with validation methods performed in the laboratory: beside experiments based upon varying temperature courses (compare fig. 8) it would be very promising to conduct experiments with fluctuating temperatures.

Project results can definitely be refined. Anybody interested in the topic of population dynamics of exotic pest insect will find useful description of breeding, overwintering experiments and of a procedure how to simulate their overwintering chances. Our work would be of special interest for working groups dealing with overwintering of vectors of human diseases.

B) Projektdetails

6 Methodik

The work was structured by several work (WP) packages which are shortly characterized here:

- WP 1: Insect breeding: prerequisite for any experimental activity.
- WP2: Adaptation of insects to cold temperatures: finding of appropriate (diapause, quiescence) overwintering stages and their optimal adaptation for laboratory experiments.
- WP3: Mortality experiments under constant low temperatures; identification of statstically secured values of LT_{100} for series of low temperatures
- WP4: Fitting of winter survival models: development of time and temperature based overwintering models, which describe observed mortalities as functions of experienced temperatures.
- WP5: Evaluation of the winter survival model in the field
- WP6: Application of climate scenarios for prediction of winter survival
- WP7: Calculation of the winter mortality under various climate scenarios and interpretation of the modelling results

<u>WP1</u> (title: insect breeding; milestone: lab colonies producing sufficient numbers of test specimens): sufficient numbers of test specimens of *H. armigera*, *T. granarium* and *T. absoluta* were produced with the help of entomological standard methods in climate chambers, …… .

<u>WP2</u> (title: adaptation of insects to cold temperatures; milestone: determination of hibernation stages and freezing points): Identification of insect stages suitable for hibernation of *T. absoluta* and *T. granarium*. This was achieved by determination of super cooling points (SCP) and survival under selected exposure conditions (temperature, duration). SCPs and melting points were identified by recording the heat of crystallisation at the freezing point respectively the heat of fusion at the melting point.

<u>WP3</u> (title: mortality experiments under constant low temperatures; milestone renamed: determination of LT_{100} for 3 experimental species in a set of low temperatures): Acclimatized overwintering stages of test insects were exposed to series of constant low temperatures (0°, -2°, -4°, -6° -8°, -10°C and lower) for various time spans. Durations were determined in preliminary tests in order to obtain mortalities of 85% to 100%. These mortality data were statistically analysed according a method of Kaplan-Meier which led to the definition of LT_{100} and confidence intervals containing 95% of the entire values. A lot of basic work on experimental design was necessary to enable successful completion of these experiments. The most important features were the definition of suitable adaptation conditions, the prevention of sudden temperature shocks by limitation of chilling or heating rates to 1 centigrade per hour and the definitely diagnosis of death.

<u>WP4</u> (title: fitting of winter survival model; milestone renamed: Development of overwintering models for 3 experimental species): activities comprise mathematical calculations but no experiments. A mathematical model in which weighted hourly chill units are accumulated according winter temperatures was developed. A new method for calculation of the mortality values had to be used.

WP5 (title: evaluation of the winter survival model in the field; milestone renamed: adaptation of winter survival model to actual mortalities in the field): In order to evaluate and eventually adapt the winsurv overwintering model it was necessary to test it with real overwintering data. For that purpose experimental insects in their proper overwintering stage were stored in sheltered small boxes together with temperature data loggers in 5 locations during 3 years: Andau (Burgenland, 120 m, 2012/13), Zwettl (Niederösterreich, 520 m, 2012/13, 2013/14, 2014/15), Mönichkirchen (Niederösterreich 957 m, 2012/13, 2013/14, 2014/15), Ramingstein (Salzburg, ~1000 m, 2013/14), Rax (Niederösterreich, 1547 m, 2014/15), Breitenlee (Wien, 160 m, 2012/13, 2013/14). According to predefined time schedules insect samples were taken from these shelters and stored in the laboratory to record survival. A calibration of the mathematical model was attempted by mathematical dilation or reduction of the calculated mean mortality-temperature curve along the x- and y-axes. By varying the dilation / reduction parameters also the amount of accumulated weighted chill units [%] will change. Thus it should be possible to move modelled mortalities in relation to recorded real mortalities. Corresponding “survival equations” which define hourly mortality as a function of temperature will be listed.

WP6 (title: Application of climate scenarios for prediction of winter survival; milestone provision of a temperature data set): data sets of observed daily mean temperature time series (1951 – 2010) and ensembles of possible future climate scenarios from regional climate model output data bases (A1B, rcp25 and rcp85) have been prepared (Figure 13). Via a quantile mapping method the dynamically downscaled temperature time series have been adjusted to the local station temperature time series. A simple soil model has been selected and adjusted to simulate the soil climate, which is typical for insects overwintering in a litter and soil environment.

WP7 (title: calculation of the winter mortality under various climate scenarios and interpretation of the modelling results; milestone: prediction of overwintering survival on present and future climatic scenarios) Observed and scenario temperature time series were fed into the winter mortality models to calculate the potential spatial and temporal variability of the insect winter mortality in Europe. Via a comparison of the observed with the scenario winter mortalities expected winter mortality trends for the future were deducible.

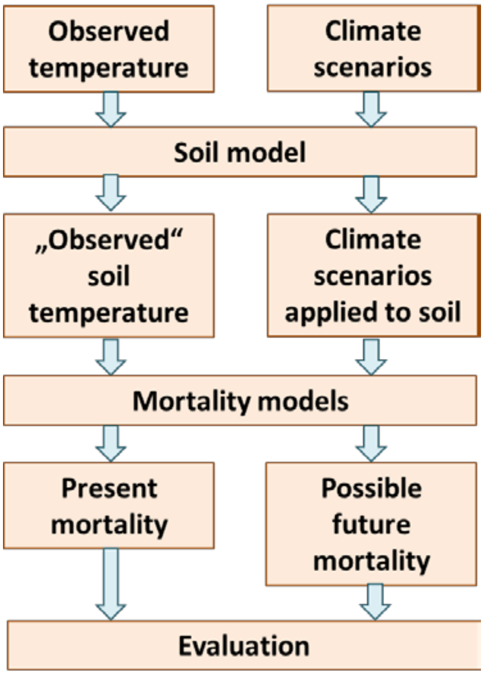

Figure 13: Sketch of the project components.

7 Arbeits- und Zeitplan

Year	(07/2011-06/2012)				(07/2012-06/2013)				(07/2013-06/2014)				(07/2014-06/2015)			
Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
WP 1																
WP 2																
WP 3																
WP 4																
WP 5																
WP 6																
WP 7																

Work packages are defined in item B6

8 Publikationen und Disseminierungsaktivitäten

Kahrer, A., Egartner, A., Moyses, A., Scheifinger, H., Matulla, Ch., Zuvela-Aloise, M. (2013): Abschätzung des Überwinterungserfolgs exotischer Insekten unter künftigen Klimabedingungen in Österreich, Poster, 14. Österreichischer Klimatag, Wien

Kahrer, A., Egartner, A., Moyses, A., Scheifinger, H., Matulla, Ch., Zuvela-Aloise, M. (2014): Abschätzung des Überwinterungserfolgs exotischer Insekten unter künftigen Klimabedingungen in Österreich. Vortrag am 15. Österreichischen Klimatag am 2. – 4. April 2014, Innsbruck.

Moyses, A. & Kahrer, A., 2013: Überwinterung von *Helicoverpa armigera* in Österreich unter zukünftigen Klimabedingungen: Akklimatisierung der Versuchstiere, Vortrag, 68. ALVA-Tagung, Klosterneuburg

Moyses, A., Kahrer, A., 2014: Kann *Helicoverpa armigera* in Österreich überwintern?, Vortrag, 69. ALVA-Tagung, Wieselburg
Moyses, A., Kahrer, A., 2015: Overwintering of *Helicoverpa armigera*in Austria, Vortrag, IOBC-WPRS meeting of the Working Group "Integrated Protection in Field Vegetables", 4.-7.10.2015, Hamburg

The most important results were obtained just at the end of the project. As a reason of time it was not possible to publish them adequately since now – but it is aimed at doing so.