Distribution, Life History, Food Choice and Chemical Ecology of the Invasive Box-Tree Pyralid *Cydalima perspectalis*

Inauguraldissertation

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Prof. Dr. Jörg Schibler
Dekan

S isch intressant, wo s Partyvolk sich trifft – und schifft:
An d Muur, uf d Stääge und in d Yfahrt näbem Lift.
Die halbi Stadt wird jeden Oobe überschwemmt,
ganz enthemmt. – Vo wäge Ryssverschluss wo klemmt!
D Bäi usenander stöhn si aane, wie sich s ghört
In äinere Räihe an der Gartehecke dört.
Äin vo der Stadgärtnerei, anstatt si z biesse,
git lut s Kommando: Achtung, fertig, löönds lo fliesse!
– Jä Sii, das isch, so sait zue mir dä Oberbrünzler,
die neui Strategie gege der Buchsbaumzünsler.

Singvogel (Comité-Schnitzelbangg)
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SUMMARY

The box-tree pyralid *Cydalima perspectalis* (Walker 1859) (Lepidoptera: Pyralidae) is a new invasive moth species in Europe. Its larvae feed on box-tree leaves and can cause severe damage within short time. Therefore, this species is of great concern for park and public garden manager in Europe.

In the context of my PhD thesis, I examined the spread in the region of Basel, the national distribution as well as the biology of this moth. The natural dispersal speed was assessed using a public survey in the region of Basel. The number of adult moths caught with light traps allowed the determination of two distinct periods of appearance of adult moths: the first in July and the second, less pronounced, from September to mid-October.

In collaboration with CABI Switzerland in Delémont, a geographic model based on literature data from Europe and Asia as well as field and laboratory data was developed. It shows the potential distribution, life-history and relative abundance of the box-tree pyralid in Europe. The model suggests that the box-tree pyralid might spread across most of Europe, except North Fenno-Scandinavia, Northern Scotland and high mountain regions, where the overall temperatures are too low to allow the completion of an entire generation per year. In most parts of Central Europe, two generations of *C. perspectalis* may occur, whereas in Northern and North-Western Europe, low temperatures allow only one yearly generation.

In an experiment, a potential preference of the box-tree pyralid for a particular box-tree variety was investigated. Female moths deposited their eggs preferably on varieties with large leaves. Larval growth and survival did not differ between the five most frequently planted box-tree varieties in Central Europe, suggesting that the box-tree pyralid has a broad food acceptance in Europe. The discrepancy between adult preference and larval performance could be explained by the fact that the larval generation which was tested in this experiment was only about the tenth in Central Europe, and that the adaptation to the new ecosystem is not fully accomplished yet.

Another study involving chemical analyses showed that larvae take up toxic compounds (alkaloids) from the box-tree leaves on which they feed and therefore become unpalatable for predators. Young larvae contain twice as much alkaloids as larvae in later instars. In box-tree leaves, the concentration of alkaloids doubles between one year-old leaves and older leaves. This finding suggests a preference of larvae for leaves containing a high concentration of alkaloids, which may explain why damage on a box-tree most often starts in the lower part, where the oldest leaves are found.

Finally, an instrument for decision-making when facing the choice of long-term care and replacement of large box-tree plantations was developed using the example of the cemetery “am Hörnli” in Riehen, close to Basel. The cemetery “am Hörnli” would suffer a great financial damage due to the loss of box-trees, having estimated 3.3 km of box-tree hedges and 650 solitary trees. Since a replacement of all box-tree plants does neither come into consideration financially nor materially, the municipal parks and garden department chose a financially more attractive solution for the clearance, care, and replacement of box-trees, following a concept of commensurability and efficiency: important trees from a heritage point of view are management and functionally less important trees are cleared and replaced by optically different but less pricy plants or not replaced at all. This strategy proves to be the most cost-efficient: after only four years this strategy is financially more attractive than a hypothetical care of all box-tree plants.

The findings of this doctoral thesis suggest that it will become unavoidable to monitor box-trees in gardens, parks and in natural sites and to treat them with pesticides in case of need.

ZUSAMMENFASSUNG

Der Buchsbaumzünsler *Cydalima perspectalis* (Walker 1859) (Lepidoptera: Pyralidae) ist eine in Europa neu auftretende, invasive Schmetterlingsart, dessen Raupen sich von Buchsblättern ernähren. Diese können innert kurzer Zeit grosse Schäden anrichten und bereiten daher den Verantwortlichen für Parks und Grünanlagen in Europa grosse Sorgen.

Im Rahmen meiner Dissertation untersuchte ich die regionale und nationale Ausbreitung sowie die Biologie des Falters. Mittels einer Umfrage bei der Bevölkerung der Region Basel wurde die natürliche Ausbreitungsgeschwindigkeit des Buchsbaumzünslers erfasst. Mithilfe der Aufzeichnung von Lichtfängen an zwei Standorten in Basel konnten zwei deutlich getrennte Perioden des Erscheinens der Falter festgestellt werden: die erste im Juli und die zweite, weniger stark ausgeprägte, von Anfang September bis Mitte Oktober.

In Zusammenarbeit mit CABI Switzerland in Delémont wurde ein geographisches Modell erstellt, welches anhand von Literaturdaten aus Europa und Asien sowie Feld- und Labor-Daten die potentielle Verbreitung, die relative Abundanz sowie den jährlichen Rhythmus des Buchsbaumzünslers in Europa simuliert. Das Modell lässt darauf schliessen, dass sich der Buchsbaumzünsler in ganz Europa ausbreiten kann, mit Ausnahme vom nördlichen Fennoskandinavien, dem Norden Schottlands und hohen Bergregionen wo die Temperaturen insgesamt zu niedrig sind, um eine komplette Generation pro Jahr auszubilden. In weiten Teilen Mitteleuropas bildet der Schädling zwei jährliche Generationen aus. In Nordeuropa erlauben die tieferen Temperaturen hingegen nur eine einzige Generation pro Jahr.

Mit einem Experiment wurde eine allfällige Buchsbaum-Sortenpräferenz des Buchsbaumzünslers untersucht. Legebereite Weibchen zeigten eine Vorliebe für grossblättrige Buchssorten. Die Wachstumsrate und Sterblichkeit der Raupen zeigten keine Unterschiede zwischen den häufigsten Sorten in Mitteleuropa, was darauf schliessen lässt, dass der Buchsbaumzünsler in Europa eine breite Futterpflanzenakzeptanz aufweist. Die Abweichung zwischen der Präferenz der Falter und der Wachstumsrate der Raupen kann daraus herrühren, dass die

getesteten Raupen und Falter erst etwa die zehnte Generation ist, welche in Mitteleuropa auftritt, und die Anpassung an das neue Ökosystem noch nicht optimiert wurde.

In einer weiteren Untersuchung konnte anhand von chemischen Analysen gezeigt werden, dass die Raupen giftige Stoffe (Alkaloide) aus den Buchsblättern aufnehmen und speichern und deshalb für Frassfeinde ungeniessbar sind. Junge Raupen weisen einen doppelt so hohen Alkaloidgehalt auf wie spätere Stadien. Beim Buchs sind die Alkaloidkonzentration in einjährigen Blättern nur etwa halb so gross ist wie in älteren Blättern. Diese Beobachtung legt nahe, dass Raupen Blätter mit hohem Alkaloidgehalt vorziehen, was erklären mag weshalb der Frass an einer Buchspflanze meist im unteren Bereich anfängt, wo die ältesten Blätter sind.

Es wurde ebenfalls ein Instrument zur Entscheidungsfindung zwischen Ersatz oder langjähriger Pflege grosser Buchsbestände anhand des Friedhofes am Hörnli in Riehen bei Basel entwickelt, da dieser mit seinen geschätzten 3.3 km Buchshecken und 650 Solitärbuchsbäumen von einem erheblichen finanziellen Schaden durch den Verlust von Buchs betroffen ist. Da ein Ersatz aller Buchspflanzen weder finanziell noch materiell in Frage kommt, wird von der Stadtgärtnerei – entsprechend dem Konzept der Verhältnismässigkeit – ein weit niedrigerer Betrag für Rodung, Ersatz und Pflege aufgewendet: Denkmal-pflegerisch wichtige Buchspflanzen werden erhalten und gepflegt, funktionell weniger wichtige Buchspflanzen gerodet und je nach Situation gar nicht oder durch optisch verschiedene aber dafür günstigere Straucharten ersetzt. Diese Strategie erweist sich als die rentabelste: bereits nach vier Jahren ist diese Strategie finanziell attraktiver als eine hypothetische Pflege des gesamten Buchsbestandes.

Aufgrund der verschiedenen Erkenntnisse, welche im Verlauf dieser Doktorarbeit gewonnen wurden, wird es in Zukunft unvermeidlich sein, Buchspflanzen in Gärten, Parkanlagen und an natürlichen Standorten zu überwachen und notfalls mit Insektenschutzmitteln zu behandeln.

GENERAL INTRODUCTION

Non-native species introduced into new habitats may spread and threaten the natural ecosystem. Species extinctions and range expansions are natural processes over an evolutionary time scale, during an ice age, after a volcanic eruption or when continents shift. Human-caused introductions and extinctions, however, exceed the background rate of extinction by far (OFFICE OF TECHNOLOGY ASSESSMENT, 1993). Human-mediated species displacements allow species to reach even the most remote areas. Although only a small proportion of introduced species succeeds in establishing and spreading in the new environment (WILLIAMSON & FITTER, 1996), they may have negative and sometimes even dramatic impacts on the native ecosystem. Alien species can threaten the biodiversity of the invaded area by predating on or competing with native species, by altering ecosystem properties such as the habitat structure, nutrient cycling or trophic dynamics or by producing hybrids with native species (WOOTTON, 1994). Some invasive species may have a deep impact on local economy particularly when they affect agriculture or public health (PIMENTEL et al., 2000).

Human-mediated introductions

Humans have always displaced species during their history of trade and travel by carrying crops and livestock across Eurasia, Africa and America. Intentional and unintentional introduction of species across the world may have begun as early as in the 16th century with the worldwide sailing expeditions of Viking, Chinese and Portuguese explorers and traders. Familiar species are brought from home when settling in a new area and exotic species are sent home for food, sport, or ornamental purposes (LEVER et al., 1992). The economic benefit of marketable non-native species runs to hundreds of billions of dollars per year only in the United States of America (PIMENTEL et al., 2000). Other species reach their final destination passively by attaching themselves to the hulk of ships, or within solid ballast made of rock and soil, water ballast or packing material (see for example CARLTON & GELLER, 1993; CARLTON & HODDER, 1995). New sea routes, such as the

Suez and Panama Canals opened ways for new species invasions. Such intentional and unintentional species introductions take place on a number of spatial scales: global traffic by sea or air, regional travel by road or train, local planting of non-native shrubs. All these species present a potential risk of becoming invasive when released into the wild. Due to a lack of historical records, many ancient invaders remain unknown, and the rate of new introductions will only increase as a result of faster travel, increased size of transportation means, and new combinations of route sources and destinations (GROOM et al., 2006).

Spread and adaptation to a new environment

Species introduced to a new environment must overcome a number of obstacles in order to become invasive: Once the biogeographic barrier is passed, they must be able to tolerate the physiological properties of the new environment, and be successful in their interactions with native species (CARLTON, 1985). Of thousand species introduced to a new environment, theoretical models predict about hundred to survive without human cultivation, ten to spread into natural habitats and one to become a serious pest species (WILLIAMSON & FITTER, 1996). Some species characteristics facilitate the successful establishment: high fecundity, vegetative spread or parthenogenesis, a broad diet and a wide physiological tolerance. Particularly invasive insects are characterized by the ability to adjust their food preference to newly encountered plants. One of the most prominent examples is the weevil *Rhinocyllus conicus* deliberately introduced to the United States for the biological control of the invasive thistles of the genus *Carduus*, which altered its diet to native *Cirsium* species and brought some of these native populations close to extinction (LOUDA, 1998).

However, not all species with the above traits become invasive, and some successful intruders lack part or all of those characteristics. Hospitable climatic conditions and empty ecological niches or unused natural resources favour the successful establishment of non-native species which may be able to use these resources in a new or different way (LONSDALE, 1999). If competition in the invaded area is low or a functional group of species is missing, native species may not be able to cope with the contact to a new competitor or predator (ELTON, 1958). Moreover, non-native species may benefit from a loss of natural enemies, parasites and diseases.

Focus of this study

The box-tree pyralid *Cydalima perspectalis* (Walker 1859) (formerly *Diaphania* or *Glyphodes perspectalis*, see MALLY AND NUSS, 2010) (Lepidoptera: Pyralidae), native to Korea, Japan and China (INOUE et al., 1982), has been recorded in Europe for the first time in south-western Germany and in the Netherlands in 2007 (KRÜGER 2008; LEUTHARDT et al., 2010; VAN DER STRATEN and MUUS, 2010). The species spread rapidly to north-western Switzerland and France (FELDTRAUER et al., 2009). Most recently, it has also been recorded in England and Austria (VAN DER STRATEN and MUUS, 2010). In the invaded regions, the box-tree pyralid causes severe damage to box-trees (*Buxus* sp.) in private and public gardens as well as in semi-natural box-tree forests. The larvae of *C. perspectalis* feed on the leaves of box-trees but can attack the bark of the trees, causing them to dry out and die. The **aim of this study** was to investigate the distribution and spread, the biology and life-history, as well as the food choice and chemical ecology of this new invasive species in order to be able to plan target-specific control actions which are both financially attractive and cause the least damage to the environment.

Since the initial invasion of the box-tree pyralid most probably occurred in 2006 but its occurrence was confirmed in a number of different locations across Europe more than a year later (LEUTHARDT et al., 2010), it is hardly possible to eradicate this species in Central Europe. **Chapter 1** shows the results of an investigation about the distribution, spread speed and biology of the box-tree pyralid in Switzerland. Knowing the current distribution of the invader, control actions can be undertaken before severe damage has occurred, and treating the larvae at the right time during the year avoids treatment failure because larvae are not present or protected in their overwintering cocoon.

On a wider spatial scale, it might be of great interest to countries or regions not yet invaded by the box-tree pyralid to know the potential geographic distribution and generation cycles of the box-tree pyralid in order to be able to respond rapidly to the arrival of the box-tree pyralid. In **chapter 2**, a bioclimatic model was developed in collaboration with CABI Switzerland in Delémont to predict the potential distribution range the box-tree pyralid might attain within the near future as well as the varying life-history and relative abundance under different climatic conditions. This model allows a better understanding of how climate affects *C. perspectalis* populations (NACAMBO et al., 2013).

Because many invasive insects are characterized by the ability to grow and reproduce on plant species unknown in their native range, **chapter 3** focuses on an experimental study about oviposition preference and larval performance on the five most common European box-tree varieties. Box-tree pyralid damage has been recorded on all box-tree species and varieties examined in Europe, indicating a successful adjustment of the food preference and a broad diet of this invader (LEUTHARDT & BAUR, 2013).

Invasive species often benefit from a loss of native predators, parasites and diseases. New predators in the invaded range may take over this function although the delay between the establishment of an invasive species and the successful adjustment of native predators' diets may be too long to ensure an effective control. In **chapter 4**, the potential toxicity of box-tree larvae and moths was investigated in collaboration with the Chemical Analyses Service of the Swiss plant Science Centre. The results of high-pressure liquid chromatographic analyses showed that larvae do indeed take up part of the alkaloids present in their food, making them at least unpalatable for vertebrate predators (LEUTHARDT et al., 2013). Newly-emerged moths do not contain any traces of alkaloids, but more research is needed to assess if they are able to absorb alkaloids during their adult life-stage.

Invasive species may not only have deleterious effects on native biodiversity, some may also cause enormous economic damage. This occurs principally in the case of agricultural pest, but some native species used for sport or ornamental purposes are of great financial value as well. Box-trees are an example of such very expensive ornamental plants. They are of great structural importance in public gardens including historical parks and cemeteries, but because of their slow growth, it takes years or even decades to grow a solitary tree or a box-tree hedge at costs reaching more than 1000 CHF for a single tree. When faced with the threat of severe damage caused by an invasive species, it is very useful to establish a model of the potential financial loss and the cost generated by the prevention of the damage to the plants. **Chapter 5** provides an instrument for decision-making used by managers of large box-tree plantations in parks and public gardens when faced with the choice of long-term care or replacement of a large number of box-trees.

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CHAPTER 1

Ausbreitung des Buchsbaumzünslers *Diaphania perspectalis* (Lepidoptera, Pyralidae) in der Region Basel – eine für die Schweiz neue Schädlingsart

Florine L. G. Leuthardt, Wolfgang Billen und Bruno Baur

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Ausbreitung des Buchsbaumzünslers *Diaphania perspectalis* (Lepidoptera, Pyralidae) in der Region Basel – eine für die Schweiz neue Schädlingsart

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Abstract: Spread of the Box-Tree Pyralid *Diaphania perspectalis* (Lepidoptera: Pyralidae) in the region of Basel – a pest species new for Switzerland. – The spread of *D. perspectalis* has been examined in the region of Basel over a period of six months. First results suggest the occurrence of three generations per year. Due to its rapid spread, *D. perspectalis* might colonize all suitable habitats in Switzerland within a few years.

Zusammenfassung: Die Ausbreitung des neu in der Schweiz vorkommenden Buchsbaumzünslers *Diaphania perspectalis* (Lepidoptera, Pyralidae) wurde über einen Zeitraum von sechs Monaten in der Region Basel erfasst. Erste Ergebnisse lassen auf drei Generationen pro Jahr schliessen. Aufgrund seiner raschen Ausbreitung dürfte *D. perspectalis* innerhalb weniger Jahre sämtliche geeigneten Lebensräume in der Schweiz besiedelt haben.

Résumé: La dispersion de la pyrale du buis *Diaphania perspectalis* (Lepidoptera, Pyralidae) a été étudiée dans la région de Bâle pendant une période de six mois. Les premiers résultats indiquent trois générations par année. Au vu de sa rapide dispersion, *D. perspectalis* pourrait coloniser tous les habitats appropriés à son développement en Suisse en l'espace de quelques années.

Keywords: *Diaphania perspectalis*, Pyralidae, *Buxus*, Basel

Diaphania perspectalis (Photo H. Ziegler)

EINLEITUNG

Im April 2007 wurde im Siedlungsgebiet von Weil am Rhein nördlich von Basel erstmals der aus Ostasien stammende Buchsbaumzünsler *Diaphania perspectalis* (Walker 1859) (Lepidoptera: Pyralidae) entdeckt. Am 18. April 2007 stellte Walter Schön eine Raupenbestimmungsanfrage von Colette Walter aus Weil am Rhein auf die Lepidopteren-Homepage Lepiforum (www.lepiforum.de). Eckard Krüger konnte die Raupe am 4. Mai 2007 bestimmen (Krüger 2008). Da die Raupen zu jenem Zeitpunkt schon massenweise auftraten und die Schäden schon beträchtlich waren, dürfte die Einschleppung bereits 2006 oder gar 2005 stattgefunden haben. Im Sommer 2007 wurde der Buchsbaumzünsler auch in Riehen (Kanton Basel-Stadt) nachgewiesen. Die Raupen dieses Kleinschmetterlings fressen in kurzer Zeit Buchsbäume (*Buxus* sp.) kahl. So sollen sie nicht nur die Blätter, sondern auch die grüne Rinde abfressen, so dass viele der Buchsbäume sich nicht mehr erholen können und absterben.

Es wird vermutet, dass der Buchsbaumzünsler aus China nach Europa eingeführt wurde. Weil die ersten Befallsherde durchwegs in der Nähe von Binnenhäfen (D-Weil am Rhein, D-Kehl am Rhein) lagen, wurde angenommen, dass der Schädling in mit Granitsteinen beladenen Containern nach Europa gelangte. Im Sommer und Herbst 2009 wurden aber wiederholt mit Raupen infizierte Buchspflanzen auf den Verkaufsregalen von Gartenzentren in der Region Basel gefunden. So konnte gezeigt werden, dass der Grosshandel von (teilweise befallenen) Buchspflanzen zur Verbreitung von *D. perspectalis* beiträgt. Die neu eingeführte Art konnte die Winter überstehen und sich in weniger als 3 Jahren im Raum Basel, im angrenzenden Elsass sowie in weiten Teilen Süd- und West-Deutschlands etablieren. Auch in den Niederlanden

Abb. 1. Frassbild des Buchsbaumzünslers *Diaphania perspectalis* (Foto Y. Richard)

und in England sind inzwischen Vorkommen des Buchsbaumzünslers bekannt (siehe www.lepiforum.de).

Das hohe Schadenspotenzial macht den für Grünanlagen verantwortlichen Personen grosse Sorgen. Für die Bekämpfung der Raupen von *D. perspectalis* wurden verschiedene Insektizide ausprobiert. Gewisse Pestizide zeigten keine Wirkung, bei anderen ist die Wirkung sehr variabel. Dazu kommt, dass der Einsatz von chemischen Pflanzenschutzmitteln in Hausgärten problematisch ist. Die Bekämpfung ist zusätzlich erschwert, weil Kenntnisse über die Biologie dieses neuauftretenden Schädlings weitgehend fehlen. Nachfolgend werden die Ergebnisse einer Studie über die Ausbreitungsgeschwindigkeit von *D. perspectalis* vorgestellt. Zudem werden erste Angaben zur Phänologie dieser Art mitgeteilt.

METHODEN

Biologie

In Tokyo, Japan, fanden die Forscher bei *D. perspectalis* bis zu drei Generationen pro Jahr, wie es auch in Europa beobachtet wurde (Maruyama & Shinkaji 1987). Studien zur Larvalentwicklung von *D. perspectalis* zeigten eine ausgeprägte Temperaturabhängigkeit: Bei 30° C dauert die Entwicklung mindestens 17 Tage, bei 15° C bis zu 84 Tage. Die Anzahl der Larvenstadien variiert ebenfalls mit der Temperatur: Bei 25° C gab es in der Regel sechs Stadien, bei 20° C manchmal nur fünf Stadien (Maruyama & Shinkaji 1991). In diesen Untersuchungen wurden die Larven auf *Buxus microphylla* gezüchtet. Bei *Buxus sempervirens* und *B. microphylla* var. *insularis* wurde in einigen Fällen ein siebtes Larvenstadium beobachtet. Diese Pflanzen dürften nicht das optimale Futter darstellen. So ist auch die kürzeste Entwicklungszeit auf *B. microphylla* beobachtet worden (Maruyama 1993).

Ausbreitung

Die Ausbreitung des Buchsbaumzünslers in der Region Basel wurde mit Hilfe einer Umfrage bei der Bevölkerung erfasst. Dazu wurde ein Fragebogen zum Vorkommen beschädigter Buchsbäume und zum Schadensbild sowie Umfang des Schadens entwickelt. Der Fragebogen wurde zwischen August und Oktober 2009 zusammen mit Informationen über den Buchsbaumzünsler in verschiedenen regionalen Zeitungen sowie im Internet veröffentlicht. Die folgenden Zeitungen baten die Bevölkerung um Mithilfe bei der Umfrage: Basler Zeitung (27.8.09, 1.9.09, 14.10.09); Badische Zeitung (8.09.09); Allschwiler Wochenblatt (16.10.09); Binninger Anzeiger (15.10.09); Birsigtal-Bote (8.10.09); Biel-Benkemer Dorf Zytig (30.10.09). Auf www.conservation.unibas.ch/news/buchsbaum konnte der Fragebogen als Word- oder pdf-Dokument heruntergeladen werden. Zudem wurde der Fragebogen an Gemeindeverwaltungen und Gärtnereien in der Region geschickt. Die eingegangenen Schadensmeldungen wurden analysiert und anschliessend für die gesamtschweizerische Auswertung an die Arbeitsgruppe Waldschutz an der WSL in Birmensdorf weitergeleitet. Die regionalen Schadensmeldungen wurden auf eine Karte übertragen, und das Verbreitungsgebiet in jedem Jahr mit Hilfe von ArcGIS berechnet.

Generationenabfolge

Um die Generationenabfolge zu dokumentieren, wurden ab Mitte Juli 2009 Pheromonfallen an zwei verschiedenen Standorten (Friedhof Hörnli, CH-Riehen und botanischer Garten Merianpark Brüglingen, CH-Münchenstein) installiert. Dabei wurden pro Standort je vier Replikate von sechs verschiedenen Lockstoff-Kombinationen aufgestellt. Die noch in der Testphase stehenden Pheromone wurden von der Firma PheroBank in Wageningen (Holland) zur Verfügung gestellt. Bei den Fallen handelt es sich um Delta-Fallen mit einer Kleb-Einlage und jeweils einer Pheromon-Kapsel (Abbildung 2). Die Fallen wurden in einem Abstand von 7 Tagen nach Faltern überprüft. Die Pheromonkapseln wurden nach jeweils 8 Wochen durch neue ersetzt.

Abb. 2. Delta-Fallen mit Kleb-Einlage und Lockstoff-Kapsel (Foto F. Leuthardt)

ERGEBNISSE

Ausbreitungsgeschwindigkeit

Zwischen dem 27. August und 1. Dezember 2009 sind insgesamt 293 Schadensmeldungen via Fragebogen eingegangen. Sieben Schadensmeldungen bezogen sich auf das Jahr 2007, 53 auf 2008, und 231 auf 2009. Unmittelbar nach Berichten in der Basler Zeitung konnte ein erhöhtes Eintreffen von Antworten festgestellt werden. Nach Berichten in Lokalzeitungen konnte ebenfalls ein leicht erhöhter Eingang von Meldungen festgestellt werden. Dieser wirkte sich aber nur gering auf die gesamten Meldungen aus (Abbildung 3).

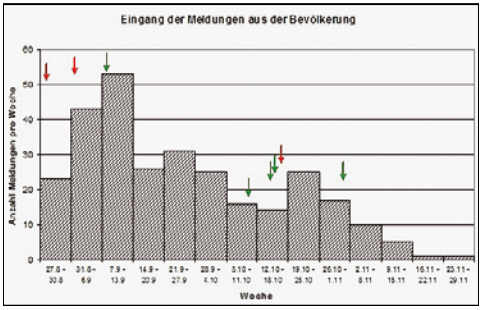

Abb. 3. Anzahl der pro Woche eingegangenen Meldungen aus der Bevölkerung, nach Datum geordnet. Rote Pfeile bezeichnen das Erscheinen von Artikeln in der Basler Zeitung, grüne Pfeile bezeichnen Artikel in lokalen Zeitungen.

Die gemeldeten Schäden betrafen vor allem *B. sempervirens*, den einheimischen Buchs. *Buxus rotundifolia* wurde weniger stark befallen, ein Sachverhalt der möglicherweise auf seine dickeren und resistenteren Blätter zurückgeführt werden kann. Neuere Buchs-Sorten, darunter auch panaschierte, wurden ebenfalls befallen.

Der Buchsbaumzünsler hat sich in der Region Basel rasch ausgebreitet (Abbildung 4). Im Jahre 2007 lagen alle Fundorte innerhalb einer Fläche von 25 km^2 mit Zentrum in CH-Riehen (Kanton Basel-Stadt) und D-Weil-Lörrach. Ein Jahr später (2008) umfasste das Ausbreitungsgebiet bereits 386 km^2 und 2009 schon 642 km^2. Die letzte Flächenschätzung ist vermutlich zu klein, wurde doch der Buchsbaumzünsler im Jahre 2009 auch dem Oberrhein entlang bis Neuenburg gefunden, und die Bevölkerung im Elsass (Frankreich) wurde nicht explizit befragt.

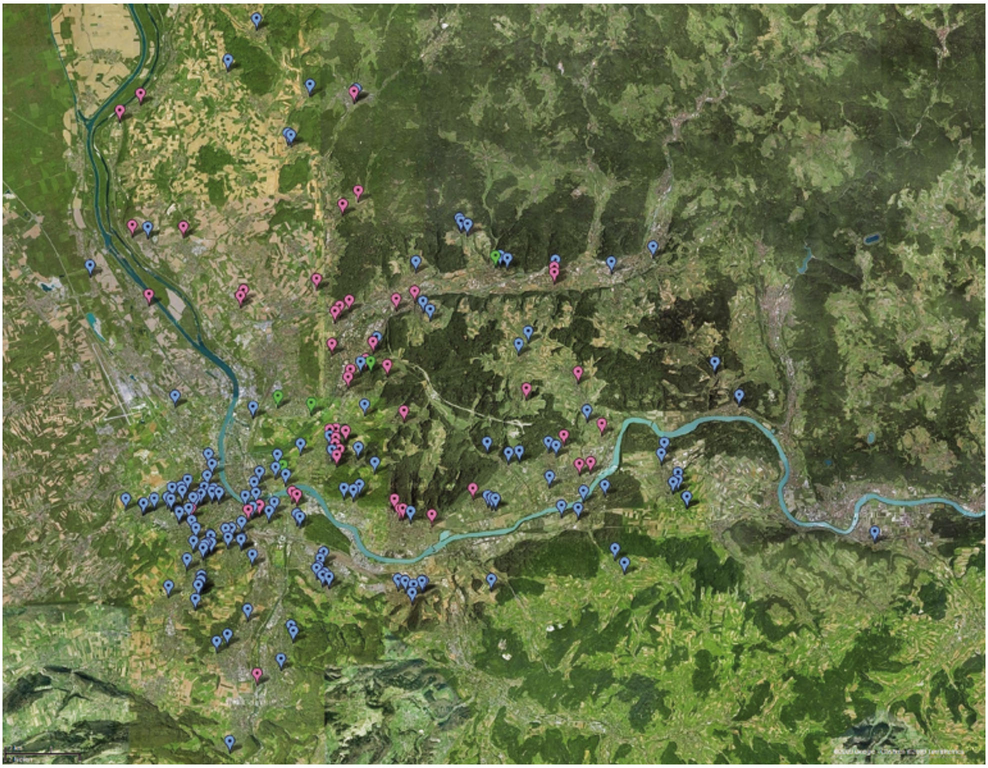

Abb. 4. Verbreitung der Schadensmeldungen in der Region Basel. Grün: 2007; Rosa: 2008; Blau: 2009. (Karte: Google maps, 18.11.09 – © 2009 Google-Kartendaten © 2009 Teleatlas)

Inzwischen wurde *D. perspectalis* in verschiedenen Regionen der Schweiz nachgewiesen (Abbildung 5). Da diese Vorkommen aber vom bisherigen Hauptverbreitungszentrum in der Region Basel isoliert sind, dürfte eine passive Verschleppung via infizierte Pflanzen zur Ausbreitung des Schädlings beigetragen haben. Dies kann durch den Grosshandel mit Buchspflanzen sowie durch den Verkauf von befallenen Pflanzen geschehen. Um diese Möglichkeit zu überprüfen wurden zwischen Juli und Oktober 2009 sechs Besuche in verschiedenen Gartenzentren durchgeführt und sämtliche zum Verkauf angebotenen Pflanzen auf Buchsbaumzünsler-Befall überprüft. Dabei wurden bei allen sechs Besuchen Frassschäden entdeckt, bei drei Besuchen sogar lebende Raupen auf den Pflanzen.

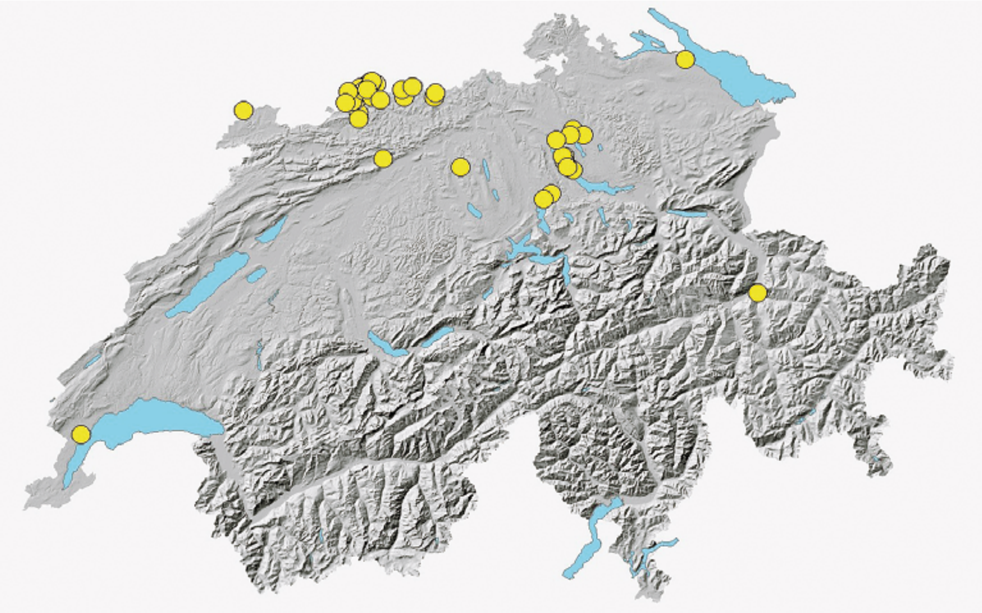

Abb. 5. Meldungen über das Vorkommen von *Diaphania perspectalis*; Waldschutz Schweiz, WSL, Birmensdorf, Stand November 2009.

Generationen

Ab Mitte Juli 2009 (dem Beginn der Studie) wurden zwei deutlich getrennte Perioden des Erscheinens der Falter von *D. perspectalis* festgestellt. Eine in der zweiten Juli-Hälfte, die andere, weniger deutlich ausgeprägt, von Anfang September bis Mitte Oktober. Sehr wahrscheinlich entsprechen diese Perioden gehäuften Auftretens der zweiten und dritten Generation von Faltern. Die geringe Bestandsgrösse der dritten Generation in unserer Region kann an den verschiedenen Temperatur-Verhältnissen liegen, an die dieses Insekt noch nicht angepasst ist.

DISKUSSION

Buchshecken sind von grosser kulturhistorischer Bedeutung, bilden sie doch ein wesentliches Gestaltungselement in grossen Garten- und Parkanlagen. Auch in privaten Gärten werden Buchshecken gepflegt. Zudem gibt es Buchsvorkommen in zahlreichen Schweizer Wäldern. Die Buchswald-Bestände der Nordwestschweizer Wälder sind bisher vom Buchsbaumzünsler noch kaum befallen. Laut Angaben von Förstern weisen die Buchswälder bei D-Grenzach und CH-Liestal (Kanton Baselland) nur am äusseren Rand geringe Frassschäden von *D. perspectalis* auf. Der Buchswald bei Grenzach ist hingegen stark vom Pilz *Cylindrocladium buxicola* betroffen. Möglicherweise sagen die schwachen Lichtverhältnisse im Waldinneren dieser Falterart nicht zu.

Der Buchsbaumzünsler hat sich innerhalb von 3 Jahren in der Region Basel stark ausgebreitet und an verschiedenen Orten grosse Schäden angerichtet. Sein Vorkommen muss als ein ernstes Problem betrachtet werden. Da anthropogene Verfrachtungen von befallenen Pflanzen weiterhin vorkommen, dürften innerhalb weniger Jahre sämtliche geeigneten Lebensräume in der Schweiz von *D. perspectalis* besiedelt sein. Damit in Parkanlagen und Gärten möglichst umweltschonende Bekämpfungsmassnahmen durchgeführt werden können, sollte die genaue Generationenabfolge des Schädlings im Jahresablauf bekannt sein. Durch verschiedene Beobachtungen und aufgrund des Wissens von Pflanzenschutz-Experten kann angenommen werden, dass der Buchsbaumzünsler in Zentraleuropa mit drei Generationen pro Jahr auftritt. Der Zeitpunkt des jeweiligen Auftretens kann jedoch von Jahr zu Jahr variieren. Natürliche Frassfeinde des Buchsbaumzünslers sind bis anhin in Mitteleuropa kaum bekannt. Zwar wurden vereinzelt Kohlmeisen und Gartenrotschwänze mit Raupen oder Falter im Schnabel beobachtet, doch wurden vor allem die Raupen nach dem Verzehr wieder ausgewürgt. Dies weist darauf hin, dass die Raupen und möglicherweise auch die Falter von *D. perspectalis* wegen ihrer giftigen Futterpflanze für Vögel ungeniessbar sind.

Danksagung

Wir danken der Stadtgärtnerei Basel und der Christoph Merian Stiftung Basel für finanzielle Unterstützung, der WSL in Birmensdorf für die Daten von Abbildung 5, der PheroBank Wageningen für die Pheromon-Kapseln und Renato Joos für Kommentare zum Manuskript.

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www.lepiforum.de – Lepiforum: Bestimmung von Schmetterlingen (Lepidoptera) und ihren Präimaginalstadien.

CHAPTER 2

Development Characteristics of the Box-Tree Moth *Cydalima perspectalis* and its Potential Distribution in Europe

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ORIGINAL CONTRIBUTION

Development characteristics of the box-tree moth *Cydalima perspectalis* and its potential distribution in Europe

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Keywords
bioclimatic model, CLIMEX, *Cydalima perspectalis*, diapause, invasive species, temperature requirements

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Abstract

The box-tree moth *Cydalima perspectalis* (Walker) is an invasive pest causing severe damage to box trees (*Buxus* spp.). It is native to Japan, Korea and China, but established populations have been recorded in a number of locations across Europe since 2007 and the spread of the insect continues. The developmental investigations suggest that larvae overwinter mainly in their 3rd instar in Europe and that diapause is induced by a day length of about 13.5 h. One and a half to 2 months in the cold are necessary to terminate diapause. Threshold temperatures for development and number of degree-days to complete a generation are slightly different from those calculated in previous studies in Japan. A bioclimatic (CLIMEX®) model for *C. perspectalis* in Europe was developed, based on climate, ecological and developmental parameters from the literature and new field and laboratory studies on diapause termination, thermal requirements and phenology. The model was then validated with actual distribution records and phenology data. The current distribution and life history of *C. perspectalis* in Europe were consistent with the predicted distribution. The climate model suggests that *C. perspectalis* is likely to continue its spread across Europe, except for Northern Fenno-Scandinavia, Northern Scotland and high mountain regions. The northern distribution of *C. perspectalis* is expected to be limited by a number of degree-days above the temperature threshold insufficient to complete a generation, whereas its southern range is limited by the absence of a cold period necessary to resume diapause. The model predicts relatively high Ecoclimatic Indices throughout most of Europe, suggesting that the insect has the potential of becoming a pest in most of its predicted range. However, damage is likely to be higher in Southern and Central Europe where the moth is able to complete at least two generations per year.

Introduction

Non-native species introduced to new habitats may spread and threaten the natural equilibrium of the invaded ecosystem. Species extinctions and range expansions are natural processes over an evolutionary timescale, but human-caused introductions and extinctions significantly exceed the background rate (Clavero and García-Berthou 2005; Simberloff and Rejmánek 2011). Although only a small proportion of introduced species succeed in establishing and spreading in new environments (Williamson and Fitter

1996), they may have negative and sometimes even dramatic impacts on ecosystems (Kenis et al. 2009). Some invasive species also have a severe impact on local economies, particularly when they affect agriculture or public health (Pimentel et al. 2000).

The box-tree moth *Cydalima perspectalis* (Walker) (formerly *Diaphania* or *Glyphodes perspectalis*, see Mally and Nuss 2010) (Lepidoptera: Crambidae) is native to East Asia (Inoue 1982). It has been reported from Japan and Korea, where it is probably distributed throughout the countries (Maruyama and Shinkaji 1993; M. Kenis, unpublished data) and parts of China, the Russian Far East and India (Hampson 1896; Inoue 1982; Kirpichnikova 2005; Zhou et al. 2005; Park 2008; Streltzov 2008). The presence of the moth in India refers to an old reference only (Hampson 1896). The distribution of *C. perspectalis* in Asia is also largely related to some species of its host plant genus, *Buxus* spp., which have been extensively planted as ornamental tree in recent years. Therefore, the absence of records in some regions may be due to the absence or scarcity of its host plants or to a lack of surveys rather than to climatic unsuitability, whereas the absence of naturally occurring *Buxus* spp. in Northern China and Russian Far East (Min and Brückner 2008) suggests that it was recently introduced in these regions with ornamentals.

The moth was recorded in Europe for the first time in south-western Germany and in the Netherlands in 2007 (Krüger 2008; Van der Straten and Muus 2010). The species spread rapidly to Switzerland (Leuthardt et al. 2010) and France (Feldtrauer et al. 2009) and is now present in several other European countries (Lepiforum 2013): England (Salisbury et al. 2012), Austria (Lepiforum 2013), Belgium (Lepiforum 2013), Croatia (Koren and Črne 2012), Czech Republic (Šumpich 2011) Hungary (Sáfián and Horváth 2011), Italy (Lepiforum 2013), Romania (Székely et al. 2011), Slovenia (Seljak 2012), Slovakia (Slamka 2010) and Turkey (Hizal et al. 2012). In all newly invaded regions, *C. perspectalis* causes severe damage to box trees (*Buxus* spp.). The larvae of *C. perspectalis* feed principally on leaves but may also attack the bark. Total defoliation causes the death of the trees. Box trees are of great structural importance in public gardens including historical parks and cemeteries. They also constitute a unique habitat when growing naturally in the understory of European broadleaf forests (Di Domenico et al. 2012). Within a period of only 2 years (2010–2012), *C. perspectalis* devastated large areas of native box trees (*Buxus sempervirens*) in forests in the region of Basel (Switzerland), which have not been able to regenerate (Kenis et al. 2013). *C. perspectalis* has been shown to feed on all of the most frequently planted box-tree species and varieties in Central Europe (Leuthardt and Baur 2013), suggesting that its spread across Europe is not limited by food resources. Its ability to have multiple generations per year, as observed in its native area (Maruyama and Shinkaji 1993; Zhou et al. 2005), increases its spread capacity. Furthermore, it is easily introduced accidentally with its host plant, which is extensively traded over Europe (Leuthardt et al. 2010; Van der Straten and Muus 2010). Finally, it experiences only small, if any, competition by other herbivores and mortality by natural enemies (S. Nacambo, F. Leuthardt, T. Haye and M. Kenis, unpublished data). Thus, the main factors limiting its dispersal must be abiotic, such as temperature, day length and humidity. Its ecological impact may become particularly important when it reaches the main areas of natural occurrence of *Buxus* spp. in Europe, for example, the southern part of the Massif Central in France and the Pyrenees, where the European box-tree *B. sempervirens* is an essential component of unique forest ecosystems (Di Domenico et al. 2012; Kenis et al. 2013).

Winter diapause occurs in the larval stage. Its induction has been studied in Japan (Maruyama and Shinkaji 1993) and is mediated by day length and temperature experienced by young larvae. In contrast, little is known about diapause termination. Thermal requirements, that is, the number of degree-days required to complete a particular developmental stage and the threshold temperatures for development (Herms 2004), were studied with native populations in Japan (Maruyama and Shinkaji 1987). However, it was essential to repeat these experiments with the invasive population in Europe because it is likely that it derives from another population in Asia adapted to different climatic conditions. We also examined in which larval instar diapause occurs in the European population, as first observations in Switzerland suggested it to be different from Asian populations (Maruyama and Shinkaji 1991). Finally, the phenology of the moth was studied in north-western Switzerland over 3 years to validate the thermal requirement data obtained in the bioclimatic model.

The main objective of this study was to develop a bioclimatic model, using the CLIMEX software (Sutherst et al. 2007), to predict the potential range and relative abundance of *C. perspectalis* in Europe. The model may also help to develop a better understanding of how climate affects *C. perspectalis* populations. Model parameterization was based on data on development characteristics and climatic constraints gathered from Asian literature as well as from new laboratory and field studies on diapause termination, thermal requirements and phenology in Europe.

Material and Methods

Developmental investigations

Diapause

Cydalima perspectalis overwinters as larva, protected in a cocoon spun between *Buxus* leaves. To assess in which instar the larvae overwinter in western Switzerland, the width of the head capsules of diapausing larvae was measured in a sample of 120 larvae collected in their overwintering cocoons at Muttenz (Basel-Landschaft, 47°31′43″N/7°38′32″E), Delémont (Jura, 47°22′23″N/7°19′30″E) and Prangins (Vaud, 46°23′43″N/6°14′58″E) in September/October 2011. These measurements were taken using a stereomicroscope and compared to a control sample of 49 larvae obtained from eggs laid in the laboratory and raised at 25°C from the first instar to pupation. All control larvae were reared singly in PVC-Petri dishes (diameter 90 mm) lined with wet filter paper that prevented the box-tree leaves provided as food to dry out. The head capsule width was measured after each moult.

Two studies were carried out to assess parameters of diapause termination. In a first study, 14 lots of 17–34 overwintering larvae of *C. perspectalis* were collected on a *B. sempervirens* hedge in Delémont at regular intervals from 15 September 2011 to 16 March 2012. Larvae were kept in their overwintering cocoon in 1.3 l ventilated plastic cylinders in the laboratory at 24 ± 1°C and 16-h day length. Box-tree twigs were partly immersed into water to keep them fresh. Larval development was monitored daily and, when larvae were observed feeding, fresh box-tree twigs were provided twice a week. Overwintering larvae frequently initiated feeding several days or weeks after incubation but were not able to complete development. The success of diapause termination was therefore measured by the proportion of larvae per lot reaching the adult stage.

In a second study, 7 lots of 19–27 diapausing larvae were collected in Prangins on 26 October 2011, placed in 1.3-l ventilated plastic cylinders in a large, humid container and stored in a cold room at 2°C. A first lot of larvae was incubated one month later at 24 ± 1°C, and 16-h day length and new lots were incubated every 2 weeks. Larvae in their overwintering cocoons were placed on a fresh twig partly immersed into water in a 1.3-l plastic cylinder. The larval development and the diapause termination were observed and assessed as described above.

Threshold temperatures and degree-days

A degree-day is a measure of the amount of heat that accumulates above a threshold temperature for development during a 24-h period (Herms 2004). To assess the threshold temperature for the development and the number of degree-days required for development of each stage, overwintered larvae were collected at several sites situated between Muttenz and Delémont in March 2012. They were reared in the laboratory at 24 ± 1°C and 16-h day length, in plastic and gaze cages (30 cm × 30 cm × 30 cm) containing branches of *B. sempervirens* collected on a nearby hedge and partly immersed in water. Branches were replaced twice a week. Adults obtained with this rearing method were placed in similar cages in groups of ca. 10 males and 10 females. Fresh branches of *B. sempervirens* partly immersed in water were provided as oviposition substrate, and moistened cotton with honey was provided as food source. Branches were examined twice a day for freshly laid eggs. Egg patches laid in the same half day were mixed and placed randomly in Petri dishes (5 cm diameter) in a humid container in incubators at 15, 17.5, 20, 22.5, 27.5 and 30°C (±0.5°C) and 16-h day length. At least 100 eggs from different females were kept at each temperature. Hatching was monitored twice a day, and the developmental duration was measured for each egg. Larvae that hatched in the same half day were mixed and distributed among the above incubators in 1.3-l plastic cylinders containing partly immerged twigs of *B. sempervirens*, changed at least twice a week. The development of the larvae was monitored once a day until pupation and the developmental duration were recorded for each larva. Pupae in different containers and incubators obtained in the same day were mixed and placed in 0.2-l plastic cups distributed among the above incubators. The development of each pupa was monitored daily to measure its duration. For each development stage and temperature, the median and quartiles of the developmental duration (t25%, t50% and t75%) were calculated.

Due to temperature fluctuations observed at the beginning of the measurements of the pupal development, temperature data loggers (HOBO) recording hourly temperatures were placed inside each incubator. The mean temperature during the period of pupal development was calculated for all incubators (16.3, 17.4, 20.1, 22.7, 24.8 and 29.5°C) and used in the pupal development assessment instead of the previously fixed temperatures used to assess egg and larval development. Following a technical breakdown of two incubators, the development times of eggs at 25°C and of larvae and pupae at 27.5°C were omitted.

The threshold temperatures for development of eggs, larvae and pupae were estimated by the *x*-intercept method (Arnold 1959). This method assumes that, in the medium range of temperatures suitable for development, the relationship between growth rate (1/development time) and temperature is linear. From the linear regressions between the median growth rate and temperature, we calculated the value of the *x*-intercept, representing the threshold temperature for development, that is, the temperature below which no development occurs. The reciprocal of the slope of the regression lines represented the sum of degree-days required for 50% of the individuals to complete development.

Phenology in north-western Switzerland

The occurrence of adult *C. perspectalis* was measured weekly with the help of two UV-light traps positioned in Basel (47°32′17″N/7°36′48″E) and Riehen, 5 km NE of Basel (47°33′45″N/7°38′27″E) between mid-July 2009 and September 2012. These data allowed the assessment of the number of degree-days needed by diapausing larvae to reach the adult stage and for the summer generation to complete a full cycle from oviposition to adult emergence. Calculations were based on climate data from the National Oceanic and Atmospheric Administration's Climate Data Center (NCDC), which includes hourly temperature data for the location of Basel. The dates of the two yearly generation peaks were calculated using the median weekly catch of adults minus 7 days to account for the 7-day observation rhythm and assuming that the adults found in the traps were on average 3–4 days old. Based on the data obtained in the laboratory experiments of this study, we set the minimum threshold temperature for development at 9.5°C to calculate the number of degree-days with a modified average method (Herms 2004). This method allows using only the relevant daily temperatures lying between the maximum and minimum temperature threshold for the development of the insect.

Climatic model

Bioclimatic models were developed using CLIMEX 3.0 (Sutherst et al. 2007) that integrates the weekly responses of a population to climate using a series of annual indices. The bioclimatic modelling process has been previously described in numerous publications (Vera et al. 2002; Sutherst and Maywald 2005; Poutsma et al. 2008; Olfert et al. 2012), and thus, only a brief description of the CLIMEX program and parameters is provided here. CLIMEX uses an annual Growth Index (GI) to describe the potential for population growth as a function of temperature and soil moisture during favourable conditions, and stress indices (cold, wet, hot, dry) to determine the effect of abiotic stress on survival in unfavourable conditions. The weekly GI is a function of temperature (TI), diapause (DI) and moisture (MI). The growth and stress indices are calculated weekly and then combined into an overall annual index of climatic suitability, the Ecoclimatic Index (EI), which ranges from EI = 0 for locations where the species is not able to persist to EI = 100 for locations that are optimal for the species. However, in temperate climates, the maximum EI value is rather close to 50 and values of >20 are sufficient to support substantial population densities (Sutherst et al. 2007).

Fitting CLIMEX parameters

Initial parameter values were based on the temperate template in the CLIMEX model. Then, values were modified according to information obtained either from the literature, mainly from the extensive work of Maruyama and Shinkaji (1987, 1991, 1993), or from our own observations on development presented in this article. Values that were not precisely known were adjusted in an iterative manner until the model closely fitted the known distribution in Asia and the phenology (H. Wan and H. Li, unpublished data) at sites where it had been studied. The model was validated by applying it to the known distribution and phenology in Europe.

The CliMond 10 spatial resolution climate data set was used as input into the model (Kriticos et al. 2012). The CliMond data set was developed for species bioclimatic modelling, including both correlative and process-based mechanistic models. The 10′ gridded data set includes a hybrid historical data set (based on CRU CL2.0 and WorldClim; centred on 1975).The model was validated by comparing model output to reported distributions, seasonal phenology and tested for consistency with empirical data. Two methods were used to validate the model. First, the model was validated by comparing model output with reported geographic distributions. Model parameterization was conducted for Asia, primarily eastern China. The model was then applied to predict the population distribution of *C. perspectalis* in East Asia and Western Europe. Second, the number of generations was compared to published reports in Asia and Europe, and the model output for the phenology was compared with field observations in Switzerland.

Results and Discussion

Developmental investigations

Diapause

The measurement of head capsule width in laboratory rearing showed that there is substantial overlap between larval instars (fig. 1). However, there was little variability in the head capsule width of diapausing larvae. Their measurement showed that the caterpillars mainly diapause and overwinter as third instar larvae in Europe (fig. 1). In China, the majority of larvae overwinter as 2nd–4th instar larvae (Tang 1993; She and Feng 2006). However, overwintering in mature instars is not uncommon, particularly in southern provinces in China (Huang and Li 2001; Xiao et al. 2011; H. Wan, unpublished data), suggesting that the larvae still develop after having experienced a decreasing day length (Xiao et al. 2011). In Japan, larvae enter diapause in the 4th or 5th instar larvae (Maruyama and Shinkaji 1991).

Diapause termination is induced by a period of cold temperature, as in most insects (Tauber et al. 1986). Field collection of overwintering larvae at regular intervals showed that only 13.3% of larvae collected mid-September and 39% of larvae collected between late September and mid-November resumed development and reached the adult stage when incubated in the laboratory. The rate of successful development reached 78.1% for the larvae collected from 30 November on (fig. 2). After this date, up to 35% of the larvae died in rearing, no matter the date of collection. The rearing success of overwintering larvae kept at 2°C increased gradually with the time spent in the cold (fig. 3). After 1 month at 2°C, only 10% of the larvae developed successfully. The rate of successful development reached 50% after a cold period of 1.5 months and 90% after 3.5 months in the cold. The rearing experiments conducted with larvae from the invasive population in Europe suggest that the life cycle of *C. perspectalis* includes an obligate diapause of at least 1.5 months. Therefore, its distribution

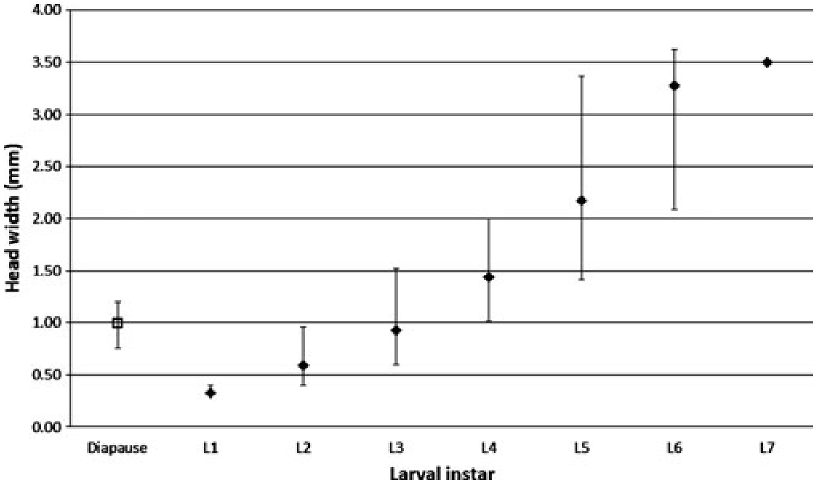

Fig. 1 Mean head capsule width in mm of all instars of *Cydalima perspectalis* larvae in the laboratory. Error bars represent the 95% confidence interval. The first data point represents the mean head width of diapausing larvae in North-Western Switzerland, indicating that the greatest proportion of larvae overwinter in their 3rd instar.

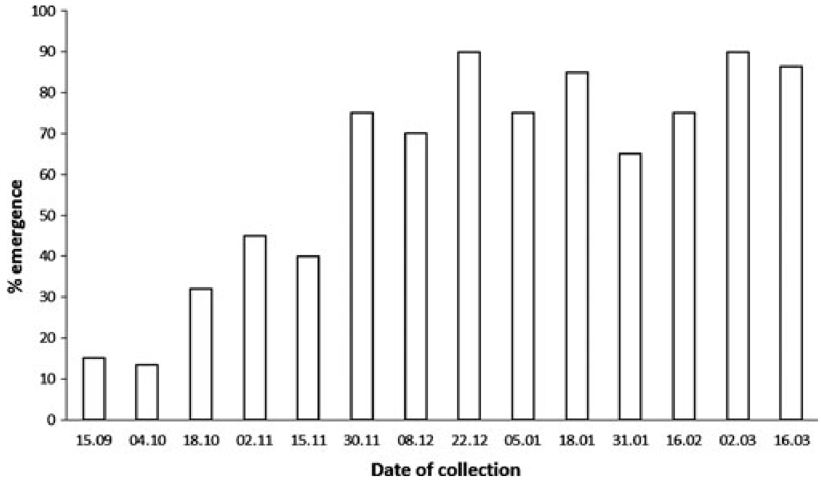

Fig. 2 Percentage of successful development to the adult stage in overwintering larvae collected in the field at regular intervals from 15 September 2010 to 16 March 2011 and incubated at 24°C and 16 h daylight.

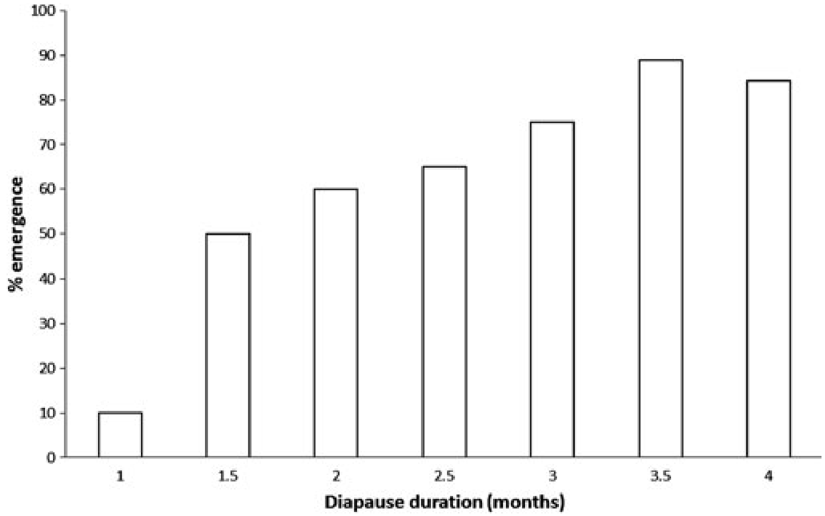

Fig. 3 Percentage of successful development to the adult stage in overwintering larvae collected in the field on 26 October 2011 and kept 1–4 months at 2°C.

potential is possibly limited in southern latitudes, where temperatures below the developmental threshold do not occur for long enough. However, *C. perspectalis* has been observed in Southern China, as far south as Fujian Province, where the climate is subtropical and temperatures rarely fall below 10°C (Wang 2008). This could be due to the occurrence of geographic biotypes that are able to develop without diapause or to unusually high temperature thresholds for diapause induction and completion. The latter hypothesis is corroborated by our observation that diapause is theoretically completed in western Switzerland in late November, that is, at a time larvae have not yet been exposed to long periods of low temperatures. Unfortunately, very little is known about the development *C. perspectalis* in subtropical areas.

Threshold temperatures and degree-days

The median growth rate linearly increased between 15 and 30°C for eggs and pupae, with R^2 of 0.986 and 0.998, respectively, indicating an excellent adjustment to the regression line. In larvae, the linear adjustment was better when the 30°C data point was omitted (R^2 = 0.981 vs. 0.950), probably because, at that temperature, the growth rate does not increase linearly anymore. Therefore, the 30°C data point was removed from the regression and consequently from the assessment of the threshold temperature and the degree-days. Threshold temperatures for development and degree-days that allow 50% of the individuals to complete development are shown in Table 1 for eggs, larvae and pupae, respectively. Threshold temperatures obtained using the European population were slightly lower than those observed by Maruyama and Shinkaji (1987) in Japan. This discrepancy could indicate that the invasive European population originates from a colder region than the populations investigated by Maruyama and Shinkaji.

Table 1 Linear regression parameters estimates describing the relationship between temperature and development rate (1/t50%) of *Cydalima perspectalis* stages

Stage	Intercept	Slope	R^2	Lower threshold (°C)	Degree-days
Eggs	−0.2248	0.0206	0.9858	10.91	48.54
Larvae	−0.026	0.0031	0.9815	8.38	322.58
Pupae	−0.0863	0.0075	0.9995	11.5	133.33

Phenology in north-western Switzerland

In north-western Switzerland, *C. perspectalis* develops two clearly separated generations per year. The first flight period starts in late June or early July and peaks in July. The second flight period starts in the second half of August and can last until early October. The exact dates of appearance may vary according to more or less favourable climatic conditions (fig. 4). The first yearly generation in Basel needed on average 518 degree-days from the overwintering stage to the adult stage (2010: 520 DD, 2011: 513 DD, 2012: 520 DD). The constancy of this value over the 3 years of observations suggests that, in the future, it could be considered as a tool to predict the period of the first flight and consequently of treatments against newly hatched larvae before severe damage can occur. The

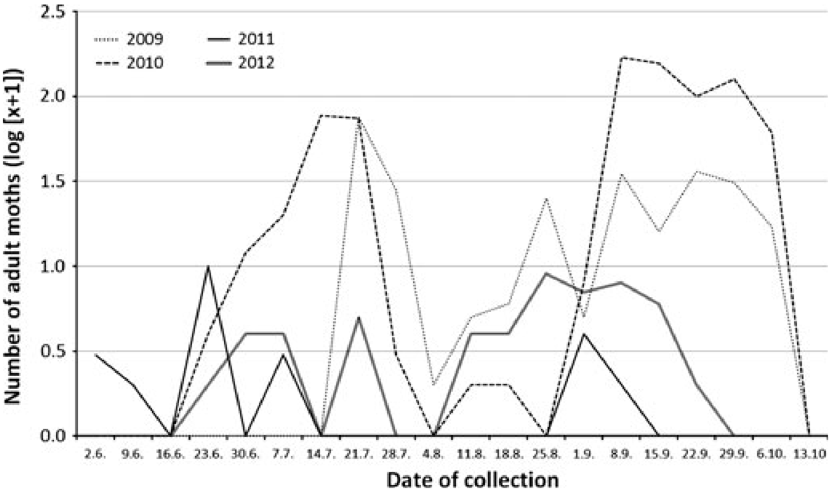

Fig. 4 Number of adult moths of *Cydalima perspectalis* (expressed in Log [x + 1]) captured by two UV-light traps in Basel and Riehen (north-western Switzerland) at weekly intervals during four consecutive years. The captures of the two traps were counted together.

summer generation surprisingly needed fewer degree-days than the overwintering generation: 430 on average (2009: 402 DD, 2010: 443 DD, 2011: not calculated because of small sample size (n = 3), 2012: 446 DD). Although the longer developmental duration of the overwintering generation is contradictory to the measurements by Maruyama and Shinkaji (1987) in Japan, the difference in the number of degree-days necessary for the development of the overwintering generation between locations may be due to differences in the larval instars which overwinter.

Observing adults flying as late as early October despite the fact that diapause induction occurs in early September is not that surprising because only young larvae are receptive to diapause induction (Maruyama and Shinkaji 1993), and thus, individuals that are in the late larval or pupal stages in early September will develop to the adult stage. However, it is not clear whether late-flying adults will be able to lay eggs and whether these eggs can develop to overwintering 3rd-instar larvae.

Climatic model

Model development

All parameter values selected for the model are listed in Table 2. Temperature values for DV0 (lower temperature limit), DV1 (lower optimal temperature), DV2 (upper optimal temperature) and DV3 (upper temperature limit) were mainly obtained from our own observations. DV0 was set to 9.5°C as an average temperature threshold observed throughout the developmental stages. DV1 and DV2 were set to 15°C and 27.5°C because in our rearing, we observed higher mortality below and above these temperatures (S. Nacambo and M. Kenis, unpublished data). There are no data available for DV3, that is, the temperature at which development ceases. However, we observed that the development rate for larvae is linear until 27.5°C, suggesting, by comparison with similar studies (e.g. Olfert et al. 2006), that development may cease ca. 7.5°C above this temperature, that is, at around 35°C. DV1, DV2 and DV3 were iteratively tested in simulations, but these tests had very little impact on the distribution and EI values in Asia.

Similarly, we gave a PDD (degree-days per generation) value of 540 based on the degree-days calculated in the present study, taking into account that approximately 40 degree-days are needed for the pre-oviposition period of the female (Maruyama and Shinkaji 1987). We chose these values over the temperature threshold of 10.5°C and the PDD value of 615 measured by Maruyama and Shinkaji (1987) in Japan because the model is built primarily to assess the potential distribution in Europe. Considering that different geographic biotypes may show different developmental responses, priority should be given to data obtained with European populations. When a PDD value of 615 was applied to the model, the number of potential generations in north-western Switzerland went below 2, which does not match the observed life cycle. However, it should be kept in mind that a precise modelling of the distribution of *C. perspectalis* in Asia may require other values, obtained with Asian populations.

Soil moisture indices are supposed to be of limited direct impact on the moth because none of the developmental stage occurs in the soil. However, it

Table 2 Values for parameter settings for the CLIMEX® model projecting *Cydalima perspectalis* distribution in Europe

Index	Parameter	Description	Value
Temperature	DV0	Limiting low temperature	9.5°C
	DV1	Lower optimal temperature	15.0°C
	DV2	Upper optimal temperature	27.5°C
	DV3	Limiting high temperature	35.0°C
Moisture	SM0	Limiting low soil moisture	0.01
	SM1	Lower optimal soil moisture	0. 1
	SM2	Upper optimal soil moisture	1.5
	SM3	Limiting high soil moisture	2.5
Diapause	DPD0	Diapause induction day length	13.5
	DPT0	Diapause induction temperature	20
	DPT1	Diapause termination temperature	0
	DPD	Diapause development days	45
	DPSW	Diapause indicator for winter diapause	0
Cold Stress	TTCS	Cold stress threshold	−20°C
	THCS	Cold stress temperature rate	−0.001
Heat Stress	TTHS	Heat stress temperature threshold	40
	THHS	Heat stress temperature rate	0.005
Degree-days above DV0	DV0		9.5
	DV3		35
	MTS	Model step time	7
Degree-days above DVCS	DVCS		8.0
	DV4		100
	MTS	Model step time	7
Degree-days above DVHS	DVHS		31
	DV4		100
	MTS		7
Degree-days per generation	PDD	Minimum degree-days above DV0 to complete one generation	540

certainly influences the distribution of the moth indirectly through an effect on host plant distribution (see for example Olfert et al. 2006). To begin, moisture indices of the CLIMEX temperate climate template were used, but these indices did not allow the occurrence of *C. perspectalis* in Central and North-East China, where winter and spring are very dry and the moth is commonly observed. Lowering SM0 (limiting low soil moisture) to 0.01 and SM1 (lower optimal soil moisture) to 0.1 allowed a better match of abundance in winter-dry areas in Central and North-East China. SM2 (upper optimal soil moisture) and SM3 (limiting high soil moisture) remained as in the template at 1.5 and 2.5, respectively. It cannot be ruled out, however, that the occurrence of the moth and its host plant in the driest areas of their distribution is restricted to watered gardens.

Diapause indices were the parameters that most strongly influenced distribution patterns in Asia. Maruyama and Shinkaji (1991, 1993) and Xiao et al. (2011) measured a diapause induction day length (DPD0) of 13 h50 – 14 h20 and 12 h50 – 13 h36, respectively. Thus, for the model, we choose a DPD0 of 13 h30, which matches our observations in north-western Switzerland where larvae cease their development and build overwintering cocoons in early September. The photoperiodic response of *C. perspectalis* is temperature-sensitive. Maruyama and Shinkaji (1993) and Xiao et al. (2011) found higher day length thresholds when larvae were reared at 15°C than at 25°C, and at 25°C than at 28°C, respectively. After iteration tests, DPT0 (Diapause induction temperature) was set to 20°C, which allowed us to account for this difference between temperate and warmer regions. In addition, a lower DPT0 would have prevented a development in Southern Chinese cities such as Guangzhou, where the insect is known to occur (Qiu et al. 2005). Following the results obtained in the present study, DPD (Diapause development days) was set to 45 days. DPT1 was set to 0°C because there is no reason to believe that a minimum weekly temperature in spring is needed to break diapause as diapause has been broken by a short period of cold in autumn and early winter. Instead, it will be the time above DV0 in spring that will determine the development. Our personal observation of larval feeding in early March, when temperature is favourable, corroborates this choice.

Abiotic stress indices (heat, cold, wet, dry and their interactions) are proposed by CLIMEX to place limits on the potential range of the species based on its ability to survive under unfavourable conditions. TTHS (heat stress temperature threshold) was set to 40°C. This high value is justified by the fact that *C. perspectalis* is a pest in Central China, where summer mean temperatures commonly go above 35°C for several consecutive days. THHS (Heat stress temperature rate) was set up at 0.005, as for the CLIMEX temperate climate template. TTCS (cold stress temperature threshold) was set at −20°C, with THCS (cold stress temperature rate) at −0.001. *C. perspectalis* is known to survive in foliage in areas where winter temperatures reaching −30°C are not uncommon (e.g. Northern China and Russian Far East). In winter 2011–2012, average temperatures in north-western Switzerland went below −15°C during several consecutive days, with extreme lows at −25°C, with no apparent impact upon population densities in spring 2012 (S. Nacambo and M. Kenis, unpublished data). Iteration tests showed that a realistic distribution and EI in Asia was provided with these parameter values. Nevertheless, even large changes in temperature stress indices did not substantially modify the distribution and EI in Asia. Wet and dry stress do not seem to play an important role in *C. perspectalis* distribution because it is found in very humid areas in Southern China as well as in rather dry areas in Central China. Thus, these stress indices were not used in the models nor were interactions of stress indices proposed in the program.

Model validation and predicted distribution of C. perspectalis in Europe

The CLIMEX map of Asia for *C. perspectalis* is shown in fig. 5. The predicted distribution is limited in the

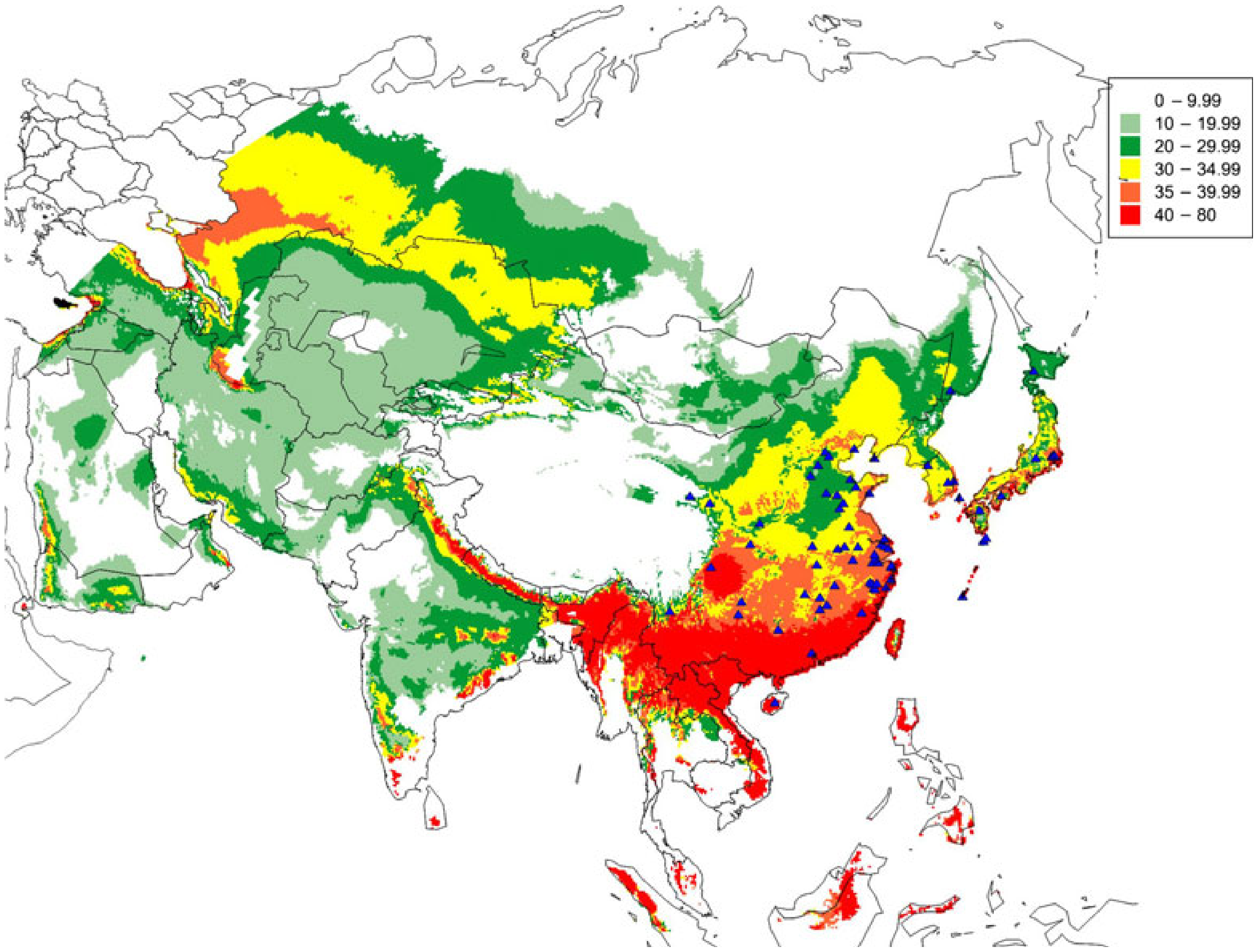

Fig. 5 CLIMEX map of predicted distribution of *Cydalima perspectalis* and relative abundance (Ecoclimatic Index) in Asia. Triangles represent the known distribution of *Cydalima perspectalis* in Asia from the literature (see references in the text) and unpublished observations by H. Wan and M. Kenis.

north by the insufficient degree-days to complete one generation and/or by cold stress. In the south, it is limited by the diapause requirements which, in tropical areas, are met only at higher altitudes. The predicted distribution includes all known distribution records of the moth, and the high EI values through most of the known range illustrate its ability to develop high populations. The model also predicts high EI values outside its known distribution range, that is, in sub-tropical and tropical areas of South and South-East Asia. Many *Buxus* spp. occur naturally in these regions (Köhler 2007; Min and Brückner 2008), but these are not or rarely used as ornamentals and it is not known whether they are suitable for *C. perspectalis*. So far, all records of *C. perspectalis* in Asia are related to plantations of temperate *Buxus* spp. in urban areas, and it would be worth surveying natural *Buxus* spp. stands to assess the ecological distribution of the moth in Asia.

When the same parameters were applied to climate data from stations across Europe, the CLIMEX EI map suggests that most of Europe is suitable for *C. perspectalis*, with the exception of Central and North Fenno-Scandinavia, northern Scotland and high mountain regions (fig. 6), where the number of degree-days above the temperature threshold is insufficient to complete one generation per year. The model accurately predicts the occurrence of *C. perspectalis* in all European countries where it is presently recorded and all regions where it occurs in very high densities (e.g. low elevation areas in Switzerland and Austria, Southern Germany, Northern Italy and Eastern France (Leuthardt et al. 2010; Van der Straten and Muus 2010; M. Kenis, personal observations) show high EI values). The model predicts that, in north-western Switzerland, development starts in early to mid-March and stops in early September (fig. 7) with two generations per year, which perfectly match our observations both in terms of development start and diapause induction (S. Nacambo, F. Leuthardt and M. Kenis, unpublished data).

The relatively high EIs throughout most of Europe suggest that the insect has the potential of becoming a pest in most of its predicted range. However, in Northern and North-Western Europe, the temperature will not allow the moth to complete two generations, which may prevent severe outbreaks. Biotic and abiotic mortality rates are probably higher in overwintering generations than in summer generations, as observed with other bi- or multivoltine insects

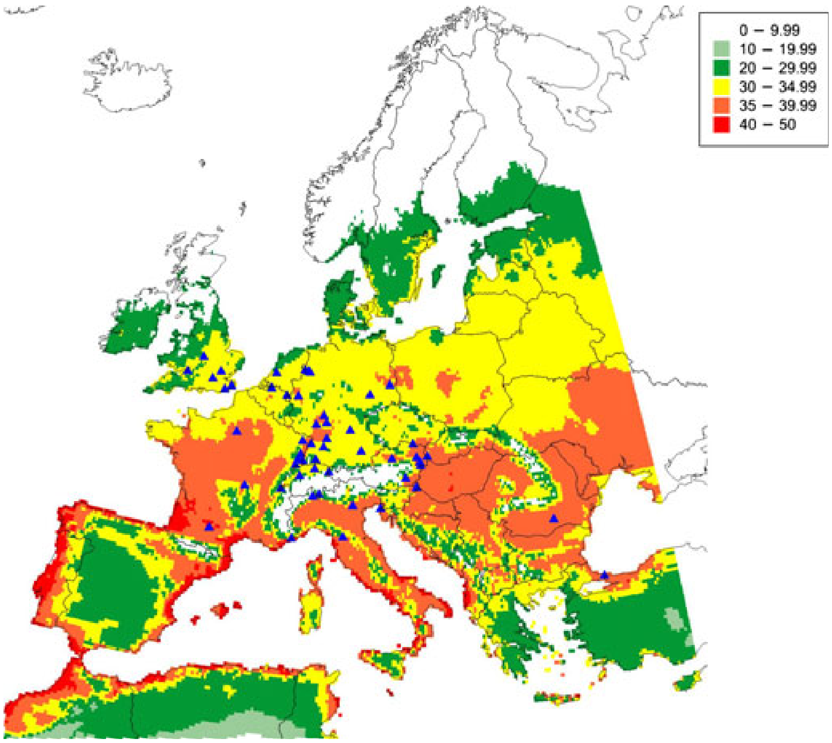

Fig. 6 CLIMEX map of predicted distribution and relative abundance (Ecoclimatic Index) of *Cydalima perspectalis* in Europe. Triangles represent the published distribution of *Cydalima perspectalis* in Europe in 2012 (see references in the text). In heavily infested areas, triangles may represent several notifications.

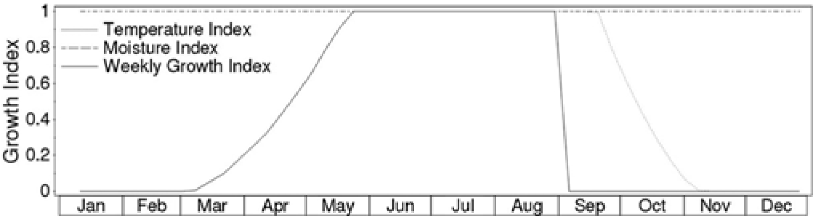

Fig. 7 CLIMEX graph showing the climatic data and the predicted temperature, moisture and weekly growth indices for *Cydalima perspectalis* in Basel (north-western Switzerland).

(Girardoz et al. 2007), and the impossibility of completing a second generation in late summer may disturb diapause induction. Thus, it is likely that, at present climatic conditions, the highest damage will occur in the southern half of Europe.

Conclusions

A number of differences have been observed between the invasive population of *C. perspectalis* and the native populations in Asia. Temperature thresholds as well as degree-days required for the development of eggs, larvae and pupae were consistent among European studies but differed from studies carried out in Japan (Maruyama and Shinkaji 1987, 1991, 1993). Such differences may occur because different geographic biotypes show different developmental responses (Maruyama and Shinkaji 1993). Furthermore, it cannot be ruled out that cryptic species occur, for example, in Japan and continental Asia. Molecular studies may reveal cryptic complexes of species in Lepidoptera (Hebert et al. 2004), and a phylogeographic study could help interpreting the physiologic differences found among populations of *C. perspectalis.* Although the precise pathway of introduction of the invasive population of *C. perspectalis* is not known, it is likely that it reached Europe on horticultural box-tree plants imported from China, because nearly all non-European imports of *Buxus* spp. to Europe come from there (EPPO 2012). However, there is little information available on the region of production of *Buxus* spp. in China. Furthermore, plants may also become infested after having left the nursery, during transportation or storage.

Modelling the potential geographic distribution of *C. perspectalis* in Europe provides the opportunity to anticipate the spread of the insect and put into place management and control plans in countries not yet invaded in order to respond rapidly and effectively to the arrival of the invader. However, climatic factors as modelled by CLIMEX are not the only determinant factors for the distribution of an invasive species. In particular, the occurrence of food plants, as well as competition, predation and parasitism can significantly influence the suitability of a particular geographic area (Baker et al. 2000). Box trees are abundantly planted as ornamentals in most climatically suitable European regions; thus, the availability of host plants should not be a limiting factor. Although predation and parasitism seem to occur in *C. perspectalis* in Europe, few studies have covered these topics to date (Zimmermann and Wührer 2010; S. Nacambo, unpublished data). Other approaches, such as physiologically based demographic models (Gutierrez et al. 2008, 2012), may address some of the shortcomings of simple climate models by explicitly capturing the mechanistic weather-driven biology of the species and of relevant interacting species in its food chain or web. However, these models require extensive data that are presently not available for the box-tree moth system.

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CHAPTER 3

Oviposition Preference and Larval Development of the Invasive Moth *Cydalima perspectalis* on Five European Box-Tree Varieties

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Oviposition preference and larval development of the invasive moth *Cydalima perspectalis* on five European box-tree varieties

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Abstract

The box-tree pyralid *Cydalima perspectalis* (Walker 1859) (Lepidoptera: Pyralidae), native to Eastern Asia, is a newly introduced species causing severe damage to box-trees (*Buxus* sp.) in private and public gardens as well as in semi-natural box-tree forests in Central Europe. It is so far not known whether different box-tree subspecies (or varieties) are similarly affected by this invasive moth. In a choice experiment offering branches of five different box-tree varieties as oviposition sites, we found a preference of female moths for laying their egg clusters on the variety 'Rotundifolia', while other varieties were less frequently considered. The preference for 'Rotundifolia', the variety with the largest leaves in the tests, remained when intervariety differences in foliar area (mean leaf size × number of leaves) were taken into account. Feeding larvae on leaves of either of the five box-tree varieties revealed a significant effect of the seasonal generation of *C. perspectalis* on the growth rate of individuals but no influence of the box-tree variety. Larvae from the spring generation show the highest growth rate, those from the summer generation a moderate and those from the autumn generation the lowest growth rate. The moths used in the experiments may belong to the 10th to 12th generation present in Europe. The time elapsed since their introduction may be too short for an optimal adaptation to the partly novel diet encountered by the invasive moth.

Introduction

Intentionally and unintentionally introduced alien species may become invasive, creating great ecological and economical damage in their new environment. Prominent examples include *Scolytus* species (EPPO 1983) as vectors of the Dutch elm disease (Webber 2007), the giant red palm weevil *Rhynchophorus ferrugineus* (EPPO 2008) and the Colorado potato beetle *Leptinotarsa decemlineata* (EPPO 1981).

The box-tree pyralid *Cydalima perspectalis* (Walker 1859) (formerly *Diaphania* or *Glyphodes perspectalis*, see Mally and Nuss 2010) (Lepidoptera: Pyralidae), native to Korea, Japan and China (Inoue et al. 1982), has been recorded in Europe for the first time in south-western Germany and in the Netherlands in 2007 (Krüger 2008; Leuthardt et al. 2010; Van der Straten and Muus 2010). The species spread rapidly to north-western Switzerland and France (Feldtrauer et al. 2009). Most recently, it has also been recorded in England and Austria (Van der Straten and Muus 2010). In the newly invaded regions, the box-tree pyralid causes severe damage to box-trees (*Buxus* sp.) in private and public gardens as well as in semi-natural box-tree forests. Box-trees are of great structural importance in public gardens including historical parks and cemeteries. The larvae of *C. perspectalis* feed on the leaves of box-trees but can attack the bark of the trees, causing them to dry out and die.

Many invasive insects are characterized by the ability to grow and reproduce on plant species unknown in their native range. Several species altered the diet by adjusting their food preference to newly encountered plants in the introduced region. One noticeable example is the weevil *Rhinocyllus conicus* deliberately introduced into the United States for the biological control of the invasive thistles of the genus *Carduus*, which ended up feeding on several native *Cirsium* species (Louda 1998). In its native range, the box-tree pyralid is specialized to one plant genus (*Buxus* sp.). In Europe, there are several species and varieties of the genus *Buxus* that do not occur in the native range of *C. perspectalis*. However, damage has been recorded on all box-tree species and varieties examined in Europe, indicating a successful adjustment of the food preference of this invader (F. L. G. Leuthardt, G. Glauser and B. Baur, unpublished data).

The oviposition choice of females and the food preference of larvae of the box-tree pyralid have been examined in its native range (Maruyama 1993; Maruyama and Shinkaji 1987, 1991, 1993). Similar information is not available for *C. perspectalis* in Europe. The aim of our study is to investigate the oviposition preference and larval performance of *C. perspectalis* on five common European box-tree varieties. We conducted two experiments to answer the following questions: (i) do female moths of *C. perspectalis* show an oviposition preference for a particular box-tree variety; (ii) do larvae feeding on different box-tree varieties show differential performances (survival, growth rate); and (iii) does female oviposition preference match the larval performance of the moth?

Materials and Methods

Insects and plants

Adults of *C. perspectalis* were obtained from a light-trap positioned near a box-tree hedge (*Buxus sempervirens sempervirens)* in Riehen, 5 km NE of Basel, Switzerland (47°33′45″N/7°38′27″E). Moths were collected between 23 June and 21 July 2010. Additionally, larvae from the culture of an entomologist (R. Joos), which emerged in the beginning of June 2010, were added to the culture. Our culture was set up in an outdoor cage (1 m × 1 m × 2 m, wooden structure covered with netting) containing a wild *B. sempervirens* tree of 1.2 m height. The resulting overlapping generations of *C. perspectalis* allowed us to conduct experiments throughout the summer, without interruption.

Five varieties of box-trees belonging to two species were tested in the two experiments: *B. sempervirens* 'Sempervirens', *B. s.* 'Rotundifolia', *B. s.* 'Argenteovariegata', *B. s.* 'Aureovariegata' and *B. microphylla* 'Faulkner'. These varieties are the most frequently planted in public and private gardens in Northwestern Switzerland. The 10- to 15-year-old trees of each variety (1–1.8 m high) grew outside the distribution area of *C. perspectalis* and were never treated with chemicals. Trees were kept outdoor and protected with nets against oviposition by *C. perspectalis*.

Oviposition preference

To examine whether female moths show a preference for depositing their eggs on a specific variety of box-trees, we conducted a multiple-choice test. Pairs consisting of a female and a male, both newly hatched (12–18 h), were placed in a cage (25 cm × 25 cm × 50 cm high) covered with netting. One branch of 15 cm length of each box-tree variety was stuck in a sand-filled container on the bottom of the cage. The positions of the five branches were randomly assigned in a circle. The sand was moistened for the time of the experiment to keep the plants fresh. A small dish with water and a dish with a 50% honey solution were placed in the centre of the cage as a food source. The tests were run at a temperature of 22–24°C under natural light conditions from June to September 2010. For the spring generation in March 2011, an artificial light source was additionally used (JBL Solar Natur, 30-watt full spectrum light; see below). The set-up of test plants was turned clockwise by 90 ° every day to exclude any influence of sunlight or spatial position. Egg clusters consist of a gelatinous mass containing 5–20 eggs. We recorded daily the number of egg clusters deposited on the branch of each box-tree variety over the entire lifespan of individual females (1–16 days; see Results). Single eggs could not be counted because they are arranged in layers in the gelatinous matrix. Plant material for oviposition was replaced as soon as approximately one-third of the leaves began to show withering signs (after 5–6 days) or when about 20% of the leaves had been removed due to oviposition on them. Up to five multiple-choice assays were run simultaneously, with a total of 18 replicates.

To account for a potential effect of different leaf size in the five box-tree varieties, we assessed the foliar area for each variety. Using a stratified sampling procedure, we scanned the surface of 15 randomly chosen leaves of each variety and calculated the mean leaf area using the image program ImageJ (version 1.46d, Rasband 1997–2011). Furthermore, we counted the number of leaves on 15 haphazardly chosen branches, each measuring 15 cm in length, for all five box-tree varieties. Mean leaf area multiplied by mean leaf number provides an estimate of the total foliar area for the branches used in the choice test. The five box-tree varieties also differed slightly in leaf colour and shape, but these differences were hardly quantifiable.

Larval performance on different box-tree varieties

To investigate whether the growth and survival of *C. perspectalis* larvae are influenced by the type of food plant, we conducted no-choice feeding tests using the five box-tree varieties described earlier. We applied a siblings-split design, in which larvae from the same egg cluster were assigned to each of the five box-tree varieties. Newly hatched (12–18 h) caterpillars were placed singly in a PVC-Petri dish (diameter 90 mm) lined with wet filter paper which prevented the food to dry out. Equal proportions of young and old leaves of the different box-tree varieties were offered as food to account for different toughness and nutrient levels of leaves. Food was offered in larger quantities than needed by the larvae and replaced with fresh leaves every 48 h. Temperature conditions were identical to those in the oviposition preference experimental design. The caterpillars were weighed to the nearest 0.01 mg every 48 h until pupation. Pupae and adults were not weighed because of their rapid weight loss. The number of larval stages was recorded and the head capsules from each larval stage were measured to the nearest 0.1 mm in a subsample of 14 larvae per variety.

To examine seasonal effects on growth, we conducted three experimental runs testing larvae from three generations: larvae tested in January 2011, hatched from eggs deposited in October 2010, overwintered under natural conditions, hereafter referred to as spring generation. Larvae tested in August 2010, from eggs deposited in July 2010, hereafter called summer generation. Finally, larvae tested in October 2010, from eggs deposited in September 2010 and prevented from hibernating due to their early development, hereafter referred to as autumn generation. Those larvae developed 1–2 weeks earlier than larvae in the field as a result of optimal climatic conditions in the laboratory. The summer and autumn generations had natural light (on average of 16.2 h natural light per day for the summer generation and 12.6 h per day for the autumn generation). The spring generation was exposed to an artificial light source (14 h of full spectrum light). For each box-tree variety, the growth of 20 singly kept larvae was recorded resulting in a total of 100 individuals (spring generation: n = 30; summer generation: n = 30; autumn generation: n = 40). However, due to larval mortality, data from only 81 individuals could be used in the statistical analyses (spring generation: n = 27; summer generation: n = 30; autumn generation: n = 24). The sample sizes for each variety are given in Fig. 2.

Statistical analysis

Statistical analyses were conducted using R (R Development Core Team 2011) and the R package Sciplot (Morales 2011). Individual moths varied greatly in total number of egg clusters deposited. We therefore calculated a relative measure of oviposition frequency using the formula n*100/N, where n is the number of egg clusters laid by a particular moth on a specific box-tree variety, and N is the total number of egg clusters deposited by the same moth on all five box-tree varieties. In this way, females with different oviposition performance have the same weight in the analysis. Deviations from the null hypothesis that moths deposit their egg clusters in equal frequencies on each box-tree variety were examined using log-likelihood test (G-test; Sokal and Rohlf 1981). The same analysis was conducted using oviposition frequency data corrected for an equal foliar area of 100 cm^2 in each box-tree variety.

We also analysed oviposition preference on the level of individual females. Fourteen of 18 females laid more than 42 egg clusters. We used a contingency test (Chi-square) for each of these 14 moths to examine whether the female deposits her egg clusters in equal frequency on each box-tree variety.

We used a nested linear model to examine how box-tree variety, seasonal generation of larvae and mother (nested in seasonal generation) influenced larval growth rate. We started with a complete model (main effects plus interactions). Then, the minimal adequate model was selected based on Akaike's Information Criterion (AIC). Correlation analyses showed that growth rate, developmental time and number of larval stages were highly intercorrelated (see Results). We therefore considered only growth rate in the analyses. We examined potential differences in survival among seasonal generations as well as among larvae kept on the five box-tree varieties using chi-square tests.

Results

Oviposition preference

Female moths varied greatly in number of egg clusters laid (range: 6–491, median = 12.5, n = 18). The period during which they deposited egg clusters ranged from 1 to 16 days (median = 7, n = 18). The number of egg clusters laid was positively correlated with the duration of the egg deposition period (Spearman correlation: $r_s = 0.54$, $n = 18$, $P < 0.001$).

Female moths deposited their egg clusters in different frequencies on different *Buxus* varieties (Fig. 1;

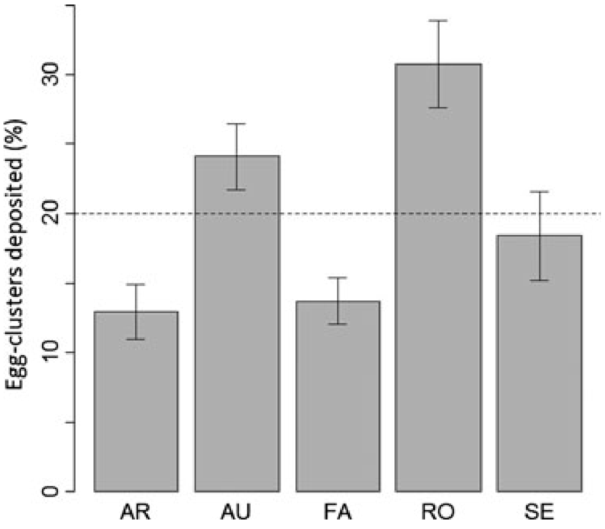

Fig. 1 Oviposition preference of *Cydalima perspectalis* in a multiple-choice test offering five box-tree varieties. Values shown are mean percentage (±SE) of the number of egg clusters deposited by 18 females for each box-tree variety. AR, Argenteovariegata; AU, Aureovariegata; FA, Faulkner; RO, Rotundifolia; SE, Sempervirens. The horizontal line indicates the null hypothesis of equal egg cluster distribution on each box-tree variety.

$G = 10.91$, d.f. = 4, $P = 0.031$). Moths showed a preference for depositing eggs on the variety 'Rotundifolia' ($P < 0.05$). In contrast, females laid less frequently eggs on the varieties 'Argenteovariegata' ($P < 0.05$) and 'Faulkner' ($P < 0.05$) than expected from a uniform distribution (Fig. 1). However, the different box-tree varieties differed significantly in leaf size (table 1). The variety 'Rotundifolia', which was preferred for oviposition, had the largest leaves (mean area of a leaf: 2.52 cm^2). In contrast, the two box-tree varieties which were less frequently considered for oviposition than expected had the smallest leaves ('Argenteovariegata': 0.67 cm^2; 'Faulkner': 0.92 cm^2; table 1). The mean leaf size of the different box-tree varieties was highly negatively correlated with the mean number of leaves that a 15-cm long branch carries (table 1; $r = -0.96$, $n = 5$, $P = 0.0054$). Thus, box-tree varieties with small leaves have more leaves than those with large leaves. The resulting foliar area (amount of leaf material available) of 15-cm long branches is similar in all varieties examined (table 1). The preference for oviposition on the variety 'Rotundifolia' remained (G-test, $P < 0.05$), even when the percentage of egg clusters deposited on the different box-tree varieties was related to an equal foliar area of 100 cm^2 for each variety. The foliar area-corrected data revealed also a preference for oviposition on the variety 'Aureovariegata' ($P < 0.05$), while females less frequently laid eggs on the variety 'Faulkner' ($P < 0.05$).

Considering individual females, 15 of the 18 females distributed their egg clusters over all five box-tree varieties, albeit in different frequencies. Fourteen of the females laid more than 42 egg clusters, which allowed for further analyses. An unequal distribution of egg clusters among box-tree varieties was found in 9 of the 14 females (Contingency test, in all 9 cases, $P < 0.01$). Seven of these 9 females deposited most egg clusters on the variety 'Rotundifolia' and two on the variety 'Aureovariegata'.

Larval performance on different box-tree varieties

The individual growth rate of *C. perspectalis* larvae ranged from 7.52 mg/day to 32.73 mg/day (grand mean = 17.34 mg/day; n = 81). The growth rate was affected by the seasonal generation (Fig. 2). Statistical model selection removed the factors box-tree variety and mother (nested in seasonal generation) as well as the interactions, keeping only the factor seasonal generation in the minimum adequate model (ANOVA: $F_{2,78} = 31.19$, $P < 0.001$). Individual growth rates were highest in the spring generation, had an intermediate value in the summer generation and were lowest in the autumn generation (Fig. 2).

Table 1 Leaf size, number of leaves and total foliar area of five box-tree varieties

	Box-tree variety						
	AR	AU	FA	RO	SE	$F_{4,70}$	P
Leaf size (cm^2)	0.67 ± 0.08	1.59 ± 0.25	0.92 ± 0.10	2.52 ± 0.37	1.07 ± 0.15	11.46	<0.0001
Number of leaves	176.5 ± 12.6	85.5 ± 6.1	153.7 ± 22.8	59.0 ± 6.8	145.8 ± 14.3	12.70	<0.0001
Foliar area (cm^2)	118.7	136.0	141.2	148.9	156		

Mean values ± SE are presented, n = 15 in all cases. Number of leaves and foliar area relate to 15-cm long branches. Foliar area is the product of leaf size and number of leaves.

AR, Argenteovariegata; AU, Aureovariegata; FA, Faulkner; RO, Rotundifolia; SE, Sempervirens.

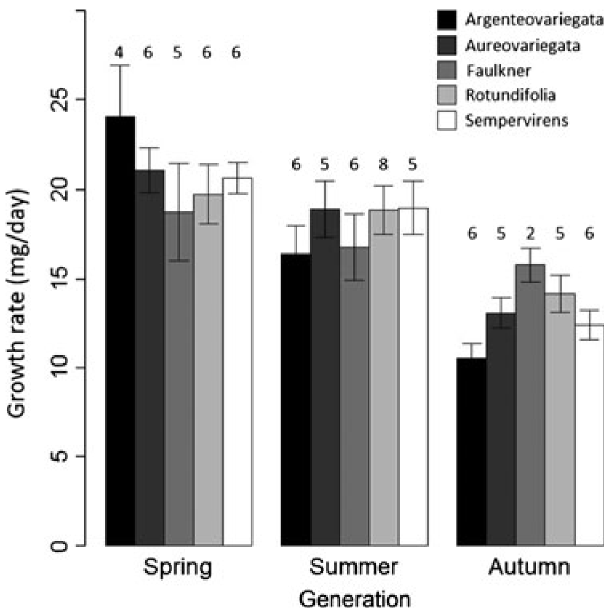

Fig. 2 Mean growth rate (mg/day) of *C. perspectalis* larvae fed on five box-tree varieties for the three seasonal generations. Values shown are means ± SE, $n_{spring} = 27$, $n_{summer} = 30$, $n_{autumn} = 24$. Figures above the bars indicate sample sizes.

Larval survival averaged 81% during the growth experiment, but differed among generations (chi-square test: $\chi^2 = 20.08$, d.f. = 2, $P < 0.001$). Survival was high in the spring and summer generations (90% and 100%), but relatively low in the autumn generation (60%). However, larval survival was not affected by the different box-tree varieties ($\chi^2 = 4.76$, d.f. = 4, $P = 0.32$).

The maximum weight of larvae did not differ significantly between the three generations (ANOVA: $F_{2,78} = 2.99$, $P = 0.06$) indicating that the growth rate is essentially influenced by the developmental time.

The developmental time of the caterpillars from egg hatching to pupation varied between 14 and 30 days (grand mean = 20.1 days; n = 81). Development time was negatively correlated with individual growth rate (Pearson correlation: $r = -0.79$, $n = 81$, $P < 0.001$). Individuals from the spring generation had the shortest developmental time (median = 16 days), whereas those from the autumn generation showed the longest development (median = 24 days). Similarly, the number of larval stages, which ranged from 3 to 7 (grand mean = 5.7; n = 81), was negatively correlated with individual growth rate (Spearman correlation: $r_S = -0.32$, $n = 81$, $P = 0.0036$). Individuals from the spring and autumn generations had 5–7 larval stages (median = 6 in both generations), whereas those from the summer generation had 3–7 larval stages (median = 5). The mean increase in the size of head capsules was positively correlated with the mean growth rate (Pearson's correlation: $r = 0.99$, $n = 15$ measurements over 30 days, $P < 0.0001$).

Discussion

Oviposition preference

Our results indicate that females of *C. perspectalis* prefer for oviposition the box-tree variety 'Rotundifolia' over other varieties. This preference could not be explained by differences in the foliar area of the different box-tree varieties. Oviposition preference may be determined by either physical or chemical cues, or a combination of both (Renwick and Chew 1994). Physical cues involve colour, leaf toughness as well as leaf shape. However, the cues used by *C. perspectalis* are so far not known, as the pre-oviposition behaviour was not investigated in this study.

Newly introduced herbivorous insect species are often forced to adapt to new host plants (Thompson and Pellmyr 1991). In its native range in Eastern Asia, more than 15 box-tree species or subspecies occur, including *Buxus microphylla* var. *japonica* and *Buxus microphylla* ssp. *sinica* (von Balthazar et al. 2000). Larvae of *C. perspectalis* have been reported to feed on *B. microphylla* in Japan (Maruyama 1993) and on *B. sinica* in China (Chen et al. 1993). Larvae of *C. perspectalis* were reared on *B. microphylla* var. *japonica* and *B. m.* var. *insularis* as well as on *B. sempervirens* under laboratory conditions in Japan (Maruyama 1993). We are aware of the general limitations of multiple-choice experiments. Our results may be influenced by the range of varieties examined in the oviposition tests. However, we considered the five box-tree varieties which occur most frequently, representing the most probable choice ovipositing females of *C. perspectalis* encounter in Central Europe.

In previous studies, it has been observed that female pyralids tend to match the number of eggs or egg clusters deposited on a plant with the available resources for the larvae (Damman 1991). Therefore, plants with many leaves are preferred over plants with few leaves for oviposition. In the present study, females of *C. perspectalis* distributed their egg clusters regularly on all sizes of leaves offered for oviposition within a single branch (F. L. G. Leuthardt, data not shown). An ovipositing female may reduce competition among the offspring by distributing her egg clusters at equal distances. Neighbouring branches may also be considered for egg laying provided they do not exhibit pronounced differences in leaf quality.

This could partly explain the fact that females deposited egg clusters on more than one branch in the experiment. If larger branches were offered in the tests, we would expect less variation in oviposition site choice.

An ongoing study investigating the alkaloid composition of leaves of the five box-tree varieties indicates that the oviposition preference of *C. perspectalis* females is not influenced by the composition of secondary compounds, especially alkaloids. The two box-tree species *B. sempervirens* and *B. microphylla* differ in alkaloid composition as do – to a lower extent – the different varieties of *B. sempervirens* (F.L.G. Leuthardt, G. Glauser, B. Baur, data not shown). In the present study, female moths showed a preference for the variety 'Rotundifolia' and a weaker preference for 'Aureovariegata', both belonging to the species *B. sempervirens*. The variety 'Faulkner', which was less frequently considered for egg laying, belongs to the species *B. microphylla*. In its native range, *C. perspectalis* encounters the box-tree species *B. microphylla* in its wild form (Global Biodiversity Information Facility database, www.gbif.org; accessed January 2012) but may also find horticultural breeds of *Buxus sempervirens*. We do not know whether and in which frequency the varieties examined in the present study occur in Eastern Asia.

Larval performance on different box-tree varieties

Our experiment revealed no differences in growth rate and survival of *C. perspectalis* larvae raised on the five box-tree varieties. Similarly, a study testing the larval performance of *C. perspectalis* larvae reared on an artificial diet containing *B. microphylla* leaf powder did not show any significant decrease in larval growth or developmental duration on the artificial diet (Kawazu et al. 2010). These findings suggest a broad acceptance of food plant varieties for larvae of *C. perspectalis*. However, an interesting reversal of the growth rate on the five box-tree varieties between the spring and the autumn generation was observed. This result is not statistically significant and the reason for this finding remains to be investigated. It could also be an experimental artefact.

We found a strong seasonal variation in both growth rate and survival. Larvae from the spring generation grew faster than larvae from the autumn generation. This variation cannot be explained by temperature because temperature was kept constant during the experiment. However, the three generations of larvae were exposed to slightly different light conditions in our experiment. Day length decreased in the period the autumn generation was raised. This could explain the lower larval performance in terms of reduced growth rate and increased developmental duration and mortality in this generation. In Japanese populations, the critical photoperiod for the induction of larval diapause has been shown to be around 14 h (Maruyama and Shinkaji 1993). *C. perspectalis* larvae of the spring generation kept under controlled laboratory conditions required less degree-days (238.1 degree-days at 20°C) for their development than larvae of the summer and autumn generations (365.0 degree-days) (Maruyama and Shinkaji 1987). Our study confirms these finding for *C. perspectalis* introduced to Europe.

Plant chemistry is the most important factor influencing the evolution of insect diet breadths (Thompson 1994). Feeding larvae need to be physiologically capable of digesting host tissues and dealing with any allelochemicals present. Studies investigating the composition of secondary compounds in leaves and other plant organs of box-trees revealed that alkaloids accumulate especially during the fruiting phase in summer (Vachnadze et al. 2009). Larvae of different seasonal generations are therefore exposed to different concentrations of alkaloids. Larvae of *C. perspectalis* sequester certain alkaloids present in box-tree leaves (F. L. G. Leuthardt, G. Glauser, B. Baur, unpublished data). Leaves of different box-tree varieties and tissue of larvae fed on those varieties showed similar patterns of alkaloid composition (F. L. G. Leuthardt, G. Glauser, B. Baur, unpublished data). A part of the seasonal variation in larval performance may therefore also be attributable to the seasonal changes in composition and concentration of secondary compounds.

Conclusions

The present study showed a preference of ovipositing females of *C. perspectalis* for the box-tree variety 'Rotundifolia' which cannot be explained by foliar area. However, larval performance was similar on five box-tree varieties but varied between generations. This suggests that there is no dependency between female oviposition choice and larval performance, as has been reported in numerous lepidopteran species (see for example Wiklund 1975; Singer et al. 1988). Different hypotheses have been proposed to explain divergences between oviposition preference and larval performance: they include time, patch dynamics, parasite versus grazer lifestyle and enemy-free space (Thompson 1988). The moths used in our experiments belong approximately to the 10th to 12th generation present in Europe, as the first individuals might have reached the continent in 2006 (Leuthardt et al. 2010). This period may be too short for adaptation, indicated by the slight divergence between oviposition preference and larval performance, as reported from other introduced species (Berdegué et al. 1998).

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CHAPTER 4

Composition of Alkaloids in Different Box Tree Varieties and their Uptake by the Box Tree Moth *Cydalima perspectalis*

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Composition of alkaloids in different box tree varieties and their uptake by the box tree moth *Cydalima perspectalis*

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Abstract Larvae of the moth *Cydalima perspectalis* are specialized on box trees (*Buxus* spp.). Native to eastern Asia, the moth has been introduced to Europe in 2007 and is nowadays causing severe damage to box trees in private and public gardens, as well as in semi-natural box tree forests. Box trees contain highly toxic triterpenoid alkaloids which may be sequestered by specialized herbivores such as *C. perspectalis.* We determined the alkaloid composition in leaves of the five most common box tree varieties in Europe belonging to two *Buxus* species using liquid chromatography–mass spectrometry (LC–MS) metabolite profiling. We also examined whether larvae and moths of *C. perspectalis* accumulate alkaloids from the different box tree varieties. The differences in alkaloid composition observed between the box tree species *Buxus sempervirens* and *Buxus microphylla* were mirrored in the tissue of *C. perspectalis* larvae fed on either of the different box tree species, indicating uptake of alkaloids. The larvae stored large amounts of dibasic alkaloids in their body, while monobasic alkaloids were metabolized and/or excreted. Newly emerged adult moths contained no traces of alkaloids.

Keywords *Buxus* · Lepidoptera · Chemical defence · Triterpenoid alkaloids · Plant–insect interactions · UHPLC–QTOFMS

Introduction

Many secondary plant metabolites are produced by plants as toxins that deter herbivores, but they may also have an important effect on the next trophic level, i.e. on predators and parasitoids of herbivores (Schaffner et al. 1994). Sequestration of these chemical compounds occurs in a wide array of herbivores (Rothschild 1972; Blum 1981; Bernays and Graham 1988; Rowell-Rahier and Pasteels 1992; Opitz and Müller 2009). Alkaloids are a particularly important class of plant toxins occurring in many different plant taxa (Hegnauer 1988) and are well suited for sequestration by insect herbivores for their own defence against predators due to their high level of deterrence (Rothschild et al. 1979; Blum et al. 1981; Gfeller et al. 1995). In this study, we investigated whether the invasive box tree moth *Cydalima perspectalis* takes up alkaloids occurring in its host plant.

Cydalima perspectalis (Walker 1859) (Lepidoptera: Crambidae) (formerly *Diaphania* or *Glyphodes perspectalis*, see Mally and Nuss 2010) is a new alien species in Europe, causing severe damage to box trees (*Buxus* spp.) in private and public gardens as well as in semi-natural box tree forests in the region of Basel (Switzerland), south-western Germany, France and the Netherlands since 2007 (Krüger 2008; Feldtrauer et al. 2009, Leuthardt et al. 2010; van der Straten and Muus 2010). Native to Korea, Japan and China (Inoue 1982), this moth has rapidly spread in Europe over the past 5 years. The larvae of *C. perspectalis* feed on leaves, but can also attack the bark of the trees,

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causing them to dry out and die. The light green larvae have black stripes with white dots and hairs, which might be a form of aposematic colouring, warning of certain toxicity. The larvae hatched from one single egg cluster spread over an area of 20–25 cm diameter on a tree until pupation. This is easily visible by the feeding damage, especially when only a few egg clusters have been deposited, as well as by the conspicuous behaviour of the larvae which do not hide under the leaves or inside the tree during daytime from the third larval stage on (Figure in Online Resource 1).

The genus *Buxus* belongs to the family Buxaceae, morphologically considered as one of the most primitive angiosperms, and comprises about 90 species occurring in most tropical regions and in the Mediterranean area (Balthazar et al. 2000). More than 15 box tree species or subspecies, including *Buxus microphylla* var. *japonica* and *B. microphylla* ssp. *sinica*, occur in the native range of *C. perspectalis* (Balthazar et al. 2000). Larvae of the invasive *C. perspectalis* are specialized on *Buxus*. In their native range in Japan, they have been reported to feed on *B. microphylla* in Japan (Maruyama 1993) and on *B. microphylla* ssp. *sinica* in China (Chen et al. 1993). Larvae of *C. perspectalis* were reared on *B. microphylla* var. *japonica* and *B. microphylla* var. *insularis*, as well as on *B. sempervirens* under laboratory conditions (Maruyama 1993).

Invasive herbivores may encounter known food plants in the introduced region, but often they are also successful in adjusting their diet to new plant species, related species or varieties of the original plant (Thompson and Pellmyr 1991). In Europe, there are several horticultural breeds of *Buxus* sp. that do not occur in Eastern Asia, particularly varieties of *B. sempervirens*, the native European box tree species which does not occur in high densities in the native range of *C. perspectalis*. Box tree moth-inflicted damage has been recorded on all box tree varieties examined in Europe (Leuthardt and Baur 2013), indicating a successful adjustment of the diet of the invader from *B. microphylla* to *B. sempervirens*.

Birds were not observed to feed on *C. perspectalis*, although encounters between this potential predator (especially *Parus* spp.) and larvae have been observed in the field (Leuthardt, unpublished data). On the rare occasions where larvae were picked up by birds, they were either killed and left aside or regurgitated. Field observations indicate that bird predators avoided not only the larvae but also the adults. A possible explanation could be that the moth accumulates toxic compounds from box tree leaves. Chemical compounds of the family Buxaceae have been the subject of numerous pharmacological studies in the past decades because of their biological activity as cholinesterase inhibitors (Choudhary et al. 2003), and antibacterial (Naeem et al. 1996; Mothana and Lindequist 2004), antiviral (Mothana et al. 2006) and antileishmanial activities (Devkota et al. 2008). Alkaloids constitute one of the main classes of defence secondary compounds found in Buxaceae. *Buxus* alkaloids are a unique class of triterpenoid alkaloids possessing a cycloartenol-type skeleton in which one or two nitrogen atoms are incorporated as side chains (Dildar 1990). The diversity of *Buxus* triterpenoid alkaloids is extremely rich with more than 300 different structures reported (Devkota et al. 2008). The concentration of the main alkaloids is virtually the same in all plant organs (1–2 % dry weight, DW), increasing with the age of the box trees (Khodzhaev and Shakirov 2000). Previous studies have focused essentially on wild-growing *Buxus* species. The composition of secondary compounds in horticultural breeds of both *B. sempervirens* and *B. microphylla* has, to our knowledge, never been investigated.

To determine whether the specialization of *C. perspectalis* on *Buxus* species is linked to the potential defence system of the larvae against vertebrate predators, we investigated the alkaloid content of *Buxus* leaves and *C. perspectalis* larvae using liquid chromatography–mass spectrometry (LC–MS). We addressed the following questions: (1) Do five common box tree varieties found in central Europe differ in their alkaloid composition? (2) Do larvae of *C. perspectalis* store *Buxus* alkaloids and are the variety-specific alkaloid compositions in box tree leaves mirrored in the tissue of larvae and moths fed on either of those box tree varieties?

Methods and materials

Plant and insect material

Five varieties of box trees belonging to two species were examined: *B. sempervirens* var. “Sempervirens” (further referred to as SE), *B. sempervirens* var. “Rotundifolia” (RO), *B. sempervirens* var. “Argenteovariegata” (AR), *B. sempervirens* var. “Aureovariegata” (AU) and *B. microphylla* var. “Faulkner” (FA). These varieties are the most frequently planted in public and private gardens in north-western Switzerland. One 10- to 15-year-old tree of each variety (1–1.8 m high) growing outside the distribution area of *C. perspectalis* until 2010 (Lyss, 60 km SW of Basel) and not treated with phyto-pharmaceutical substances (pesticides, herbicides, fungicides or other substances) was used. Replicate trees were not available because they were already treated with chemicals against *C. perspectalis*. The experimental trees were kept outdoors and protected with nets against oviposition by *C. perspectalis* from 2010 to 2011. From each variety, we collected four samples of 1-year-old leaves from different branches, each consisting of five to six first-year leaves representing 200–300 mg fresh mass in May 2011. To compare young and old leaves, we collected one leaf sample of 2- to 3-year-old leaves on the cultivated *B. sempervirens* var. "Sempervirens".

Newly hatched larvae of *C. perspectalis* were obtained from a culture maintained in an outdoor cage (1 m × 1 m × 2 m, wooden structure covered with netting) containing a wild *B. sempervirens* tree of 1.2 m height. The founders of the culture were captured with a light trap positioned near a box tree hedge in Riehen, 5 km NE of Basel, Switzerland (47°33′45″N, 7°38′27″E) between 23 June and 21 July 2010. Larvae were kept in groups of three to five individuals in 90 mm Petri dishes lined with moist filter paper to prevent the food from drying out. Each group was fed with only one of the five examined box tree varieties from the time of hatching. We used a sibling-split design, equally distributing larvae from the same egg cluster on all five varieties to minimize potential genetic influences. In total, ca. 100 larvae from five different females were obtained with this culture. Equal proportions of young and old leaves cut from the corresponding box tree variety were offered ad libitum as food. Food was replaced every day.

Extraction of alkaloids

We examined four larvae of the sixth larval stage kept on each of the five box tree varieties as well as eight larvae of the fourth larval stage kept on *B. sempervirens* var. "Sempervirens". Individuals were dissected and their gut was removed to exclude any influence of remaining plant material. We also examined the faeces of larvae growing on *B. sempervirens* var. "Sempervirens". Similarly, eight newly emerged adult moths were collected and analysed shortly after hatching. Each of the four replicates of plant and larvae samples as well as the unique sample of faeces and moth were lyophilized and ground into a fine powder using a mortar and pestle. 25 mg of dry powder was transferred to a microcentrifuge tube and extracted in 1 mL of a mixture of methanol:water:formic acid (70:29.5:0.5, v/v) using Eppendorf Thermomixer 5436 (Eppendorf, Hamburg, Germany). After centrifugation at 16,000 rpm for 3 min (Eppendorf 5415 R), the supernatant was collected and diluted ten times with the same extraction solvent.

Mass spectrometry analyses

Chemical analyses were carried out by ultra-high pressure liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC–QTOFMS) in the electrospray positive ionization mode using an Acquity UPLC system (Waters, Milford, USA) and a Synapt G2 QTOF mass spectrometer (Waters). The separation was performed on an Acquity BEH C18 column (50 × 2.1 mm i.d., 1.7 μm particle size; Waters) at 400 μL/min under the following conditions: solvent A, 0.05 % formic acid in water; solvent B, 0.05 % formic acid in acetonitrile; 2–40 % B in 5 min, 40–100 % B in 2 min, 100 % B for 1 min, re-equilibration at 2 % B for 1 min. The temperature of the column was maintained at 25 °C and that of the autosampler at 15 °C. The injection volume was 2.5 μL. QTOFMS parameters were: electrospray capillary voltage +2.8 kV, cone voltage +25 V, desolvation gas temperature 350 °C, desolvation gas flow 800 L/h, mass range 85–1,200 Da, scan time 0.2 s. The analyses were carried out in the MS^E mode using alternating scans at low (4 eV) and high (10–30 eV ramp) collision energies. Data were recorded using Masslynx 4.1 (Waters).

Except for cyclovirobuxine D, whose identification was ascertained by comparison with an authentic standard (Chengdu Biopurify Phytochemicals LTD, Chengdu, China) run under identical conditions, we tentatively identified the alkaloids based on high-resolution mass spectra of pseudo-molecular and fragment ions obtained by QTOFMS in the absence of available standards. For the determination of elemental compositions, the following elements were included: C (max. 500), H (max. 1,000), O (max. 200), N (max. 5), S (max. 6), P (max. 1) and Na (max. 1). Mass accuracy tolerance was set to 3 ppm, ring plus double bond equivalents limits were −1.5 to 50 and the nitrogen rule was applied. Accurate measurements of masses and isotopic ratios provided unique elemental compositions for all ions investigated with an excellent degree of confidence. Compound databases including the *Dictionary of Natural Products* (Chapman & Hall, CRC Informa, London; version 20.2) were investigated to match the found elemental compositions with potential alkaloids. MS/MS spectra obtained by collision-induced dissociation (CID) were interpreted for additional structural information.

We measured the concentration of cyclovirobuxine D in *Buxus* leaves and *C. perspectalis* larvae relative to the initial dry mass weighed for the respective tissues before extraction (i.e. 25 mg of dried tissue for both leaves and caterpillars) using external calibration. The concentrations of the calibration points were 0.02, 0.2, 1.0, 2.0, and 5.0 μg/mL. For other alkaloids, relative concentrations were measured.

Peak picking of LC–MS data was performed by Markerlynx XS (Waters) using the following parameters: initial and final retention times 0.5–7.2 min, low and high masses 85 and 1,200 Da, mass window 0.03 Da, retention time window 0.1 min, intensity threshold 1,000 counts, automatic peak width measurement, automatic peak-to-peak baseline noise, noise elimination disabled, deisotoping function enabled. This yielded a list of ‘features’ (i.e. variables characterized by mass and retention time). The obtained data were then normalized to the total ion intensity, Pareto scaled, and subjected to multivariate analysis using EZinfo (Umetrics, Umea, Sweden).

Results

Determination of alkaloids in plant tissues

UHPLC–QTOFMS analyses revealed that numerous alkaloids were present in the four varieties of *B. sempervirens* (Fig. 1a–d) as well as in *B. microphylla* (Fig. 1e). A list of the main alkaloids tentatively identified in both species is presented in Table 1. Compound **1**, which was present only in *B. sempervirens* but not in *B. microphylla*, was identified as cyclovirobuxine D based on an $(M + H)^+$ ion at *m/z* 403.3687 ($C_{26}H_{47}N_2O$) and a doubly charged ion at *m/z* 202.1886 typical of alkaloids containing two basic moieties (Fig. 2a). In the MS/MS spectrum, the loss of $-NH_2CH_3$ gave an intense fragment at *m/z* 372.3269 ($C_{25}H_{42}NO$). Another typical fragment at *m/z* 330.2795 ($C_{22}H_{36}NO$) resulted from the concomitant losses of $-CHCH_3NHCH_3$ and $-CH_3$ from the singly charged pseudo-molecular ion. The structure of cyclovirobuxine D (Fig. 3) was confirmed by injecting a pure standard under identical conditions. Fig. 2b, c shows the almost identical MS/MS spectra of compound **1** and the cyclovirobuxine D authentic standard. Following a similar scheme, compound **2** was putatively identified as either buxaminol E or cyclobuxine B, based on an $(M + H)^+$ ion at *m/z* 401.3524 ($C_{26}H_{45}N_2O$) and a doubly charged ion at *m/z* 201.1801 (Fig. 4a). The characteristic fragment at *m/z* 384.3257 ($C_{26}H_{42}NO$) in the MS/MS spectrum resulting from a loss of $-NH_3$ (primary amine) suggested the presence of buxaminol E, which contains both primary and tertiary amines (Figs. 3, 4b), rather than cyclobuxine B, which contains secondary and tertiary amines. Compound **10** was annotated as cyclobuxophylline O (Fig. 3) based on a pseudo-molecular ion at *m/z* 356.2951 corresponding to the molecular formula $C_{24}H_{38}NO$ (Fig. 4c), and a characteristic fragment ion at *m/z* 339.2685 ($C_{24}H_{35}O$) in the MS/MS spectrum resulting from the loss of $-NH_3$ (Fig. 4d). Compound **11** (*m/z* 370.3114, $C_{25}H_{40}NO$) was putatively identified as cyclobuxophylline M, an *N*-methyl derivative of **10** (Fig. 3): the fragment at *m/z* 339.2685 resulting from a loss of $-NH_2CH_3$ also occurred in the corresponding MS/MS spectrum.

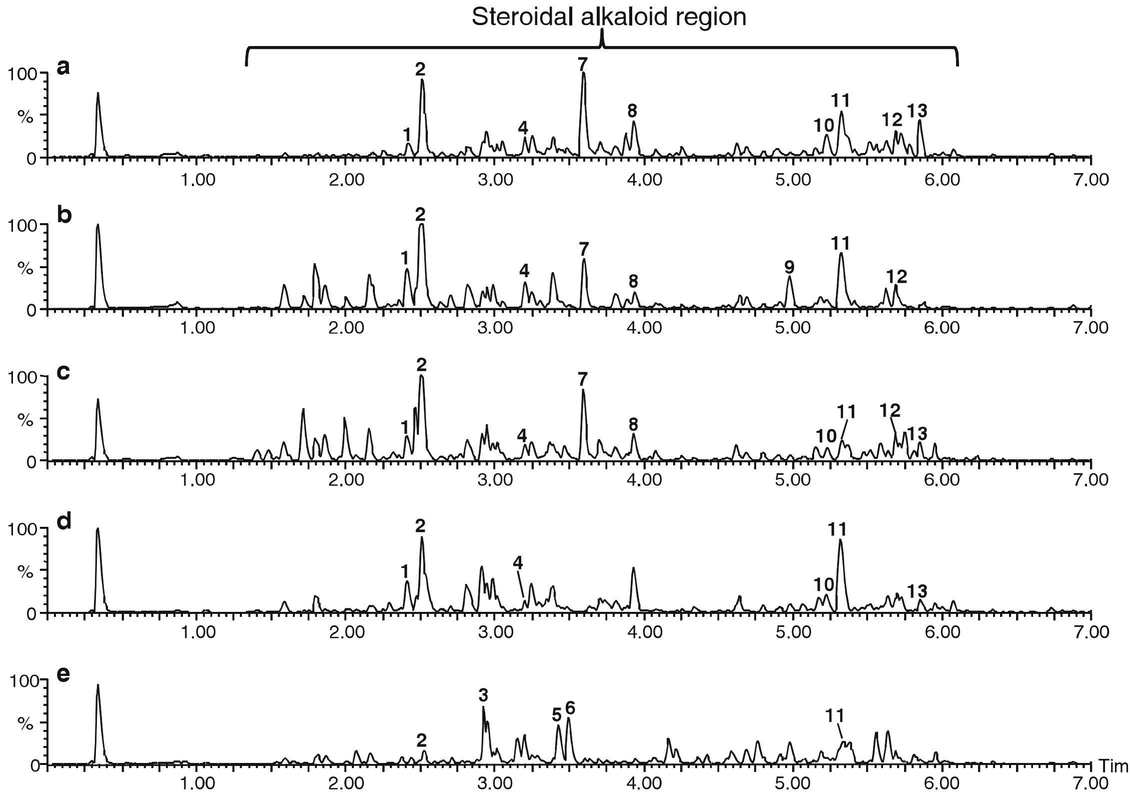

Fig. 1 Chromatograms of leaves of the five most common box tree varieties in the region of Basel, Switzerland. **a** *Buxus sempervirens* var. “Sempervirens”; **b** *B. sempervirens* var. “Rotundifolia”; **c** *B. sempervirens* var. “Argenteovariegata”; **d** *B. sempervirens* var. “Aureovariegata”; **e** *B. microphylla* var. “Faulkner”

Uptake of alkaloids by *C. perspectalis*

To determine whether *C. perspectalis* larvae feeding on box tree store alkaloids, we analysed their tissue and excrements using the same profiling approach as used for the plant material. We compared the alkaloid profile of young leaves of all five examined varieties with those of sixth instar larvae ($n = 4$) fed on either of these varieties. To receive more detailed information on the variability among samples than we could derive from visual inspection of individual chromatograms, chemometric tools were employed. A list of more than 3,000 'features' was obtained by peak picking and condensed using principal component analysis (PCA). The PCA separated the profiles of *Buxus* spp. leaves from those of larvae fed on the corresponding leaves along the first PC axis (variance explained 37 %, Fig. 5). Furthermore, the alkaloid profiles of larvae fed on either *B. sempervirens* or *B. microphylla* were separated along the second PC axis (variance explained 17 %). Interestingly, even the subtle differences in alkaloid profiles between the four *B. sempervirens* varieties were reflected in larval profiles (inset in Fig. 5). In the loadings plot, the four most contributive features to the discrimination between *B. microphylla* and *B. sempervirens* leaves were compounds **2** and **7** (typical for *B. sempervirens*) and **3** and **6** (typical for *B. microphylla*) (see loadings in Online Resource 2). However, this difference was only partly maintained in larval profiles. Indeed, although compounds **2** and **7** were still among the main contributive features to larvae feeding on *B. sempervirens*, three less dominant alkaloids from *B. microphylla* leaves became the most characteristic features of larvae feeding on *B. microphylla* (see loadings in Online Resource 2). One was tentatively identified as dihydrocyclomicrophylline F ($C_{26}H_{46}N_2O_2$) based on its MS/MS spectrum, but the two others ($C_{28}H_{48}N_2O_2$ and $C_{28}H_{48}N_2O$) could not be fully identified because of several possible isomeric derivatives reported for these molecular formulae in the literature. Yet, larval profiles could also be separated only based on the 13 predominant alkaloids identified in *Buxus* leaves (Online Resource 3). Corroborating these observations, cyclovirobuxine D accumulated only in caterpillars that fed on *B. sempervirens* leaves, while it was absent in those feeding on *B. microphylla*.

More generally, larvae stored only dibasic alkaloids (i.e. containing two basic amine groups), while monobasic alkaloids (i.e. containing only one amine group) were metabolized and/or eliminated in their excrements (Fig. 6). In particular, an intense peak of *m/z* 388.3209 eluting at 3.98 min was discovered in the excrements of larvae feeding on *B. sempervirens* (Fig. 6c). This compound could possibly be formed from the predominant compound **11**, tentatively identified as cyclobuxophylline M, by ketone reduction and hydroxylation.

A more detailed analysis of the concentration of dibasic cyclovirobuxine D revealed marked differences between young (340 µg/g DW) and old (2,090 µg/g DW) *B. sempervirens* leaves as well as between larvae of different instars. The cyclovirobuxine D concentrations in larvae of the fourth and sixth instar reared on a mixture of old and young leaves were 540 and 250 µg/g DW, respectively, indicating a strong accumulation of this alkaloid in body tissues. Even more spectacular was the heavy

Table 1 List of predominant alkaloids tentatively identified in *Buxus* spp.

#	RT (min)	$(M + H)^+$	Formula	Species	Tentative identification
1	2.40	403.3690	$C_{26}H_{46}N_2O$	*semp.*	Cyclovirobuxine D[a]
2	2.51	401.3524	$C_{26}H_{44}N_2O$	*semp.*	Buxaminol E
3	2.92	599.4050	$C_{35}H_{54}N_2O_6$	*micro.*	Buxmicrophylline H
4	3.20	372.2903	$C_{24}H_{37}NO_2$	*semp.*	Buxtauine M
5	3.43	549.4046	$C_{35}H_{52}N_2O_3$	*micro.*	Cyclomicrophyllidine A
6	3.50	523.3901	$C_{33}H_{50}N_2O_3$	*micro.*	Buxepidine
7	3.63	497.4102	$C_{32}H_{52}N_2O_2$	*semp.*	O-tigloylcyclovirobuxeine B
8	3.88	519.3944	$C_{34}H_{50}N_2O_2$	*semp.*	O30-benzoyl-16-deoxybuxidienine C
9	5.00	354.2802	$C_{24}H_{35}NO$	*semp.*	Cyclosuffrobuxinine M
10	5.23	356.2951	$C_{24}H_{37}NO$	*semp.*	Cyclobuxophylline O
11	5.32	370.3114	$C_{25}H_{39}NO$	*semp.*	Cyclobuxophylline M
12	5.68	547.3899	$C_{35}H_{50}N_2O_3$	*semp.*	Buxadienine/*N*-Benzoyl-*O*-acetylbuxodienine E
13	5.85	547.3893	$C_{35}H_{50}N_2O_3$	*semp.*	Buxadienine/*N*-Benzoyl-*O*-acetylbuxodienine E

RT Retention time

semp. sempervirens, *micro. microphylla*

[a] confirmed by an authentic standard

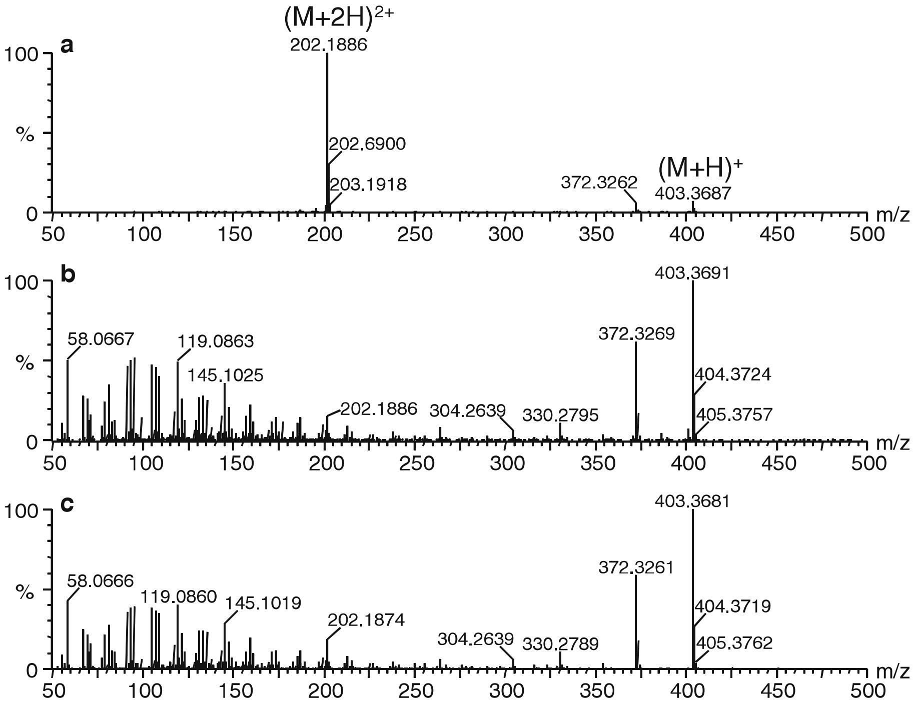

Fig. 2 High-resolution mass spectra obtained for cyclovirobuxine D in *B. sempervirens* extract and comparison with an authentic standard. **a** Mass spectrum obtained at low collision energy from *B. sempervirens* extract, **b** mass spectrum obtained at high collision energy from the same extract, **c** mass spectrum obtained at high collision energy for the authentic standard of cyclovirobuxine D

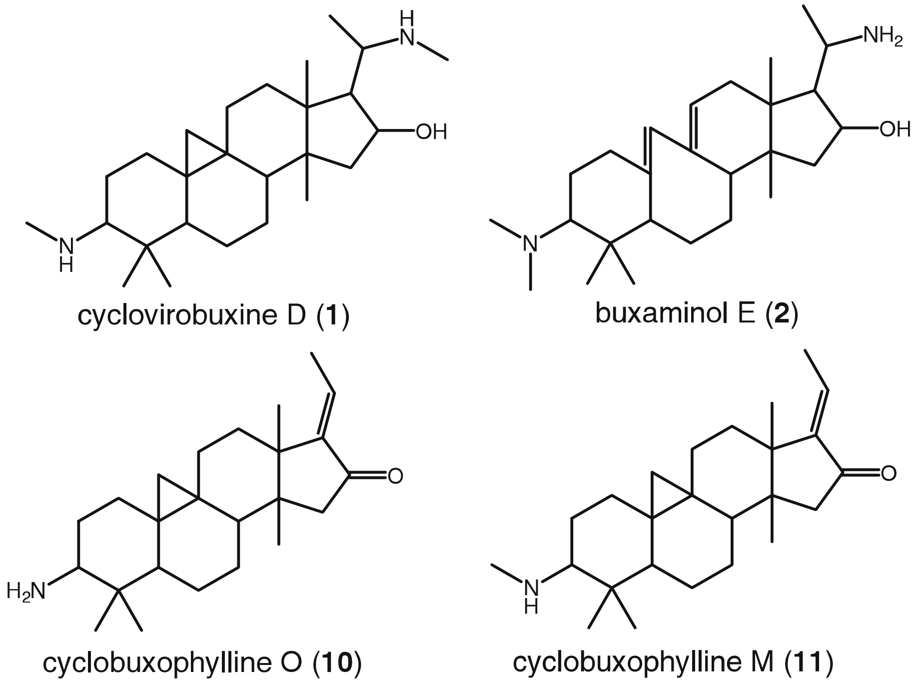

Fig. 3 Structures of triterpenoid alkaloids cyclovirobuxine D, buxaminol E, cyclobuxophylline O and cyclobuxophylline M. Numbers in *brackets indicate* the respective numbering in Table 1

sequestration of putative dihydrocyclomicrophylline F and the two other unidentified alkaloids ($C_{28}H_{48}N_2O_2$ and $C_{28}H_{48}N_2O$) whose relative amounts were 1.5 to 20 times higher in larval bodies than in *B. microphylla* (Fig. 7). In contrast, the relative amount of monobasic putative cyclobuxophylline M in larvae represented less than 0.5 % of that found in *B. sempervirens* leaves. Finally, the adult moths examined did not contain any traces of alkaloids, indicating that alkaloids do not persist after metamorphosis.

Discussion

This study is, to our knowledge, the first to investigate the uptake of *Buxus* alkaloids in the highly specialized larvae

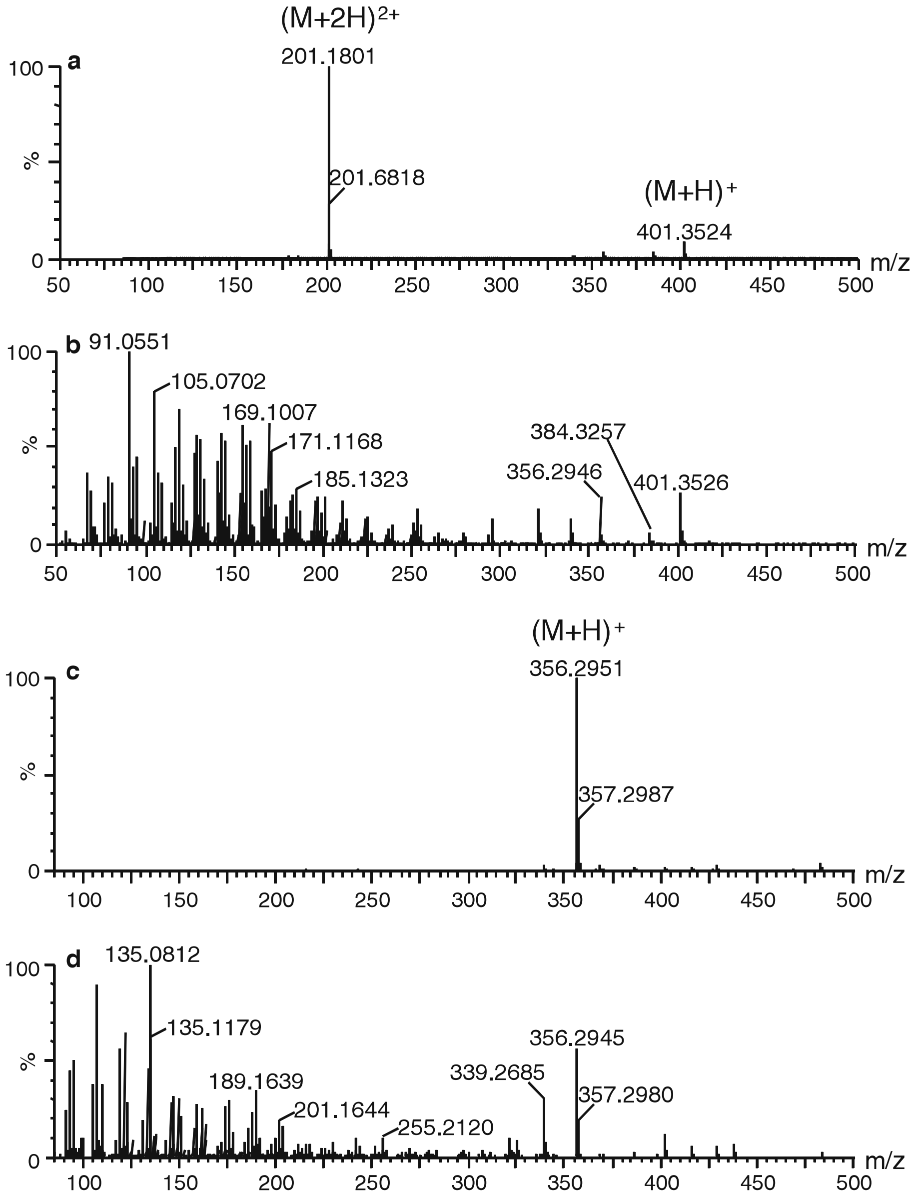

Fig. 4 Representative mass spectra of tentatively identified buxaminol E (compound **2**) and cyclobuxophylline O (compound **10**). **a** Mass spectrum of **2** obtained at low collision energy. **b** Mass spectrum of **2** obtained at high collision energy. **c** Mass spectrum of **10** obtained at low collision energy. **d** Mass spectrum of **10** obtained at high collision energy

of the moth *C. perspectalis*. The fact that alkaloid profiles of a box tree variety were mirrored in the tissue of larvae feeding on its leaves suggests that larvae of *C. perspectalis* store alkaloids. A detailed analysis of the absolute or relative amounts of specific alkaloids in *Buxus* leaves and larvae confirmed this hypothesis. This may explain why larvae of *C. perspectalis* are avoided or regurgitated by birds that commonly feed on Lepidoptera (F. Leuthardt, unpublished data). Specialized herbivores such as the sawfly *Rhadinoceraea nodicornis* are able to metabolize toxic polycyclic alkaloids and store them for their own predator defence (Schaffner et al. 1994). So far, the accumulation of triterpenoid alkaloids by Lepidoptera has not been investigated, in contrast to that of other alkaloid types such as pyrrolizidine alkaloids (PA), quinolizidine alkaloids and tropane alkaloids (Nishida 2002; Opitz and Müller 2009).

Insects sequestering plant secondary compounds often specialize on a specific group within a class of compounds (Rowell-Rahier and Pasteels 1992). The uptake of triterpenoid alkaloids by the box tree moth seems to follow this pattern. We found that the levels of certain alkaloids in larvae feeding on *B. microphylla* were up to 20 times higher than those present in the corresponding leaves. A number of possible explanations can be suggested for the selective sequestration of given alkaloids. First, there may be a trade-off between storing the compounds most effective for defence and the potential of these toxins to harm the insect itself. Indeed, feeding on suboptimal food sources such as toxic plants may reduce individual growth rate,

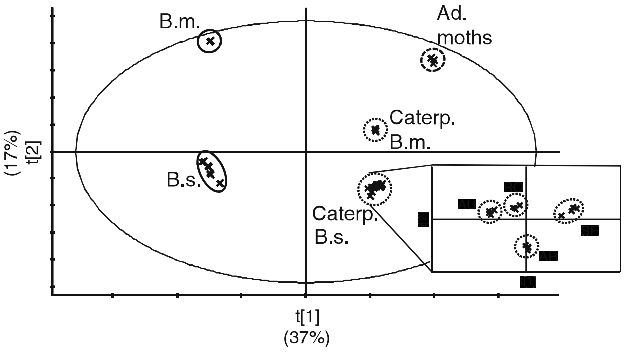

Fig. 5 Principal component analysis (PCA) of leaves of the five box tree varieties (B.s. respectively B.m.) and sixth instar caterpillars fed on the corresponding varieties (Caterp. B.s. respectively Caterp. B.m.), as well as adult moths from larvae fed on *Buxus sempervirens* var. "Sempervirens" ($n = 4$ replicates per variety). t[1] and t[2] are first and second principal components, with their percentage of variance explained. Inset: PCA based on larvae fed on the four different varieties of *B. sempervirens* (AR, *B. s.* var. "Argenteovariegata"; AU, *B. s.* var. "Aureovariegata"; RO, *B. s.* var. "Rotundifolia"; SE: *B. s.* var. "Sempervirens")

but this might be outweighed by a lower mortality due to avoidance by predators, provided that the food source presents either a protected feeding site or contains secondary compounds which can be sequestered (Damman 1987). However, comparative data on the toxicity of the box tree alkaloids for the sequestering insect and for its predators are not yet available. Second, there might be chemical and physical properties such as lipophilicity that make some compounds easier to diffuse through the gut membrane (Rowell-Rahier and Pasteels 1992). Indeed, compounds that are highly polar or highly hydrophobic cannot easily cross biological membranes passively and thus are expected to be more difficult to absorb unless they can be actively transported by specific carriers (Duffey 1980). Additionally, the pH in the insect gut may play an important role in their absorption. The pH of the midgut of Lepidoptera is usually very alkaline, exceeding 12 in certain species (Dow 1984). At such high pH, triterpenoid alkaloids are certainly deprotonated, and hence particularly hydrophobic. Dibasic *Buxus* alkaloids are typically more polar than monobasic alkaloids (as confirmed by their elution order in reverse-phase HPLC). It is therefore conceivable that, under the pH conditions in the midgut of *C. perspectalis*, dibasic alkaloids, unlike monobasic alkaloids, are within the acceptable range of polarity for passive diffusion through the cell membranes. Alternatively, it is possible that specific carrier transport favours the uptake of polar dibasic alkaloids over monobasic alkaloids.

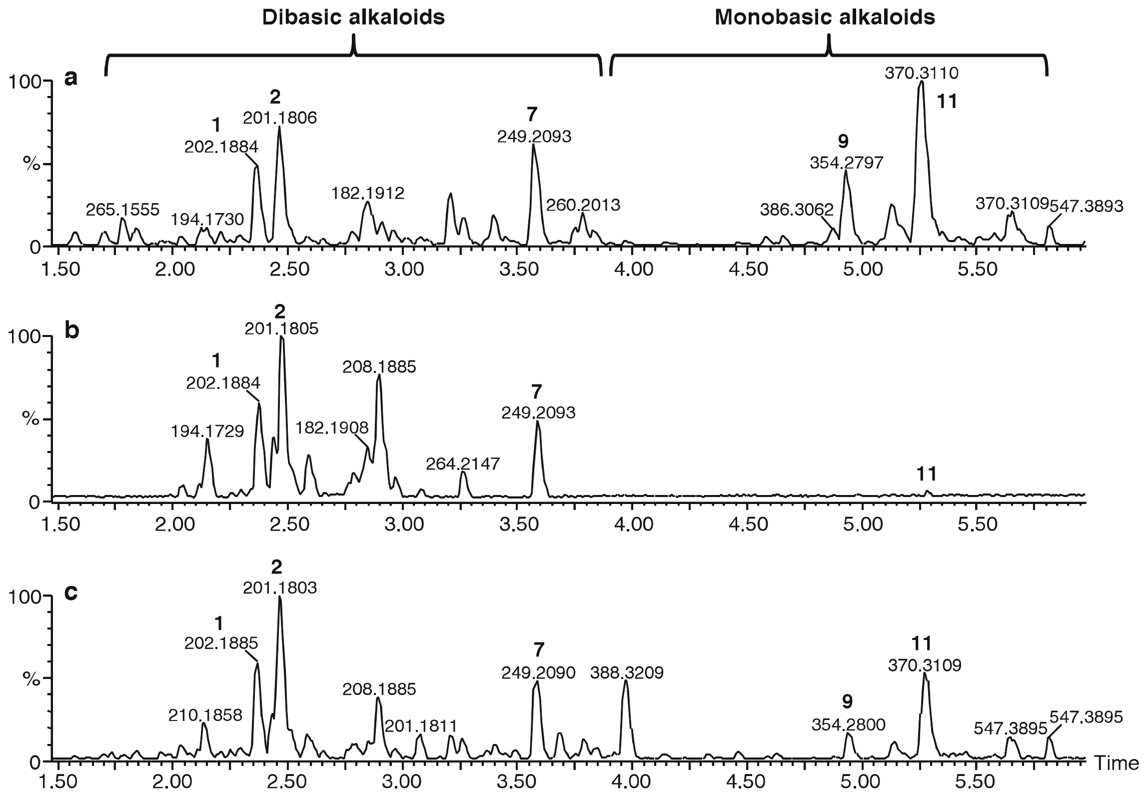

Fig. 6 Chromatograms of **a** 1-year-old leaves of *Buxus sempervirens* var. "Sempervirens", **b** larval tissue and **c** excrements of larvae. Predominant peaks are labeled according to their numbers in Table 1 and/or *m/z* base peak, either singly charged (e.g. compounds **9** and **11**) or doubly charged (e.g. compounds **1**, **2** and **7**)

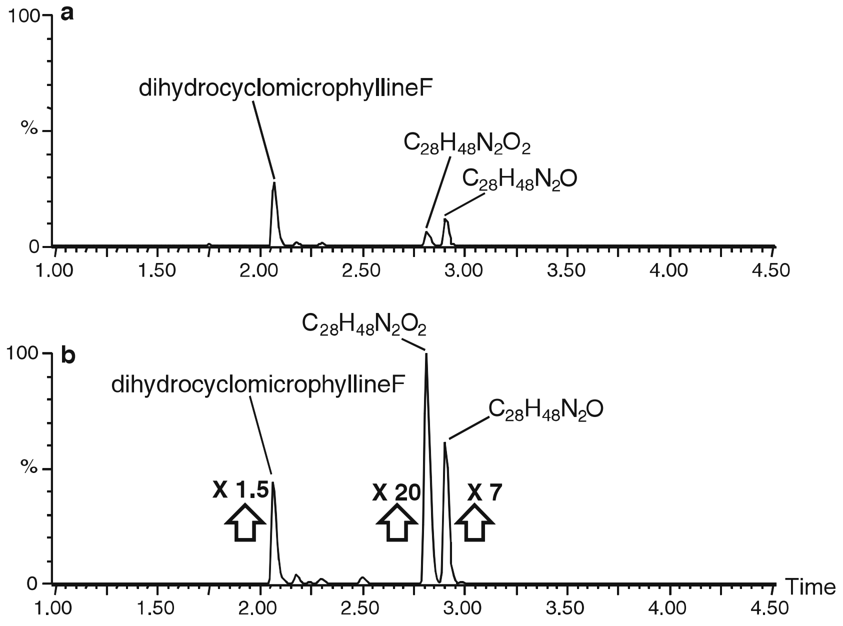

Fig. 7 Extracted ion chromatograms (EIC) showing a strong relative increase in the levels of three specific alkaloids in larvae compared to *B. microphylla* leaves. **a** EIC obtained from *B. microphylla* leaf extract, **b** EIC obtained from *C. perspectalis* extract

We observed a strong difference in cyclovirobuxine D concentration between young and old larvae and leaves: younger instars contained a twofold concentration of cyclovirobuxine D related to last-instar larvae, whereas the concentration of this alkaloid in box tree leaves was sixfold higher in 2- to 3-year-old leaves compared to 1-year-old leaves. This suggests a preference of young larvae for leaves containing a high concentration of alkaloids, i.e. older leaves. Indeed, under natural conditions, young larvae tend to feed on old leaves at the bottom of box tree plants (F. Leuthardt, unpublished data). This behaviour permits a fast uptake of large amounts of alkaloids within a short time to increase protection against predators, before maximizing the larval growth rate. However, this observation remains to be confirmed experimentally.

Newly emerged individuals prevented from any contact with box trees did not contain any traces of alkaloids. Nevertheless, field observations indicated that bird predators avoided not only the larvae, but also the adults. This suggests that adults become deterrent at a later time, which could be explained by a certain level of pharmacophagy. It is well known that several lepidopteran species sequester PA as larvae or obtain them pharmacophagously as adults (Nishida, 2002), but there are no data on this phenomenon concerning triterpenoid alkaloids. This hypothesis remains to be investigated.

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CHAPTER 5

Kostenermittlung des Buchsbestandes und der Pflege der Buchsbäume hinsichtlich des Buchsbaumzünslers im Friedhof am Hörnli, Kanton Basel-Stadt

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Auftraggeber:
Bau- und Verkehrsdepartement Basel – Stadtgärtnerei
Oktober 2011

Kostenermittlung des Buchsbestandes und der Pflege der Buchsbäume hinsichtlich des Buchsbaumzünslers im Friedhof am Hörnli, Kanton Basel-Stadt


Buchshecken im Friedhof am Hörnli (Photo K. Levy)

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Oktober 2011

Einleitung

Buchsbäume zeichnen sich durch eine grosse kulturhistorische Bedeutung aus und bilden ein wesentliches Gestaltungselement in grossen Gartenanlagen, so auch im Raum Basel. Dies trifft besonders auf folgende Anlagen zu: Friedhof am Hörnli, Kannenfeldpark, St. Alban-Anlage, Merianpark. Der seit 2007 in der Region Basel beobachtete invasive Buchsbaumzünsler (*Cydalima perspectalis*, Walker 1859, Lepidoptera: Pyralidae) verursachte bisher grosse Schäden in Park- und Gartenanlagen sowie in wildwachsenden Buchsbeständen. Die Raupen dieses Kleinschmetterlings fressen in kurzer Zeit Buchsbäume (*Buxus sp.*) kahl (Leuthardt et al., 2010). Nicht nur die grünen Blätter, sondern auch die Rinde der Pflanzen werden abgefressen, was rasch zum Absterben der Pflanze führt. Das hohe Schadenspotenzial des Buchsbaumzünslers bereitet den für Grünanlagen verantwortlichen Personen grosse Sorge.

Bäume gelten trotz ihres Wachstums und Absterbens als Sachen im Rechtssinne. Sie gehören nach dem sachenrechtlichen Akzessionsprinzip gemäss Art. 667 Abs. 2 ZGB dem Eigentümer des Grundstücks, auf dem sie wachsen. Ihre Beschädigung oder Zerstörung beeinflusst daher den Wert des Grundstücks, dessen Bestandteil sie bilden.

Auch der Friedhof am Hörnli als eine der grössten Parkanlagen der Stadt Basel ist von einer solchen Wertminderung durch den Befall vom Buchsbaumzünsler betroffen. Mit seinen geschätzten 3.3 km Buchshecken, wovon die meisten als strukturell wichtige Unterteilungselemente fungieren, kann er als Paradebeispiel für die Berechnung des vom Buchsbaumzünsler verursachten Schadens angesehen werden. Daraus soll ein Instrument zur Entscheidungsfindung für ein weiteres Vorgehen entwickelt werden. Die Wahl zwischen dem Ersatz ausgewachsener Pflanzen oder der langjähriger Pflege ausgewählter Buchsbäume in Basler Park- und Gartenanlagen soll so vereinfacht werden.

Nachfolgend wird in einem ersten Schritt die Parkanlage des Friedhofs am Hörnli mit seiner Entwicklungs- und Gestaltungsgeschichte vorgestellt. Danach wird das Sachwertverfahren, eine Methode zur Wertermittlung von Gehölzen, erklärt. Zum Schluss werden in mehreren Schritten verschiedene Pflege-Szenarios im Friedhof am Hörnli anhand einer vom Sachwertverfahren abgeleiteten Methode berechnet und verglichen. Dazu zählt auch die Berechnung des gesamten Buchsbaumwertes an diesem Standort.

Der Friedhof am Hörnli: Geschichte

Nachdem die Untersuchungen des Sanitätsdepartements ergaben, dass sich bei den bestehenden Gottesäckern Kannenfeld, Kleinhüningen, Horburg und Wolfgottesacker keine oder nur beschränkte Erweiterungsmöglichkeiten boten, wurden ab 1905 erste Überlegungen zur Anlage eines Zentralfriedhofes angestrengt (Voss, 1997). Die Regierung Basel-Stadt entschied sich 1919 für die Anlegung eines Zentralfriedhofes am Hörnli.

Das 1925 genehmigte Bauprojekt der Architektengemeinschaft zeigt eine hierarchisch aufgebaute und weitgehend symmetrisch angeordnete Anlage. Strukturiert wird die Anlage durch die Stützmauern und Böschungen, die das nach Norden und Osten stark ansteigende Gelände terrassieren. Breite kompakte Baumpflanzungen umschliessen Gebäude und Abteilungen. Im Innern sind die Abteilungen durch Heckenpflanzungen gerahmt und so unterteilt, dass sich die Gräberflächen schalenförmig um das jeweilige Zentrum einer Abteilung legen: nach innen die Reihengrabfelder und nach aussen die Familiengräber. Ein enges orthogonales Wegenetz teilt die Flächen in eine Vielzahl kleinerer Grabfelder auf. Das Areal für die Gärtnerei liegt im Süden der Friedhofanlage.

Das Ziel der Architekten war, einen auf Sachlichkeit aufgebauten Friedhof zu erstellen, der übersichtlich ist und eine schnelle Orientierung ermöglicht. Das bestimmende Prinzip war das des „Friedhofs im Walde"; der den Ausblick auf die Stadt offen lässt. Die Bepflanzung sollte einheitlich sein und sich nicht in Details verlieren; sie sollte einen ruhigen grünen Rahmen für die Grabsteine und die blütenreichen Grabfelder bilden.

Nach sechsjähriger Bauzeit wurde der Friedhof am Hörnli am 1. Juni 1932 offiziell eröffnet und ersetzte die Friedhöfe im Kannenfeld, in Kleinhüningen und Horburg. Während der Bauarbeiten wurden 307'000 m^3 Erde bewegt, 67'000 m^2 Wegflächen und 79'000 m^2 Waldflächen angelegt. Dabei wurden 43'000 Hainbuchen, 600 Linden, über 16'000 Eiben, 9'000 Rottannen und 12'000 Buchsbäume angepflanzt. Das Gelände umfasst insgesamt 482'000 m^2. Im Laufe der Zeit wurden weitere Abteilungen dem Bedarf gemäss ausgebaut. Mit dem Ausbau der Abteilung 12 „Im Finstern Boden" als Schlussetappe war die Gesamtfriedhofanlage 1963 fertiggestellt. Ein Grossteil der zurzeit als visuelle Unterteilung der Grabfelder und Friedhofareale fungierenden Buchshecken (Länge: 3'290 m), sowie 610 der 650 aktuell auf dem Friedhof vorzufindenden Solitärbäume wurden in den Jahren um 1960 oder früher angepflanzt (P. Goepfert, pers. Mitt., dazu eigene Beobachtungen der Autorin). Im Jahr 2010 umfasst der Friedhof am Hörnli insgesamt 540'000 m^2 Gesamtfläche, wovon 110'000 m^2 Waldfläche sind.

Das Sachwertverfahren

Das Sachwertverfahren bezeichnet eine Wertermittlungsmethode, welche auf der Kostenrechnung aufbaut und darauf abzielt, die Kosten der Herstellung bzw. der Wiederbeschaffung des zu bewertenden Objektes zu eruieren. Das Verfahren eignet sich hauptsächlich zur Festsetzung von Verkehrswertentschädigungen oder Schadenersatzleistungen und kommt dann zur Anwendung, wenn weder ein Vergleichswertverfahren angewendet werden kann noch ein Ertrag in betriebs- oder volkswirtschaftlichem Sinne erwirtschaftet wird. Dies gilt insbesondere bei öffentlichen Grundstücken (Koch, 1974).

Bei einem Schadensereignis wird der Herstellungswert eines Gehölzes nach den Kosten einer Pflanzung berechnet, durch die derjenige Zustand wiederhergestellt wird, welcher ohne das schädigende Ereignis voraussichtlich bestanden hätte. Dieser Anforderung wird laut BGE 127 III 73 eine Lösung gerecht, welche die Kosten eines Baumes berechnet, der dem Ausgewachsenen möglichst entspricht, im Handel noch erhältlich und von seinem Alter her nicht ungeeignet ist, an den vorgesehenen Ort verpflanzt zu werden. Dabei wird grundsätzlich von gewöhnlichen Herstellungskosten inkl. Baunebenkosten ausgegangen. Anders als bei baulichen Anlagen, wo man annehmen kann, dass diese in einem Zuge fertiggestellt werden, weist das Sachwertverfahren bei Gehölzen diesbezüglich eine Besonderheit auf: die sofortige Herstellung der ursprünglichen Grösse und Form ist unter Umständen mit erheblich höheren Kosten verbunden. Dies ist jedoch nur in besonderen Situationen vertretbar, wie zum Beispiel das Bewahren eines bestimmten Stadt- beziehungsweise Grundstücksbildes. Beim Schadenersatz von Sachwerten wird von der Naturalrestitution, also der Wiederherstellung des vor dem Schadenereignis vorhandenen Zustandes ausgegangen. Als Alternative bietet sich an, je nach Bedeutung des Standortes, den Anforderungen an die Funktion des Gehölzes und dem jeweils wirtschaftlich vertretbaren Aufwand, kleinere Pflanzgrössen zu verwenden, welche zwar nicht teuer sind, aber eine zum Teil lange Herstellungszeit an Ort mit entsprechendem Risiko mit sich bringen. Es werden in der Folge längere Wuchszeiten mit entsprechendem Risiko in Kauf genommen und die Wiederherstellung wird erst mit Erreichen der objektiv richtigen Grösse erreicht. Um einen Herstellungswert zu erhalten, welcher der entsprechenden Lage angemessen ist, wird von der Pflanzgrösse ausgegangen, die am jeweiligen Standort üblicherweise verwendet wird und somit je nach Funktion des Gehölzes variieren kann. Trotzdem hat sich die Schadenersatzermittlung hinsichtlich Warengruppe und Erziehungsform der Baumschulware zwingend an die Situation des geschädigten Baumes zu halten (VSSG, 2010). Dabei können folgende Aufgaben von Gehölzen unterschieden werden: Hygienische Funktion (Lärmschutz, Schattenwirkung, Staubfilterung oder Luftverbesserung), ingenieurbiologische Funktion (Festigung von erosionsgefährdeten Flächen, Schutzpflanzungen gegen Wind, Schnee oder Steinschlag, oder Strassenleitpflanzungen als Blendschutz, zur optischen Führung oder als Mittel gegen ermüdende Eintönigkeit) und schlussendlich die ästhetisch-architektonische als Element zur Schaffung einer erholsamen, freundlichen Atmosphäre, als Mittel zur Schaffung von Park- und Grünanlagen oder zur Gliederung von freier Landschaft, als Eingrünung von Spielplätzen, Sportplätzen, Friedhöfen, sowie bei Wiederbegrünungen.

Berechnung des Buchsbaumwertes nach Sachwertverfahren im Friedhof am Hörnli

Bei einer Anlage, welche wie der Friedhof am Hörnli über 80 Jahre alt ist, ist es mit einem vernünftigen Aufwand nicht möglich, die Anschaffungs- und Folgekosten des Buchsbaumbestandes seit 1932 zu beziffern, da zumal das hierzu notwendige Zahlenmaterial weitestgehend fehlt und die erwähnten Kosten die aktuelle Preisstruktur für solche Gehölze nicht widerspiegelt. Daher ist es grundsätzlich irrelevant, wie die damalige Herstellung tatsächlich verlaufen ist – ob mit stärkeren oder schwächeren Pflanzstärken. Entscheidend in einem solchen Fall ist jeweils die gegenwärtige und zukünftige Funktion des Gehölzes (Koch, 1974). Somit werden nachfolgend, analog zum BGE 127 III 73, die Kosten für Ankauf und Neupflanzung des gesamten Buchses im Friedhof am Hörnli zur Verkehrswertschätzung miteinbezogen.

Da es sich beim Friedhof am Hörnli um eine Anlage handelt, in der das Bewahren des Grundstückbildes eine grosse Rolle spielt, und zudem von einem Schadenersatzfall auszugehen ist, wird in diesem Fall von Naturalrestitution, also der Pflanzungen eines möglichst gleich grossen Ersatzes ausgegangen (Koch, 1974). Somit fällt die Berechnung der Herstellungskosten bis zur vollen Funktion der Pflanze weg. Ebenso kann die Wertminderung durch das Altern der Pflanze vernachlässigt werden: Ausgehend von der mittleren Lebensdauer eines Buchsbaumes von ca. 500 Jahren, einem Alter der vollständigen Gehölzherstellung von ca. 20 Jahren und einer Standdauer von ca. 80 Jahren aus, ergibt sich nach der Parabelformel (anzuwenden auf besonders langlebige Pflanzen) eine Alterswertminderung von nur 0.04 % (Standdauer^2/(Lebenserwartung-Alter der Pflanze)^2) für eine Buchspflanze, welche im Jahr 1931 angepflanzt wurde.

Die Kosten, welche bei einer Pflanzung anfallen, werden grundsätzlich wie folgt berechnet (Koch, 1974, sowie Stadtgärtnerei Basel):

- Kosten der Pflanze, nach geltendem Katalog einer repräsentativen Baumschule (in diesem Falle Hauenstein Rafz Baumschulen);
- Etwaige Frachtkosten;
- Kosten der Pflanzarbeit. Dies beinhaltet folgende Leistungen:
    - Transport von der Entlade- zur Baustelle
    - Lagerung und Einschlag

        - Pflanzgruben-Aushub
        - Pflanzvorgang
        - Wässern
        - Verankerung
        - Schutz vor Austrocknung
        - Wildschutz.
    - Im städtischen Bereich: Auswechseln des alten Bodens, Einbau von Belüftungs- und Bewässerungsröhren, Wiederherrichtung von Kleinpflaster und ähnlichen Befestigungen.
    - Bei architektonisch gestalteten Pflanzungen: Leistungen des Garten- und Landschaftsarchitekten

Fracht- und Pflanzkosten betragen bei einfachen grossflächigen Pflanzungen ca. 30 % des Katalogpreises.

Die Anwachspflege eines Gehölzes beinhaltet üblicherweise die folgenden Leistungen:

- Lockern und Säubern der Pflanzfläche,
- Erhalten der Giessmulden,
- Entfernen von Steinen und Unrat,
- Schneiden der Gehölze,
- Düngen und Wässern,
- Durchführen des Pflanzenschutzes,
- Erhaltung der Verankerungen.

Durch eine sorgfältige Pflanzung und eine ebenso sorgfältige Anwachspflege kann das Anwachsrisiko in Bezug auf den Erfolg der Pflanzung erheblich verringert, aber dennoch nicht ausgeschlossen werden. Die Kosten für Anwachspflege und Risiko der ersten zwei Jahre werden mit etwa 20% der Pflanzarbeit zu den Pflanzkosten dazugerechnet (Stadtgärtnerei Basel, 2011).

Werden alle Buchsbäume im Friedhof am Hörnli in die Berechnung des Buchsbaumwertes im Sinne des Sachwertverfahrens miteinbezogen, ergibt sich eine Gesamtanzahl von 9060 Pflanzen, davon 8410 in Hecken verschiedener Höhe und 650 solitäre Bäume (Siehe Anhang 1 und 2). Dies entspricht einem heutig aktuellen Katalogpreis von insgesamt 1'793'747 CHF. Werden hingegen nur die nach den Rodungsmassnahmen vom Winter 2010 – 2011 noch bestehenden Pflanzen berücksichtigt, entspricht dies 5960 Heckenpflanzen und 200 solitären Bäumen (Total = 6160) und einem Preis von 890'897 CHF. Somit kann der gesamte Wiederbeschaffungswert der Buchspflanzen im Friedhof am Hörnli inklusive der Pflanz- und Anwachskosten auf 2'690'620 CHF im Jahr 2010 bzw. 1'336'345 CHF im Jahr 2011 geschätzt werden.

Effektive Schadenssumme des Buchsbaumzünslers im Friedhof am Hörnli

Im Sinne des Sachwertverfahren ergibt sich ein Wiederbeschaffungswert der vom Buchsbaumzünsler vernichteten Pflanzen von 1'354'275 CHF. Für Behandlung und Ersatz der betroffenen Buchspflanzen wurde jedoch von der Stadtgärtnerei Basel, Kreis Hörnli, ein weit niedrigerer Betrag aufgewendet. Dies entspricht dem Konzept der Verhältnismässigkeit. Die oben genannten Zahlen zum Verkehrswert des auf dem Friedhof am Hörnli stehenden Buchses sind aber nicht ohne weiteres zur Berechnung der reellen Schadenssumme anwendbar, da bis zum heutigen Zeitpunkt der grössere Teil der Buchspflanzen, welche im Winter 2010 – 2011 entfernt wurden, nicht oder durch andere Pflanzenarten ersetzt wurden. Es soll demnach eine der Situation angepassten Rechnungsweise angewendet werden, welche nicht einzig auf dem Ersatzwert der Pflanzen aufbaut, sondern die effektiv anfallenden Kosten berücksichtigt, die durch den Buchsbaumzünsler verursacht wurden. Bei den entfernten Pflanzen wird in dieser Rechnung vom Totalschaden ausgegangen, da ihre Beeinträchtigung aus biologischer Sicht solchermassen fortgeschritten war, dass sie ihre Funktion auch als Teil des Grundstückbildes nicht mehr zu erfüllen vermochten.

Somit ergibt sich eine Berechnung, welche die Kosten für die Rodung der beschädigten Pflanzen, die jährliche Pflege der zu bewahrenden Buchsbäume sowie die an einigen Stellen erfolgte Neupflanzung anderer Arten miteinbezieht. Die Kosten für die Rodung setzten sich aus Lohn- und Materialkosten sowie Kosten für die Abfuhr des Schnittgutes zusammen. Die Kosten für die Pflege der verbleibenden Buchspflanzen beinhalten anstelle der Abfuhrkosten die Kosten für die Anwendung eines Pflanzenschutzmittels. Bei der Neupflanzung werden Lohn- und Materialkosten sowie der Ankauf der Ersatzpflanzen summiert. Zahlen dazu wurden von der Stadtgärtnerei Basel, Kreis Hörnli, zur Verfügung gestellt und ermöglichten eine Näherungsrechnung der bei diesen Aufgaben anfallenden Kosten.

Die Näherungswerte für die Rodung der Pflanzen, welche durch den Buchsbaumzünsler einen Totalschaden erlitten hatten, betragen ca. 42‘776 CHF (siehe Anhang 3). Die Kosten für die jährliche Pflege unterschieden sich je nach Zeitpunkt, ob vor oder nach den grossflächigen Rodungsmassnahmen von Dezember 2010 – Januar 2011. Im Jahr 2010 beliefen sich diese Kosten auf ca. 23‘303 CHF und im Jahr 2011, bedingt durch die kleinere Anzahl an Buchspflanzen, auf ca. 11‘561 CHF. Dazu wurden Neupflanzungen in Höhe von ca. 11‘000 CHF ausgeführt bzw. für einen späteren Zeitpunkt budgetiert. Diese Kosten summieren sich zu einer totalen effektiven Schadenersatzsumme von ca. 45‘860 CHF, geltend Ende 2011.

Vergleich von Pflege- und Ersatzkosten im Friedhof am Hörnli

Wie weiter oben deutlich gemacht wurde, sind die Kosten für eine Wiederbeschaffung des gesamten Buchsbestandes im Friedhof am Hörnli weit höher als die Kosten, welche jährlich für die Pflege dieser Bäume anfallen würden. Würde der gesamte Buchsbestand infolge der Bedrohung durch den Buchsbaumzünsler durch andere, optisch und funktionell ähnliche Pflanzenarten ersetzt, um langjährige Pflegekosten zu vermeiden, könnte dies langfristig eine deutliche Kostenersparnis bedeuten. Durch die Pflanzung günstigerer Arten, welche – zurzeit – keiner Bedrohung im Ausmass des Buchsbaumzünslers ausgesetzt sind, wären die Pflanzkosten je nach Pflanzenart bereits nach weniger als 30 Jahren gegenüber den Pflegekosten des gesamten Buchsbestandes amortisiert. Hier wird ein Beispiel präsentiert, in welchem vom Ersatz des gesamten Bestandes durch Liguster (*Ligustrum vulgare*) oder Eibe (*Taxus baccata*) ausgegangen wird. Auch hier wurde zur Berechnung der Pflanzkosten das Sachwertverfahren angewendet, indem zum Katalogpreis der jeweiligen Pflanzentypen (Hecke oder solitäre Pflanze) und Pflanzgrössen die prozentualen Kosten für Pflanzung und Anwachspflege dazugerechnet wurden. Nach Sachwertverfahren betragen die Kosten für den Ersatz des gesamten Buchsbaumbestandes im Friedhof am Hörnli (Bestandgrösse vor den Rodungsmassnahmen vom Winter 2010 – 2011) mit Liguster 443'320 CHF und mit Eibe 2'464'320 CHF (siehe Anhang 4). Die Pflegekosten werden anhand der langjährigen Pflege des gesamten Buchsbaumbestandes vor den Rodungsmassnahmen im Winter 2010 – 2011 aufsummiert, wobei angenommen wird, dass diese in einem vergleichbaren Rahmen wie in den letzten zwei Jahren weitergeführt werden. Es ergibt sich aus dieser Rechnung, dass ein kompletter Ersatz mit Liguster bereits nach 20 Jahren günstiger wäre als die hochgerechneten Kosten für die jährliche Pflege des gesamten Buchsbaumbestandes. Bei einem Ersatz mit Eiben würde solch ein Fall erst nach ca. 105 Jahren eintreten, eine Zahl welche durch fluktuierende wirtschaftliche Verhältnisse nur schwer vorherzusagen ist. Werden die langfristigen Pflegekosten auch noch mit dem gesamten Wiederbeschaffungswert des Buchsbestandes verglichen, würde sich der Ersatz erst nach geschätzten 120 Jahren auszahlen (Abbildung 2).

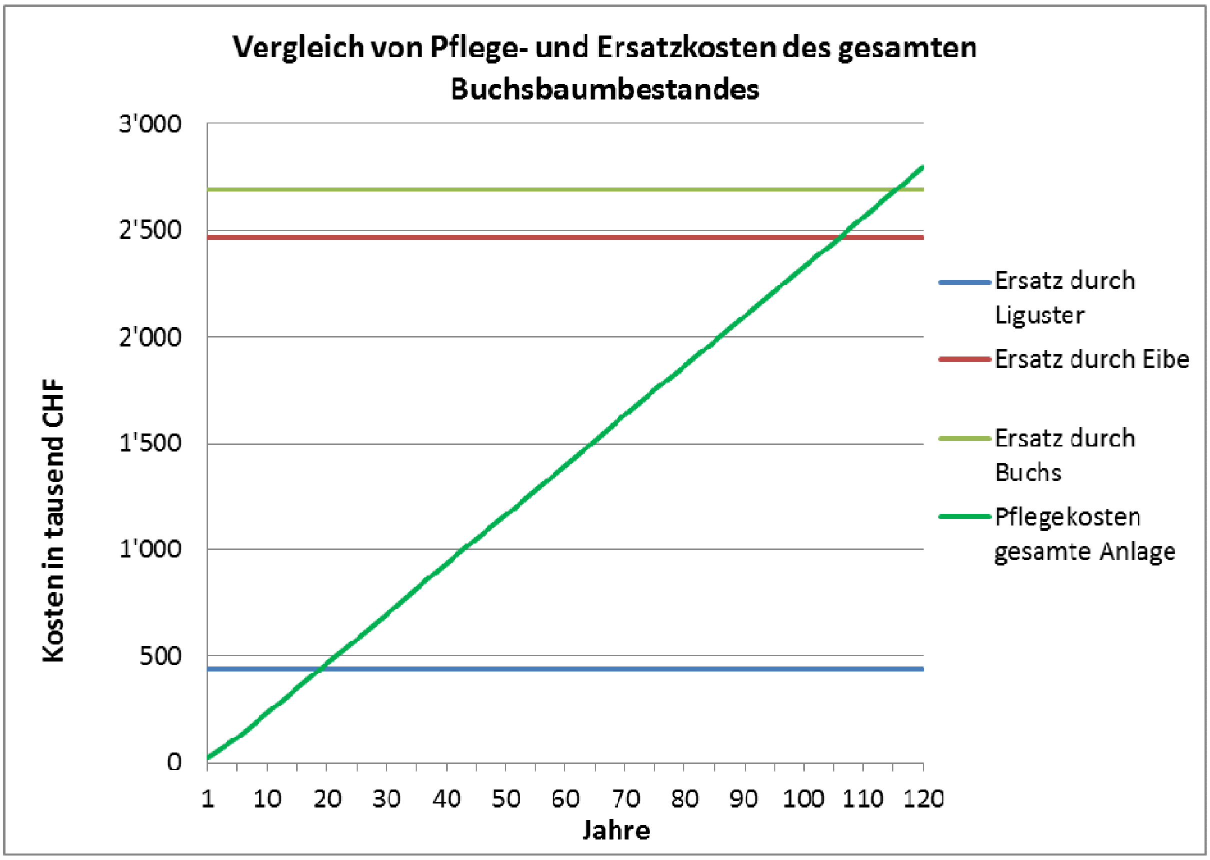

Abbildung 2: Vergleich von Pflege und Ersatz durch Liguster (*Ligustrum vulgare*), Eibe (*Taxus baccata*), sowie mit dem Wiederbeschaffungswert der gesamten Buchspflanzen, welche sich vor den Rodungsmassnahmen im Winter 2010 – 2011 im Friedhof am Hörnli befanden.

Die Strategie, welche von der Stadtgärtnerei Basel im Friedhof am Hörnli angewandt wird, namentlich Erhalt und Pflege von funktionell und denkmalpflegerisch wertvoller Buchspflanzen und Rodung bzw. Ersatz der als nicht zwingend notwendig erachteten Pflanzen, wird in einem weiteren Rechnungsbeispiel mit verschiedenen Varianten dieser Strategie verglichen. Dabei werden die Kosten für die Rodung der vernichteten Buchsbäume mit den Kosten für deren Ersatz durch Liguster bzw. Eibe und den Pflegekosten der verbleibenden Buchsbäume aufsummiert. Die Strategie der Stadtgärtnerei wird in diesem Beispiel so miteinbezogen wie sie derzeit ausgeführt bzw. budgetiert ist (siehe „Effektive Schadenssumme des Buchsbaumzünslers im Friedhof am Hörnli“), wobei die jährlich anfallenden Pflegekosten der verbleibenden Buchsbäume ebenfalls dazugerechnet werden. Diese Rechnungsbeispiele werden zum Verständnis des allgemeinen Zusammenhangs mit den Pflegekosten verglichen, welche für den bis Winter 2010 – 2011 vorhandenen Buchsbestand anfallen würden. Dabei wird ersichtlich, dass wenn aller Buchs, welcher im Winter 2010 – 2011 entfernt wurde, durch Liguster ersetzt würde, dies die gesamten Pflegekosten innert 21 Jahren amortisieren würde (Abbildung 3). Bei einem Ersatz der entfernten Buchspflanzen mit Eibe als einziger Ersatzpflanze wäre dies erst nach ca. 105 Jahren der Fall. Die zurzeit angewandte Strategie der Stadtgärtnerei erweist sich in diesem Vergleich durchaus als die rentabelste, da sie bereits nach 4 Jahren günstiger ist als die hypothetische Pflege des gesamten Buchsbaumbestandes.

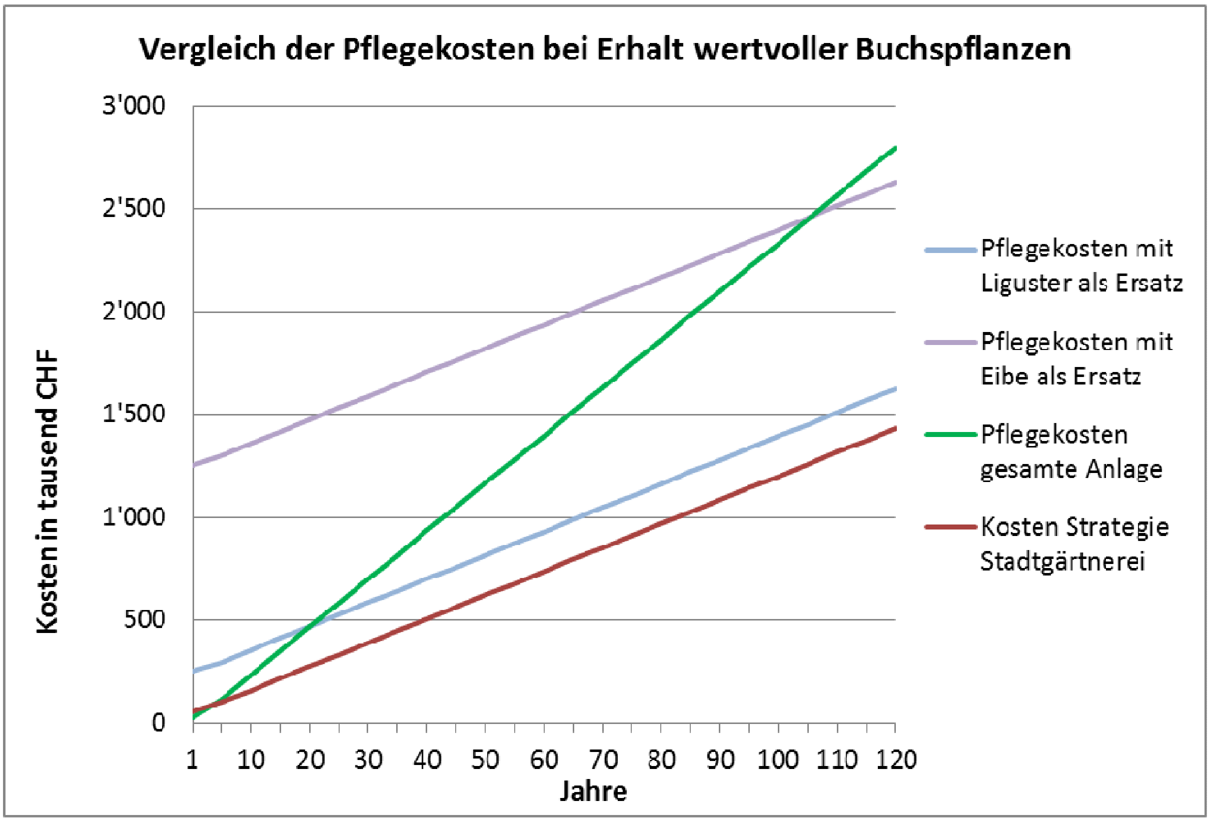

Abbildung 3: Vergleich der Pflegekosten verschiedener möglichen Strategien nach den Rodungsmassnahmen im Winter 2010 – 2011 mit den Pflegekosten, welche für den bisherigen gesamten Buchsbaumbestand anfallen würden.

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Anhang

<u>Anhang 1</u>

Plan des Friedhofs am Hörnli mit eingezeichneten Buchshecken und Solitärpflanzen, deren Pflanzdatum, sowie im Jahr 2010 entfernte Pflanzen. (PDF-Datei)

<u>Anhang 2</u>

Aufstellung der Anzahl vor und nach 2010 im Friedhof am Hörnli vorzufindenden Buchspflanzen mit Alter, Grösse, Laufmeter, sowie Katalogpreis und Pflanzenwert inklusive Pflanz- und Anwachskosten der entsprechenden Pflanzen.

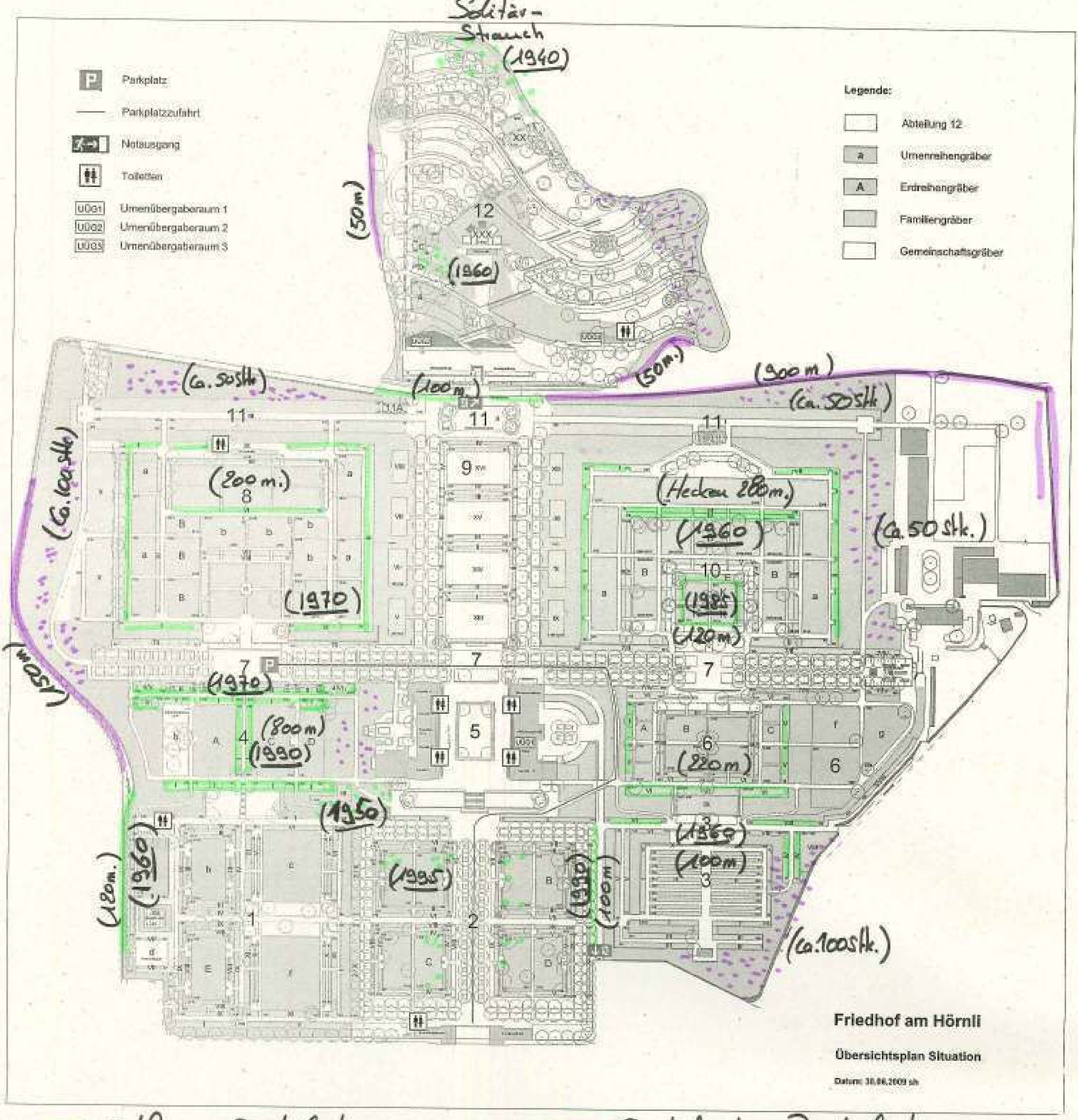
<u>Anhang 3</u>

Aufstellung der zur Rodung sowie zur Pflege der Buchsbäume auf dem Friedhof am Hörnli anfallenden Kosten, gegliedert nach bestehendem und entferntem Buchs.

<u>Anhang 4</u>

Vergleich der Pflegekosten der Buchsbäume auf dem Friedhof am Hörnli mit den Kosten, welche durch den Ersatz mit verschiedenen Pflanzenarten anfallen würden.

Anhang 1: Plan des Friedhofes am Hörnli mit entfernten und noch bestehenden Buchspflanzen



▬ Entfernung Buchshecken ▬ Bestehende Buchshecken.

∴ Entfernung von Buchsbäumen (—) Pflanzjahr +/-

∴ Solitärstrauch

Anhang 2: Berechnung des Buchswertes im Friedhof am Hörnli

Standort	Pflanzjahr	Gerodet	Hecke	Höhe in cm	Laufmeter	Pflanzen pro Meter	Anzahl Pflanzen	Preiskategorie *Buxus sempervirens*	Einkauf *Buxus sempervirens*	Total *Buxus s.* inkl. Pflanzkosten
1 - Strasse	1960	nein	ja	200	120	2	240	258.00	61'920.00	92'880.00
10 - Aussen	1960	nein	ja	50	280	3	840	59.00	49'560.00	74'340.00
10 - Innen	1985	nein	ja	110	120	2	240	194.00	46'560.00	69'840.00
12 - unten	1960	nein	ja	200	100	2	200	258.00	51'600.00	77'400.00
2 - rechts	1990	nein	ja	150	100	2	200	258.00	51'600.00	77'400.00
3 - Mitte	1960	nein	ja	90	100	2	200	125.00	25'000.00	37'500.00
4 - Innen	1990	nein	ja	70	200	3	600	90.00	54'000.00	81'000.00
4 - Innen	1970	nein	ja	100	600	2	1200	194.00	232'800.00	349'200.00
6 - Innen	1960	nein	ja	30	220	7	1540	33.30	51'282.00	76'923.00
6 - Rand	1960	nein	ja	200	50	2	100	258.00	25'800.00	38'700.00
8 - Innen	1970	nein	ja	50	200	3	600	59.00	35'400.00	53'100.00
1 - WC	1960	nein	nein	200			60	1'070.00	64'200.00	96'300.00
12 - oben	1940	nein	nein	150			5	725.00	3'625.00	5'437.50
2 - Mitte	1995	nein	nein	200			40	1'070.00	42'800.00	64'200.00
3- - Oben	1960	nein	nein	250			35	1'070.00	37'450.00	56'175.00
3 - Rand	1950	nein	nein	150			20	725.00	14'500.00	21'750.00
4 - Wald	1950	nein	nein	300			10	1'070.00	10'700.00	16'050.00
Gärtn. links	1960	nein	nein	250			30	1'070.00	32'100.00	48'150.00
11 - Strasse	1960	ja	ja	200	150	2	300	258.00	77'400.00	116'100.00
11 & Gärtn.	1960	ja	ja	160	900	2	1800	258.00	464'400.00	696'600.00
12 - links	1960	ja	ja	120	50	2	100	194.00	19'400.00	29'100.00
12 - rechts	1960	ja	ja	100	50	3	150	194.00	29'100.00	43'650.00
12 - unten	1960	ja	ja	200	50	2	100	258.00	25'800.00	38'700.00
11 - links	1960	ja	nein	100			50	330.00	16'500.00	24'750.00
11 - rechts	1960	ja	nein	100			50	330.00	16'500.00	24'750.00
11 - Strasse	1960	ja	nein	150			100	725.00	72'500.00	108'750.00
3 - Rand	1950	ja	nein	150			100	725.00	72'500.00	108'750.00
4 - Wald	1960	ja	nein	150			100	725.00	72'500.00	108'750.00
Gärtn. links	1960	ja	nein	150			50	725.00	36'250.00	54'375.00

Totalwert Buchs in CHF vor Entfernung	2'690'620.50
Totalwert Buchs in CHF nach Entfernung	1'336'345.50

Anhang 3: Effektive Schadenssumme des Buchsbaumzünslers im Friedhof am Hörnli

Standort	Pflanzjahr	Gerodet	Hecke	Höhe in cm	Laufmeter	Pflanzen pro Meter	Anzahl Pflanzen	Kubikmeter	Nur Roden	Jährliche Pflegekosten
8 - Innen	1970	nein	ja	50	200	3	600	25.00		134.50
6 - Rand	1960	nein	ja	200	50	2	100	100.00		538.00
6 - Innen	1960	nein	ja	30	220	7	1540	9.90		53.26
4 - Innen	1990	nein	ja	70	200	3	600	49.00		263.62
4 - Innen	1970	nein	ja	100	600	2	1200	300.00		1'614.00
3 - Mitte	1960	nein	ja	90	100	2	200	40.50		217.89
2 - rechts	1990	nein	ja	150	100	2	200	112.50		605.25
12 - unten	1960	nein	ja	200	100	2	200	200.00		1'076.00
10 - Innen	1985	nein	ja	110	120	2	240	72.60		390.59
10 - Aussen	1960	nein	ja	50	280	3	840	35.00		188.30
1 - Strasse	1960	nein	ja	200	120	2	240	240.00		1'291.20
Gärtn. links	1960	nein	nein	250			30	208.33		1'120.83
4 - Wald	1950	nein	nein	300			10	120.00		645.60
3 - Rand	1950	nein	nein	150			20	30.00		161.40
3- - Oben	1960	nein	nein	250			35	243.06		1'307.64
2 - Mitte	1995	nein	nein	200			40	142.22		765.16
12 - oben	1940	nein	nein	150			5	7.50		40.35
1 - WC	1960	nein	nein	200			60	213.33		1'147.73
12 - unten	1960	ja	ja	200	50	2	100	100.00	1'960.00	538.00
12 - rechts	1960	ja	ja	100	50	3	150	25.00	490.00	134.50
12 - links	1960	ja	ja	120	50	2	100	36.00	705.60	193.68
11 & Gärtn.	1960	ja	ja	160	900	2	1800	1'152.00	22'579.20	6'197.76
11 - Strasse	1960	ja	ja	200	150	2	300	300.00	5'880.00	1'614.00
Gärtn. links	1960	ja	nein	150			50	75.00	1'470.00	403.50
4 - Wald	1960	ja	nein	150			100	150.00	2'940.00	807.00
3 - Rand	1950	ja	nein	150			100	150.00	2'940.00	807.00
11 - Strasse	1960	ja	nein	150			100	150.00	2'940.00	807.00
11 - rechts	1960	ja	nein	100			50	22.22	435.56	119.56
11 - links	1960	ja	nein	100			50	22.22	435.56	119.56

Totalkosten Rodung in CHF	42'775.91

Jährliche Pflegekosten in CHF vor 2011	23'302.87
Jährliche Pflegekosten in CHF ab 2011	11'561.32

Anhang 4: Vergleich von Pflege- und Ersatzkosten der Buchspflanzen im Friedhof am Hörnli

Sektor	Pflanzjahr	Gerodet	Hecke	Höhe in cm	Laufmeter	Anzahl Pflanzen	Kubikmeter	Preiskategorie *Ligustrum vulgare*	Total *Ligustrum v.* inkl. Pflanzkosten	Preiskategorie *Taxus baccata*	Total *Taxus b.* inkl. Pflanzkosten	Preiskategorie *Buxus sempervirens*	Total *Buxus s.* inkl. Pflanzkosten	Nur Roden	Jährliche Pflegekosten
8 - Innen	1970	nein	ja	50	200	600	25.00	12.60	11,340.00	37.90	34,110.00	59.00	53,100.00	490.00	134.50
6 - Rand	1960	nein	ja	200	50	100	100.00	59.50	8,925.00	412.00	61,800.00	258.00	38,700.00	1,960.00	538.00
6 - Innen	1960	nein	ja	30	220	1540	9.90	12.60	29,106.00	30.10	69,531.00	33.30	76,923.00	194.04	53.26
4 - Innen	1990	nein	ja	70	200	600	49.00	12.60	11,340.00	57.00	51,300.00	90.00	81,000.00	960.40	263.62
4 - Innen	1970	nein	ja	100	600	1200	300.00	18.60	33,480.00	128.00	230,400.00	194.00	349,200.00	5,880.00	1,614.00
3 - Mitte	1960	nein	ja	90	100	200	40.50	15.20	4,560.00	87.00	26,100.00	125.00	37,500.00	793.80	217.89
2 - rechts	1990	nein	ja	150	100	200	112.50	59.50	17,850.00	264.00	79,200.00	258.00	77,400.00	2,205.00	605.25
12 - unten	1960	nein	ja	200	100	200	200.00	59.50	17,850.00	412.00	123,600.00	258.00	77,400.00	3,920.00	1,076.00
10 - Innen	1985	nein	ja	110	120	240	72.60	18.60	6,696.00	128.00	46,080.00	194.00	69,840.00	1,422.96	390.59
10 - Aussen	1960	nein	ja	50	280	840	35.00	12.60	15,876.00	37.90	47,754.00	59.00	74,340.00	686.00	188.30
1 - Strasse	1960	nein	ja	200	120	240	240.00	59.50	21,420.00	412.00	148,320.00	258.00	92,880.00	4,704.00	1,291.20
Gärtn. links	1960	nein	nein	250		30	208.33	98.00	4,410.00	1,360.00	61,200.00	1,070.00	48,150.00	4,083.33	1,120.83
4 - Wald	1950	nein	nein	300		10	120.00	98.00	1,470.00	2,360.00	35,400.00	1,070.00	16,050.00	2,352.00	645.60
3 - Rand	1950	nein	nein	150		20	30.00	53.00	1,590.00	390.00	11,700.00	725.00	21,750.00	588.00	161.40
3- - Oben	1960	nein	nein	250		35	243.06	98.00	5,145.00	1,360.00	71,400.00	1,070.00	56,175.00	4,763.89	1,307.64
2 - Mitte	1995	nein	nein	200		40	142.22	98.00	5,880.00	810.00	48,600.00	1,070.00	64,200.00	2,787.56	765.16
12 - oben	1940	nein	nein	150		5	7.50	53.00	397.50	390.00	2,925.00	725.00	5,437.50	147.00	40.35
1 - WC	1960	nein	nein	200		60	213.33	98.00	8,820.00	810.00	72,900.00	1,070.00	96,300.00	4,181.33	1,147.73
12 - unten	1960	ja	ja	200	50	100	100.00	59.50	8,925.00	412.00	61,800.00	258.00	38,700.00	1,960.00	538.00
12 - rechts	1960	ja	ja	100	50	150	25.00	18.60	4,185.00	128.00	28,800.00	194.00	43,650.00	490.00	134.50
12 - links	1960	ja	ja	120	50	100	36.00	18.60	2,790.00	128.00	19,200.00	194.00	29,100.00	705.60	193.68
11 & Gärtn.	1960	ja	ja	160	900	1800	1152.00	59.50	160,650.00	264.00	712,800.00	258.00	696,600.00	22,579.20	6,197.76
11 - Strasse	1960	ja	ja	200	150	300	300.00	59.50	26,775.00	412.00	185,400.00	258.00	116,100.00	5,880.00	1,614.00
Gärtn. links	1960	ja	nein	150		50	75.00	53.00	3,975.00	390.00	29,250.00	725.00	54,375.00	1,470.00	403.50
4 - Wald	1960	ja	nein	150		100	150.00	53.00	7,950.00	390.00	58,500.00	725.00	108,750.00	2,940.00	807.00
3 - Rand	1950	ja	nein	150		100	150.00	53.00	7,950.00	390.00	58,500.00	725.00	108,750.00	2,940.00	807.00
11 - Strasse	1960	ja	nein	150		100	150.00	53.00	7,950.00	390.00	58,500.00	725.00	108,750.00	2,940.00	807.00
11 - rechts	1960	ja	nein	100		50	22.22	40.10	3,007.50	195.00	14,625.00	330.00	24,750.00	435.56	119.56
11 - links	1960	ja	nein	100		50	22.22	40.10	3,007.50	195.00	14,625.00	330.00	24,750.00	435.56	119.56

Totale Kosten in CHF für die ganze Friedhofsanlage	443,320.50	2,464,320.00	2,690,620.50	84,895.22	23,302.87
Kosten für den Ersatz der gerodeten Buchspflanzen	237,165.00	1,242,000.00	1,354,275.00	42,775.91	
Pflegekosten für die nicht gerodeten Buchspflanzen					11,561.32

GENERAL DISCUSSION

Consequences of the introduction of alien species

Invasive species can alter an ecosystem in many different ways. They may interact directly with native species as predators, herbivores, competitors, parasites or diseases. Key-stone predators can induce cascading effects through the food chain, as this is experienced when domestic cats are introduced on an island ecosystem (EBENHARD, 1988). Indirect effects of non-native species include resource competition, habitat modification, and alteration of trophic dynamics, the nutrient cycle or of the disturbance regime. The introduction of the North-American beaver (*Castor canadensis*) to the Cape Horn in order to create a fur-industry had devastating consequences for the ecosystem, as the native plant species were not able to recolonize the deforested and flooded wetlands created by the beavers (ANDERSON et al., 2006). Non-native plants with nitrogen-fixing symbionts alter the nutrient input in the soil, causing the extinction of indigenous low-nitrogen species (VITOUSEK and WALKER, 1989), and grass species invading a prairie habitat can increase the frequency and intensity of fires (D'ANTONIO and VITOUSEK, 1992). Direct consequences of invasive species for the human society include economic damage as well as public health issues occurring for example when diseases are spread across the planet (cholera, AIDS or avian flu being only selected examples).

Impacts of the box-tree pyralid

The box-tree pyralid caused the loss of two natural box-tree forests in the region of Basel which were so severely damaged that complete regeneration is very unlikely. Due to the defoliation and dying of box-trees in the understory of mixed forests, the light regime, soil stability and nutrient cycling are affected in a way which is not yet assessable precisely. Trophic cascade and animal biodiversity might also be modified by the growth of a great number of broadleaf saplings which did not have the ability to grow under the light-regime determined by the dense standings of box-trees. Although the exact ecological function of box-trees in Central Europe is not clearly defined, we face the disappearance of a unique ecosystem, ultimately related to its intrinsic value. When referring to the economic consequences of box-tree loss due to the impact of the box-tree pyralid, direct and indirect values are concerned: Ornamental value, on one hand, and the indirect value or service of confinement and visual protection offered by box-tree hedges in private gardens or cemeteries on the other hand. However, none of these values are easily measurable and differ significantly depending on the subjective point of view. The material asset of purchase price and care expenses summed up is much easier to calculate, being on a monetary scale. This can also be expressed by a substitute cost approach, calculating the costs of substitution of the existing plants with similar-looking plants still available for sale (see **chapter 5**). One must keep in mind whatsoever that the values calculated through the substitution costs and the material asset value are often largely underestimated because of the monetarily inestimable sentimental value of box-trees, especially old individuals which can reach an age of several centuries.

Control of non-native species

The control of invasive species would be most effective if an invasion could be prevented. This involves the identification and regulation of invasion pathways but relies on lists of low- and high-risk species (OFFICE OF TECHNOLOGY ASSESSMENT, 1993). These may not always reflect the reality, based on the difficulty to predict a species' behaviour in a new environment, its interactions with native species and the particular traits of the sample of individuals which pursued the invasion. If invasion pathways can be identified and propagule pressure reduced under a threshold permitting population survival in the invaded area, a subsequent eradication of already established individuals can be rendered unnecessary. If the invasion pathway cannot be controlled or if no clear pathway exists, control or regulation of invaders must be undertaken via physical, chemical or biological control. This includes digging, trapping, hunting, treatment with herbicides or pesticides, or the release of predators, parasitoids, herbivores or diseases from the native range of the invader to the new habitat. Using the above-mentioned methods, a harmful impact on nontarget species should be avoided as much as possible and control programs meticulously planned. A rapid response to the invasion of a new alien species is crucial for its effective control or removal (PARKER et al., 1999). Once an invasive species has started spreading, it is almost impossible to eradicate it and the only solution remains the control to a tolerable level for the native ecosystem.

Solutions for the control of the box-tree pyralid

Based on the results of this study, it seems not to be possible to eradicate the box-tree pyralid *Cydalima perspectalis* from Europe. The box-tree pyralid has established successfully in different locations across Europe and spreads rapidly, mainly by human trade as described in **Chapter 1** (LEUTHARDT et al., 2010). Moreover, climatic modelling suggests that the box-tree pyralid may spread across most parts of Europe (**Chapter 2**, NACAMBO et al., 2013). Experiments showed that the box-tree pyralid has a broad diet. Consequently, there is no control via the available food source, i.e. planting some varieties of box-tree rather than others (**Chapter 3**, LEUTHARDT and BAUR, 2013). There is little chance that the box-tree pyralid might encounter a natural enemy in Europe in the near future, because of the unpalatability of the larvae, showed by the chemical analyses in **Chapter 4** (LEUTHARDT et al., 2013).

On a local scale, control by physical or chemical means may be sufficient to preserve the optical aspect of box-tree plants in spite of the presence of the box-tree pyralid. These methods include the removal of larvae by hand, washing down the plants with a strong water jet, or using a vacuum cleaner. After the first severe damages in 2007, a number of pesticides have proven efficacy and their use against the box-tree pyralid was authorized by Swiss law. The biological control of the box-tree pyralid in Central Europe using either indigenous parasitoids or organisms from the native range of the invader has not been investigated profoundly up to now. Preliminary experiments suggest that there is no parasitoid capable of taking over that role in Europe under natural conditions yet. Although oviposition on larvae of the box-tree pyralid occurred, it resulted with the death of both the box-tree pyralid and the parasitoid (ZIMMERMANN and WÜHRER, 2010). Other means of biological control include the release of genetically modified sterile insects or mating disruption via pheromone traps disorienting male moths. Pheromones have been developed in the native range of the box-tree pyralid (KAWAZU et al., 2007), but were not effective in Europe (F. LEUTHARDT, personal observations). Although the above solutions are very promising in terms of low negative ecological effects, they imply a lot of scientific work until they will be effective.

A combination of different control approaches often proves to be most effective. Actions taken by the government, local authorities as well as private people should be combined to achieve the greatest possible success. Additionally to the methods presented previously, the identification and subsequent regulation of international, regional and local invasion pathways reduce propagule pressure and increase long-term control of the invader.

Conclusions and implications for management

As a consequence of the rapid spread and successful establishment of the box-tree pyralid in Central Europe, rethinking of the management of wild and cultured box-trees is essential. The financial and material expenses of the care of box-trees during their whole life-span must be included into the budget when setting up gardens or parks with box-trees. However, although a natural regulation of population densities does not seem to exist at this point of time, the prediction of long-term impacts of the box-tree pyralid is difficult due to co-evolution between the invader, its food-plant and potential predators, parasites or diseases. Local population densities also depend on the reaction of people faced with damaged box-trees. Their choice of leaving the plants to their own fate, to treat them using different methods or to remove them will influence the net population size of the box-tree pyralid as well as the availability of food-plants.

The increasingly global human activity increases the overall number of introduced species in all parts of the world. The effects of invasive species are primarily a function of the degree at which humans modify the environment by fragmentation, disturbance or species extinctions (LUGO, 2006). Rapid environmental change induced by humans also generates adaptation and evolution, both for native and non-native species. Human-mediated species displacement is not inherently bad, as it can allow some threatened species to find refuge in a new location (GROOM et al., 2006). Regulation and management of introduced species is a process which will eventually become an integral part environmental management. It requires an increasing awareness of the public for environmental matters, and a reduction of negative impacts of human activity on the environment. To achieve successful invasion control, it is essential to increase the knowledge about invasion mechanisms and management techniques both among scientists as in the public. In the relatively young but very active research field of invasive species, it is of crucial importance to improve the global communication between scientists around the world and speaking different languages in order to be able to induce rapid response and effective management of non-native species with potentially severe impacts on invaded ecosystems.

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Personal Information

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Education

2010 - 2012	**PhD in Zoology,** Department of Environmental Sciences University of Basel
2007 - 2009	**MSc in Ecology**, Department of Environmental Sciences University of Basel
2004 - 2007	**BSc in Biology with major in Animal and Plant Sciences** University of Basel
2003	**Cambridge Certificate in Advanced English** Elanguest Language School, Malta
2000 - 2003	**Swiss Federal Maturity** Lycée cantonal, Porrentruy/JU

Recent Research

2010 - 2012	Department of Environmental Sciences, University of Basel **PhD Thesis** "Food Choice, Life History and Chemical Ecology of the Invasive Box-Tree Pyralid *Cydalima perspectalis*", *Magna cum Laude* Supervision: Prof. Bruno Baur
2007 - 2009	Department of Environmental Sciences, University of Basel **Master Thesis** "Habitat model for the orb-web spider *Cyclosa oculata*", final grade: 5.3 Supervision: Prof. Bruno Baur

Work Experience

2012	**Survey** of the occurrence of *Cydalima perspectalis* in the Canton Jura, **local dissemination of information** regarding this pest. CABI – Europe Switzerland
2010 – 2012	**Development and presentation of talks** about the box-tree pyralid *Cydalima perspectalis*
2007 - 2012	**Teaching and coaching of Bachelor students** in fieldwork, data analysis and writing, **development and organisation of student projects** in ecology Institute of Conservation Biology, University of Basel

Professional Skills

Scientific	Knowledge of ecologic processes
	Animal and plant determination (especially arthropods)
	Scientific writing
	Analytical statistics using R Statistics
	Geo-modelization using ArcGIS
Methodical	coordination and overview of workflows, setting priorities
	Systematic and analytic work
	Logical and abstract thinking, sense of numbers
	Contact and communication faculty

Languages

German	Oral and written with very good proficiency (native: Swiss German)
French	Native
English	Oral and written with good proficiency (CAE, Grade A)

Publications

2013	Development Characteristics of the Box-Tree Moth *Cydalima perspectalis* and its Potential Distribution in Europe – Nacambo S., Leuthardt F.L.G., Wan H., Li H., Haye T., Baur B., Weiss R.M. & Kenis M. –Journal of Applied Entomology, DOI: 10.1111/jen.12078
2013	Composition of Alkaloids in Different Box Tree Varieties and their Uptake by the Box Tree Moth *Cydalima perspectalis* – Leuthardt F.L.G., Glauser G. & Baur B. – Chemoecology 23: 203-212
2013	Oviposition Preference and Larval Development of the Invasive Moth *Cydalima perspectalis* on five European Box-Tree Varieties – Leuthardt F.L.G. & Baur B. – Journal of Applied Entomology 137: 437-444
2012	Der Buchsbaumzünsler *Cydalima perspectalis* – eine neue invasive Art in Mitteleuropa – Leuthardt F.L.G. – Regio Basiliensis 53: 127-133
2011	Kostenermittlung des Buchsbestandes und der Pflege der Buchsbäume hinsichtlich des Buchbaumzünslers im Friedhof am Hörnli, Kanton Basel-Stadt – Leuthardt F.L.G. – Auftraggeber: Bau- und Verkehrsdepartement Basel, Stadtgärtnerei
2011	Der Buchsbaumzünsler *Diaphania perspectalis* – Auftreten, Ausbreitung und Bedeutung eines neuen Schädlings an Buchsbaum am Beispiel der Schweiz – Leuthardt F.L.G. & Ramin S. – Jahrbuch der Baumpflege 2011, Deutsche Baumpflegetage (Wissenschaftlicher Kurzbericht)
2010	Ausbreitung des Buchsbaumzünslers *Diaphania perspectalis* (Lepidoptera: Pyralidae) in der Region Basel - eine für die Schweiz neue Schädlingsart – Leuthardt F.L.G., Billen W. & Baur B. – Entomo Helvetica 3: 51-57

Talks and Poster

27.01.2012	„Neue Erkenntnisse zum Auftreten und der Biologie des Buchsbaumzünslers“ – Talk at the NLU conference „Naturschutz in und um Basel“, Basel
08.09.2011	„Der Buchsbaumzünsler in der Schweiz: Sind unsere Buchswälder in Gefahr?“ – Talk at the Seminary „BioInfo“, Zentrum für Weiterbildung, University of Zurich
03.05.2011	„Der Buchsbaumzünsler *Diaphania perspectalis* – Auftreten, Ausbreitung und Bedeutung eines neuen Schädlings an Buchsbaum am Beispiel der Schweiz“ – Poster at the „Deutsche Baumpflegetage“, Augsburg, with Stephan Ramin
23.03.2011	„Der Buchsbaumzünsler in der Schweiz: Sind unsere Buchswälder in Gefahr?“ – Talk at the information event on the box-tree pyralid held by the „Bund für Naturschutz“, Grenzach/D
28.01.2011	„Der Buchsbaumzünsler in der Schweiz: Sind unsere Buchswälder in Gefahr?“ – Talk at the NLU conference „Naturschutz in und um Basel“, Basel
18.01.2011	„Der Buchsbaumzünslers: eine für die Schweiz neue Schädlingsart“ – Talk at the „Vogelschutzverein“, Pratteln/BL
07.11.2010	„Der Buchsbaumzünsler – eine für die Schweiz neue Schädlingsart” – Talk at the „Rosengruppe Graubünden, Brunch zum Jahresende“, Malans/GR
27.10.2010	„Der Buchsbaumzünsler – eine für die Schweiz neue Schädlingsart“ – Talk at the „Naturforschende Gesellschaft Baselland“, Liestal, with Stephan Ramin
07.10.2010	„Der Buchsbaumzünsler – Eine für die Schweiz neue Schädlingsart” – Talk at the „Gartencenter Meier“, Tann/ZH
29.10.2010	Radio interview on the box-tree pyralid and the damages it causes, in „Wissen aktuell” on SRF DRS1
09.09.2010	Live radio interview on the box-tree pyralid, its biology and potential control strategies, on Radio Basel
25.08.2010	TV interview on the box-tree pyralid, especially the damages causes in the box-tree forest in Grenzach/D, with Stephan Ramin, Renato Joos and Markus Dischinger, in „Schweiz aktuell” on SRF SF1
19.08.2010	„Der Buchsbaumzünsler – eine für die Schweiz neue Schädlingsart” – Talk at the JardinSuisse Seminary „Problematische Krankheiten und Schädlinge an Freiland-Pflanzen“, Wädenswil/ZH
26.06.2010	TV Interview on the box-tree pyralid with focus on lab work, in „7 vor 7” on TeleBasel
12.04.2010	TV interview on the box-tree pyralid with focus on the cemetery „am Hörnli”, in „7 vor 7” on TeleBasel
08.02.2010	„Bedroht der Buchsbaumzünsler (*Diaphania perspectalis*) unsere einheimischen Buchsbestände?” – Talk at the monthly assembly of the Entomological Society of Basel, with Christoph Wicki
29.01.2010	„Bedroht der Buchsbaumzünsler unsere einheimischen Buchsbestände?” – Talk at the NLU conference „Naturschutz in und um Basel“, Basel

Basel, 31. 09. 2012

Ich erkläre, dass ich die Dissertation

„Food Choice, Life History and Chemical Ecology of the Invasive Box-Tree Pyralid Cydalima perspectalis"

nur mit der darin angegebenen Hilfe verfasst und bei keiner anderen Universität und keiner anderen Fakultät der Universität Basel eingereicht habe.

Florine Leuthardt