

Stefanie Göttig, Annette Herz

## Observations on the seasonal flight activity of the box tree pyralid *Cydalima perspectalis* (Lepidoptera: Crambidae) in the Rhine-Main Region of Hessa

Erfassung der saisonalen Flugaktivität des Buchsbaumzünslers *Cydalima perspectalis* (Lepidoptera: Crambidae) im Rhein-Main-Gebiet in Hessen

157

### Abstract

The seasonal activity of wild populations of the invasive box tree pyralid *Cydalima perspectalis* Walker (Lepidoptera: Crambidae) was observed between 2012 and 2015 in the Rhine-Main region in Hessa. The moth flight was detected by a light trap (2012–2014) and several pheromone traps (2013–2015) at two locations. Two main flight periods were identified annually, which indicate the existence of two generations. The first flight phase was relatively weak. It began in mid June and reached its peak in mid July. It was followed by a much extended flight phase of the second generation from mid August to October with a peak in early September (calendar week 36/37). In addition to the surveillance of the flight, basic data on the sex ratio and the appearance of the two main phenotypes of *C. perspectalis* (white and melanic morph) were obtained. It could be shown that neither the proportions of females nor the amount of melanic moths differ significantly between the two flight phases. The proportion of melanic moths was on average  $14\% \pm 2.9\%$  and differed only slightly over the years. Overall, it can be stated that both trap systems are suitable for the observation of the flight activity and thus also for the proper timing of control measures. The detection of females by using light traps is not necessary for this purpose.

**Key words:** Box tree moth, light- and pheromone traps, flight activity, melanic morph

### Zusammenfassung

Zwischen 2012 und 2015 wurde die saisonale Flugaktivität von Freilandpopulationen des invasiven Buchsbaumzünslers *Cydalima perspectalis* Walker (Lepidoptera: Crambidae) im Rhein-Main-Gebiet in Südhessen erfasst. Der Falterflug wurde an zwei Standorten durch eine Lichtfalle (2012–2014) sowie mehrere Pheromonfallen (2013–2015) aufgezeichnet. Es konnten zwei deutliche Hauptflugzeiten identifiziert werden, die auf zwei Generationen im Jahr schließen lassen. Die erste Flugphase war stets schwach ausgeprägt, begann Mitte Juni und gipfelte Mitte Juli. Es folgte eine sehr umfangreiche und intensive Flugphase der zweiten Generation von Mitte August bis Oktober, mit einem Höhepunkt Anfang September (Kalenderwoche 36/37). Darüber hinaus wurden grundlegende Daten zum Auftreten der Geschlechter sowie der zwei Hauptphänotypen (weiße und melanierte Morphe) der angelockten *C. perspectalis* Falter erarbeitet. Weder die Proportion der Geschlechter noch die des Auftretens der braunen Morphe unterschieden sich signifikant im Vergleich der beiden Flugphasen. Mit der Lichtfalle konnte gezeigt werden, dass weibliche Falter ihren Flug im Frühjahr nicht vor dem der Männchen beginnen. Der Anteil an Faltern der braunen Farbvariante betrug im Durchschnitt  $14\% \pm 2,9\%$  und unterschied sich über die Jahre hinweg nur gering. Insgesamt kann festgehalten werden, dass sich beide Fallensysteme zur Erfassung der Flugaktivität und somit auch zur Terminierung

### Institute

Julius Kühn-Institut (JKI) – Federal Research Centre for Cultivated Plants, Institute for Biological Control, Darmstadt, Germany

### Correspondence

Dr. Annette Herz, Julius Kühn-Institut (JKI), Federal Research Center for Cultivated Plants, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany, E-Mail: Annette.herz@julius-kuehn.de

### Accepted

2 December 2016

von Bekämpfungsmaßnahmen eignen. Die Aufzeichnung der Weibchen mit Lichtfallen ist dazu nicht zwingend notwendig.

**Stichwörter:** Buchsbaumzünsler, Licht- und Pheromonfallen, Flugaktivität, braune Morphe

**Introduction**

Ten years ago, in 2006, the box tree pyralid *Cydalima perspectalis* (WALKER, 1859) (Lepidoptera: Crambidae) syn. *Glyphodes perspectalis* Guenée, *Palpita perspectalis* Hübner and *Diaphania perspectalis* Hübner (MALLY and NUSS, 2010) arrived in Europe (BILLEN, 2007; KRÜGER, 2008) and became highly invasive. Until now more than 20 European countries are affected (NACAMBO et al., 2014). In Europe, *Buxus* plants are one of the most popular ornamental shrubs with great cultural significance. Most frequently planted species are different varieties of *Buxus sempervirens* L. and *B. microphylla* Sieb. & Zucc.. Thousands of plants in private gardens, cemeteries, public parks and palace grounds are affected by feeding damage of the new pest or already had to be replaced. Besides the cultural and economic effects, potential ecological effects may occur due to the additional threat to the natural *Buxus* stands. *B. sempervirens* L. in Central and Southern Europe, *B. balearica* Lam. in the Mediterranean area (DI DOMENICO et al., 2012) and *B. colchica* Pojark, in the Caucasus, especially in the forests of Georgia (MATSIAKH, 2016) are highly endangered. It can be assumed that no *Buxus* species or variant exists, which is not serving as potential host plant for *C. perspectalis* and a lot of the rare natural stands are already concerned.

*C. perspectalis* is a monophagous pest and its life cycle occurs completely on host plants of the genus *Buxus*. Moths are flying in the late evening and night. After mating and dispersal adult females lay their eggs in batches mostly on the underside of the leaves. Eggs are going to hatch out within ten days. Larvae emerge and undergo a feeding period of several weeks. At the beginning small

larvae feed aggregated, causing damage on the exterior leaf layer and form loose nests in the plant. Later on, they spread and feed solitary on the foliage, typically sparing the vein and often additionally attacking the bark. Six larval instars are usually passed until the pupation. Larvae spin a cocoon among a few leaves and evolve to a pupa from which the moth will emerge and the cycle begins anew. Larvae of the next generation emerge in autumn. They feed until the third larval instar and overwinter as small larvae in a closely spun cocoon between two leaves. In spring, feeding activity restarts. There are different statements to the number of occurring generations in Germany. ALBERT and LEHNEIS (2010) assumed at least three generations in Baden-Württemberg, but also two generations were postulated (CABI, 2013; ZIMMERMANN, 2014). However, there is still a lack of detailed and documented information on the flight phenology.

It is known that different types of wing color varieties exist in *C. perspectalis* populations. The typical morph is white with a dark brown margin and small characteristically crescent-shaped white marks on it. The body is white with a brownish abdominal segment (Fig. 2 A). Body and wings of the melanic morph are almost uniform



Fig. 1. Trap types. Light trap (left) and pheromone trap, funnel type (right).



Fig. 2. Morphological varieties and sex dimorphism of *C. perspectalis*. (A) moth; typical white morph, (B) moth; intermediate morph, (C) moth; melanic morph, (D) pupae; typical white (above) and melanic morph (below), (E) Male, hair-pencil present on the tip of the abdomen (left) Female, abdomen without hair-pencil (right).

dark brown with the exception of the two white marks (Fig. 2 C), also described by SÁFIÁN and HORVÁTH (2011) and SZÉKELY (2011). In addition, an intermediate phenotype exists with an extra brown margin at the forewings (Fig. 2 B), also recognized by PAN et al. (2011) in China, but not considered in this investigation. The melanic morph can already be identified at the pupal stage (Fig. 2 D).

Monitoring is an important component when dealing with invasive species, according to assess the spread, population levels and the seasonal flight activity per year (VALLES et al., 1991). The determination of the adult flight phases per year can lead to conclude the number of completed generations and additionally the phases of egg deposition and larval feeding. It enables to time diverse control measures adapted to the occurring developmental stage in field. This can reduce dispensable insecticide applications, resulting in a more efficient pest management system. The investigation of the occurrence and the proportion of the sexes and morphological varieties in *C. perspectalis* populations offer information on potential seasonal differences which may lead to a developmental advantage, for instance on overwintering. This could be indicated by an earlier flight of a particular sex or melanic moths. LAURENT and FRÉROT (2007) reported problems of monitoring the European corn borer (*Ostrinia nubilalis* Hübner, Crambidae) with pheromone traps. It remains unclear whether flight curves plotted from pheromone trap captures truly reflect moth phenology because of the earlier flight of the female moths which cannot be lured by sex pheromone trapping. DE JONG et al. (1996) postulated that the melanic morph of the ladybird (*Adalia bipunctata* Linnaeus, Coccinellidae) exhibits higher body temperature, tending to warm up slightly faster than the non-melanic ones. Morphs appeared to be the principal factor influencing activity.

The emphasis of this study was to observe *C. perspectalis* populations for consecutive years with two monitoring systems, pheromone and light trapping (Fig. 1). We wanted to find out exact information on the seasonal flight activity patterns and whether there are differences in the temporal flight sequence of the sexes and of the two major morphological varieties (Fig. 2).

## Material and Methods

The study was conducted at two sites in the Rhine-Main region nearby Frankfurt in South Hesse, Germany. The “Convent Garden Seligenstadt” (50°2′38.75″N 8°58′31.25″E) has an area of about 3 ha and contains more than 3.5 km *Buxus* hedges as bed enclosures. The historical “Old Cemetery Darmstadt” (49°51′53″N 8°40′6″E) has a ground area of 13.5 ha and includes numerous individual *Buxus* plants or groups on graves and the enclosure of the irrigation systems. On both sites, infection with *C. perspectalis* was high and no insecticide treatment occurred, except of *Bacillus thuringiensis kurstaki* applications at Seligenstadt in 2013 and 2014.

The **light trap** (Figure 1) was installed for three consecutive years (2012, 2013 and 2014) in the herb garden of the cloister Seligenstadt, South Hesse, which has an area of 600 m<sup>2</sup>. It was purchased from the company Fiebig-Lehrmittel (Berlin, Germany). The total height of the trap was 1.3 m. A square case (length 51 × width 51 × height 40 cm) was forming the standing base of the trap. In the middle of this main chamber, a plastic catch tray (135 × w 29 × h 18 cm) was placed with a small hole below the funnel (diameter 26 cm, 7 cm at the tail). Above the funnel, the baffle (h 70 cm) and the light sources (two NARVA Colourlux plus bulbs) was rising out of the case, protected by a plate (d 52 cm) on top against rain. Moth flight was surveyed from May/June until September/October (Table 1). Trap was daily checked and the content was examined. The number of caught moths, sex and the morphological variety of the wing color (white and brown) was determined. Sex was assessed by recognizing abdominal dimorphism. Males could be identified on their hair-pencil at the last abdominal segment (Fig. 2 E).

**Pheromone trapping** was conducted between 2013 and 2015 in Darmstadt and Seligenstadt, to survey the seasonal moth flight activity. Investigations on the pheromone compositions, lures and traps were already carried out in 2013 and 2014 in cooperation with the company Pherobank B.V. (NL). Thus, at the end of 2014 a combination of a reliable pheromone composition and a valuable lure was discovered for optimal capturing of *C. perspectalis* (data will be published elsewhere). Funnel traps (Figure 1) proved to be more suitable on moth trapping than the delta trap type and were appropriate for an effective monitoring (unpublished data). Pheromone lures were based on the two main active components (Z)- and (E)-11-hexadecenal (KAWAZU et al., 2007; KIM and PARK, 2013) and offered by polyethylene vials. Lures were exchanged after 3–4 weeks. In our trials, pheromone traps (d 16.5 cm, h 21.5 cm) were set up in approximately 1.6 m height on trees or shrubs with the greatest possible distance to each other (about 25 m in Seligenstadt and more than 100 m in Darmstadt). 8–12 traps were used per comparison phase and site. They were assessed weekly and the local position was randomized. In 2015, observation could only be conducted in Darmstadt. In Seligenstadt the experiment had to be stopped because of repeated vandalism on the pheromone traps.

Statistics were done with RStudio (Version 0.99.489 – ©2009–2015 RStudio, Inc.; R Version 3.2.2). To analyze the count data of caught moths (distribution of sex and morphological variety) within one year, Chi-squared tests for given probabilities were used. To compare mean proportions [%] 2-sample tests for equality of proportions with continuity correction were done.

## Results

### Seasonal flight activity

In general, moth catch was summarized to calendar weeks (cw) and could be observed from June (rarely

May/cw 22) until October (cw 42). Two main flight periods were clearly identified with both trapping systems. The first flight phase arose from mid June (cw 24) until late July (cw 29). At this time, the adults of the overwintering generation emerged. Catch decreased at late July/early August (cw 31/32) in both trapping systems. Then a much extended second flight phase occurred from mid August (cw 33) until October (cw 42) where the major flight was performed with a peak in September (cw 36) (Fig. 3 and 4). Thus, according to these results two main flight periods were identified annually. In 2014, a decreased number of trapped moths were documented for pheromone trapping in Seligenstadt, resulted from repeated vandalism on the traps. But also for light trapping a lower population was recorded, probably due to the *Bacillus thuringiensis* applications conducted in 2013 and 2014.

**First catch**

In 2012, only the light trap in Seligenstadt was installed and the first catch (Table 1) was registered in early June (cw 24). In 2013, the pheromone trapping started and the first moth catch occurred in late June/early July (cw 27) for both sites. First catch of the light trap was shown one week earlier (cw 26), but the trap installation was

realized one week earlier as well. In 2014, a single male was caught in a pheromone trap in Seligenstadt very early, in May (cw 22). Other first catch in 2014 were observed in early June (cw 23) caught by the light trap and one week later in cw 24 caught by a pheromone trap in Darmstadt, which was not installed before cw 23. There was no major difference between the temporal recordings of the first catch in the two trap systems. First females were caught together with first males in the light trap but in less number. Early captured moths were mostly white and a potential earlier flight of the melanic moths in spring was not documented.

**Sex distribution**

Light trapping enabled to record the sex distribution of caught moths. In total, sex could be determined for a number of 743 moths. For a small amount of moths (4%) determination was not possible, because catch were partially soaked by rain and/or the abdomen was damaged. To examine the occurrence of possible temporal differences in the sex distribution, catch was analyzed separately for the two flight phases per year (first: June-July and second: August-October). Mean distribution (2012–2014) of the first flight phase was 54% ± 17% males and

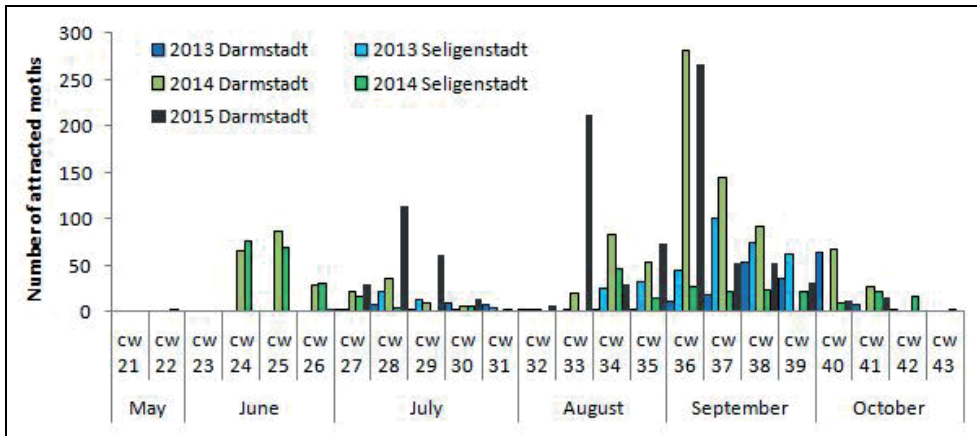


Fig. 3. Seasonal flight activity detected by pheromone trapping in the years 2013–2015 at Seligenstadt and Darmstadt (cw = calendar week).

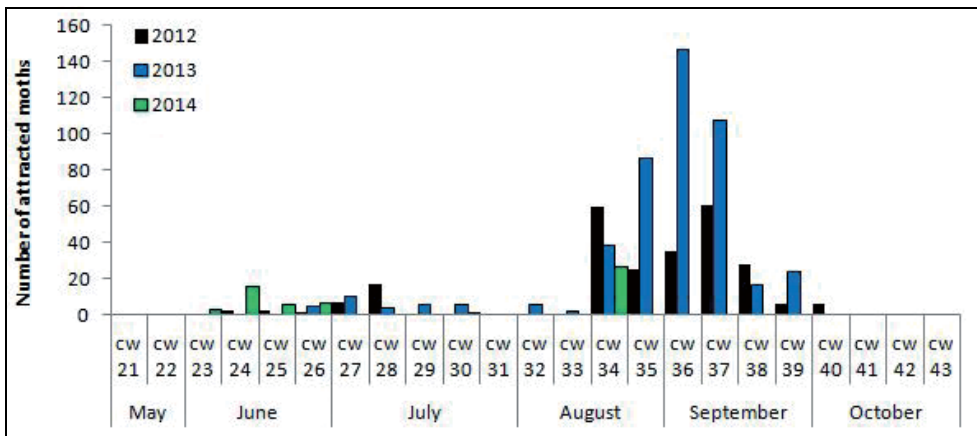


Fig. 4. Seasonal flight activity detected by one light trap in the years 2012–2014 at Seligenstadt (cw = calendar week).

46% ± 17% females, whereas we found 60% ± 3.8% males and 40% ± 3.8% females in the second flight phase (Table 2). There is no significant difference in the proportions of sex in the two flight phases ( $\chi^2 = 0.51$ ,  $df = 1$ ,  $p = 0.4751$ ). First females were recorded together with first males in the light trap (Table 1).

However, the statistical analyses of observed moth catch per year showed a significant surplus of caught males during the first flight phase in 2014 ( $\chi^2 = 4.84$ ,  $df = 1$ ,  $p = 0.03$ ), the second flight phase in 2012 ( $\chi^2 = 4.74$ ,  $df = 1$ ,  $p = 0.03$ ) and in 2013 ( $\chi^2 = 9.94$ ,  $df = 1$ ,  $p = 0.002$ ). Sex ratios (males: females) ranged between 0.6 and 2.6 (Table 2). Similar ratios were found if considering only the brown variety; ranging between 1.3 and 1.7. Only in 2012 a surplus of females could be detected for the first flight phase (sex ratio 0.6), but the difference was not significant ( $\chi^2 = 2$ ,  $df = 1$ ,  $p = 0.1573$ ). There is no evi-

dence that *C. perspectalis* females begin their flight earlier than males.

#### Morphological variety

Early caught moths were mostly white and an earlier flight of the brown morph in spring became not apparent (Tab. 1). The proportions of the brown moth variety per month summarized per trap system (and site within pheromone trapping) ranged between 3% and 27%. Using pheromone traps, a mean proportion of 84% white to 16% ± 0.1% brown lured male moths per year was observed in Seligenstadt and 89% white and 11% ± 3.4% brown moths in Darmstadt over the years of the study. Light trapping detected a similar ratio of 84% white to 16% ± 1.9% brown moths in Seligenstadt. Thus, on average, a proportion of 14% ± 2.9% melanic moths per year could be expected (Fig. 5).

**Table 1. First catch of *C. perspectalis* during the observation of seasonal flight activity (2012–2015) by light and pheromone trapping at the two study sites (SE = Seligenstadt, DA = Darmstadt)**

Trap type and site	Trap installation period	First catch*	Number and sex	Morph
Light trap (SE)	24.05.-09.10.2012	cw 24	1 male, 2 females	white
Light trap (SE)	18.06.-10.10.2013	cw 26	4 males, 1 female	white
Light trap (SE)	22.05.-19.10.2014	cw 23	2 males, 1 female	white
Pheromone traps (SE)	25.06.-03.10.2013	cw 27	3 males	white
Pheromone traps (SE)	22.05.-23.10.2014	cw 22	1 male	white
Pheromone traps (DA)	25.06.-16.10.2013	cw 27	3 males	white
Pheromone traps (DA)	05.06.-16.10.2014	cw 24	60 males, 5 males	white, brown
Pheromone traps (DA)	19.06.-30.10.2015	cw 25	4 males	white

\* cw = calendar week

**Table 2. Occurrence of sex (males and females) and morphological varieties (white and brown) of *C. perspectalis* (proportion [%] and number) during the two flight phases (first: June-July and second: August-October) detected by light trapping (2012–2014). Asterisks indicate statistical significance<sup>a</sup>**

Year	First flight phase				Second flight phase			
	males	females	sex ratio	$\chi^2$	males	females	sex ratio	$\chi^2$
2012	38 (12)	62 (20)	0.6	2.0	57 (124)	43 (92)	1.3	4.7*
2013	53 (16)	47 (14)	1.1	0.1	58 (238)	42 (174)	1.4	9.9*
2014	72 (18)	28 (7)	2.6	4.8*	64 (18)	36 (10)	1.8	2.3
mean ± sd	54 ± 17	46 ± 17			60 ± 3.8	40 ± 3.8		
	white	brown		$\chi^2$	white	brown		$\chi^2$
2012	91 (30)	9 (3)		22.1*	88 (194)	12 (27)		126.2*
2013	81 (26)	19 (6)		12.5*	82 (354)	18 (76)		179.7*
2014	81 (26)	19 (6)		12.5*	75 (21)	25 (7)		7.0*
mean ± sd	84 ± 5.8	16 ± 5.8			82 ± 6.6	18 ± 6.6		

<sup>a</sup>  $\chi^2$ -test for given probabilities ( $\chi^2 > 3.8$ ,  $df = 1$ ,  $p < 0.03$ )

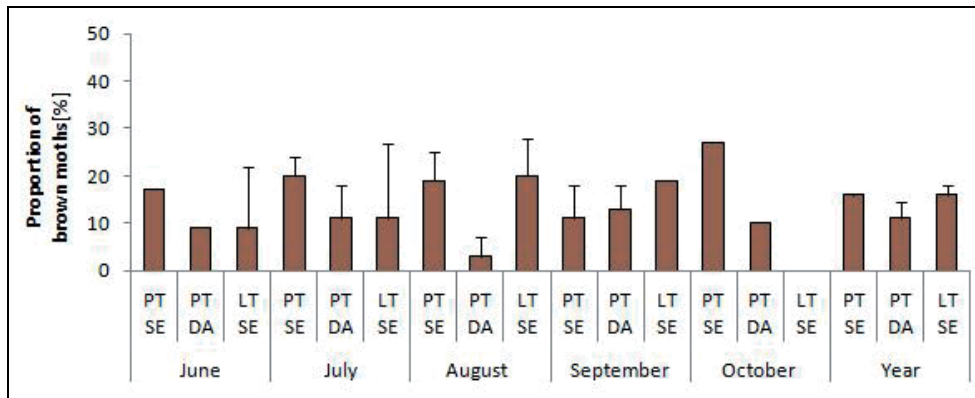


Fig. 5. Mean proportion [%] of the brown variety of *C. perspectalis* motlis per month. Caught motlis (2013 and 2014) were summarized per trap system (Pheromone trap = PT and Light trap = LT) and study site (Seligenstadt = SE and Darmstadt = DA).

In addition, the proportion of caught melanic moths per flight phase evaluated per light trapping displayed similar range. Mean distribution (2012–2014) of the first flight phase was  $84\% \pm 5.8\%$  white and  $16\% \pm 5.8\%$  brown colored moths. In the second flight phase  $18\% \pm 6.6\%$  brown *C. perspectalis* could be observed. There is no significant difference in the proportions at the two flight phases ( $\chi^2 = 0.035436$ ,  $df = 1$ ,  $p = 0.8507$ ) (Table 2). Mean percentage of melanic moths of the first flight phase detected by pheromone trapping in **Seligenstadt** (2013 and 2014) was  $20\% \pm 4.2\%$ . In the second flight phase  $12\% \pm 4.9\%$  brown moths could be observed. However, the difference in the proportions of the morphological varieties of the two flight phases is not significant ( $\chi^2 = 1.8229$ ,  $df = 1$ ,  $p = 0.177$ ) (Table 3). In **Darmstadt** a percentage of  $13\% \pm 5.7\%$  brown moths could be determined for the first flight phase and  $11\% \pm 2.8\%$  for the second flight. There is no significant difference in the proportions of the morphological varieties of the two flight phases ( $\chi^2 = 0.047348$ ,  $df = 1$ ,  $p = 0.8277$ ) (Table 3), so there is no incidence for earlier flight of the melanic moths in spring and no significant surplus of melanic moths observed in the first flight phase.

### Discussion

It is well known, that reliable monitoring is based on a specific, effective and easy applicable trapping system. Pheromone trapping enables a selective observation of a target organism and can provide high catch if the right pheromone is used. One major drawback might be that only males can be lured. LAURENT and FRÉROT (2007) reported problems of monitoring the European corn borer (*Ostrinia nubilalis* Hübner, Crambidae) with pheromone traps. It remained unclear whether flight curves plotted from pheromone trap captures truly reflect moth phenology because of the earlier flight of the *Ostrinia* female moths which cannot be lured by sex pheromone trapping. Additionally blends lose their attractiveness or attract different species if the pheromone components are not combined in the exact suitable proportions. When using light traps there is the major advantage, that both sexes are attracted. This is indispensable to acquire data on biological characteristics, like the timely occurrence and proportions of both sexes in field. Drawbacks are the dependence on electricity, the bulkiness and the attraction of different non-target and beneficial insects. Also maintenance effort is very high.

Table 3. Occurrence of morphological varieties (white and brown) of *C. perspectalis* (proportion [%] and number) during the two flight phases (first: June–July and second: August–October) detected by pheromone trapping (2013–2014) at the two study sites (SE = Seligenstadt, DA = Darmstadt). Asterisks indicate statistical significance<sup>a</sup>

Site/Year	First flight phase			Second flight phase		
	white	brown	$\chi^2$	white	brown	$\chi^2$
SE/2013	77 (34)	23 (10)	13.1*	84 (291)	16 (54)	162.8*
SE/2014	83 (170)	17 (35)	88.9*	91 (316)	9 (33)	229.5*
mean $\pm$ sd	$80 \pm 4.2$	$20 \pm 4.2$		$88 \pm 4.9$	$12 \pm 4.9$	
DA/2013	83 (25)	17 (5)	13.3*	87 (173)	13 (26)	108.6*
DA/2014	91 (230)	9 (22)	108.6*	91 (704)	9 (67)	526.3*
mean $\pm$ sd	$87 \pm 5.7$	$13 \pm 5.7$		$89 \pm 2.8$	$11 \pm 2.8$	

<sup>a</sup> $\chi^2$ -test for given probabilities ( $\chi^2 > 3.8$ ,  $df = 1$ ,  $p < 0.0005$ )

This study provides new information about the moth flight activity of *C. perspectalis* per year and the usability of different trap types to monitor the pest. Evaluations with light and funnel traps at two sites in the Rhine-Main region showed two clearly separated flight phases with both trap types (Fig. 3 and 4). The highest flight activity of this invasive pest occurs in mid July and early September. That agrees with the observed seasonal development of *C. perspectalis* postulated by ZIMMERMANN (2014) and by the CABI (2013) for South Germany. Data on the flight phenology, including the number of generations a year, have also been published for different populations in Asia and Europe (Table 4). Three to five generations were described for eleven different Chinese populations depending on the climatic conditions of the particular regions (WAN et al., 2014). WANG (2008) showed emergence periods of the first, second, third and fourth generation starting on the middle ten days of May, the first ten days of July, the last decade of September and the middle and last ten days of November, respectively. Serious damage has been observed in China from May to September. In Japan, three generations were described for a Tokyo population where adults appeared from mid-May to late June, from late July to late August and from late August to mid-September (MARUYAMA and SHINKAJI, 1987). In Korea two generations were observed, first from early June to late June and second from mid- August to early September (PARK, 2008), similar to the pattern that we found here. In Russia, also two generations were recognized. The first generation remained almost unnoticed, flight of the second generation was observed in the first ten-day period of September (KARPUN and IGNATOVA, 2013). In Europe, two to four generations occur. Three generations are published for Italy (SANTI et al., 2015) and the region of Basel in Switzerland (LEUTHARDT et al., 2010). In warmer areas of Switzerland even four generations per year may occur (KENIS et al., 2013). Populations in northern Switzerland (NACAMBO et al., 2014) and Cro-

atia (MATOŠEVIĆ, 2013) were monitored and two generations per year were described. In Georgia, the exact number of generations could not be defined until now but the flight period was observed in summertime (late July) as well as in late October (MATSIAKH, 2016).

This study also gives first results concerning abundance of the melanic moths and proportions of sex in field populations. There are many insect orders and moth species with natural incidence of melanism, i.e. the occurrence of morphological variants that are mostly or completely dark in pigmentation. Common lepidopteran examples are the Peppered moths (*Biston betularia* Linnaeus, Geometridae) from the UK and the tiger swallow-tails (*Papilio glaucus* Linnaeus, Papilionidae) from Florida (TRUE, 2003). In recent decades a wide range of explanations on the cause of melanic morphs and the effect on fitness have been suggested and discussed. The attention was mainly focussed on the genetic origin and inheritance in connection with changing environmental conditions as well as predation. For example COOK and SACCHERI (2013) reviewed the ideas about industrial melanism and its evolutionary changes in peppered moth populations, because in some cases melanic mutants became dominant. LIU et al. (2015) reported on the association of beet armyworm (*Spodoptera exigua* Hübner, Noctuidae) pupal melanism and fitness heightening. Our results detect no dominance of the melanic moths in the trapped populations; proportions were nearly constant for both trap types, in every year, for both flight phases and at both sites and ranged between 11% and 20% (Table 2 and 3). The mean proportion of melanic moths per year was  $14\% \pm 2.9\%$ . Separated per month, mean proportions (2013 and 2014) had a wider range (between 3% and 27%) but there was no indication on a raised amount of melanic moths in spring after overwintering or in one of the two flight phases.

In most lepidopteran species the appearance of males occurs earlier in the season than of females of the same

**Table 4. Number of *C. perspectalis* generations per year for different countries and regions**

No. of generations	Country	Region	Reference
up to five	China	Zhejiang province	SHE and FENG (2006)
up to four	China	Youxi Fujian	WANG (2008)
three	China	Shanghai	TANG et al. (1990)
three	China	Xian	CHEN et al. (1993)
three	Japan	Tokyo	MARUYAMA and SHINKAJI (1987)
three	Italy	Verona	SANTI et al. (2015)
two	Korea	Seoul	PARK (2008)
two	Russia	Sochi	KARPUN and IGNATOVA (2013)
two	Switzerland	Northern	NACAMBO et al. (2014)
up to four	Switzerland	Southern	KENIS et al. (2013)
two	Croatia	Varaždin	MATOŠEVIĆ (2013)
three	Switzerland	Basel	LEUTHARDT et al. (2010)

species (protandry) (WIKLUND and FAGERSTRÖM, 1977; IWASA et al., 1983; ZONNEVELD, 1992). WIKLUND and FAGERSTRÖM (1977) advanced hypotheses which explain the incidence of protandry, indicating that males emerge before females to maximize reproductive success. Early occurring males seem to stand a greater chance of mating with one or several virgin females than do males emerging later, maintaining females mate only once (monandry), whereas males are capable of multiple matings (polyandry). But earlier emergence of females (protogyny) additionally occurs among lepidopteran species, for example in the mating system of the diamondback moth (*Plutella xylostella* Linnaeus, Yponomeutidae) (UEMATSU and MORIKAWA, 1997). Thus, it is being also part of the complex matter of sexual selection and mating systems of individual lepidopteran species. In our study, one aim was to examine whether *C. perspectalis* females may do begin their flight earlier than males and furthermore to assess if there are differences in the temporal flight sequence of the sexes. First females were recorded together with first males in the light trap and the mean proportion (2012–2014) of females during the first flight phase was  $46\% \pm 17\%$  and  $40\% \pm 3.8\%$  during the second. Thus, we can assume that obviously no protogyny occurs and flight curves plotted from pheromone trap captures truly reflect the exact moth flight of both sexes.

The present findings regarding the use of light and pheromone traps clarified the usability of pheromone traps to survey the seasonal flight activity of *C. perspectalis*. It can be stated that both trap systems are suitable for exact observation. The additional consideration of the females by using light traps is not necessary.

The flight monitoring by traps lead to conclude two completed generations per year and additionally can support the early detection of egg deposition and larval feeding phases. Thus, diverse control measures can be adapted to the occurring developmental stage in field, for example releasing egg parasitoids like *Trichogramma* wasps (Trichogrammatidae) during the time of egg deposition or to time insecticides precisely that are directed to first larval instars. This can reduce dispensable insecticide applications, resulting in a more efficient pest management system. Additionally, the documentation of the regular occurrence of melanic moths provided useful insights into the biology of this invasive organism.

### Acknowledgements

We would like to express our gratitude towards the Arthur and Aenne Feindt-Foundation (Hamburg), for the generous support of this work as a part of the project “Development of friendly methods for monitoring and regulating the box tree pyralid, *Cydalima perspectalis* (Lepidoptera: Crambidae), an invasive pest in ornamentals”. Furthermore we would like to thank Uwe KRIENKE, head of the Convent Garden Seligenstadt and his gardener’s team for maintaining the light trap. We sincerely

thank Frans GRIEPINK, director of Pherobank B.V. (Wijk bij Duurstede, The Netherlands) for providing the pheromone lures. We also would like to thank Simon FEIERTAG (JKI Darmstadt) for his technical assistance.

### References

- ALBERT, R., T. LEHNEIS, 2010: Der Buchsbaumzünsler, ein neuer Problemschädling in Baden-Württemberg [The Box tree moth, a new pest causing problems in Baden-Württemberg]. Landinfo. 3/10, 40-45.
- BILLEN, W., 2007: *Diaphania perspectalis* (Lepidoptera: Pyralidae) – ein neuer Zünsler in Europa. [*Diaphania perspectalis* (Lepidoptera: Pyralidae) – a new Pyralidae in Europe]. Mitteilungen der Entomologischen Gesellschaft Basel 57 (2-4), 135-139.
- CABI, 2013: Centre for Agriculture and Biosciences International. Annual Report 2012 (Biological control of the box tree caterpillar, *Cydalima perspectalis*) Delémont, Switzerland, 52.
- CHEN, X., Z. ZHANG, Y. ZHANG, 1993: Studies on the spatial distribution model, biology and control of box-tree pyralid. Scientia Silvae Sinicae 29, 77-80 (summary).
- COOK, L.M., I.J. SACCHERI, 2013: The peppered moth and industrial melanism: evolution of a natural selection case study. Heredity (Edinb.) 110 (3), 207-212.
- DE JONG, P.W., W.S. GUSSEKLOO, P.M. BRAKEFIELD, 1996: Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spotted ladybird beetles (*Adalia bipunctata*) under controlled conditions. Journal of Experimental Biology 199, 2655-2666.
- DI DOMENICO, F., F. LUCCHESI, D. MAGRI, 2012: *Buxus* in Europe: Late Quaternary dynamics and modern vulnerability. Perspectives in Plant Ecology, Evolution and Systematics 14 (5), 354-362.
- IWASA, Y., F.J. ODENDAAL, D.D. MURPHY, P.R. EHRlich, A.E. LAUNER, 1983: Emergence patterns in male butterflies: A hypothesis and a test. Theoretical Population Biology 23, 363-379.
- KARPUN, N.N., Y.A. IGNATOVA, 2013: The first report about *Cydalima perspectalis* Walker on the black sea coast of Russia. The State Research Institution All-Russian Scientific and Research Institute of Floriculture and Subtropical Crops of the Russian Academy of Agricultural Sciences, Sochi, Russia, www.rusnauka.com/31\_NNM\_2013/Biologia/7\_146134.doc.htm, Retrieved on 28.10.2016.
- KAWAZU, K., H. HONDA, S. NAKAMURA, T. ADATI, 2007: Identification of sex pheromone components of the box tree pyralid, *Glyphodes perspectalis*. Journal of chemical ecology 33 (10), 1978-1985.
- KENIS, M., S. NACAMBO, F.L.G. LEUTHARDT, F. DI DOMENICO, T. HAYE, 2013: The box tree moth, *Cydalima perspectalis*, in Europe: horticultural pest or environmental disaster? Aliens 33, 38-41.
- KIM, J., I.-K. PARK, 2013: Female sex pheromone components of the box tree pyralid, *Glyphodes perspectalis*, in Korea: Field test and development of film-type lure. Journal of Asia-Pacific Entomology 16, 473-477.
- KRÜGER, E.O., 2008: *Glyphodes perspectalis* (Walker, 1859) – neu für die Fauna Europas (Lepidoptera: Crambidae) [*Glyphodes perspectalis* (Walker, 1859) - new for the European fauna (Lepidoptera: Crambidae)]. Entomologische Zeitschrift 118, 81-83.
- LAURENT, P., B. FRÉROT, 2007: Monitoring of European Corn Borer with Pheromone-Baited Traps: Review of Trapping System Basics and Remaining Problems. Journal of Economic Entomology 100 (6), 1797-1807.
- LEUTHARDT, F.L.G., W. BILLEN, B. BAUR, 2010: Ausbreitung des Buchsbaumzünslers *Diaphania perspectalis* (Lepidoptera, Pyralidae) in der Region Basel – eine für die Schweiz neue Schädlingart [Spread of the Box-Tree Pyralid *Diaphania perspectalis* (Lepidoptera: Pyralidae) in the region of Basel - a pest species new for Switzerland] Entomo Helvetica 3, 51-57.
- LIU, S., M. WANG, X. LI, 2015: Pupal melanization is associated with higher fitness in *Spodoptera exigua*. Scientific Report 5, 10875.
- MALLY, R., M. NUSS, 2010: Phylogeny and nomenclature of the box tree moth, *Cydalima perspectalis* (Walker, 1859) comb. n., which was recently introduced into Europe (Lepidoptera: Pyraloidea: Crambidae: Spilomelinae). European Journal of Entomology 107 (3), 393-400.
- MARUYAMA, T., N. SHINKAJI, 1987: Studies on the life cycle of the box-tree pyralid, *Glyphodes perspectalis* (Walker) (Lepidoptera: Pyralidae). I. Seasonal adult emergence and developmental velocity. Japanese Journal of Applied Entomology and Zoology 31, 226-232 (in Japanese with english summary).



- MATOŠEVIĆ, D., 2013: Box tree moth (*Cydalima perspectalis*, Lepidoptera; Crambidae), new invasive insect pest in Croatia. South-East European Forestry 4 (2), 89-94.
- MATSIKHI, I., 2016: Assessment of Pests and Diseases in Native Boxwood Forests of Georgia-Final report. Tbilisi, Forestry Department, Ukrainian National Forestry University (Lviv).
- NACAMBO, S., F.L.G. LEUTHARDT, H. WAN, H. LI, T. HAYE, B. BAUR, R.M. WEISS, M. KENIS, 2014: Development characteristics of the box-tree moth *Cydalima perspectalis* and its potential distribution in Europe. Journal of Applied Entomology 138 (1-2), 14-26.
- PAN, S.-B., Q.-H. LUO, J.-K. LONG, Z.-Y. ZHANG, D.-Z. MANG, M. SHU, 2011: Discovery of melanous adult of *Diaphania perspectalis* in Guizhou, China and preliminary survey of the wing colour stripes change of normal adults. Chinese Journal of Applied Entomology 48 (1), 212-213 (in Chinese).
- PARK, I., 2008: Ecological characteristics of *Glyphodes perspectalis*. Korean Journal of Applied Entomology 47 (3), 299-301 (in Korean with english summary).
- SÁFIÁN, S., B. HORVÁTH, 2011: Box tree moth - *Cydalima perspectalis* (Walker, 1859), new member in lepidoptera fauna of Hungary (Lepidoptera: Crambidae). Natura Somogyiensis 19, 245-246.
- SANTI, F., P. RADEGHIERI, G. SIGURTA, S. MAINI, 2015: Sex pheromone traps for detection of the invasive Box tree moth in Italy. Bulletin of Insectology 68 (1), 158-160.
- SHE, D., F. FENG, 2006: Bionomics and Control of *Diaphania perspectalis*. Journal of Zhejiang Forestry Science and Technology 26 (6), 59 (in Chinese with english summary).
- SZÉKELY, L., 2011: *Cydalima perspectalis* (Walker, 1859), a new species for the Romanian fauna (Lepidoptera: Crambidae: Spilome-linae). Buletin de informare entomologică 22 (3-4), 73-77.
- TANG, S., H. QIN, W. SUN, 1990: Studies on the bionomics of *Diaphania perspectalis*. Journal of Shanghai Agricultural College 8 (4), 312 (summary).
- TRUE, J.R., 2003: Insect melanism: the molecules matter. Trends in Ecology & Evolution 18 (12), 640-647.
- UEMATSU, H., R. MORIKAWA, 1997: Protogyny in diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Japanese Journal of Applied Entomology and Zoology 41 (4), 217-223 (in Japanese with english summary).
- VALLES, S.M., J.L. CAPINERA, P.E.A. TEAL, 1991: Evaluation of pheromone trap design, height, and efficiency for capture of male *Diaphania nitidalis* (Lepidoptera: Pyralidae) in a field cage. Environmental entomology 20 (5), 1274-1278.
- WALKER, F., 1859: Part XVIII. Pyralides – List of Specimens of Lepidopterous Insects in the Collection of the British Museum. London, British Museum (Natural History), Departement of Zoology 18, 509-798.
- WAN, H., T. HAYE, M. KENIS, S. NACAMBO, H. XU, F. ZHANG, H. LI, 2014: Biology and natural enemies of *Cydalima perspectalis* in Asia: Is there biological control potential in Europe? Journal of Applied Entomology 138 (10), 715-722.
- WANG, Y.-M., 2008: The biological character and control of a new pest (*Diaphania perspectalis*) on *Murraya paniculata*. Journal of Fujian Forestry Science and Technology 35 (4), 161-164 (in Chinese with english summary).
- WIKLUND, C., T. FAGERSTRÖM, 1977: Why do males emerge before females. Oecologia 31, 153-158.
- ZIMMERMANN, O., 2014: Hinweise zur Pflanzengesundheit – Buchsbaumzünsler [Notes on plant health – Box tree moth]. Landwirtschaftliches Technologiezentrum Augustenberg (LTZ).
- ZONNEVELD, C., 1992: Polyandry and Protandry in Butterflies. Bulletin of Mathematical Biology 54 (6), 957-976.