Comparison of some aspects of the bionomy of *Ostrinia nubilalis* Hbn. (Lep., Crambidae) on Bt and non-Bt maize in south-eastern Poland

Porównanie wybranych elementów bionomii *Ostrinia nubilalis* Hbn. (Lep., Crambidae) na kukurydzy Bt i non-Bt w południowo-wschodniej Polsce

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ABSTRACT

A study on the dynamics of the oviposition and hatching of *Ostrinia nubilalis* Hbn. larvae on transgenic maize line MON 810 (DKC 3421YG cultivar) and isogenic maize (DKC 3420 cultivar) was carried out in 2008-2011 in Głuchów (south-eastern Poland). No significant differences were found between the number of egg clusters deposited by *O. nubilalis* on Bt and non-Bt maize based on the carried out observations. A small, statistically significant difference in the number of egg clusters between the studied cultivars was found only in 2011 and was most likely associated with weather changes. Female *O. nubilalis* did not significantly prefer any of the cultivars when depositing eggs. In addition, we found no differences in the dynamics of oviposition and larvae hatching between Bt and non-Bt maize. The first egg clusters, their maximum number, and the last egg clusters were found on similar dates for both studied cultivars. Empty egg clusters proving larval hatching were also found on similar dates for Bt and non-Bt maize.

Key words: *Ostrinia nubilalis*, seasonal dynamics, Bt, non-Bt, maize, oviposition

STRESZCZENIE

Badania nad dynamiką składania jaj i wylęgu gąsienic *Ostrinia nubilalis* Hbn. na kukurydzy transgenicznej linii MON 810 (odmiana DKC 3421YG) i kukurydzy izogenicznej (odmiana DKC 3420) wykonano w latach 2008-2011 w Głuchowie (południowo-wschodnia Polska). Na podstawie wykonanych obserwacji nie stwierdzono istotnych różnic w liczbie złóż jaj składanych przez *O. nubilalis* na kukurydzy Bt i non-Bt. Niewielka, statystycznie istotna różnica w liczbie złóż jaj pomiędzy badanymi odmianami wystąpiła jedynie w 2011 roku, co było najprawdopodobniej związane z przebiegiem warunków meteorologicznych. Samice *O. nubilalis* nie wykazały istotnej preferencji w składaniu większej liczby jaj na żadnej z odmian. Nie stwierdzono także różnic w dynamice składania jaj oraz wylęgu gąsienic na kukurydzy Bt i non-Bt. Terminy pojawu pierwszych złóż jaj na roślinach, maksimum ich liczebności oraz koniec występowania były podobne na obu badanych odmianach. Również terminy występowania pustych złóż jaj świadczące o wylęgu gąsienic były podobne na kukurydzy Bt i non-Bt.

Słowa kluczowe: *Ostrinia nubilalis*, dynamika występowania, Bt, non-Bt, kukurydza, składanie jaj

DETAILED ABSTRACT

Celem badań było porównanie preferencji samic *Ostrinia nubilalis* Hbn. do składania jaj na kukurydzy transgenicznej linii MON 810 wytwarzającej toksyczne dla gatunku białko Cry 1Ab oraz na kukurydzy izogenicznej nie chronionej insektycydami. Wyznaczono dynamikę składania jaj oraz wylęgu gąsienic omacnicy prosowianki na obu rodzajach kukurydzy reprezentowanych przez dwie odmiany średniowczesne (FAO 250): DKC 3420 (non-Bt) oraz DKC 3421YG (Bt).
W latach 2008-2011samice omacnicy prosowianki na 400 oznakowanych i każdorazowo analizowanych 2-3 razy w tygodniu roślinach kukurydzy złożyły w sezonie wegetacyjnym od 114 (2011 rok) do 280 (2008 rok) złóż jaj. Najwięcej złóż jaj (powyżej 200 sztuk) na obu badanych odmianach kukurydzy stwierdzono w 2008 i 2010 roku, natomiast najmniej w 2011 roku, na co duży wpływ miały warunki meteorologiczne.
Szczegółowa analiza statystyczna wyników z poszczególnych lat wykazała, że jedynie w 2011 roku średnia liczba złóż jaj *O. nubilalis* stwierdzona na odmianie Bt była istotnie niższa w stosunku do odmiany non-Bt średnio o -0,26 sztuki; na co najprawdopodobniej wpłynęły warunki pogodowe w postaci intensywnych i przedłużających się opadów deszczu. Biorąc pod uwagę średnie wyniki z czterech lat badań nie stwierdzono istotnej różnicy w preferencji omacnicy prosowianki do składania jaj na żadnej z odmian. Średnia liczba złóż jaj *O. nubilalis* stwierdzona na odmianie DKC 3420 i na odmianie DKC 3421YG w analizowanym czteroleciu była na porównywalnym poziomie, wynoszącym odpowiednio 1,68 i 1,64 sztuki.
Terminy pojawu pierwszych złóż jaj na roślinach, maksimum ich liczebności oraz koniec występowania na roślinach kukurydzy izogenicznej i transgenicznej były zbliżone. Pierwsze jaja stwierdzano w drugiej lub na początku trzeciej dekady czerwca. Maksimum ich liczebności przypadało w drugiej dekadzie lipca, a jedynie w 2011 roku na początku trzeciej dekady tego miesiąca. Ostanie złoża jaj na roślinach samice omacnicy prosowianki składały do trzeciej dekady sierpnia, a jedynie w 2011 roku do pierwszych dni września.
Nie stwierdzono istotnych różnic w dynamice składania złóż jaj na analizowanych odmianach. Analiza statystyczna wykazała, że niezależnie od terminu oceny liczba złożonych jaj przez omacnicę prosowiankę na odmianie non-Bt i Bt była porównywalna (nieistotna interakcja czas × odmiana, wysoka wartość $p = 0,91$).
Na podstawie obserwacji obecności pustych złóż jaj na roślinach, nie stwierdzono wyraźnych różnic w terminach wylęgu gąsienic *O. nubilalis* na odmianie izogenicznej oraz transgenicznej. Pierwsze puste osłonki jajowe stwierdzano w trzeciej dekadzie czerwca. Maksimum ich liczebności świadczące o masowym wylęgu gąsienic w

stadium rozwojowym L1 przypadało zwykle w drugiej lub w trzeciej dekadzie lipca. Ostatnie puste złoża jaj stwierdzano od trzeciej dekady sierpnia do drugiej dekady września.

INTRODUCTION

The European corn borer (ECB) (*Ostrinia nubilalis* Hbn.; Lep., Crambidae) is one of the major maize pests (*Zea mays* L.) in Europe and North America. According to Mason et al. (1996) and Calvin and Van Duyn (1999), in North America annual economic loss in maize yield caused by the feeding larvae of this pest, and the costs for their chemical control are estimated at one billion USD. The level of loss largely depends on the number of pest generations developing within a year, and this may be from one to six (Calvin and Van Duyn, 1999).

In Poland *O. nubilalis* has been found on maize since 1950 (Kania, 1962), and it produces one or sometimes two generations in a year (Dubniak and Kania, 1961; Lisowicz and Tekiela, 2004; Żołnierz and Hurej, 2007; Bereś, 2012a). In regions with intense maize cultivation ECB larvae damage up to 80% plants (locally up to 100%), causing a 40% loss in maize grain yield (Lisowicz and Tekiela, 2004).

Because of the great harmfulness of the pest, farmers control it using various chemical and non-chemical methods, which are recommended in integrated plans for maize protection (Lisowicz and Kot, 1999; Mazurek and Hurej, 1999; Lisowicz, 2003; Bereś and Lisowicz, 2005; Bereś, 2008; Bereś and Pruszyński, 2008).

In some countries one of the methods to prevent damage caused by *O. nubilalis*, and to ensure high maize yield and grain quality involves the cultivation of transgenic cultivars from the MON 810 line that synthesize Cry1Ab protein toxic to *Lepidoptera* pests over the entire maize vegetation period (Ostlie, et al., 1997; Baute, et al., 2002). In 2009-2011 the official acreage of GMO maize in Poland was stable and accounted for about 3,000 ha (Report, 2010; 2011; 2012), while studies on the resistance of MON 810 maize to damage caused by ECB have been carried out since 2006 (Bereś and Gabarkiewicz, 2007; Haliniarz and Bojarczyk, 2007; Twardowski, et al., 2008; Bereś, 2010). However, previous experiments did not include detailed observations on the effect of MON 810 maize on the selected aspects of the bionomy of *O. nubilalis.*

The presented studies were focused on potential differences in: (a) preference or non-preference of the ECB females for oviposition, (b) dynamics of egg laying by *O. nubilalis* females, and (c) larval hatching on the GM (cv. DKC 3421YG) versus the non-GM (cv. DKC 3420) maize cultivars.

MATERIAL AND METHODS

Studies were carried out in 2008-2011 in Głuchów, near Łańcut (south-eastern Poland), on a 4 ha field of fodder maize. The experiment was established using the random block method in four replications on 8 plots, 1,600 m^2 (40 x 40 m) each. Observations were carried out on isogenic maize cultivar DKC 3420 (FAO 250), not protected with insecticides, and on transgenic maize line MON 810, cultivar DKC 3421YG (FAO 250) synthesizing Cry1Ab protein toxic to *Lepidoptera* pests.

On each plot in five sites we marked 20 plants diagonally in a row, and inspected them 2-3 times a week, starting from June. In total 400 marked plants of each cultivar were inspected each time. We searched for new egg clusters deposited by ECB and recorded the number of empty egg shells, signifying larval hatching. A cluster was considered empty if larvae hatched from 90% or more eggs. Observations ended when there were no newly hatched pests from identified egg clusters for 2-3 weeks. To prevent duplicate counting each newly found egg cluster was marked with a waterproof marker. Each new empty egg cluster was marked in the same way.

Changes in the number of egg clusters and empty egg shells on Bt and non-Bt maize in different maize developmental stages according to BBCH scale (Adamczewski and Matysiak, 2011) were presented as a mean number calculated for 100 plants.

We carried out a statistical analysis of results obtained for the number of egg clusters deposited on transgenic and isogenic maize. We analyzed variance for the repeated measures model, where the maize cultivar (DKC 3420 and DKC 3421YG) was an experimental factor, and time was the factor of repeated measures. The time factor was 31 dates on which the number of ECB egg clusters were measured on maize within the vegetation period. The significance of differences was analyzed by the F-test (Fisher-Snedecor) and Tukey's test at p = 0.05. All calculations were carried out using Statistica© 10.0 PL software (GLM – ANOVA model with repeated measures).

Meteorological data for the test area were obtained from the Institute of Meteorology and Water Management of the National Research Institute in Jasionka near Rzeszów situated 15 km north of the test field.

RESULTS

Changes in weather conditions during the study period were presented only for the months when the flight of *Ostrinia nubilalis* moths, oviposition and larvae hatching (developmental stage L1) took place.

In 2008-2011 weather conditions (temperature and precipitation) varied significantly, but were the least favourable for moth flight and oviposition in 2008-2009. Although in both years there was a relatively high number of very windy days, and in 2008 there was also intense rainfall, these factors did not limit the flight of ECB moths or oviposition considerably. However, particularly unfavourable weather conditions for pest development were recorded in a part of 2010, and throughout 2011. In both these years, in June, July and early August (2010) there was heavy rainfall, which disturbed moth flight, oviposition and larvae hatching (Table 1).

Table 1. Weather conditions in 2008-2011 in Głuchów
Tabela 1. Warunki meteorologiczne w latach 2008-2011 w Głuchowie

Month Miesiąc	Decade Dekada	Daily average air temperature [°C] Średnia dobowa temperatura powietrza [°C]				Rainfall sum [mm] Suma opadów [mm]				Number of days with precipitation Liczba dni z opadami deszczu			
		2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
June Czerwiec	I	17.9	14.9	18.0	19.6	1.4	17.8	102.6	29.2	1	5	4	6
	II	16.2	15.2	18.7	17.4	40.0	77.9	20.9	23.2	3	7	4	6
	III	19.7	19.3	16.9	17.2	45.3	50.7	2.6	36.1	6	6	2	6
Mean monthly – Średnia miesięczna		17.9	16.4	17.8	18.0	–	–	–	–	–	–	–	–
Sum monthly – Suma miesięczna		–	–	–	–	86.7	146.4	126.1	88.5	10	18	10	18
July Lipiec	I	17.8	20.0	19.2	16.5	35.9	65.4	73.5	96.5	6	6	4	9
	II	19.2	20.1	23.7	18.8	43.3	9.0	9.2	82.2	6	4	2	6
	III	19.1	19.9	19.3	17.3	38.4	23.6	117.5	55.1	6	4	7	9
Mean monthly – Średnia miesięczna		18.7	20.0	20.7	17.5	–	–	–	–	–	–	–	–
Sum monthly – Suma miesięczna		–	–	–	–	117.5	98.0	200.2	233.8	18	14	13	23
August Sierpień	I	19.6	19.6	20.7	18.2	21.2	8.1	4.7	15.7	4	2	2	4
	II	19.9	18.2	20.6	19.0	18.2	0.8	33.7	12.9	3	1	5	5
	III	17.3	18.3	17.0	21.5	15.9	12.9	60.2	0.7	4	4	7	1
Mean monthly – Średnia miesięczna		18.9	18,7	19.4	19.5	–	–	–	–	–	–	–	–
Sum monthly – Suma miesięczna		–	–	–	–	55.3	21.8	98.6	29.3	11	7	14	10
September Wrzesień	I	19.3	15.9	11.6	15.7	5.0	22.7	55.1	8.3	2	5	8	3
	II	9.0	15.5	12.7	16.3	84.1	0.0	13.7	0.3	9	0	5	2
	III	10.7	14.2	12.0	13.4	14.1	2.8	28.7	0.0	5	1	5	0
Mean monthly – Średnia miesięczna		13.0	15.2	12.1	15.1	–	–	–	–	–	–	–	–
Sum monthly – Suma miesięczna		–	–	–	–	103.2	25.5	97.5	8.6	16	6	18	5

In 2008 the first egg clusters of *O. nubilalis* were found on the DKC 3421YG cultivar on 21 June, when maize had 9 leaves developed (BBCH 19), while on the DKC 3420 cultivar this was on 24 June. From the third decade of June the number of newly deposited egg clusters increased rapidly, and reached its maximum on 11 June, when plants were developing tassels (BBCH 51). The population peak was followed by a gradual decrease in the number of newly deposited egg clusters, both on isogenic and transgenic cultivars. The last eggs were found in the third decade of August, when plants were at the early stage of kernel maturity (BBCH 83) (Figure 1).

In total in 2008 females of *O. nubilalis* deposited 280 egg clusters on 400 analyzed plants of non-Bt, and 274 egg clusters on Bt maize (Table 2).

Observations on the dates of ECB larvae hatching from the deposited eggs demonstrated that in 2008 the first empty egg shells occurred on plants of both studied cultivars on 30 June, when maize was developing the first node (BBCH 31). The highest number of empty egg clusters demonstrating mass hatching of the pest was recorded on 19 July, when plants began flowering (BBCH 61). The last empty egg shells were found on both cultivars on 1 September, when plants were at the stage of full kernel maturity (BBCH 85) (Figure 1).

In total in 2008 on 400 analyzed plants of the non-Bt cultivar, larvae of *O. nubilalis* hatched from 276 egg clusters, and from 260 egg clusters on the Bt cultivar. We found that on plants of the isogenic cultivar larvae failed to hatch from 4 egg clusters, and from 14 egg clusters on the transgenic cultivar. This concerned the total number of egg clusters deposited in the relevant vegetation season. These egg clusters were either destroyed by natural predators (the presence of Trichogramma spp. and Orius spp. was observed) or fell on the ground because of the effect of weather conditions (Table 2).

In 2009 the first egg clusters of *O. nubilalis* were found on both cultivars on 17 June, when plants had 7 leaves developed (BBCH 17). Because of the intense rainfall that had occurred at the end of June and in the first days of July, the number of new egg clusters increased slowly. Yet when weather conditions improved from the second half of July, the number of deposited egg clusters increased and reached its maximum in the first half of July. During that time maize plants were developing tassels (BBCH 51). The last eggs were found in the third decade of August, when plants were at the early stage of kernel maturity (BBCH 83) (Figure 2).

In 2009, for all 400 analyzed plants, ECB females deposited 172 egg clusters on the non-Bt cultivar, and 203 egg clusters on Bt cultivar (Table 2).

The first empty egg shells were found in 2009 on both studied cultivars on 22 June, when maize had 9 leaves developed (BBCH 19). The number of egg clusters increased slowly but steadily, reaching a single peak when plants began shooting tassels (BBCH 61). The last empty egg shells were found on the Bt cultivar on 31 August, and on the non-Bt cultivar on 6 September, when plants were at the stage of kernel maturity (BBCH 85) (Figure 2).

A comparison of the number of empty egg shells versus the total number of eggs deposited on 400 marked plants demonstrated that in 2009, on the DKC 3420 cultivar, larvae hatched from 157 out of all 172 found egg clusters, while on the DKC 3421YG cultivar from 191 out of all 203 egg clusters deposited by *O. nubilalis* females. Larvae did not hatch from 15 egg clusters on the non-Bt cultivar and from 12 egg clusters on the Bt cultivar (Table 2).

In 2010 female ECB began oviposition at the end of the second decade of June, when plants had 9 leaves developed (BBCH 19). Because of intense rainfall in early July, the increase in the number of newly deposited egg clusters was slow and reached its maximum for both cultivars on 16 July, when plants were developing tassels (BBCH 53). The last eggs were found on both cultivars in the third decade of August, when plants were at the early stage of kernel maturity (BBCH 83) (Figure 3).

In 2010, on all 400 analyzed plants, we found 223 egg clusters on the DKC 3420 cultivar, and 210 egg clusters on the DKC 3421YG cultivar (Table 2).

In 2010 the first empty egg clusters were found in the third decade of June, when plants had the first node developed (BBCH 31). The increase in the number of empty egg clusters was gradual and reached its maximum for both cultivars on 23 July, when maize plants began shedding pollen (BBCH 63). In the analyzed year the last empty egg clusters of ECB were found in the first decade of September, when maize plants were at the stage of kernel maturity (BBCH 85) (Figure 3).

In 2010, on 400 plants of each cultivar, the larvae of *O. nubilalis* hatched from 214 out of 223 of all deposited egg clusters on the non-Bt cultivar, and from 205 out of 210 egg clusters on the Bt cultivar. The difference between the number of egg clusters deposited by females and the number from which larvae hatched successfully (the same as in previous years), resulted from the effect of natural enemies (mainly Trichogramma spp.) and eggs falling on the ground (Table 2).

In 2011 the first egg clusters of ECB were found on the plants on 13 June, when maize was developing leaf 6 (BBCH 16). This was the earliest date in the analyzed four-year period. The increase in the number of newly deposited egg clusters on both cultivars was irregular, and it was affected by intense rainfall in June and July. Because of the weather conditions the maximum number of egg clusters on plants was recorded on the latest date in the analyzed four-year period, and the number of egg clusters was the lowest. The maximum number of egg clusters was recorded in the third decade of July, when plants were shedding pollen (BBCH 65). Unfavourable weather conditions significantly prolonged the oviposition period for moths. The last egg clusters were found on 30 August on the Bt cultivar and on 3 September on the non-Bt cultivar, when plants were at the stage of full kernel maturity (BBCH 85) (Figure 4).

In total in 2011, on 400 analyzed plants, females of *O. nubilalis* deposited 148 egg clusters on the non-Bt cultivar, and 114 egg clusters on the Bt cultivar (Table 2).

In 2011 the first empty egg clusters were found in the third decade of June, when plants were developing leaf 9 (BBCH 19). The number of egg clusters in the next few days increased slightly and reached a small peak in the third decade of July, when plants were shedding pollen intensely (BBCH 67). After that period we recorded a gradual decrease in the number of empty egg shells on both tested cultivars. The last empty egg shells were found in the first half of September, when plants were at the stage of full kernel maturity (BBCH 85) (Figure 4).

In 2011, on 400 analyzed plants, larvae hatched from 135 out of 148 recorded egg clusters on the DKC 3420 cultivar, and from 105 out of 114 egg clusters deposited by females on the DKC 3421YG cultivar. Larvae did not hatch from 13 egg clusters on the non-Bt cultivar and from 9 egg clusters on the Bt cultivar. Similar to previous years, egg clusters of *O. nubilalis* were destroyed by natural enemies or fell on the ground due to weather conditions (Table 2).

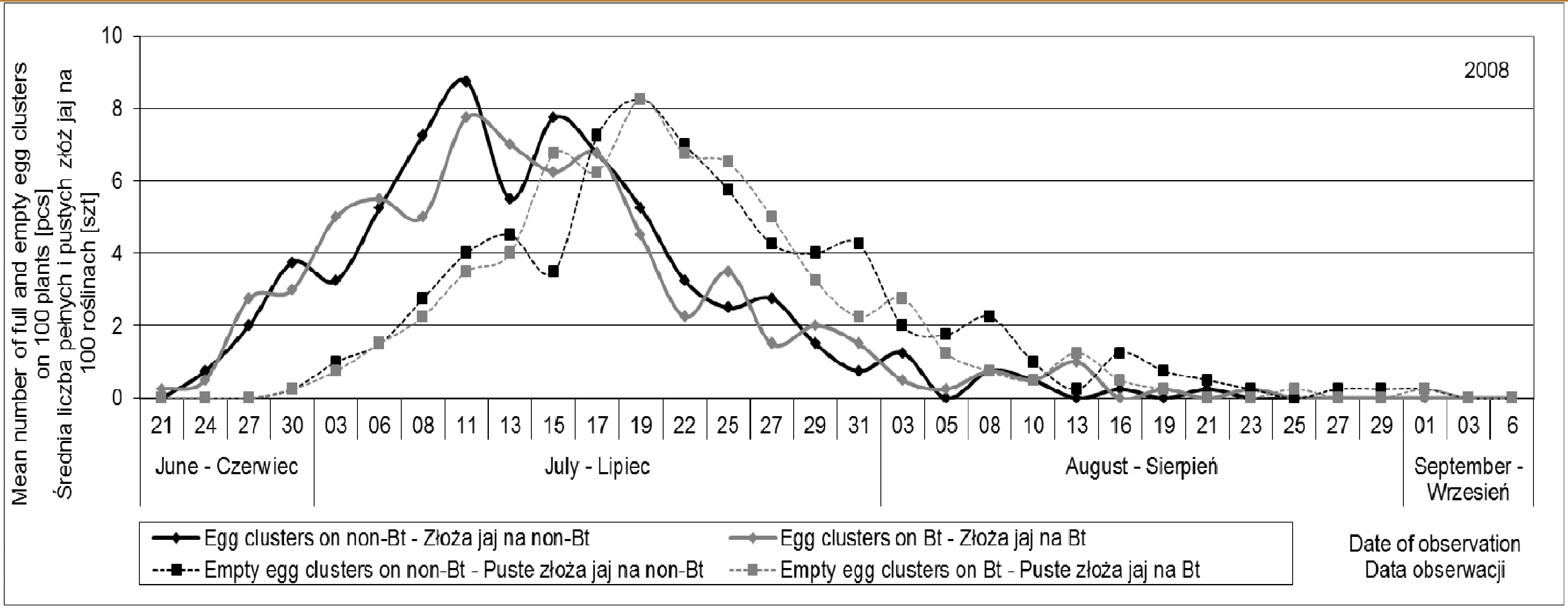

Figure 1. Dynamics of oviposition and larvae hatching for *Ostrinia nubilalis* on non-Bt and Bt maize in 2008
Rysunek 1. Dynamika składania jaj i wylęgu gąsienic *Ostrinia nubilalis* na kukurydzy non-Bt i Bt w 2008 roku

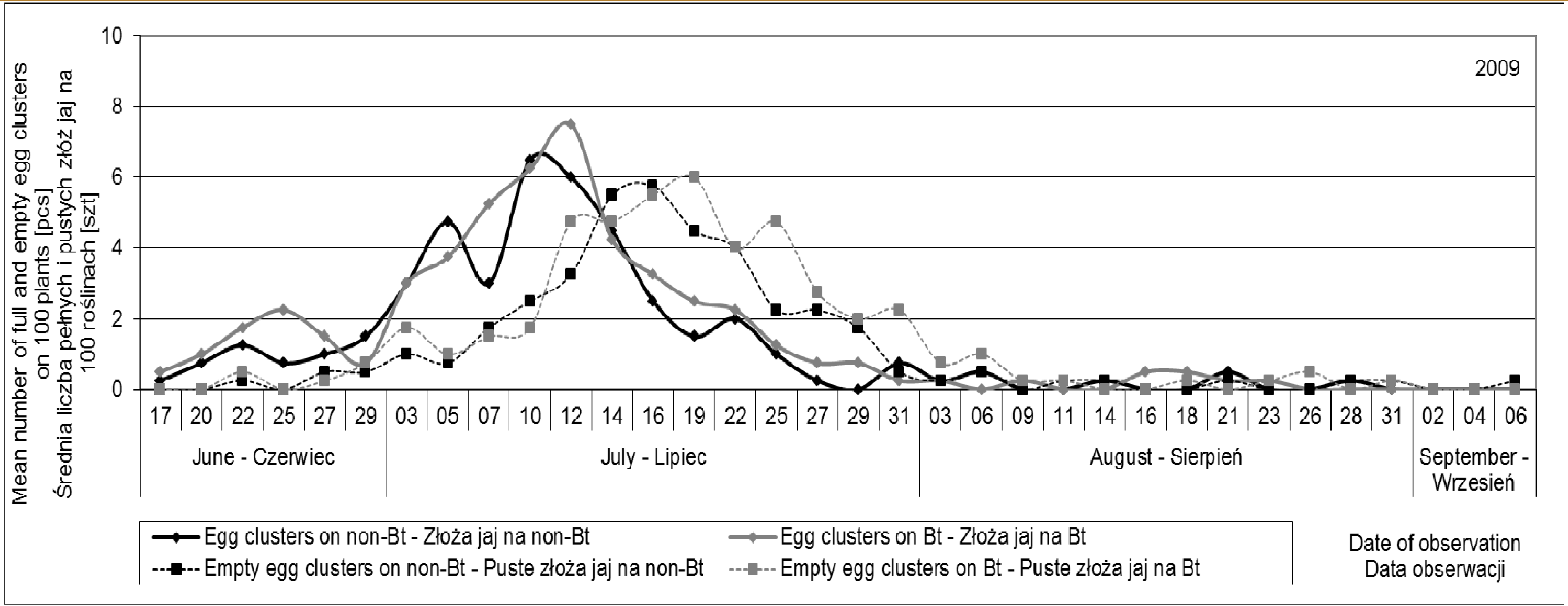

Figure 2. Dynamics of oviposition and larvae hatching for *Ostrinia nubilalis* on non-Bt and Bt maize in 2009
Rysunek 2. Dynamika składania jaj i wylęgu gąsienic *Ostrinia nubilalis* na kukurydzy non-Bt i Bt w 2009 roku

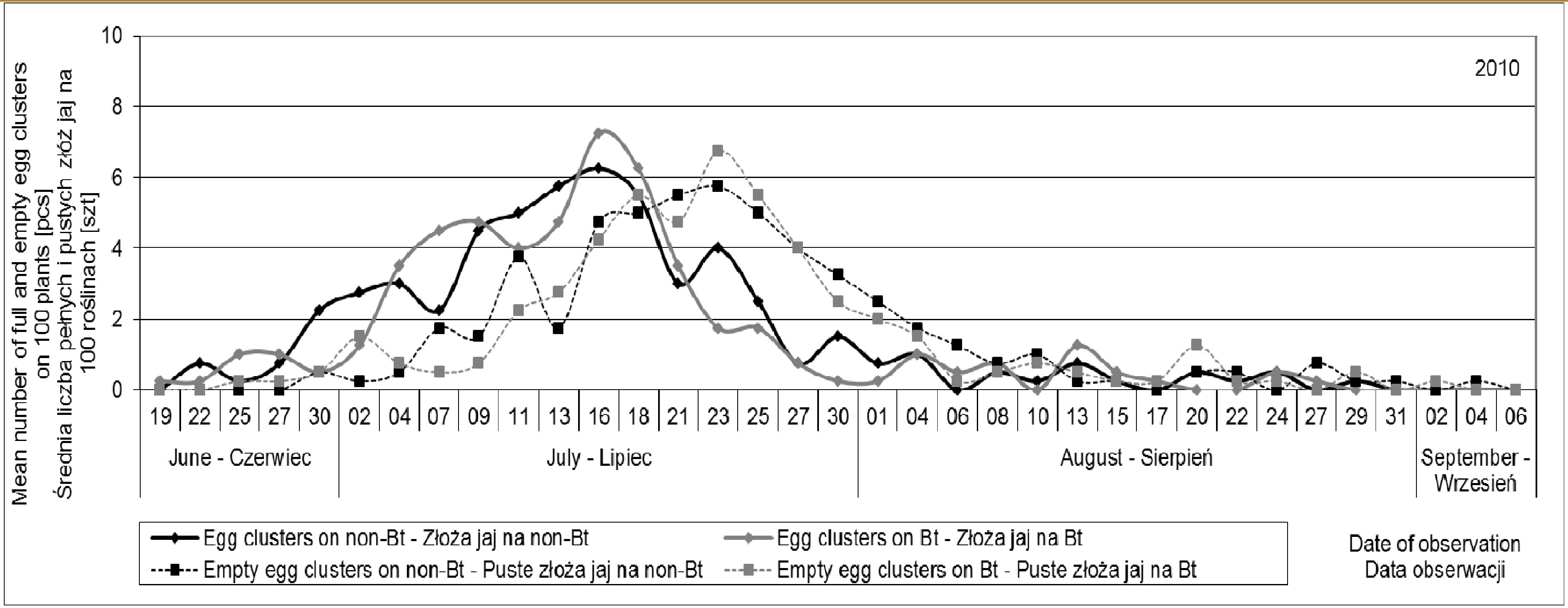

Figure 3. Dynamics of oviposition and larvae hatching for *Ostrinia nubilalis* on non-Bt and Bt maize in 2010
Rysunek 3. Dynamika składania jaj i wylęgu gąsienic *Ostrinia nubilalis* na kukurydzy non-Bt i Bt w 2010 roku

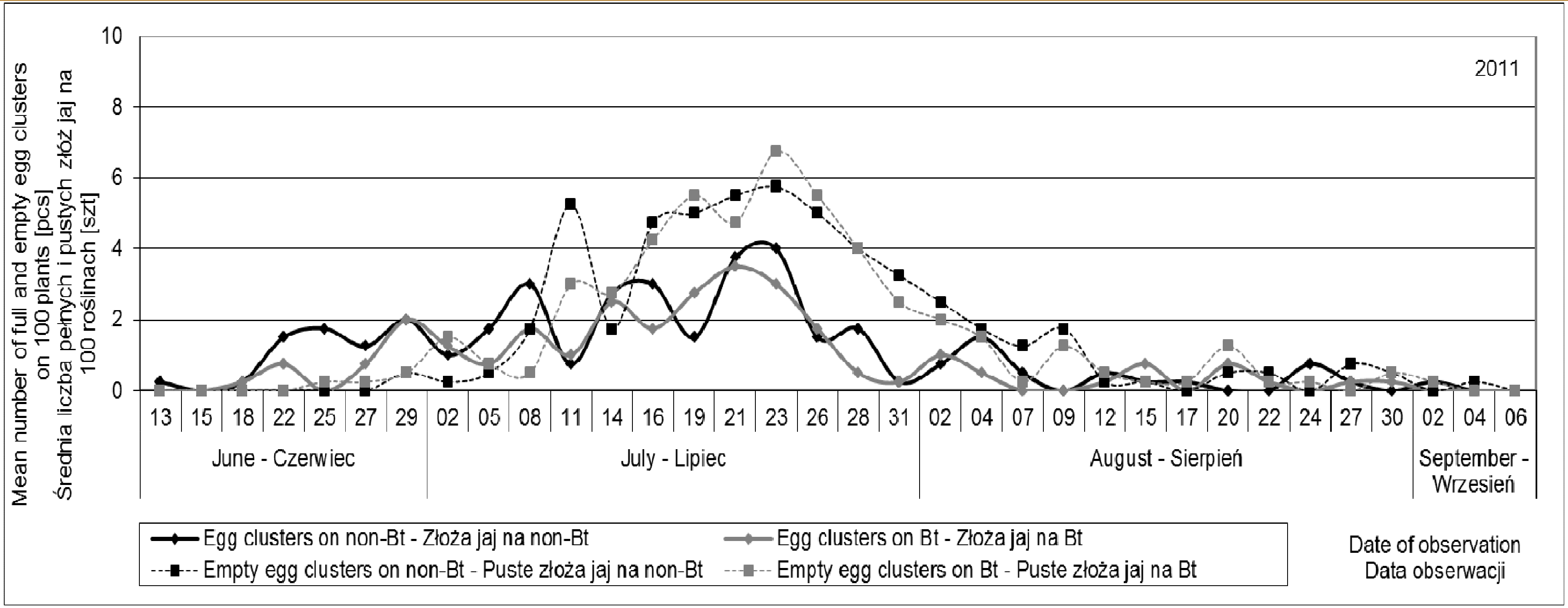

Figure 4. Dynamics of oviposition and larvae hatching for *Ostrinia nubilalis* on non-Bt and Bt maize in 2011
Rysunek 4. Dynamika składania jaj i wylęgu gąsienic *Ostrinia nubilalis* na kukurydzy non-Bt i Bt w 2011 roku

Table 2. Number of egg clusters and empty egg shells of *Ostrinia nubilalis* on non-Bt and Bt maize in 2008-2011

Tabela 2. Liczebność złóż jaj oraz pustych osłonek jajowych *Ostrinia nubilalis* na kukurydzy non-Bt i Bt w latach 2008-2011

Year Rok	Cultivar Odmiana	Total number of egg clusters on 400 plants [pcs] Całkowita liczba złóż jaj na 400 roślinach [szt.]	Number of egg clusters per plant [pcs] Liczba złóż jaj na roślinie [szt.]	Total number of empty egg clusters on 400 plants [pcs] Całkowita liczba pustych złóż jaj na 400 roślinach [szt.]	Number of empty egg clusters per plant [pcs] Liczba pustych złóż jaj na roślinie [szt.]	Total number of egg clusters destroyed in study year [pcs] Całkowita liczba zniszczonych złóż jaj [szt.]
2008	DKC 3420	280	0.70	276	0.69	4
	DKC 3421YG	274	0.69	260	0.65	14
2009	DKC 3420	172	0.43	157	0.39	15
	DKC 3421YG	203	0.51	191	0.48	12
2010	DKC 3420	223	0.56	214	0.54	9
	DKC 3421YG	210	0.53	205	0.51	5
2011	DKC 3420	148	0.37	135	0.34	13
	DKC 3421YG	114	0.29	105	0.26	9

Detailed analysis of results obtained in individual years demonstrated that only in 2011 was the mean number of egg clusters of *O. nubilalis* recorded on the transgenic cultivar (DKC 3421YG) significantly lower than that on the isogenic cultivar (DKC 3420), by -0.26 pcs. on average. In 2008 and 2010 a reduction in the number of ECB egg clusters on the genetically modified cultivar was observed, while in 2009 the trend was reversed (Table 3).

Analyzing mean results for four study years, we found no statistically significant difference in preference or non-preference of the ECB females for oviposition on transgenic and isogenic maize. The mean number of *O. nubilalis* egg clusters recorded on the DKC 3420 and DKC 3421YG cultivars was comparable, and accounted for 1.68 and 1.64 pcs. respectively. No significant differences were found for the dynamics of oviposition by females on both cultivars. Statistical analysis demonstrated that, regardless of the evaluated date, the number of eggs deposited by the ECB on isogenic and transgenic cultivars was comparable (non-significant interaction time × cultivar, high value of $p = 0.91$) (Table 3).

Table 3. Results of variance analysis for the number of egg clusters deposited by *Ostrinia nubilalis* on non-Bt and Bt maize in 2008-2011
Tabela 3. Wyniki analizy wariancji dotyczące liczby składanych złóż jaj przez *Ostrinia nubilalis* na kukurydzy non-Bt i Bt w latach 2008-2011

Year Rok	Cultivar Odmiana	Mean number of egg clusters [pcs] Średnia liczba złóż jaj [szt.]	Interaction: time × cultivar Interakcja: czas × odmiana
2008	DKC 3420	2.29 a	n.s. (p = 0.92)
	DKC 3421YG	2.25 a	
2009	DKC 3420	1.41 a	n.s. (p = 0.99)
	DKC 3421YG	1.66 a	
2010	DKC 3420	1.81 a	n.s. (p = 0.40)
	DKC 3421YG	1.70 a	
2011	DKC 3420	1.20 a	n.s. (p = 0.78)
	DKC 3421YG	0.94 b	
2008–2011	DKC 3420	1.68 a	n.s. (p = 0.91)
	DKC 3421YG	1.64 a	

Mean values in rows identified with the same letters do not differ significantly (F-test, p = 0.05)
Mean values in rows identified with different letters differ significantly (F-test, Tukey test, p = 0.05)
n.s. – non-significant interaction (F-test, p = 0.05)
Średnie w wierszach oznaczone tymi sami literami nie różnią się istotnie (test F, p = 0,05)
Średnie w wierszach oznaczone różnymi literami różnią się istotnie (test F, test Tukeya, p = 0,05)
n.s. – interakcja nieistotna (test F, p = 0,05)

DISCUSSION

The MON 810 line maize, with a gene determining resistance of plants to damage caused by Lepidoptera pests, is the only type of transgenic maize approved for growing in the European Union. In 2011 GM maize varieties were grown in six European Union member states: Spain, Portugal, Czech Republic, Poland, Slovakia and Romania, on an acreage of 114.49 K ha (James, 2011). The almost total resistance of MON 810 maize to damage caused by *O. nubilalis* was confirmed by many researchers, e.g. Ostlie et al. (1997); Beaute et al. (2002); Kaiser-Alexnat et al. (2005), Twardowski et al. (2008) and Bereś (2010).

However, because of the intense debates and controversies associated with the cultivation of GMO plants in Europe, detailed studies are being carried out in many countries, e.g. on the effects of GM maize on non-target organisms (NTO). Studies in this area have also been carried out in Poland. A part of the carried out experiments was to determine whether under the soil and climate conditions of south-eastern Poland Bt maize plants are threatened by infestation with *O. nubilalis* eggs to the same degree as isogenic maize.

Studies carried out on macroplots confirmed that transgenic maize plants are threatened by infestation with the European corn borer to the same degree as isogenic maize plants. The only statistically confirmed difference in the number of *O. nubilalis* egg clusters between Bt and non-Bt maize concerned 2011, but this was most likely associated with the effect of weather conditions, particularly intense rainfall, which disturbed moth flight and oviposition.

Thompson and Pellmyr (1991) indicated that oviposition by female moths (Lepidoptera) is affected by many factors. Chiang et al. (1960) showed that in North America second generation females of ECB avoid oviposition on severely damaged maize plants. Harmon et al. (2003) demonstrated, in their study carried out on microplots, that ECB deposit a significantly lower number of egg clusters on plants previously damaged by the larvae of this pest than on plants free from this type of damage. This may suggest that in countries where *O. nubilalis* produces more than one generation in a year, Bt maize plants can be infested with a higher number of egg clusters because they are free, or almost free, from damage caused by larvae, and therefore are preferred by females laying eggs. However, this correlation was not confirmed in studies by Orr and Landis (1997) and Pilcher and Rice (2001), who found that the number of eggs deposited by ECB on transgenic and isogenic maize is similar. Hellmich et al. (1999) reported that Bt maize has no impact on the reproductive behaviour of *O. nubilalis.* In four out of five experiments carried out in cages and in two field experiments they did not demonstrate differences in oviposition by *O. nubilalis* between Bt and non-Bt maize. Only in one cage experiment did ECB moths deposit more eggs on GM maize than on plants containing no Cry1Ab protein.

Detailed field research on the effect of Bt maize on arthropods carried out in the Czech Republic did not demonstrate differences in the number of eggs deposited by *O. nubilalis* on Bt and non-Bt maize (Habuštová and Sehnal, 2007). According to the quoted authors GM plants did not affect the oviposition by ECB females, and eggs were deposited randomly both on Bt plants and non-Bt plants. It was found that larvae hatch from eggs deposited on both types of maize, which indicates that oviposition and larvae hatching is not correlated with the presence of Bt toxin in plant tissues. The quoted authors suggested that young ECB larvae on Bt maize must therefore die shortly after hatching, because no feeding tunnels were found in tissues of plants containing Cry1Ab protein. Differences were also not found between the number of egg clusters deposited by *O. nubilalis* on genetically modified maize and its conventional variety in the study carried out by Venditti and Steffey (2003) in Illinois (USA). The quoted authors found no difference between the number of egg clusters deposited by first and second generation ECB on Bt and non-Bt maize. However, a statistically significant lower number of larvae were found on plants containing Bt toxin.

Because Cry1Ab protein is also toxic to other maize pests from the Lepidoptera order, studies have been carried out to compare the effect of Bt toxin on their oviposition. This problem was investigated by, e.g. Van den Berg and Van Wyk (2007), who found no differences between Bt and non-Bt maize with respect to the preference of plants for oviposition by moths *Sesamia calamistis* Hampson (Lep., Noctuidae). This suggests that the presence of Bt toxin in plants is not a factor attracting or repelling moths depositing eggs. Genetically modified maize and its isogenic forms are threatened by infestation with eggs to the same degree, and this was confirmed in our study with respect to *O. nubilalis.*

Some authors, e.g. Pilcher and Rice (2001) reported that the oviposition by the ECB moths can be affected by the date of maize sowing. They found differences in the number of egg clusters between plants sown on different dates, with the highest number of eggs recorded on maize sown on the earliest date. Based on this, we can conclude that maize sown on earlier dates is preferred by females laying eggs, and this can later result in greater damage to plants caused by larvae. A similar effect can be achieved by sowing maize cultivars of different earliness. This was confirmed in the study by Bereś and Górski (2012), who demonstrated that in regions heavily infested by *O. nubilalis* the earliest-maturing cultivars (up to FAO 220) are more susceptible to damage than medium-late cultivars.

Some researchers also reported that the number of deposited eggs can be determined by the type of grown maize. For example, the study by Tate et al. (2008) showed that *O. nubilalis* deposits a higher number of eggs on popcorn than on fodder maize. The diversified preferences of the European corn borer for host plants was also found in the study by Bereś (2012b), who demonstrated that sweet maize is the most frequently damaged plant when compared to fodder maize and sweet sorghum. This may be associated with the diversified distribution of ECB egg clusters, and also larvae hatched from these eggs on sweet maize plants.

Some researchers also indicated that the number of ECB egg clusters on maize plants may be influenced by the presence of other pests feeding on leaves. Harmon et al. (2003) found that the number of ECB egg clusters is significantly lower on plants heavily infested by aphids (Aphididae) than on plants infested by a lower number of this pest. In addition, hatched larvae had a higher survival rate on plants infested by lower numbers of aphids.

In this study, apart from the number of *O. nubilalis* egg clusters, no statistically significant differences were found between Bt and non-Bt maize in terms of the dynamics of oviposition within the entire vegetation season, particularly with respect to dates when the first eggs, the peak number and the end of occurrence on plants were recorded. The first egg clusters, their maximum number, and the last egg clusters were found on similar dates for isogenic and transgenic maize. The first eggs were found in the second decade of June or at the beginning of the last decade of June, which is consistent with previous observations by the author (Bereś, 2012c), and studies by Birova (1962) carried out in former Czechoslovakia, and by Tancik and Cagan (1998) is Slovakia. These dates, however, are different from the observations by Pieprzyk and Romankow (1960), who recorded the first eggs of *O. nubilalis* in south-western Poland as late as in the second half of July. The maximum number of egg clusters on Bt and non-Bt plants were found in the second decade of July, and only in 2011 at the beginning of the last decade of July, which is also confirmed in studies by Bereś (2012c) and Birova (1962). Our results differ from those presented by Tancik and Cagan (1998, 2004), who recorded the maximum number of egg clusters on plants much earlier, i.e. in the last decade of June and the first decade of July. The maximum number of egg clusters on sweet corn were recorded in south-western Poland at a similar time by Mazurek et al. (2003). The last egg clusters were deposited by female ECB on plants in the third decade of August, and only in 2011 was it in the early days of September. These dates are similar to those reported Bereś (2012c). In studies by Mazurek et al. (2003) the oviposition period on sweet corn usually ended in the third decade of July, and only in some years did it last until mid-August. In contrast to that, studies by Tancik and Cagan (1998) conducted on fodder maize did not demonstrate the presence of egg clusters of *O. nubilalis* in August.

No differences were found between Bt and non-Bt plants for the presence of empty egg clusters suggesting larval hatching dates. It was found that the first larvae of *O. nubilalis* usually hatched at the end of June or in early July, while the population peak was recorded in the second or third decade of July, and the last empty egg clusters were found between the third decade of August and the second decade of September. These findings are consistent with the previous observations by the author (Bereś 2012c).

CONCLUSIONS

1. In the study years, except for 2011, no statistically significant differences were found between the number of egg clusters deposited by *Ostrinia nubilalis* on isogenic and transgenic maize, which means that the studied MON 810 line cultivar is as threatened with infestation by pest eggs as conventional maize.
2. The first egg clusters, their maximum number, and the last egg clusters were found on similar dates for non-Bt and Bt maize. The first eggs were found in the second or at the beginning of the third decade of June. The maximum number of eggs on plants was usually found in the second decade of July, and only in 2011 was it at the beginning of the third decade of July. The last egg clusters were found on plants in the third decade of August, and only in 2011 was it in the early days of September.
3. No significant differences in dates of larvae hatching for *O. nubilalis* were found between non-Bt and Bt cultivars based on the presence of empty egg clusters. The first empty egg shells were found on plants from the third decade of June. The maximum number of empty egg shells proving mass hatching of L1 stage larvae was recorded in the second and third decade of July. The last empty egg clusters were found between the end of August and the first half of September.

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