STUDY ON THE FECUNDITY, EGG COLLECTION TECHNIQUE AND LONGEVITY OF *DIABROTICA VIRGIFERA* LE CONTE FEMALES UNDER LABORATORY AND FIELD CONDITIONS

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Abstract: Studies on rearing the beetles of the Western corn rootworm (WCR) (*Diabrotica virgifera* Le Conte) under laboratory and field conditions were carried out in 2008–2009. Obtained results allowed for the identification of the reproductive potential and longevity of the females of this species under different rearing conditions. The method used for rearing beetles may be used for further studies on the development and control of larvae under controlled conditions.

Key words: *Diabrotica virgifera*, Poland, females, egg collection, longevity, oviposition

INTRODUCTION

In Poland, the Western corn rootworm (WCR) (*Diabrotica virgifera* Le Conte) appeared for the first time in 2005 (Sahajdak *et al*. 2006a, b). Despite immediate phytosanitary action taken by the State Plant Health and Seed Inspection Service (SPHSIS) focused on the elimination at the first pest-infested sites, control of its expansion failed (Konefał *et al*. 2007). Up to the end of 2009, *D. virgifera* beetles were recorded in 8 Polish voivodeships: Dolnośląskie, Lubelskie, Małopolskie, Mazowieckie, Opolskie, Podkarpackie, Śląskie and Świętokrzyskie (SPHSIS 2009).

Currently in Poland, apart from the extended range of this pest's distribution, an increase in its population has been observed in the southern part of the country (Konefał *et al*. 2007; Konefał and Bereś 2009; Drzewiecki and Pietryga 2009).

In the European Union the WCR is a quarantine pest. Its occurrence in Poland created the necessity for the investigation of selected aspects of its biology, based on which a national control plan could be developed for the WCR.

Although the biology of the WCR in Europe has been studied, there may be differences in particular countries regarding the dates at which individual development forms occur. Knowledge of this is essential for the establishment of optimal dates for the control of both larvae and beetles.

Currently in Poland the majority of conducted studies are focused on the distribution of beetles, which is the most commonly recorded developmental stage of this pest (Bereś and Sionek 2007, 2008).

One generation of the Western corn rootworm occurs per year under moderate climate conditions. The wintering stage is in the egg, from which larvae hatch in spring and then feed on the corn root system in the soil. Larvae undergo three developmental stages. The adult individuals leave the pupa after pupal metamorphosis (Berger 2001). The beetle developmental stage of the pest is the most mobile. They are able to migrate long distances, both actively and passively, using terrestrial, aquatic and air transportation means (Bača 1994). In this way the beetles increase the distribution range of their species.

The expansion rate of the WCR in Europe, including Poland, and its degree of harmfulness to maize are influenced by many factors. The longevity and fertility of females is one of the most important factors. Both parameters have been the subject of studies carried out by scientists in many countries. Branson and Johnson (1973) reported, for example, that *D. virgifera* beetles can live for about 60 days. According to Chiang (1973) one female WCR within its lifespan, is able to deposit over 1 000 eggs from which, after successful winter survival, a new, more numerous pest generation will hatch.

Knowledge of these facts about the pest population in a particular area allows for the estimation of its potential to increase its distribution range and harmfulness.

In Poland, where the population of *D. virgifera* is not yet significantly large, observations on the fecundity of this species under field conditions, for example for es-

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tablishing the dynamics of oviposition, are very difficult, and at this moment impossible in practice.

Therefore, a study on rearing WCR beetles in insect chambers placed under laboratory and field conditions was undertaken in order to explore the fecundity of this pest. Our intention was also to investigate the longevity of adult individuals (females) under rearing conditions.

A technique for collecting eggs from the female *D. virgifera* was developed for the purposes of the conducted experiments. The technique allows for the observation of oviposition dynamics, but is also useful for other studies, for example on larvae.

MATERIALS AND METHODS

The study was carried out in 2008 and 2009. Because *D. virgifera* is a quarantine pest in the European Union including Poland, the experiment was planned based on permit No. WF-411d/5/2008 from the Main Inspectorate of Plant Health and Seed Inspection.

The permission allowed the rearing of a total number of 100 beetles of *D. virgifera* in one year (50 males and 50 females) in insect chambers placed under laboratory and field conditions.

The standard insect chamber used in the study was made of a wooden structure with a small-sized mesh stretched around it. The dimensions of the chamber were 1.5x1.0x1.5 meters (length x width x height). Each insect chamber had a tightly closed door, and a floor and roof made of rigid material (Plexiglas). The structure of the chamber ensured access to light, air and water vapour (humidity) inside. The chamber placed under field conditions was roofed to protect the Petri dishes, and also the beetles from direct exposure to sunshine and rain.

Laboratory observations

A total of 25 pairs of beetles (25 females and 25 males) were kept in insect chamber placed in the laboratory of the Regional Experimental Station in Rzeszów which is part of the Institute of Plant Protection – National Research Institute (IPP – NRI). The same number of insects were reared in insect chamber placed under field conditions in the village of Terliczka near Rzeszów.

The experiments started the moment beetles of *D. virgifera* occurred in maize fields (from July), and ended at the moment of their natural death in the chambers. Beetles used in the study were captured in the wild. Capture was carried out by visual inspection 2–3 times a week, starting from the first ten days of July, on 500 maize plants grown in a monoculture (100 plants in each of five cultivation places), according to the methodology described by Bereś and Sionek (2008). Only colourless juvenile individuals which had just left the pupa were selected for the study.

In the study years copulating beetle pairs of *D. virgifera* collected from the maize plants were kept on 9 cm diameter Petri dishes. There was 1 pair of beetles on each Petri dish (Fig. 1).

Fig. 1. Male and female of *D. virgifera* on Petri dish

In the laboratory beetles were kept under controlled temperature and humidity. During the study period the average air temperature in the laboratory was 22°C±6°C, and relative air humidity 70±10%.

Field observations

Similarly, 25 pairs of beetles (25 females and 25 males) captured from the wild were kept in insect chamber placed under field conditions in the village of Terliczka near Rzeszów. Only juvenile beetles which had just left the pupa were selected for the study. A total of 25 Petri dishes were used, on which the beetles were kept in pairs. Experiments began at the moment beetles of *D. virgifera* occurred in maize fields (from July), and ended at the moment of their natural death in the chamber.

In the chamber which was placed under field conditions, the temperature and air varied and depended on the weather. Changes in individual weather parameters during the study period in the field chamber, in each 10-day period, are presented in table 1.

Table 1. Average daily air temperature and average daily humidity in individual 10-day periods in 2008–2009

Year	Month	Average daily air temperature in 10-day period:			Average daily humidity in 10-day period:		
		I	II	III	I	II	III
2008	July	17.8	19.2	19.0	74.2	77.1	78.0
	August	19.6	19.9	17.3	74.3	75.4	78.1
	September	19.3	9.0	10.7	73.8	92.6	88.0
	October	9.9	11.0	8.3	89.2	85.4	74.2
	November	9.2	4.4	1.5	93.2	86.4	85.9
	December	4.8	1.0	1.9	84.8	92.1	87.8
2009	July	20.0	20.1	19.8	73.1	72.5	77.5
	August	19.6	18.2	18.3	68.9	71.6	84.5
	September	15.9	15.5	14.2	76.1	81.7	80.7
	October	11.8	4.9	7.8	77.5	93.4	91.7
	November	3.9	6.8	7.4	90.6	90.8	85.5
	December	4.9	–7.2	0.9	86.4	85.5	95.4

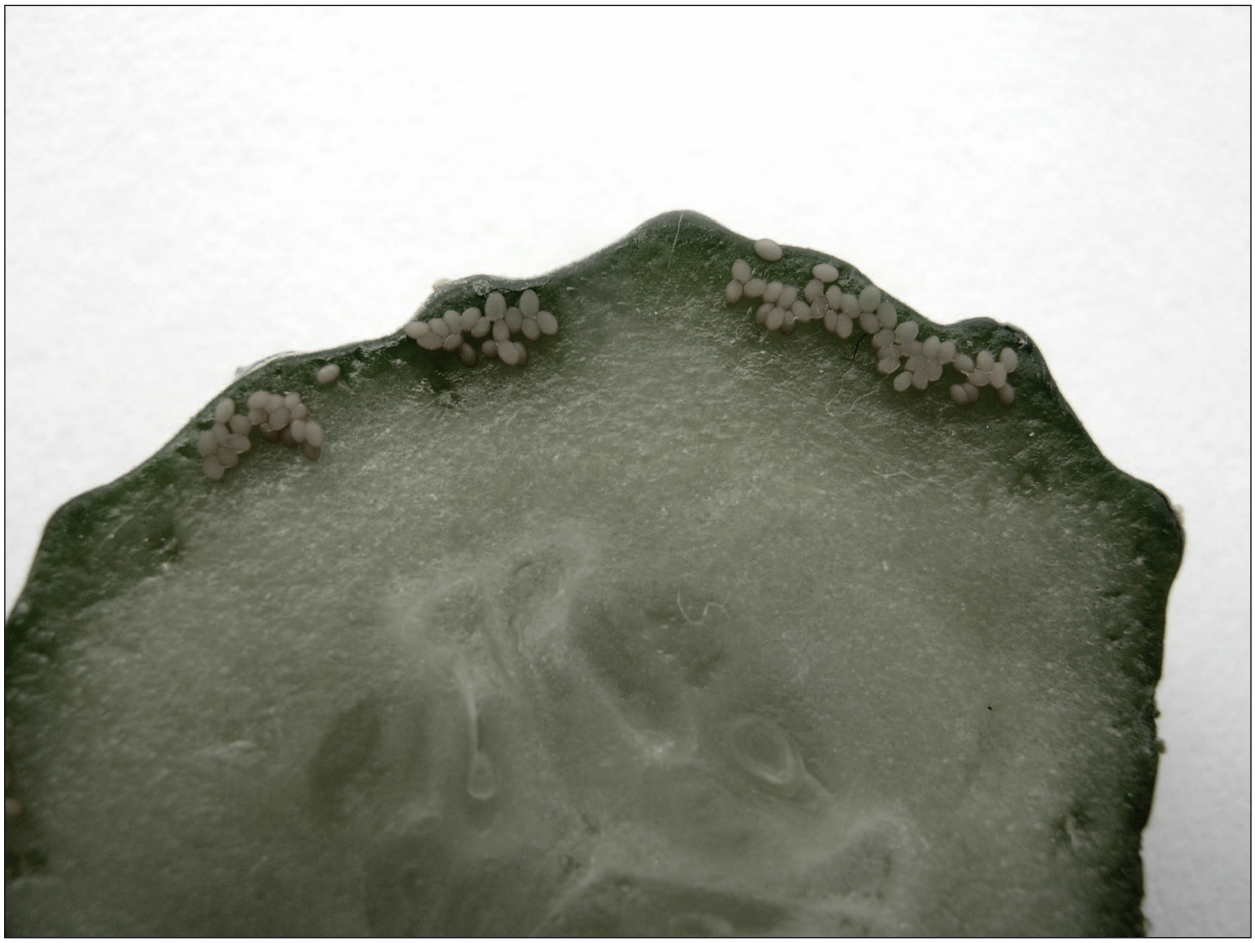

Fig. 2. *D. vifgifera* eggs on a cucumber

Food for beetles

Food was provided for the beetles on a regular basis, 3–4 times a week. Because they are polyphagous, the food consisted of: fresh maize silk, soft maize kernels and maize leaves, as well as sliced cucumber, pumpkin, courgette, summer squash and apple. Previous observations which had been carried out demonstrated that cucumber (*Cucumis sativus* L.), summer squash (*Cucurbita pepo* var. *patisoniana*), courgette (*C. pepo* convar. *giromontiina* Greb.) and pumpkin (*C. pepo* L.) were the most useful for experimental purposes, and were the feeding plants preferred by the beetles.

Egg collection technique

Observations carried out during the rearing of the beetles demonstrated that females deposited eggs on Petri dishes, especially on the food provided. During the study period the Petri dishes were observed on a regular basis every 1–2 days in search of pest eggs. Each Petri dish with food was observed under a stereoscopic microscope. Eggs deposited by females both on the bottom of the dish and on the supplied food were removed with entomological tweezers and counted (Fig. 2). The eggs of the WCR collected during rearing were destroyed as required in permit no. WF-411d/5/2008.

During the analysis the beetles were transferred onto new Petri dishes with fresh food, which was also the substrate for further oviposition.

To ensure suitable sanitary conditions during the rearing of WCR beetles, Petri dishes were regularly replaced with clean ones. Dishes contaminated with faeces and food residues were washed with detergent, thoroughly rinsed and disinfected with 96% ethyl alcohol. Food supplied to beetles was replaced with fresh food every 1–2 days to ensure its high turgor.

RESULTS

Laboratory observations

2008

In 2008 females of *D. virgifera* began oviposition on 1 August, and the last deposited eggs were observed on 12 October (Table 5). The first eggs were deposited by females 8–14 days after fertilization (usually after 9 days). Under laboratory conditions WCR females deposited over their lifespan from 250 to 858 eggs (average 528.2 eggs) (Table 4). The number of deposited eggs mainly depended on the female's health, its longevity and food quality. Under laboratory conditions females lived from 42 to 114 days (average 60.0 days) and deposited eggs for 18–63 days (average 40.4 days) (Table 2). A total of 13 206 eggs were collected from 25 female *D. virgifera* (Table 4).

2009

In 2009 the first eggs of WCR in the laboratory were observed on 29 July, and the last on 4 December (Table 5). Similarly to 2008, females began egg deposition 5–18 days after fertilization (usually after 8 days). Under relatively constant temperature and humidity, females over their life span deposited from 230 to 1 462 eggs (average 695.9 eggs) (Table 4), and their longevity in the laboratory was from 42 to 135 days (average 87.6 days) (Table 2). The oviposition period in 2009 was from 34 to 122 days long (average 73.3) (Table 2). In total 17 398 eggs were deposited by females in 2009 (Table 4).

Analysis of the oviposition process under laboratory conditions in individual years demonstrated that in 2008 it began in the first ten days of August, and in 2009 in the third then days of July. In 2008 the maximum number of eggs was deposited in the last ten days of August, and in 2009 in the second ten days of August. The oviposition process ended in 2008 in the second ten days of October, and in 2009 in the first ten days of December.

Field observations

2008

Under field conditions WCR females began depositing eggs on Petri dishes on 5 August and ended on 9 October (Table 5). Oviposition began 6–26 days after fertilization (on the average after 11 days). The fertility of females under field conditions was from 13 to 583 eggs (average 267.8 eggs) (Table 4). The longevity of females under field conditions was 21–67 days (average 53.3 days), and the oviposition period was from 6 to 53 days (on the average 30.8 days) (Table 3). A total of 6 695 eggs were collected from 25 females (Table 4).

2009

In 2009 *D. virgifera* females began depositing eggs 5–14 days after fertilization (on the average after 7 days). The first eggs were observed on Petri dishes on 5 August, and the last on 27 October (Table 5). The fertility of females in the insect chamber placed under field conditions was from 285 to 817 eggs (average 473.6 eggs) (Table 4). The longevity of females was from 50 to 91 days (average 77.1 days), and the oviposition period was from 45 to 81 days (average 61.9 days) (Table 3). In 2009, 25 WCR females deposited a total of 11 841 eggs in the field chamber (Table 4).

In 2008 the longevity of beetles under field conditions was limited by large temperature changes despite the fact that beetles of *D. virgifera* are quite resistant to unfavourable weather conditions. In 2009 temperatures were considerably more favourable to beetles, which resulted in their extended longevity, as well as a higher number of eggs deposited by females. Under field conditions beetles of *D. virgifera* survived night temperature drops to 4°C. At night when the temperature dropped to –2°C, only males survived, while the females, weakened by intensive oviposition, usually did not survive.

In the chamber placed under field conditions in 2008 and 2009 the beginning of oviposition was observed in the first ten days of August, and the maximum number of deposited eggs was in the last ten days of August. Oviposition ended in 2008 in the first ten days of October, and in 2009 in the last ten days of this month.

In 2008–2009, 50 *D. virgifera* females deposited a total of 49 140 eggs, in insect chambers of which 30 604 eggs (62.3%) were deposited under laboratory conditions, and 18 536 eggs (37.7%) under field conditions. Such a high

Table 2. Longevity and oviposition period for females of *D. virgifera* in a laboratory chamber in 2008–2009

Number of the female	2008		2009	
	longevity [in days]	oviposition period [in days]	longevity [in days]	oviposition period [in days]
1	60	37	135	122
2	43	30	99	86
3	45	36	110	98
4	61	29	81	60
5	114	63	68	57
6	51	35	107	98
7	57	42	42	36
8	42	36	110	67
9	59	47	47	34
10	48	25	110	86
11	63	55	118	102
12	61	35	92	88
13	75	59	99	82
14	59	45	53	39
15	57	38	53	43
16	67	36	102	84
17	63	36	99	80
18	70	61	102	97
19	58	47	67	35
20	71	48	95	84
21	50	30	92	77
22	69	62	81	74
23	42	18	99	88
24	60	36	53	42
25	55	24	78	74
Average	60.0	40.4	87.6	73.3

Tabela 3. Longevity and oviposition period for females of *D. virgifera* in the field chamber in 2008–2009

Number of the female	2008		2009	
	longevity [in days]	oviposition period [in days]	longevity [in days]	oviposition period [in days]
1	59	25	83	55
2	64	24	83	64
3	46	26	83	76
4	63	51	91	51
5	35	25	91	66
6	46	22	50	45
7	55	23	83	59
8	33	7	83	59
9	21	6	83	66
10	38	8	78	64
11	55	23	91	59
12	61	23	83	65
13	61	44	83	55
14	67	29	79	76
15	59	48	73	56
16	59	53	62	49
17	47	27	69	60
18	63	52	62	70
19	54	36	73	60
20	66	38	79	70
21	48	39	73	60
22	63	50	90	81
23	59	33	62	53
24	56	36	73	67
25	55	24	69	63
Average	53.3	30.8	77.1	61.9

Table 4. The number of eggs deposited by individual females in 2008–2009

Number of the female	The number of eggs deposited in laboratory chamber		The number of eggs deposited in field chamber	
	2008	2009	2008	2009
1	445	1462	123	404
2	254	701	24	817
3	461	1115	30	415
4	596	756	13	455
5	858	508	124	625
6	756	953	452	462
7	855	731	70	613
8	686	471	50	464
9	427	700	197	606
10	250	843	293	730
11	421	793	168	703
12	616	849	190	671
13	535	520	297	414
14	613	482	319	435
15	443	346	352	315
16	623	512	564	323
17	507	466	493	310
18	273	680	516	497
19	603	286	246	361
20	769	607	583	318
21	473	798	288	313
22	662	741	495	430
23	336	795	333	285
24	462	230	392	457
25	282	693	283	418
Total	13 206	17 398	6 695	11 841
Average	528.2	695.9	267.8	473.6

Table 5. The number of eggs deposited by all females in individual periods in 2008–2009

Oviposition period	The number of eggs deposited in laboratory chamber		The number of eggs deposited in field chamber	
	2008	2009	2008	2009
01–10 July	–	–	–	–
11–20 July	–	–	–	–
21–31 July	–	7	–	–
01–10 August	713	2 798	106	705
11–20 August	3 072	3 902	1 116	2 404
21–31 August	4 037	3 848	2 330	3 461
01–10 September	3 206	2 438	1 764	1 983
11–20 September	1 425	1 660	853	1 682
21–30 September	527	846	472	794
01–10 October	219	875	54	712
11–20 October	7	371	–	91
21–31 October	–	496	–	9
01–10 November	–	91	–	–
11–20 November	–	8	–	–
21–30 November	–	35	–	–
01–10 December	–	23	–	–
11–20 December	–	–	–	–
21–31 December	–	–	–	–
Total	13 206	17 398	6 695	11 841

difference between the reproductive potential of females kept in the laboratory and those reared in the field chamber were mainly determined by changes in temperature and humidity.

It was observed that under the constant temperature and humidity maintained in the laboratory chamber, the reproductive potential of female *D. virgifera* was significantly higher than that of females in field chamber.

Analysis of the oviposition process on Petri dishes demonstrated some cycles characterised by 4–6 day periods of continuous egg deposition followed by a 2–5 day break. However, this was not a constant process as some modifications in the cycle were found. Generally, in one week females deposited from 159 to 231 eggs, and 2 to 84 eggs daily.

Food for beetles

Our study demonstrated that beetles of *D. virgifera* reared under laboratory conditions were characterised by extended longevity, and also initiated copulation and oviposition more frequently. However, regular access to fresh food was found to be a factor which allowed for the creation of optimal reproductive conditions for beetles. Of the food provided, the insects preferred cucumber, courgette, summer squash and pumpkin. This food ensured that beetles had a sufficient amount of water, and also necessary nutrients. Young courgette and summer squash with hard flesh and without seeds, which lasted on the Petri dishes for a long time, were was the most suitable food types. At the end of the rearing period insects also willingly fed on pumpkin flesh.

Egg collection technique

Females deposited eggs in different locations. Initially, they deposited eggs on the top of the provided food. During subsequent days, females most frequently pressed eggs with their abdomen under a slice of cucumber, courgette, summer squash or pumpkin. Eggs were arranged into irregular chains. If the tissue of the provided food was soft, females pressed single eggs or clusters into the flesh. Sometimes females deposited eggs singly or in small clusters, directly on the bottom of the Petri dish.

It was also observed that the number of eggs and the method of egg deposition by females was influenced by the presence of a male. Females usually deposited a higher number of eggs on Petri dishes where males stayed for the longest time. Repeated copulation (observed in several cases) probably stimulated female fertility. In very rare cases the deposited eggs were damaged by beetles.

The technique for egg collection from Petri dishes and food supply to beetles demonstrated high suitability in practice. It allows for the simple, inexpensive collection of eggs from this pest, and may be further used for other experimental studies on other developmental stages of this species.

Our studies demonstrated that this method, with ensured basic hygiene standards, including frequent food replacement, allows for obtaining high numbers of eggs from females, increasing at the same time the survival rate of beetles.

DISCUSSION

According to Hamilton (1965), beetles of the Western corn rootworm are not monophagous, because apart from maize they also feed on plants classified under several other families, like Cucurbitaceae (Metcalf *et al*. 1980; Tallamy *et al*. 2005). Although Cucurbitaceae contain toxic cucurbitacins from the group of tetracyclic triterpenoids (Lavie and Glotter 1971), beetles of *Diabrotica* spp. are able to metabolize these compounds (Andersen *et al*. 1988).

Because *Cucurbitaceae* plants are suitable as a dietary component for *D. virgifera* beetles, they were included in our own studies as the basic feeding plant. The choice of Cucurbitaceae for feeding beetles in insect chamber placed in the laboratory and in field conditions was determined by the fact that this food is more available than maize pollen. Maize pollen is only found from the third ten days of July to the first half of August.

Studies demonstrated that cucumber, pumpkin, courgette and summer squash are willingly eaten by *D. virgifera* beetles. In addition, no negative influence of these plants on the longevity or fertility of beetles was found.

In their laboratory studies on WCR beetles, some authors used artificial egg-laying substrate e.g. agar (Singh and Howe 1971; Muller *et al*. 2001). According to Branson *et al*. (1975) agar media creates technical problems because, for example, they are fast drying and have a limited storage period. Another disadvantage of such media is the need for their protection against microbial growth, e.g. with antibiotics. Other authors, like Guss and Krysan (1973), used dry food combining different nutrients, including vitamins and proteins, for feeding *Diabrotica* spp. beetles. However, this type of food required water to be provided for the beetles (Branson *et al*. 1975).

The fruit of the different Cucurbitaceae plants used in our study contained, apart from a large amount of water, nutrients necessary for beetles. However, to maintain the good health of the beetles and the high quality of the provided food, the food had to be replaced with fresh food on a regular basis (every 2–4 days). Petri dishes had to be disinfected, as well, with 96% ethyl alcohol to control microbial growth. Regular replacement of food also allowed for the easy collection of beetle eggs which were deposited on the provided food. Egg deposition on food, especially on its bottom side, was also observed by Branson *et al*. (1975). Egg counting and collection, from the plant tissues which had been provided as food, was an uncomplicated procedure, but it was necessary or the food would rot.

In our study WCR beetles demonstrated high fertility, particularly under laboratory conditions. Some females deposited over 1 000 eggs within their entire lifespan. Such high fertility was also observed, for example, by Chiang (1973), Branson and Johnson (1973), and Branson *et al*. (1975). The number of eggs deposited by individual females varied significantly. In 2008–2009, *D. virgifera* females in the laboratory deposited from 230 to 1 462 eggs, while under field conditions, exposed to large changes in temperature and humidity, the number was lower and ranged from 13 to 817 eggs. The average number of eggs deposited per *D. virgifera* female in the laboratory in the

study period was from 528.2 to 695.9, while under field conditions from 267.8 to 473.6 eggs. Other authors, *e.g.* Toepfer and Kuhlmann (2006) observed that females usually deposited from 237 to 353 eggs, while Hill (1975) observed an average of 217 to 1 087 eggs.

The study also demonstrated the high longevity of WCR females. In the laboratory, females lived on the average 60.0–87.6 days, while under field conditions longevity was insignificantly shorter, on the average 53.3–77.1 days. Branson and Johnson (1973) reported that under laboratory conditions female *D. virgifera* lived on the average, for 3 months.

It was found that both in the chambers placed in the field and in the laboratory, the number of deposited eggs gradually increased to attain its maximum, and decreased slowly afterwards. This trend was also observed by Branson and Johnson (1973), who also reported that on the average 12 eggs daily can be obtained from healthy WCR females. In our study females deposited on the average from 2 to 84 eggs daily.

The carried out observations demonstrated that under laboratory and field conditions eggs can be collected to establish the fertility of female *D. virgifera.* The proposed egg collection technique can also be used for studies on future larvae rearing under controlled conditions with a focus on the aspects of their control.

CONCLUSIONS

1. Under laboratory conditions in 2008 and 2009, females lived on the average, for 60.0–87.6 days, while under field conditions on the average, for 53.3–77.1 days. In the laboratory the oviposition period was on the average, 40.4–73.3 days, and in the field insect chamber on the average, 30.8 to 61.9 days.
2. In the laboratory, oviposition began at the end of July and at the beginning of August, with its peak in the second half of August, while the end of oviposition was observed in the second ten days of October in 2008 and in the first ten days of December in 2009.
3. In the field chamber, females began oviposition at the beginning of August, with the peak at the end of the month. Females ended oviposition in the first ten days of October and at the beginning of the last ten days of October.
4. The average number of eggs deposited by *D. virgifera* females in the laboratory chamber in 2008 was 528.2, and 695.9 in 2009, while in the field chamber 267.8 eggs were deposited in 2008, and 473.6 eggs in 2009.
5. The method of rearing beetles on Petri dishes allows the opportunity to collect eggs from females of this species for future studies on larvae development under controlled conditions.
6. Plants like cucumber, courgette, pumpkin and summer squash were demonstrated to be highly suitable food in the controlled rearing of *D. virgifera* beetles and as material for the deposition of eggs by females.
7. The proposed method for rearing *D. virgifera* beetles is uncomplicated in practice, but requires regular replacement of food with fresh food, and disinfection of Petri dishes to prevent microbial growth.

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POLISH SUMMARY

BADANIA NAD PŁODNOŚCIĄ, TECHNIKĄ POZYSKIWANIA JAJ ORAZ DŁUGOŚCIĄ ŻYCIA SAMIC *DIABROTICA VIRGIFERA* LE CONTE W WARUNKACH LABORATORYJNYCH I TERENOWYCH

Badania dotyczące hodowli chrząszczy *Diabrotica virgifera* Le Conte w warunkach laboratoryjnych oraz terenowych, wykonano w latach 2008–2009. Uzyskane wyniki pozwoliły określić potencjalne możliwości rozrodu oraz długość życia samic tego gatunku w różnych warunkach hodowlanych. Zastosowana metoda hodowli chrząszczy może być wykorzystana do dalszych badań nad rozwojem i zwalczaniem larw w warunkach kontrolowanych.