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VPLYV ZAPLAVENIA NA ŽIVOTASCHOPNOSŤ VAJÍČOK KUKURIČIARA KOREŇOVÉHO (*DIABROTICA VIRGIFERA VIRGIFERA*)

INFLUENCE OF FLOODING TO THE VIABILITY OF THE WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA VIRGIFERA*) EGGS

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Laboratory trials of the Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) were conducted to determine the effects of four periods of flooding on the percentage of hatched eggs in Petri dishes and the percentage of larvae survived in pots after flooding of eggs and subsequent development of larvae on maize plants. Percentage of hatched eggs of the WCR in Petri dishes was significantly higher in treatments where eggs were watered and filter paper was maintained moist compared to treatment where paper was maintained semidry (eggs were maintained on the semi-dry filter paper in Petri dishes at 100 % relative humidity). Flooding of eggs in Petri dishes did not have negative influence to egg hatching. Percentage of hatched eggs was 82.67, 80.67, 80.67 and 73.33 after 21, 14, 28, and 7 day period of flooding, respectively. The percentage of hatched eggs achieved 70.0 and 37.33 in wet and dry control, respectively. The percentage of survived larvae from soil after period of flooding of soil with eggs was significantly different compared to control treatment. Total percentage of the larvae developed in soil was 21.00, 18.00, 12.67 and 0.00 after 7, 14, 21 and 28 days of the egg flooding period in the soil, respectively. In the same experiment, the percentage of larvae developed in control treatment was 61.33 %.

Key words: *Diabrotica virgifera virgifera*, Western corn rootworm, flooding, eggs

Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) and European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) are considered as major pests of maize in Slovakia (Cagáň, 2006). WCR is considered as a major insect pest of field maize (Meinke et al., 2009). The appearance of corn rootworm is one of the greatest plant protection challenges that European maize production has faced in its 500-year history (Marton et al, 2009). In 2000, the first occurrence of WCR was recorded in Slovakia (Sivicek, 2000). It was found that there were 1044 hectares of maize fields with economic important population of the WCR adults in Slovakia during 2004 compared to 16887 hectares during 2005 (Cagáň, 2006). Damage caused by the WCR larvae was found on 348 hectares during 2004, and on 11 728 hectares during 2007 (Cagáň, 2008).

The western corn rootworm overwinters as an egg in the soil (Krysan, 1986). Egg hatch begins from mid-May to early June the following year (Chiang, 1973). It was found the effect of maize phenology on establishment and adult emergence of the Western corn rootworm and plant damage to maize (Hibbard et al., 2008). The percentage of viable eggs of the Western corn rootworm, which survived to the adult stage was also evaluated for the effect of egg density. A broken line analysis indicated that density-dependent mortality began at approximate to 800 viable eggs per 30.5 cm (Hibbard et al., 2010).

In spring many fields were flooded in Slovakia in 2006 and in 2010 (Cagáň – unpublished data). It was the case in localities near big rivers in the southwest of Slovakia. This situation evoked the idea that flood water probably killed the WCR populations in some fields. Flooding causes immersion resulting in oxygen limitation (Baumgartl et al., 1994). Although many species of terrestrial insects can likely survive immersion little is known about their survival (Hoback and Stanley, 2001). The objective of this study was to determine the influence of flooding to the WCR egg viability.

Material and methods

Eggs of the WCR were obtained from the laboratory rearing at the Department of Plant Protection, Slovak Agricultural University in Nitra. Eggs were obtained from adults, which were collected in the field during the previous growing season. Adults were fed with fresh immature maize ears and maize silks. The laying containers with eggs were exposed to two-week prediapause period at 25 °C (Jackson, 1986). Then the containers were placed into the refrigerator and stored for 38 weeks at 5 °C, respectively. After this period, the soil was washed through the screen with water. The temperature of water was the same as eggs. The eggs were thus prevented to possible temperature shock (Jackson, 1986). The remnants of soil on the screen were transferred into the plastic dish and eggs were obtained by using a microscope and micropipette.

The trial was conducted in Petri dishes and pots with soil in climatic chamber at the temperature of 20–21 °C and light: dark period 14L : 10D.

In the first experiment, the eggs in the first control treatment (wet control treatment) were maintained on the wet filter paper in the Petri dishes at 100 % relative humidity and eggs were in contact with water on the filter paper.

The eggs in the second control tratment (semi-dry control treatment) were maintained on the semi-dry filter paper in Petri dishes at 100 % relative humidity. Eggs were not in water contact on the filter paper. "Semi-dry" in the second treatment means, that filter paper was damp, but it was not fully saturated by water. Thus the eggs could not take direct water, but they were maintained at 100 % relative air humidity.

In the treatments where eggs were exhibited in various periods of flooding, the eggs were covered with 10 millimeter layer of water. After the flooding period the water was sucked from Petri dishes and the eggs were tested for their viability. Distilled water was added to keep the filter paper in Petri dishes wet.

Percentage of egg hatching for each treatment (kept at 20–21 °C) was recorded weekly until no hatch was observed for 4 weeks. After transferring eggs from 5 °C to 20–21 °C, mass egg hatching was observed during 22–25 days.

Together 50 eggs were used in each of 3 replications. The data were analysed by analysis of variance and tested by Tuckey's multiple range test (P = 0.05). Petri dish treatments included two control treatments, then eggs + water 7 days, eggs + water 14 days, eggs + water 21 days and eggs + water 28 days.

In the second experiment the amount of 300 ml of sifted soil was placed to rearing container (size 15 cm x 18 cm with 1.28 cm of soil layer). Afterwards 50 eggs of the WCR were placed to rearing container and covered by another 300 ml of soil. These rearing containers were considered as control treatments.

In the treatments, where the eggs were exhibited to different periods of flooding, the eggs were placed on 300 ml of sifted soil. This was covered by another 300 ml sifted soil, which was watered. Water level was one centimeter above the soil level.

At the same time, 300 ml of soil was added into the other rearing containers (containers for the maize growing). 15 kernels of maize were placed on the surface of soil and covered by 300 ml of sifted soil.

After the period of flooding (7, 14, 21, and 28 days), the bottom of rearing containers was punctured and redundant water drained.

Maize plants from maize growing containers were placed together with soil into the control treatment and flooded rearing containers.

After eight weeks, the influence of flooding eggs viability of the WCR was determined by ratio between the number of larvae survived in control treatment and larvae survived in treatments of tested period of flooding. The larvae were separated from the soil by sieving. In each treatment three replicates of 50 eggs were used. The data were analyzed by analysis of variance and tested by Tuckey's multiple range test (P = 0.05).

Results and discussion

Table 1 shows the percentage of eggs hatched after the period of flooding in Petri dish experiment. The highest average of hatched eggs was observed at treatments where the eggs were flooded for 21 days (82.67 %), without significant difference to wet control (Tuckey multiple range test, P = 0.05). The number of hatched larvae was 80.67 %, 80.67 % and 73.33 % after 14, 28 and 7 days, respectively. It was not significantly different to wet control treatments where the number of hatched eggs was 70.0 %. On the other hand, when the WCR were maintained at 100 % relative air humidity on damp filter paper, their viability was significantly lower in comparison with all other treatments.

Our results show that submersion of the WCR eggs under the layer of water did not influence egg hatching. This is in agreement with the results of other authors. They found out that, the WCR eggs are highly tolerant to immersion in laboratory conditions. According to Krysan (1978), during post-diapause, the absorption of water is critical for the complete development of the WCR eggs. Eggs completed post-diapause development and neonate larvae emerged from completely submerged eggs (Meinke, unpubl. cit. Hoback et al., 2002). Hoback et al. (2002) also reported that *virgifera* species because of their obligatory diapause are likely to encounter both dry and saturated soil conditions that may have led to the evolution of egg and larval stages with a higher tolerance for adverse environmental conditions. In our experiment, significantly fewer eggs hatched in dry-control treatments in comparison with wet-control treatment and the other tratments. It is probably related to post-diapause absorption of water.

In the pot experiment, the number of the WCR larvae collected from the soil, was the highest in control treatment – 61.33 % (Table 2). It was significantly different comparing to all treatments with soil flooding. Similarly, when the soil remained saturated for prolonged periods during egg hatch, the establishment of larvae on maize roots was prevented (Sutter et al., 1989). Also the results of MacDonald and Ellis (1990) indicated that increased mortality or decreased movement can reduce larval establishment when soil is very wet or dry. According to Hoback et al. (2002), if a majority of the soil pores are filled with water when soil is saturated during a flooding event, hypoxia sensitivity of *Diabrotica* stages in soil clearly can become an important factor that affects larval survival and population dynamics over time. But, our experiments did not confirm for sure that submersion under water influenced the WCR egg mortality, or WCR larvae viability, even statistical data from pot experiment show lower number of larvae in the treatments with flooding.

Table 1 Percentage of eggs hatched after the period of flooding (eggs situated under the layer of water) – Petri dish treatment

Treatment (1)	Replication (2)			Average (3)
	I	II	III	
Control – semi-dry* (4)	40	36	36	37.33a
Control – wet* (5)	76	70	64	70.00b
7 days of flooding (6)	70	90	60	73.33b
14 days of flooding (6)	80	82	80	80.67b
21 days of flooding (6)	80	86	82	82.67b
28 days of flooding (6)	84	84	74	80.67b

Together 50 WCR eggs were used in each replication. Values with the same letter are not significantly different at the P = 0.05)
V každom opakovaní sa použilo 50 vajíčok kukuričiara koreňového. Hodnoty označené rovnakým písmenom nie sú preukazne rozdielne (Tuckeyov test; P = 0,05)
* – detailné vysvetlenie metódy je uvedené v kapitole "Materials and Methods"

Tabuľka 1 Percento vajíčok, z ktorých sa vyliahli larvy po období zaplavenia (obdobie, keď sa vajíčka nachádzali pod vrstvou vody v Petriho miske
(1) variant, (2) opakovanie, (3) priemer, (4) kontrola – polosuchá, (5) kontrola – mokrá, (6) počet dní zaplavenia

Table 2 Percentage of the WCR larvae collected from the soil in the pots after the period of egg flooding

Treatments (1)	Replication (2)			Average (3)
	I	II	III	
28 days of flooding (4)	0	0	0	0.0a
21 days of flooding (4)	16	12	10	12.67a
14 days of flooding (4)	22	12	20	18.0a
7 days of flooding (4)	22	16	34	21.0a
Control (no flooding) (5)	42	64	78	61.33b

Tabuľka 2 Percento lariev nazbieraných z pôdy v nádobových experimentoch po období zaplavenia vajíčok
(1) variant, (2) opakovanie, (3) priemer, (4) počet dní zaplavenia, (5) kontrola (nezaplavené vajíčka)

Table 3 Number of the WCR larvae hatched from 100 eggs during 5 weeks at the temperature 20–21 °C. Eggs were maintained at moist filter paper in Petri dishes

Period of the WCR egg storage in weeks (2)	Period when the eggs were at 5 °C in day.month (3)	Number of the WCR larvae hatched after 7, 14, 21, 28 and 35 days at the temperature of 20–21 °C (1)				
		7	14	21	28	35
34	9. 8.–21. 4.	0	0	0	3	41
34	9. 8.–21. 4.	0	0	0	0	50
34	9. 8.–21. 4.	0	0	0	3	49
35	9. 8.–28. 4.	0	0	0	5	43
35	9. 8.–28. 4.	0	0	0	7	47
35	9. 8.–28. 4.	0	0	0	5	52
36	9. 8.–5. 5.	0	0	0	26	36
36	9. 8.–5. 5.	0	0	0	17	36
36	9. 8.–5. 5.	0	0	0	14	34
37	9. 8.–12. 5.	0	0	0	23	31
37	9. 8.–12. 5.	0	0	0	25	24
37	9. 8.–12. 5.	0	0	0	23	31
38	9. 8.–19. 5.	0	0	1	31	16
38	9. 8.–19. 5.	0	0	0	34	23
38	9. 8.–19. 5.	0	0	0	49	18

Tabuľka 3 Počet lariev kukuričiara koreňového vyliahnutých zo 100 vajíčok počas 5 týždňov pri teplote 20–21 °C. Vajíčka boli udržiavané na vlhkom filtračnom papieri v Petriho miskách
(1) počet lariev kukuričiara koreňového vyliahnutých po 7, 14, 21, 28 a 35 dňoch pri teplote 20–21 °C; (2) obdobie skladovania vajíčok v týždňoch; (3) obdobie, keď vajíčka boli uložené pri teplote 5 °C v dňoch . mesiac

No larvae were collected from the treatment, where the soil with eggs was flooded for 28 days. The explanation of this situation seems very clear. In the experiment, majority of all eggs hatched during 28 days. After the larvae emerged from eggs, they did not find available food, because maize plants were added to pot after the period of the soil flooding, which was 28 days. The eggs stored more than 38 weeks were used for the experiment. The reason for this decision shows Table 3. From this table it is clear, that after storage during the week 34–37 in the refrigerator the eggs of the WCR started to show stabile behavior. It means that they started to hatch usually during 21–28th day after they were taken out from refrigerator.

It was supposed by other authors that delayed planting reduces the availability of food to the early-hatching larvae resulting in their mortality (Bergman, Turpin, 1984). Significant mortality of the first instar larvae occurred within 12 hours after hatching if a suitable host was not located (Strnad and Bergman, 1987). Later on, Branson (1989) reported that survival to the adult stage was significantly reduced when neonate larvae of the WCR were starved for 1 day before being placed on the roots of maize.

Together 12.67 %, 18.0 % and 21.0 % of larvae (number of collected larvae to number of eggs added × 100) were collected from the soil after the period of soil flooding during 21, 14 and 7 days, respectively. This was significantly lower compared to control treatment. The percentage of larvae collected from the soil decreased with the time of the duration of soil flooding. After 28 days of flooding no larvae emerged from eggs, so their mortality can not be explained by starvation of hatched larvae. Next explanation is that movement of hatched neonate larvae was restricted by more compacted soil after flooding. The WCR larvae primarily move through the soil profile via air-filled pores (Gustin a Schumacher, 1989; MacDonald, Ellis, 1990). Strnad and Bergman (1987a) reported that as soil bulk densities increased, larval movement was impeded. Gustin and Schumacher (1989) reported that movement towards maize roots was restricted to less than 5 cm in soil with a bulk density of 1 100 kg/m^3 and 7 % of the soil pores with a diameter of > 0.30 mm. Likewise Ellsbury et al. (1994) reported that the lowest pest survival and injury coincided with higher bulk density, lower air-filled porosity, and lower air permeability values characteristic of soil from the compacted interrow plots.

It was found that WCR larvae were stressed for food when high larval densities were present (Branson and Sutter, 1985). Riedell and Sutter (1995) concluded that saturated soil conditions during eggs hatch reduced WCR densities and root growth compared with less saturated plots. They also reported that, while water-saturated soil at specific times of the life cycle may reduce populations of the WCR, the magnitude and duration of this treatment needed to achieve this reduction may adversely affect plant growth and development. In our experiment we added developed maize plants to eggs which survived after flooding. So, the larvae hatched after flooding could not suffer because of starvation. It seems that less percentage of the received larvae from the soil could be probably ascribed only to the compacted soil after flooding, which restricted the movement of larvae to maize roots.

Súhrn

V práci sa uvádzajú výsledky laboratórnych pokusov s kukuričiarom koreňovým, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Ich cieľom bolo stanoviť vplyv štyroch rôznych období zaplavenia na percento vyliahnutých vajíčok v Petriho miskách a na percento lariev, ktoré prežili po zaplavení vajíčok a ich následnom vývine na kukuričných rastlinách v nádobových pokusoch. Percento lariev vyliahnutých z vajíčok kukuričiara koreňového v Petriho miskách bolo preukazne vyššie vo variantoch, v ktorých sa vajíčka udržiavali namočené vo vode na vlhkom filtračnom papieri ako vo variante, kde bol papier polosuchý (nenamočený vo vode, ale zároveň udržiavaný pri 100 % relatívnej vlhkosti vzduchu). Zaplavenie vajíčok nemalo žiadny vplyv na ich životaschopnosť. Percento lariev vyliahnutých po 21, 14, 28 a 7 dňoch zaplavenia dosiahlo 82,67, 80,67, 80,67 a 73,33. V kontrolnom variante bez zaplavenia bolo percento vyliahnutých lariev 70,00 (mokrý variant) a 37,33 (polosuchý variant). Percento lariev, ktoré prežili z vajíčok vystavených zaplaveniu v pôde bolo v porovnaní s kontrolou preukazne nižšie. Percento lariev vyvinutých z vajíčok vystavených záplave v pôde 7, 14, 21 a 28 dní dosiahlo 21,00, 18,00, 12,67 a 0,00. V kontrolnom variante bez záplavy sa z vajíčok vyvinulo 61,33 % lariev.

Kľúčové slová: *Diabrotica virgifera virgifera*, kukuričiar koreňový, zaplavenie, vajíčka

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