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Maize hybrid and nutrient specific evaluation of the population dynamics and damage of the western corn rootworm (*Diabrotica Virgifera Virgifera* LeConte) in a long-term field experiment

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ABSTRACT

Agricultural production is threatened by different invasive species, as their damage results in a serious loss of income. The aim of the research was the assessment of the swarming dynamics and damage of the western corn rootworm (WCR) adults and larvae. The experiment was carried out in monoculture fertilization long-term experiments and three maize hybrids compared for their reaction against WCR adult and larval damage under non-infested plots at different nitrogen levels. Differences among the hybrids have a lower effect on the damage of corn rootworm adults and larvae than the amount of applied nitrogen. The phosphorus-potassium are optimal levels, while nitrogen ranges from 0 to 300 kg and no nutrient supply took place in the control plots for 30 years. The number of adults located and feeding on the styles of the female flower recorded and the damage caused on the roots by larvae ranked on a modified Iowa scale. Nitrogen fertilization resulted in a change in the silking time. The lowest root damage observed

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in the case of the high nutrient treatment with an Iowa value of 3.18. The coincidence of the nourishment of adults and the egg-laying time with silking is a potential threat in terms of fertility. Based on the results, it found that the extent of root damage can be reduced through the optimal selection of the time and dose of nutrient supply, primarily that of nitrogen. In general, both larvae and adults can cause severe yield loss, but the method of control against them is different. The coincidence of the nourishment of adults and the egg-laying time with silking is a potential threat in terms of fertility.

KEYWORDS

damage evaluation, Diabrotica Virgifera, long term experiment, maize, monoculture

INTRODUCTION

The global challenge of our time is to maintain and improve food production standards while preserving biodiversity. Agricultural production might be threatened by various invasive species as their damage might result in tens of millions of dollars in income loss (Pimentel et al., 2000, 2003). Currently, climate change has an impact on global food production (Ray et al., 2019), which may be the driving force behind the spread and establishment of new pests in areas that used to be disadvantageous for them in the past. In the area involved in the study, the average of the summer periods between April and September of the last 5 years showed a positive temperature difference each year compared to the annual average (Gombos & Nagy, 2019). Based on the above, it can be concluded that numerous new pests might emerge that were previously not widespread in our area due to temperature-related limiting factors.

According to the studies of Tigchelaar et al. (2018), global yield and markets of cereals will become increasingly variable over the 21st century, even if temperature fluctuations remain unchanged. In the scope of agricultural research, field trials provide an opportunity to analyze and compare long-term agro-technical operations applied during cultivation and their effects.

Maize is one of the most important field crops in the world, with around 190 million hectares of production area worldwide and about 1.140 million tonnes of harvested crops (Nagy, 2006). In 2007, its production area was 8.3 million ha in the EU Member States for grain maize and 5 million ha for silage maize (Faostat, 2007). The annual crop yield and its qualitative parameters are significantly threatened by pests, pathogens, and weeds (Oreck, 2006). Most research indicates that NPK fertilizer can be increasing yield and component yield on maize (Illés et al., 2020; Mousavi et al., 2019).

With the spread of maize monoculture in modern agricultural practice, the western corn rootworm (*Diabrotica Virgifera Virgifera* LeConte) has become one of the most important insect pests of maize, which also caused significant challenges in the monoculture field experiments of agricultural research institutes (Levine & Oloumi-Sadeghi, 1991; Sappington et al., 2006). Knowledge of the nutritional biology of pests is essential for proper pest control, especially when certain pests conquer new areas after their original place of origin (Moeser & Hibbard, 2005).

According to its taxonomic classification, the western corn rootworm belongs to the class of insects (Insecta), the order of the beetles (Coleoptera), and the family of leaf beetles (Chrysomelidae) (Leconte, 1868). It is a species of tropical origin with a gene center in Mexico (Smith, 1966); its first economic damage was done to sweet corn in the early 20th century (Gillette, 1912). According to the earliest observations, its first European appearance might have taken place in 1989–1990 (Sivcev et al., 1996); however, later research suggests that 5–6 years have passed between the appearance of the first specimens and the experienced economic damage of larvae, so probably the species was in Europe already in the mid-1980s (Edwards et al., 1998; Kiss et al., 2001). During its life cycle, the development of larvae and mature specimens is also closely related to maize as their primary host (Branson & Krysan, 1981).

The larvae feed mainly on the root of maize, the L1 larvae consume the thin roots and the external tissues of the root. In stages L2 and L3, the larvae feed on the stronger roots and infiltrate the root tissue as well. Larvae often damage the brace root (Chiang, 1973). Consequently, the water balance of the plant can be disrupted (Riedell, 1990), the plants are characterized by reduced.

Nutrient intake (Kahler et al., 1985; Oleson et al., 2005) and photosynthesis (Godfrey et al., 1993). In the case of severe damage, the weakening of the root system may cause lodging (Spike & Tollefson, 1989). According to American studies, larvae damage qualifies as economic damage when its extent reaches 3.5 on the IOWA 1–6 scale (Davis, 1994). According to recent research, each root cut by larvae causes a 15% loss of yield (Tinsley et al. 2013). The movement of larvae in the soil is primarily restricted by its density (Ellsbury et al., 1994) and its pore size if it is smaller than the width of the head (Gustin & Schumacker, 1989).

Despite knowing the preference of larvae in terms of host crops, a significant proportion of European farmers do not use crop rotation in maize production, since maize is one of the most profitable arable crops. According to surveys from 2006, approximately 29.5% of maize production areas in the EU countries were not involved in crop rotation (Fall & Wesseler, 2007).

The adult of the western corn rootworm beetle mainly consumes the leaf, stigma, and pollen of the maize. After mating, the female specimens remain in the initial stock, and then migrate to other maize fields in the morning or evening after a week-long feeding period and continue to do damage there (Isard et al., 2004). Significant economic loss may occur in the case of the severe damage of styles (Culy et al., 1992). As a result, maize ears are deficient in terms of seed formation, as pollens are unable to pollinate damaged styles (Basetti & Westgate, 1993). Adults detect the volatile substance emitted by the stigma and then seek their food following it (Prystupa et al., 1988). The hazardous threshold value for the qualitative and quantitative damage of adults is 1–3 adults/ear for seed production and 9 adults/ear for industrial production (Tuska et al., 2003).

There are many different, relative and absolute methods for estimating the level of corn rootworm contamination, of which the important advantages of the former modeling methods (visual calculation methods, sticky insect traps) are their low costs and their easy and quick application. These methods provide a relative estimate for population density and they might be useful in various comparative studies as well (Tollefson, 1986).

The best defense against pests is to maintain crop rotation. Also, there are different types of insecticides, the application of which depends on the production or management system of the producer and the available equipment. The most common defense against larvae is the application of soil disinfectant at the time of sowing. Adults can be treated with wide-spectrum insecticides and insecticides with species-specific nutritional attractants, either with the aerial application or with the use of straddle tractors (Boriani et al., 2006). With the emergence of genetically modified plants, resistance against this pest has also been developed. In the United States, transgenic Bt maize expresses Cry endotoxin proteins derived from the soil bacterium Bacillus. Berliner Thuringiensis (Bt), which can significantly reduce the economic damage caused by the pest. Advantages of Bt maize include effective pest control (Storer et al., 2006), reduced use of wide-spectrum insecticides (Phipps & Park 2002), and consequently, reduction of the damage of non-target species by chemical protection (Al-Deeb & Wilde, 2003; Lundgren & Wiedenmann, 2002).

However, with the spread of Bt maize, Bt-toxin resistant populations of the past have emerged, significantly reducing the effectiveness of this pest control strategy (Gassmann et al., 2011). In Europe, where genetically modified plants are subject to more strict control and the use of additional insecticides is environmentally undesirable, biological control methods have also become significant. The classic solution of this is to import and spread natural enemies from their North American origins. A more easily available option is the Inundative method biological control approach with commercially available native antagonist organisms such as entomopathogenic nematodes (Kuhlmann & Van Der Burgt, 1998).

The development of sustainable management strategies in North America and Europe still requires responses to questions about pest population dynamics, adverse effects on maize in general, and control technologies (Meinke et al., 2009). Studies involving population dynamics and the extent of damage in field long-term experiments can provide a good basis for answering these questions.

MATERIAL AND METHODS

The experiments were carried out at the Látókép Experimental Station of the University of Debrecen (47°33′ N, 21°26′ E, 111 m asl). The climatic-meteorological factors of the experimental area characterized by continental and often extreme conditions. This is especially true for precipitation and its distribution, moreover, extreme temperatures are measured both during and outside of the growing period (Fig. 1) (Gombos & Nagy, 2019). In the scope of the experiment, in 2019 three maize (*Zea mays* L.) hybrids with different maturity groups like H1 = FAO 400, H2 = FAO 330, H3 = FAO 490 were studied in long-term experiment, where the environment promotes the large-scale proliferation without artificial infestation with western corn rootworm (WCR). The experimental area is excellent calcareous chernozem soil; Arany's plasticity index (KA = 43–45), average humus content (Hu% = 2.7–2.8), the thickness of the humus layer is approximately 80 cm (Nagy, 2019). In the examined plots, phosphorus and potassium are constantly at optimal levels for the plant, while nitrogen ranges from 0 to 300 kg (Dose 1 = 0 kg*ha^{-1} N, Dose 2 = 120 kg*ha^{-1} N, Dose 3 = 300 kg*ha^{-1} N) and no nutrient

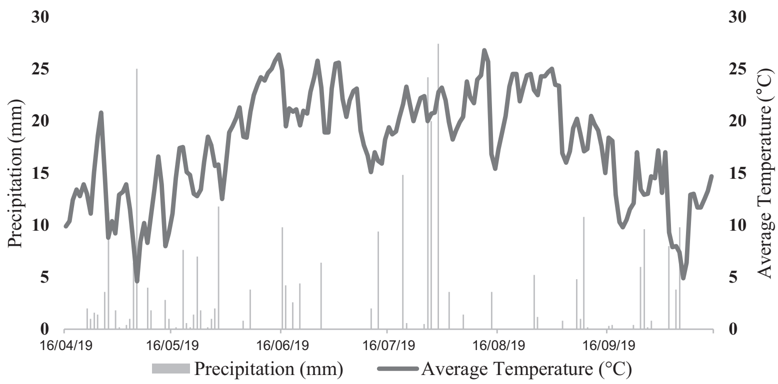

Fig. 1. Temperatures (°C) and precipitation (mm) during the growing season (Debrecen, 2019)

supply took place in the control plots for 30 years. In terms of tillage, winter plowing is applied at a depth of 30 cm. The date of sowing was 20.04.2019 when a soil disinfectant containing Teflutrin applied at a dose of 15 kg*ha^{-1} also. 73,000 plant sowing to 7.6 square meters by the randomized complete block design.

In the scope of the study, the number of adults located and feeding on the styles of the silk was recorded and the damage caused on the roots by larvae ranked on a modified Iowa scale (EPPO, 1999). Data were recorded on a silking percentage (%), the number of adults, larval damage, node lodging, and yield during three successive weeks in July. The number of adults located and feeding on the styles of the silks was recorded three times after the start of the swarming (date 1 = 02.07.2019, date 2 = 08.07.2019, date 3 = 13.07.2019) aiming at under identical environmental conditions. The time of recording was 9 a.m. after dew drying when only *Diabrotica Virgifera Virgifera* LeConte males and females were present in the area. Root damage caused by larvae was evaluated after a random selection of three plants per plots within a radius of 25 cm and a depth of 30 cm. Plants were dug, completely washed, and rated on a modified IOWA 1-6 scale (EPPO Bulletin, 1999; Hanway 1966). The IOWA 1-6 scale is a 6-point rating scale where the amount of injury on the root is classified into one of six categories, ranging from no damage or only a few minor feeding scars (rating of 1) to three or more nodes of roots pruned to within 3.8 cm of the stalk (rating of 6).

The number of lodged plants showing root was determined to damage as compared to healthy plants, too. Complex evaluation of root damage allows the monitoring of larval damage in the previous year. Grain was harvested by the machine when grain moisture was around 17%, and yields adjusted to 150 g kg^{-1}. The statistical test was carried out in the R 3.2.4. statistical environment (Team, R. C. 2020) with RStudio (Team R. 2016) graphical interface, using “G plots” (Warnes et al., 2015), “car” (Fox &Weisberg, 2011), and “Agricolae” (De Mendiburu, 2016) packages. Graphs were created with Ms. Excel 2019. The statistical analysis like analysis of variance (ANOVA), (simple regression and bivariate correlation analysis) for the studied characteristics carried out with Ms. Excel and Minitab software.

RESULTS

The result of variance showed NPK fertilizer treatments significant on one percent. On the other hand, between levels of NPK fertilizer there was a variation on the silking percentage index. The significance of the replication showed that there was variation between blocks (Table1). Nitrogen fertilization resulted in a change in the silking time of the hybrids. At the first sampling

Table 1. Mean squares (from ANOVA) for silking %, WCR adult and larval damage and yield (t ha^{-1}) (2019, Debrecen, Hungary)

Source of variation	Df	Silking %	Number of adults	Larval damage (IOWA values)	Yield (t ha^{-1})
Replication	3	0.10812	0.10812	0.0348	1.182
NPK	2	5.96230**	5.96230**	11.5078**	79.266**
Hybrid	2	0.02056	0.02056	0.1169	15.827
Error	28	0.03554	0.03554	0.0243	1.843
Total	35	**$P < 0.01$	**$P < 0.01$	**$P < 0.01$	**$P < 0.01$

date, 13.16% of the plants were silking in the dose level 1 plot, while 43% in the plots that received the highest dose of nitrogen fertilization. The rate of nitrogen fertilization influenced the silking time of the hybrids, there was a significant difference between the nutrient levels at each of the three measurement dates. Based on the examinations, it was found that nutrient supply and nitrogen fertilization affected the time of silking and thus on the host preference of the emerging adults. The earlier silking maize specimens were potentially more likely to be attacked by rootworm adults for feeding purposes (Fig. 2).

Variance analysis of adults on the silk index showed NPK treatments significant on one percent, so there had been variation between NPK fertilizer treatments. Replication was significant because of variation on each block in this index (Table1). The number of adults on the silks was different for the three nutrient treatments. There was no statistical difference between the two low doses at the 5% level of significance, however, the highest nitrogen treatment resulted in a significant difference in all cases. Based on our studies, it was found that the higher *N* treatment increased the number of adults feeding on silks at each of the three recording times, thus increasing the amount of damage caused to the stigma. The highest number of adults (3.67 pcs.*plant^{-1}) was observed at the highest nitrogen dose (Fig. 3).

There was a significant increase in the number of adults observed on the silk between the three study points. The increase between the examination times was steady, the highest value was recorded for the third time (Fig. 4).

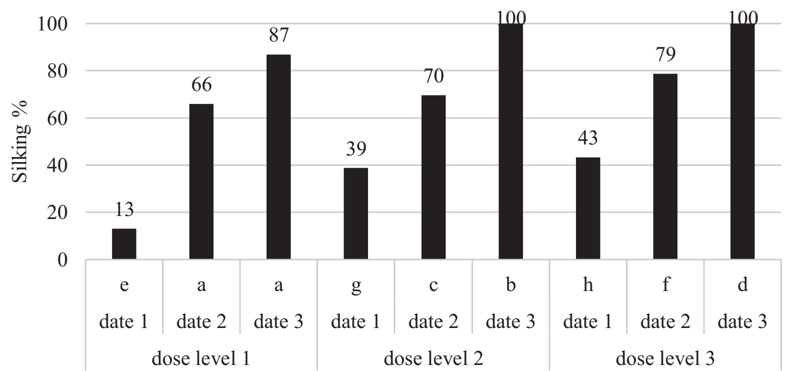

Fig. 2. Percent of silking plants under different levels of fertilizers (Treatments with the same letter are not significantly different, $n = 50$, $SzD_{5\%} = 2.7118$ (Debrecen, 2019)

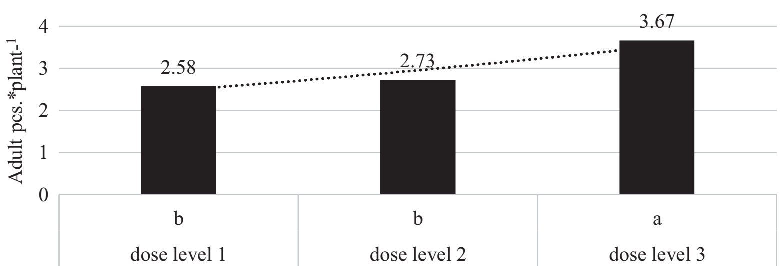

Fig. 3. Effect of nitrogen fertilization on the number of adults feeding on the silks of hybrids. Treatments with the same letter are not significantly different, $SzD_{5\%} = 0.1573$ (Debrecen, 2019)

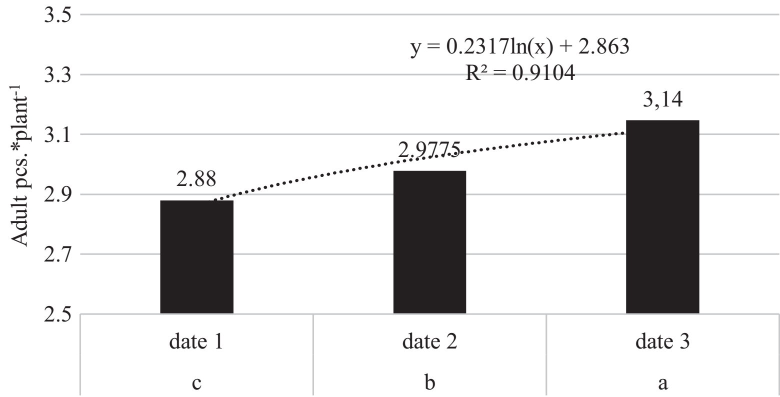

Fig. 4. Number of rootworm adults on the silk at three different dates. Treatments with the same letter are not significantly different, SzD_5=0.0572 (Debrecen, 2019)

There were significant differences in the number of adults on the silk, which was influenced by the amount of nitrogen fertilizer. The lowest number of adults (2.38 count*plant^{-1}) was measured with the dose level 1 treatment. In the case of the dose level 3 treatment, the number of adults was over 3.5 at each of the three recording dates (Fig. 5).

We examined the differences among hybrids in terms of the feeding preference of the adults, which showed that there were significant differences among hybrids in terms of the number of adults. All three hybrids had the highest number of adults at the latest recoding date and Hybrid 3 had the highest number with 3.2 count*plant^{-1} (Fig. 6).

The result of variance analysis on LOWA value showed that NPK was significant at one percent. So, variation had appeared on a different level of NPK fertilizer. Hybrids were significant at five percent and there was variation between hybrids on LOWA value too (Tables 1 and 2). Based on a complex analysis of the factors monitored during the examination of the corn rootworm, it can be concluded that all measured factors have a significant effect on the amount

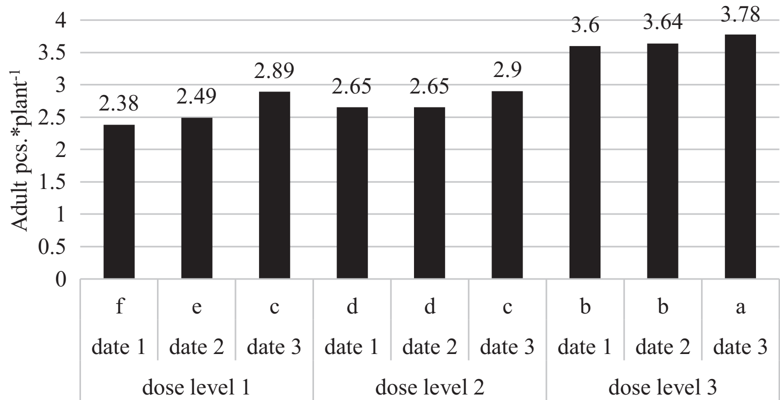

Fig. 5. Effect of nitrogen fertilization and different examination dates on the number of adults on the silk of maize hybrids. Treatments with the same letter are not significantly different, $SzD_{5\%}$ = 0.0991 (Debrecen, 2019)

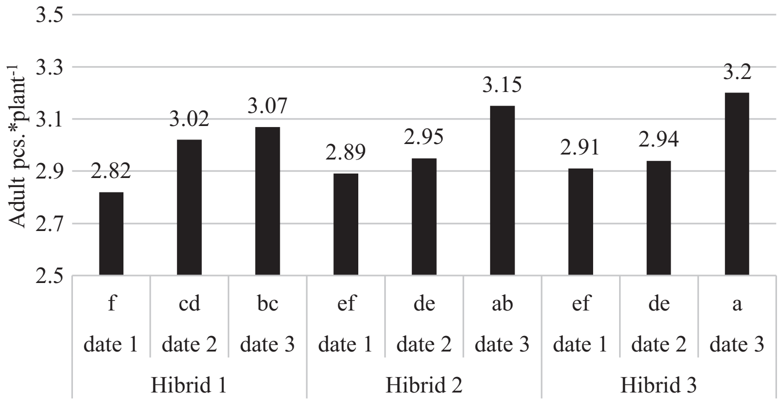

Fig. 6. Effect of hybrids and different examination dates on the development of the number of adults on the silk of maize. Treatments with the same letter are not significantly different, $SzD_{5\%} = 0.0991$ (Debrecen, 2019)

of yield, with a moderately close ($R^2 = 0.583$) correlation between the examined factors and yield results (Tables 3 and 4).

The extent of root damage caused by the pest activity was examined taking into account the time of silking, based on which it was established that the time of silking and the extent of root damage have a joint effect on the amount of yield, and the correlation was moderately close ($R^2 = 0.6226$) between the analyzed factors and yield results. The Iowa value, i.e. the feeding damage of the larvae, affects the yield more than the time of silking and the consequent fertilization problems caused by adults feeding on the style (Tables 5 and 6).

By increasing the rate of nitrogen fertilization, the extent of root damage decreased. The lowest root damage value was observed in the case of the dose level 3 nutrient treatment with a 3.18 Iowa value. As a result of nutrient treatment, the root weight increased, which affected the extent of damage caused by the feeding of larvae. According to studies by Riedell (1996), the

Table 2. Mean values of larval damage on the IOWA rating and values of root lodging on the IOWA rating scale of three maize hybrids, at three *N* doses under non-infested conditions (2019, Debrecen, Hungary)

	values of larval damage on the IOWA rating			values of root lodging on the IOWA rating		
Hybrid	0 kg ha^{-1} Nitrogen	120 kg ha^{-1} Nitrogen	300 kg ha^{-1} Nitrogen	0 kg ha^{-1} Nitrogen	120 kg ha^{-1} Nitrogen	300 kg ha^{-1} Nitrogen
H1 = FAO 400	4.26	4.17	4.08	6.75	6.02	5.9
H2 = FAO 330	4.44	4.37	4.24	5.76	5.6	5.79
H3 = FAO 490	4.22	4.15	4.07	5.96	5.1	5.44
Mean	4.3	4.23	4.13	6.15	5.57	5.71
CV (%)	2.22	2.34	1.88	6.94	6.74	3.43

Table 3. The effect of the number of adults, larval damage and node lodging on the yield (2019, Debrecen, Hungary)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Adult pcs.	1	71.45	71.45	24.4429	2.338e−05 ***
Iowa value	1	51.897	51.897	17.7538	0.0001915 ***
Lodging	1	28.46	28.46	9.7359	0.0038121 **
Residuals	32	93.541	2.923		

**$P < 0.01$; ***$P < 0.001$

Table 4. Mean values of the number of adults feeding on the silks of three maize hybrids at three *N* doses in three sampling times (2019, Debrecen, Hungary)

Hybrid	0 kg ha^{-1} Nitrogen			120 kg ha^{-1} Nitrogen			300 kg ha^{-1} Nitrogen		
	02/07	08/07	13/07	02/07	08/07	13/07	02/07	08/07	13/07
H1 = FAO 400	2.30	2.44	2.82	2.65	2.74	2.84	3.25	3.89	3.57
H2 = FAO 330	2.26	2.53	2.94	2.66	2.51	2.96	3.70	3.8	3.70
H3 = FAO 490	2.47	2.49	2.90	2.65	2.71	2.91	3.75	3.66	3.65
Mean	2.34	2.48	2.88	2.65	2.65	2.90	3.56	3.78	3.64
CV (%)	3.88	1.48	1.72	0.17	3.84	1.69	6.30	2.50	1.47

Table 5. The effect of root damage and silking percentage on the yield of three maize hybrids, at three *N* doses and at three recording times (2019, Debrecen, Hungary)

	Df	Mean Sq	F value	Pr(>F)
Iowa value	1	116.411	43.997	1.516e−07 ***
Silking %	1	41.623	15.731	0.0003702 ***
Residuals	33	2.646		

***$P < 0.001$.

Table 6. Mean values of silking % of three maize hybrids at three *N* doses in three recording times (2019, Debrecen, Hungary)

Hybrid	0 kg ha^{-1} Nitrogen			120 kg ha^{-1} Nitrogen			300 kg ha^{-1} Nitrogen		
	02/07	08/07	13/07	02/07	08/07	13/07	02/07	08/07	13/07
H1 = FAO 400	11.75	37.5	63	42.5	76.25	100	66	86.25	100
H2 = FAO 330	14.25	40.25	274	46.25	77.65	100	72.5	85	100
H3 = FAO 490	13.5	38.5	66.5	41.25	82.5	100	70.25	89.5	100
Mean	13.875	39.375	134.5	41.875	78.8	100	71.375	86.91667	100
CV (%)	7.5	2.8	7.3	5.07	3.3	0	3.7	2.18	0

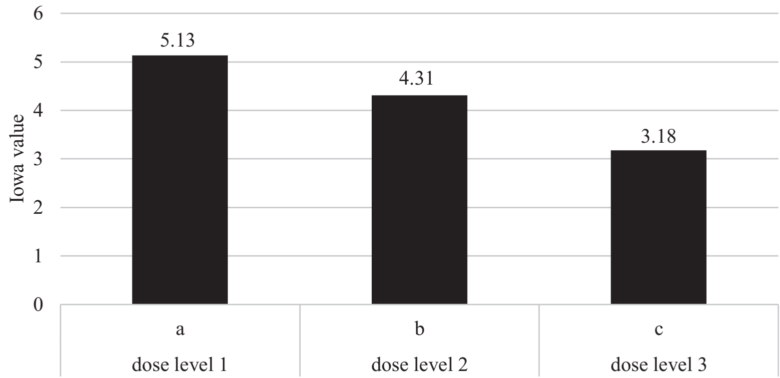

Fig. 7. Effect of nutrient treatment on the extent of root damage (Iowa value). Treatments with the same letter are not significantly different (Debrecen, 2019)

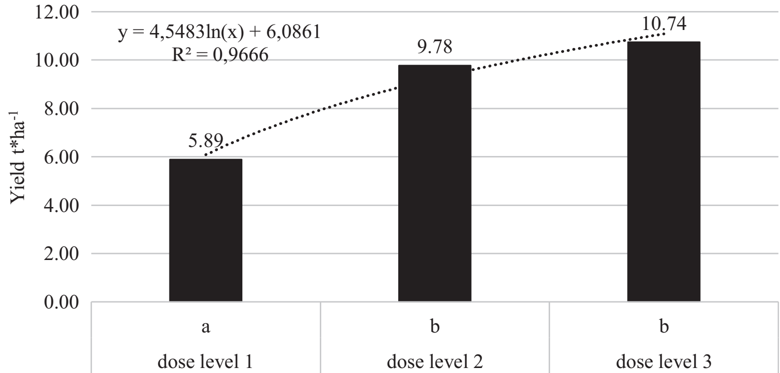

Fig. 8. Effect of nutrient treatment on the amount of yield. Treatments with the same letter are not significantly different (Debrecen, 2019)

extent of nitrogen fertilization and, in particular, its application method affects the tolerance of maize roots against larval damage (Fig. 7).

The results of the variance analysis showed that the fertilizer effect on yield was significant in one percent. So, the result showed that different levels of fertilizer had different functions as well as diversity. Also, the significant effect of genotype in one percent showed that genotypes have different yields (Table 1). Nitrogen fertilization resulted in a significant increase in yield amounts, there was a significant difference between the control and nutrient level 1, but there was no statistically confirmed increase in terms of yield between doses 2 and 3 (Fig. 8, Table 5).

CONCLUSION

The host plant preferences of the corn rootworm adults and the degree of root damage by their larvae related to the *N* supply of the plant. Differences among the hybrids have a lower effect on the damage of corn rootworm adults and larvae than the amount of applied nitrogen. The time of silking influences the host plant preference of the emerging adults, which can be modified by the amount of applied nitrogen. The silking time of maize hybrids showed a significant correlation with increasing *N* fertilizer doses. Earlier silking plants become potential host plants for earlier emerging adults. Higher doses of nitrogen fertilization might increase fertility deficiencies resulting in stigma damage caused by adults. Within the effect of the corn rootworm on crop yield, larval damage is more significant, which can be influenced by the amount of applied nitrogen, since studies have confirmed that increasing the dose of nitrogen reduces the degree of damage done by the larvae and thus their negative effect on yield. In general, both larvae and adults can cause severe yield loss, but the method of control against them is different. The coincidence of the nourishment of adults and the egg-laying time with silking is a potential threat in terms of fertility. Root damage can be reduced by the time and extent of nutrient application, primarily by nitrogen fertilization. Based on the results of the experiment, further studies are required to assess the complex effects of crop years, which may facilitate more accurate modeling and prediction of pest population dynamics and damage.

Conflict of interest statement: None.

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