Chapter

The Role of Irrigation and Nitrogen Fertilization on the Feeding Behavior of European Corn Borer

Ankica Sarajlić, Emilija Raspudić, Zdenko Lončarić, Marko Josipović and Ivana Majić

Abstract

European corn borer (ECB) creates tunnels inside the plant stalks, causing damage, which could significantly decrease yield loss. This study aimed to determine the relationship between damage caused by ECB larval feeding and different irrigation and nitrogen fertilization rates on different maize genotypes. We conducted a field experiment in Croatia from 2012 to 2014. Increased plant nitrogen adsorption was observed under irrigation only in drought years, and it was decreased in optimal or extremely wet years. We found a weak or a moderate relationship between ECB damage and nitrogen concentration, but the greatest ECB damage was in all years recorded in treatments with the highest fertilization rates. However, the highest plant nitrogen concentration was observed in the hybrid with the lowest damage from ECB larvae. The maize damage caused by ECB larval feeding was negatively affected by high plant nitrogen concentrations only when plants were under drought stress. Nitrogen uptake was increased in irrigated plots. We did not find a strong relationship between the C/N ratio or irrigation and intensity of ECB damage. In 2012, when the narrowest C/N ratio was calculated, the greatest damage by ECB was measured. Further studies are needed since we detected the significant impact of drought on intensive ECB larval feeding.

Keywords: *Ostrinia nubilalis*, nitrogen, irrigation, C/N ratio, fertilization

1. Introduction

European corn borer (ECB) is a polyphagous insect able to attack more than 200 different plant species. This pest mostly damages maize plants; however, a yield of peppers can be decreased up to 60% due to the ECB feeding activity [1]. The intensity of the ECB attack in Croatia often reaches 100% of maize plants in the last 20 years, with few exceptions, which could be related to recent global changes in climate [2]. The cultural and biological controls are mostly used for the management of ECB in maize, while chemicals are mostly applied in sweet maize and production of maize for seeds. Tolerant maize hybrids are a very important tool in integrated pest management programs against ECB. Plant breeders successfully developed hybrids with stronger stalk, a larger root system, and such plants are more tolerant to this pest. Tolerant hybrids are also characterized by the low potential of plant lodging and yield losses in conditions of the highest ECB infestation and stalk damage [3, 4]. One ECB larvae per plant on commercial hybrids can reduce yield up to 6.05% [5]. The amount of plant-available water has been studied as a potentially important factor for ECB infestation and damage. Irrigated plants have a higher yield, and grain quality is better [6]. If the water supply is satisfactory, plants will adsorb more micro and macro elements. Nitrogen is the most important essential element, and plants adsorb it in a mineral form. Agricultural soils usually contain 0.1–0.3% of nitrogen, and from this, only 1–3% is available for plants. Maize plants under nitrogen fertilization treatment are attractive for ECB females for laying eggs. Such plants contain more quality ingredients for larval nutrition. The positive correlation between nitrogen fertilization and ECB oviposition and larval feeding was found by many researchers [7–9]. C/N ratio is important for immobility and mineralization processes and varies with levels of nitrogen fertilization. If the C/N ratio is wider than 1:25, nitrogen would become immobile. However, if the ratio is narrower, the mineralization is possible. Plants alone are complex systems in which a lot of factors interact; therefore, we should elucidate damage potential of ECB in changing environments and techniques that will enable better and sustainable protection of plants against this pest.

2. European corn borer

European corn borer (*Ostrinia nubilalis* Hübner) (Crambidae, Lepidoptera) (**Figure 1**) is a destructive pest on maize, but it also occurs on many other plant species such as peppers and tomatoes. In eastern Croatia, ECBs have been studied for many years [10, 11], population dynamics and intensity of attack of ECB have been monitored since 1965 (Ivezić, personal commun.). In Croatia, ECB usually develops two generations per year; however, the third generation may occur in years with favorable agro-climatic conditions [12]. Larvae feed on aboveground parts of a plant and pass through five larval stages before attaining the pupal stage. The first larval stage tends to move toward higher moisture conditions in

Figure 1.
Female and male of European corn borer (Photo: Sarajlić, 2010).

plants, and for this reason, they feed within the whorls of leaves. When all leaves are developed, typical symptoms of larval feeding can be observed as small holes on leaves, lined in one row (**Figure 2**). Third stage larvae bore into the plant stalk or fruits and disrupt physiological processes inside the plant. Larvae pupate into the stalk holes (**Figure 3**). Larvae are the most abundant in the tasseling and silking stage of maize. During these stages, maize plants are the most attractive for insects to feed on due to the low content of flavonoids [13].

2.1 Influence of agroclimatic conditions on European corn borer

High-temperature stress has a negative impact on plant growth, productivity, and metabolism, and plants are the most vulnerable in the reproductive phase [14]. Climatic stress has a significant impact on ECB oviposition and larvae mortality. The first and second larval stages are the most sensitive to stress, and mortality rates can reach up to 62% [15, 16]. Quantity of available water can be regulated by the irrigation system, but other climatic factors, such as air temperatures, cannot be manipulated in field crops. Excessive rainfalls and lower temperatures can delay ECB appearance from 10 and more days, and the intensity of attack is lower; consequently, the damage is also lowered [2]. We are already facing often deviations from average multiyear precipitation and air temperature; therefore, models to predict the occurrence of a new generation of ECB have been proposed [17].

2.2 Potential of ECB natural enemies

During the vegetation, ECB has been exposed to different species of natural enemies, such as wasps and flies that feed on eggs or larval stages of this pest. The most investigated natural enemies of ECB are *Trichogramma* spp. Contradicting results on their effectiveness have been reported [18]. *Trichogramma* wasps are insect egg parasitoids. We have detected the natural parasitism of ECB eggs by these wasps in Croatia on the second generation of ECB. We reported low populations of natural enemies in the field crop, insufficient to significantly reduce the population of ECB and keep it under the economic threshold. Only artificial release of

Figure 2.
Leaf damage from ECB larvae (Photo: Sarajlić, 2010).

Figure 3.
ECB pupae inside maize stalk (Photo: Sarajlić, 2010).

these wasps may be considered as a reliable suppressant of ECB [19]. *Trichogramma* spp. has a great potential in biological control of sweet maize, where ECB has been controlled by chemicals that pose an environmental and toxicological threat [20].

3. The role of plant defense mechanisms against herbivory

Plants have developed direct and indirect defense mechanisms against herbivores. Direct plant defense is reflected through primary and secondary metabolites (silica, trichomes, proteinase inhibitors, polyphenol oxidases, toxic compounds, and other). The indirect defense is plants' ability to attract insect predators by emitting different volatile organic compounds [21]. Blend structure of volatiles is specific and very complex and depends on plant and herbivore species, plant developmental stage, and environmental conditions [22]. Many plants induce volatile emissions only during the photo phase, while many lepidopteran larvae are nocturnal insects and consume plant material during the night. Damaged plants may emit volatiles for the attraction of natural enemies. Adult insects may avoid these plants for egg deposition; however, lepidopteran larvae could be attracted to induced volatiles [23]. To reveal and better understand signaling pathways between plants and insects, plant defense mechanisms post herbivory attack have been in the focus of many researchers, so far. In a study of the Asian Corn Borer (*Ostrinia furnacalis*), it is found that females laid fewer eggs on damaged plants by insect larvae than mechanically damaged or healthy plants [24]. The blends of plant volatiles are tested to attract gravid females and manipulate with a population of pests as well [25]. The ECB is considered a model insect for studying the sex pheromone communication system [26, 27]. Several strains of ECB (E, Z, and E/Z) are recognized in the world [28], while in eastern Croatia Z-strain is present only [29].

4. The role of nitrogen and C/N ratio on the intensity of the ECB attack

A high amount of nitrogen in the soil system is unavailable for plants. Nitrogen fertilization is applied according to the plans of plant nutrition and expected yields. Nitrogen also rinses in groundwater, so it is necessary to ensure plants with a sufficient amount of nitrogen fertilizer. Plant dry matter contains

2–5% of nitrogen. In soils with higher pH, plants prefer ammonium form of nitrogen, and in soils with lower pH, nitrate form.

The quality of host plants influences the feeding behavior of insects. Plant nitrogen and carbon concentrations as well as other metabolites directly affect insect fertility (oviposition, size, and quality of egg masses). The quality of the host plant is being changed due to damage caused by insect feeding [30]. Plants in treatments with nitrogen fertilization have been damaged more intensively from lepidopteran larvae than plants without fertilization. The plants with the highest growth potential attract lepidopteran pests. This attractive behavior of pests is mostly affected by the plants' nitrogen concentration [31]. High nitrogen supply usually increases protein production and decreases carbohydrates, so plants have softer tissue, and they are more susceptible to the ECB attack [32]. Contradictory results are reported on the effect of sulfur and calcium concentration in maize leaves concerning plant defense mechanisms. The consensus has been made, and their interaction is very important in plant protection against herbivores [33]. Mixtures of lignin, proteins, minerals, and carbohydrates are organic compounds that contain a different ratio of carbon and nitrogen, which is usually abbreviated to the C/N ratio. Preferably, ECB attacks plants that contain higher nitrogen concentration and a narrower C/N ratio. The susceptible plants, those with the highest damages, are characterized by high lignin content and decreased C/N ratio [34]. We aimed to evaluate the role of irrigation and nitrogen fertilization on the ECB attack on maize in Croatia.

5. Experimental design

The open field experiment was set up at the Agricultural Institute Osijek, Croatia (45° 33′27.11N, 18°40′46.52E) during three vegetation seasons: 2012, 2013, and 2014. We tested the efficacy of three treatments of irrigation and nitrogen fertilization on ECB attack on four different maize genotypes. Soybean was used in crop rotation. A factorial experimental design, the split-split plot, was used with three replications. Each independent variable was one factor in experimental design. Three levels of irrigation and nitrogen fertilization are applied for each tested maize genotype 3 × 3 × 4 (**Table 1**).

Factor	A	B	C
	Irrigation	**Nitrogen fertilization**	**Hybrid**
Treatment	A1—natural rainfall (control)	B1—soil nitrogen (control)	C1—OSSK 596 C2—OSSK 613
	A2—60–100% FWC	B2—100 kg N ha^{-1}	C3—OSSK 602
	A3—80–100% FWC	B3—200 kg N ha^{-1}	C3—OSSK 552

FWC, field water capacity.

Table 1.
Factors and treatments in the experiment.

The area of the experimental field was 0.5 ha. The basic plot consisted of two maize rows 10 m long. Row spacing was 70 cm. The first three rows at the edges of fertilization plots were omitted in evaluations. Maize was sown with hand planters with two seeds per together and thinned in the phase of 4–6 leaves plants.

1. Plot size was: factor (a), irrigation, 4 hybrids 56 m^2 × 3 level of nitrogen fertilization = 168 m^2;

2. Subfactor (B), nitrogen fertilization, 4 hybrids × 14 m^2 = 56 m^2; and

3. Subsubfactor (C), maize hybrid, 14 m^2 (10 m × 2 rows × 0.7 m = 14 m^2).

5.1 Irrigation

Maize was irrigated by Typhon system (sprinkling irrigation). The range of the system was 20–25 m. Intensity and amount of water were regulated by the selection of nozzles and the speed system movement. Watermark 200SS device was used to determine the start of irrigation and to monitor the soil moisture condition. The water quality analysis was satisfactory [35].

5.2 Nitrogen fertilization

Nitrogen fertilization was applied four times per vegetation. One-third of urea (46% N) was applied in the autumn with the basic soil cultivation, and the two-thirds were added pre-sowing. Top dressing was done twice with KAN (calcium-ammonium nitrate; 27% N). The first top dressing, one sixth of N was done in phase 6–8 leaves, and the last application, one sixth of N was done in phase 8–10 leaves.

5.3 The maize genotypes

Maize genotypes used in this experiment are developed at the Agricultural Institute Osijek and presented in **Table 2**.

Hybrid	Factor	FAO group	Insect tolerance
OSSK 596	C1	590	+
OSSK 617	C2	610	+
OSSK 602	C3	620	++
OSSK 552	C4	580	—

+—Increased tolerance to diseases and insects. ++—Increased tolerance to ECB. – Not specified (Source: http://www.poljinos.hr) [36].

Table 2.
Maize genotypes.

5.4 The evaluation of ECB larval damage

Stalks were dissected at the end of the vegetation period [2], at the beginning of September in 2012 and 2013, and October in 2014 due to the unfavorable environmental conditions. In total, 1080 maize plants were evaluated for tunnel length (cm) [TL].

5.5 Chemical analysis of the maize leaves

For plant analysis, we took 10 randomly chosen leaves below the ear in the silking stage, from each plot from 1080 maize plants. Leaves were cleaned from dust and other debris, stored in paper bags, and dried at 70°C to decrease in elasticity and then further dried at 40°C for 24 hours. Dried leaves were crushed on Retsch Gmbh Germany, SM 100 mill, and from 10 crushed leaves, subsample was taken. Organic carbon was determined by oxidation of dry samples by a wet process at 135°C [37] and nitrogen content by the Kjeldahl method. The C/N ratio was calculated.

5.6 Statistics

The data were evaluated by analysis of variance after the data were subjected to a normality test using the SAS software [38]. Log transformation (log [n + 1]) was used to normalize the data. Least square means with the Tukey adjustment for multiple comparisons were calculated and reported for significance at the 95% confidence level. Back transformation was done for original values. Data in figures are presented with standard error (SE) bars. Pearson correlation coefficient is used to test relationships between variables.

5.7 Influence of irrigation on nitrogen concentration and C/N ratio

In the formation of maize reproductive tissues, heat stress occurs at 32.5°C air temperatures, and as a consequence, pollen viability is decreased and pollen tube elongated [14]. We have observed the highest air temperatures (on average 19.95°C) in vegetation season in 2012 with peaks above 33°C in July and August [2] and low natural rainfall (**Figure 4**). In 2012, plants undergone drought stress since 22% less precipitation was recorded compared to the multi-year average (62.41 mm; https://meteo.hr). In subsequent years, we noticed over multi-year average precipitations (**Figure 4**).

The data on the damage of maize stalks caused by ECB larval feeding were previously analyzed and reported [2]. The greatest damage of maize stalks was observed in 2012, ranging from 49.13 cm (A3) to 79.22 cm (A1) tunnel length per stalk (**Figure 4**). The lowest damage was recorded at the highest level of irrigation (A3). Drought in 2012 probably affected ECB survival and consequently larval damage. Excessive rainfall in 2013 and 2014 could have caused eggs to rinse from maize leaves, thus preventing the penetration of larvae inside the stalk and less plant damage.

Available nitrogen and water supply are the most important factors for plant growth and quality. Plant nitrogen concentration in maize leaves significantly differed between the irrigation plots and the control plots in 2012. Treatment with the highest nitrogen concentration was also with the highest irrigation level A3 (2.93%); compared to the control plot, it was higher for 8.87%. In the subsequent years, plant nitrogen concentrations were not affected by irrigation treatments (**Figure 5**). Soil moisture level and texture are the major factors influencing the root uptake of nitrogen [2]. Our results revealed that nitrogen uptake was not only increased under irrigation in drought year, which was characterized by high temperature and low rainfall, but also decreased in optimal or extremely wet years with a large amount of rainfall. Some authors reported that nitrogen concentration was increased in the aboveground part of irrigated plants and roots nitrogen concentration decreased in irrigation plots [39].

In 2012, the C/N ratio was the widest in control plots of irrigation (A1) (15:1), and it was significantly higher for 1.17 than in the plots with the highest irrigation level (A3). However, the C/N ratio in 2012 was narrowest comparing to the other 2 years. In subsequent years, there were no statistical differences between irrigation treatments concerning the C/N ratio (**Figure 6**). Due to the decreased nitrogen concentration during drought, the values of the C/N ratio were wider in a drought year (2012) and narrower in the other 2 years. In our study, the greatest damage by

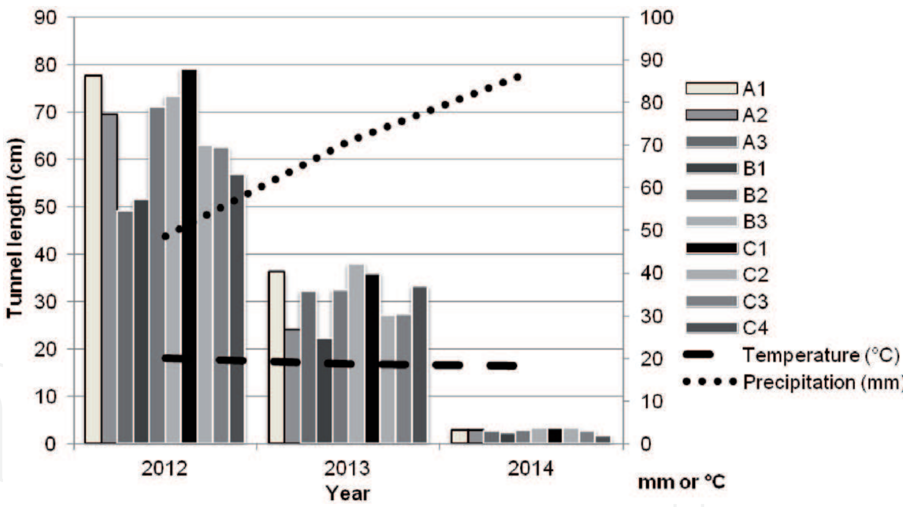

Figure 4.
Maize damage caused by ECB larval feeding and agroclimatic conditions 2012–2014.

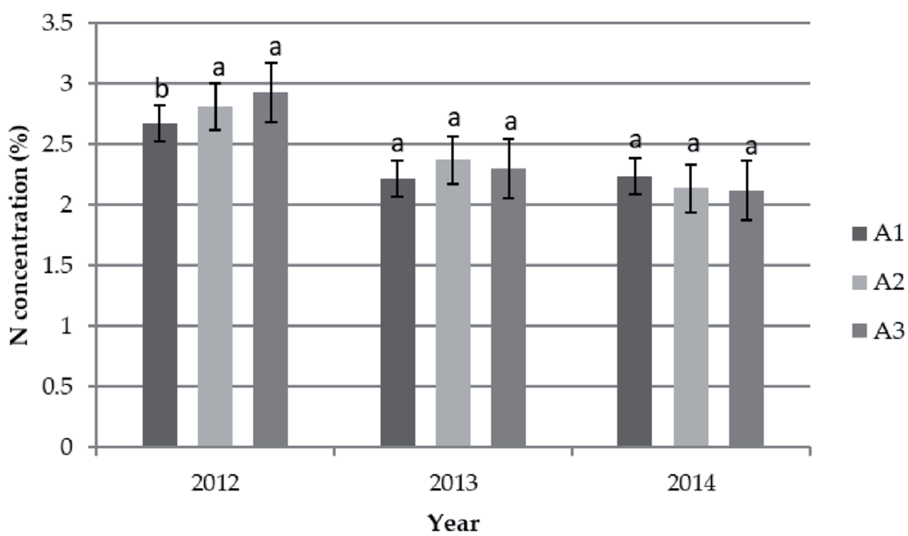

Figure 5.
Plant nitrogen concentrations in irrigation treatments presented by years of investigation.

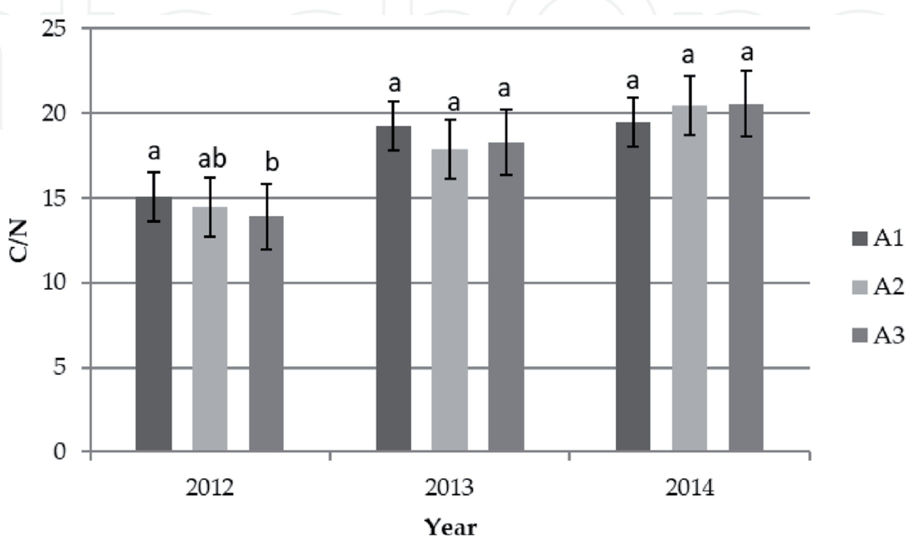

Figure 6.
The C/N ratio in irrigation treatments presented by years of investigation.

ECB was found in 2012 when the narrowest C/N ratio was calculated compared to the other 2 years.

Plant nitrogen concentration increased with an increase in fertilization rates, while the C/N ratio was narrowed. This problem has been studied by many researchers, and they also obtained similar results [40–43]. Upon herbivores attack, maize plants differently react, and it can be observed as translocation of sugars in stalk and root, increase in nutrition and photosynthesis, and other processes. All these changes can affect the C/N ratio in plant tissue [44]. The significantly highest concentration of nitrogen occurred, as it was expected in fertilization treatments with the highest rates (B3) in all years of research, compared to the control (B1) (**Figure 7**).

The B2 and B3 treatments did not differ statistically in plant nitrogen concentration. The lowest nitrogen concentrations were detected in 2012 in control plots (B1), and compared to the plots with the highest level of fertilization (B3), it was lower for 2.87%, in 2013 for 31.41%, and in 2014 for 22.26% (**Figure 7**). On average, 20.7% higher nitrogen concentration was found in plants in the B3 treatment compared to the control. Nitrogen fertilization increases ear weight and yields [45]. In all years, the greatest damage, tunnel length created by ECB larvae, was in the B3 treatment and the lowest in the control. Our data are similar to previously reported studies [8, 9]. By increasing the level of nitrogen fertilization, the C/N ratio was significantly reduced. Significant differences in C/N ratio were found in all years between the control (B1) and the other two treatments of nitrogen fertilization (B2 and B3), with the exception in 2012, when significantly differed only B1 and B3. The rate of applied nitrogen was not a significant factor for the C/N ratio since the B2 and B3 treatments did not differ statistically. In 2014, the C/N ratio was the widest (15:1) on the control treatment (B1), and it was wider for 5.47 than the B3 treatment. Similar results are obtained in 2013 (23:1) when the C/N ratio was wider for 8.38 and in 2012 (15:1) for 1.27 (**Figure 8**). On average, the widest C/N ratio (21:1) was recorded at the treatment B1, and compared to the B3 treatment, it was significantly wider for 5.04.

The highest nitrogen concentration in this research and the lowest damage from ECB larvae were observed in maize hybrid C4 (**Figure 4**) [2]. These results are contrary to the studies who reported a positive relationship between plant nitrogen and ECB damage. The insects' interactions are complex, and other compounds

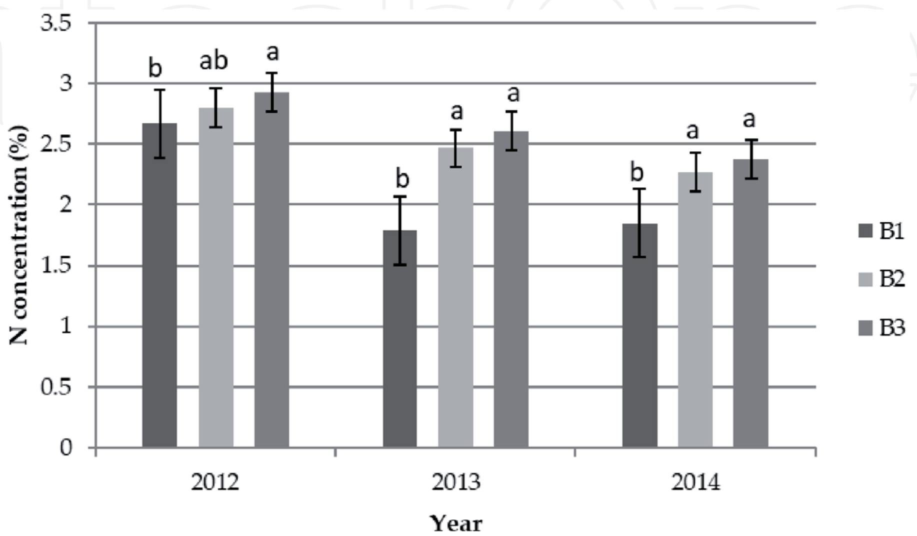

Figure 7.
Nitrogen concentrations in fertilization treatments presented by years of investigation.

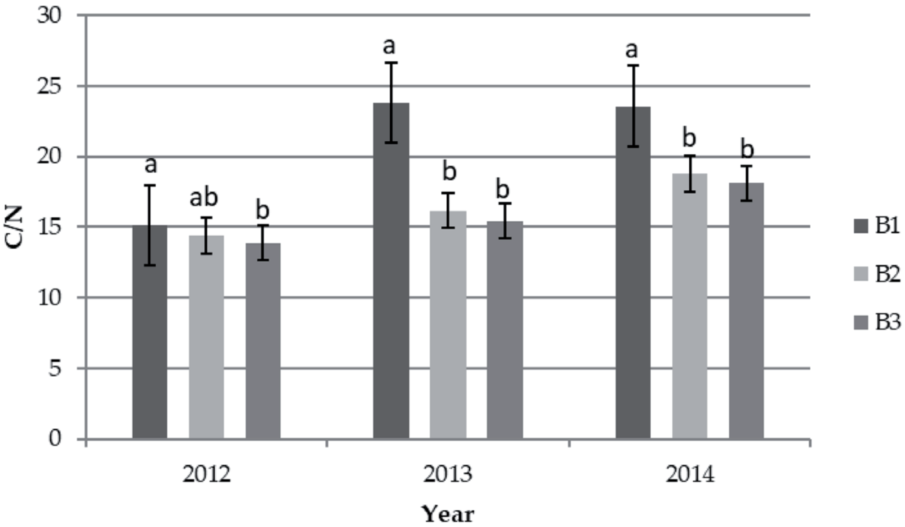

Figure 8.
The C/N ratio in nitrogen fertilization treatments presented by years of investigation.

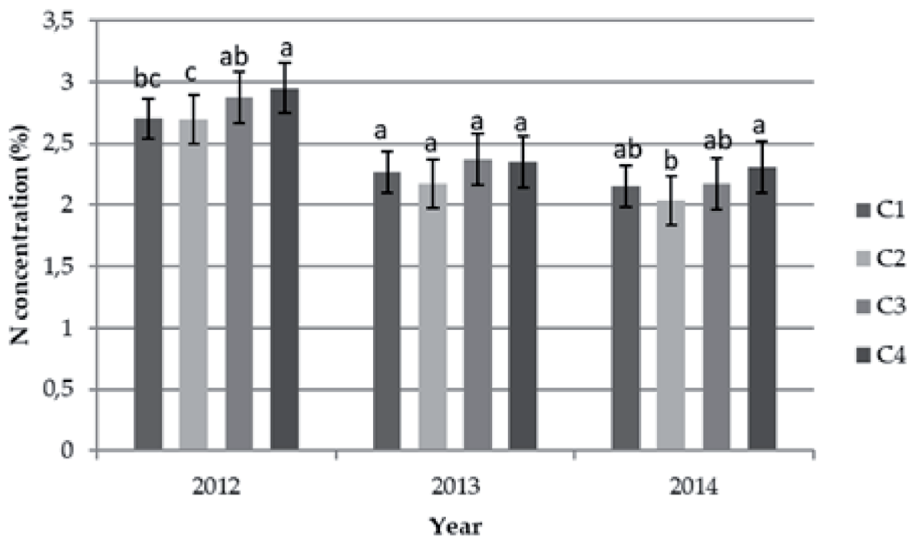

Figure 9.
Nitrogen concentrations in different maize hybrids presented by years of investigation.

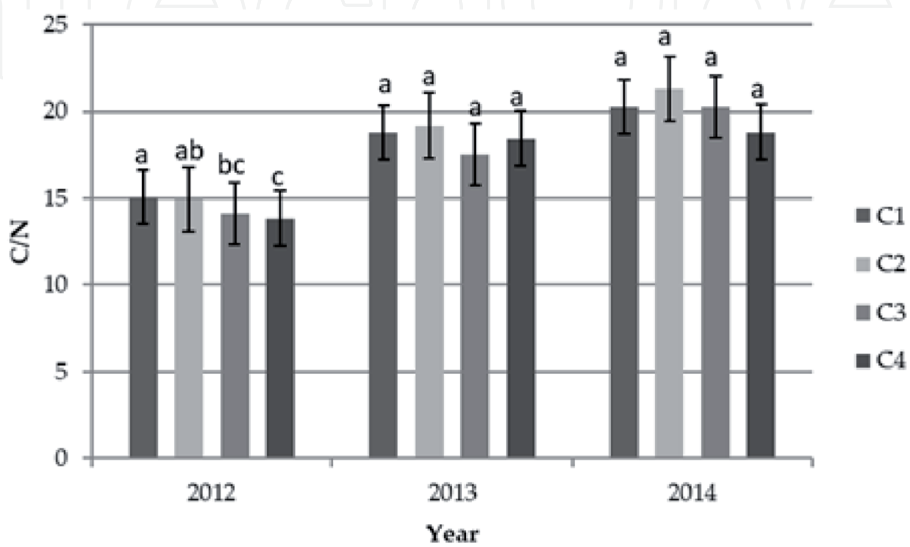

Figure 10.
The C/N ratio in different maize hybrids presented by years of investigation.

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	Irrigation			Nitrogen fertilization			Maize hybrids			
	A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**	**C4**
2012										
$R_{tl;N}$	−0.04	0.16	0.07	−0.43**	0.09	−0.26	−0.16	0.09	−0.21	0.07
$R_{tl;C/N}$	0.01	−0.15	−0.01	0.36*	−0.18	0.30	0.19	−0.08	0.24	−0.11
$R_{N;C/N}$	−0.89**	−0.91**	−0.93**	−0.85**	−0.86**	−0.95**	−0.91**	−0.91**	−0.91**	−0.89**
2013										
$R_{tl;N}$	0.09	0.27	0.09	0.07	−0.32	0.11	0.23	−0.17	0.47*	−0.14
$R_{tl;C/N}$	−0.11	−0.26	−0.09	−0.05	0.28	−0.11	−0.24	0.10	−0.49**	0.13
$R_{N;C/N}$	−0.98**	−0.99**	−0.98**	−0.99**	−0.95**	−0.99**	−0.98**	−0.99**	−0.99**	−0.99**
2014										
$R_{tl;N}$	0.01	−0.01	0.03	0.23	0.02	0.14	−0.26	0.22	0.19	−0.05
$R_{tl;C/N}$	0.01	−0.03	−0.05	−0.28	−0.05	−0.07	0.32	−0.26	−0.15*	0.02
$R_{N;C/N}$	−0.96**	−0.98**	−0.97**	−0.97**	−0.93**	−0.96**	−0.96**	−0.98**	−0.98**	−0.97**

A1—control, A2—from 60 to 100% WFC, A3—from 80 to 100% WFC; B1—control, B2—100 kg N ha^{-1}, B3—200 kg N ha^{-1}; C1—OSSK 596, C2—OSSK 617, C3—OSSK 602, C4—OSSK 552; tl, tunnel length; N, nitrogen; C/N ratio.
**$P < 0.05$.*
***$P < 0.01$.*

Table 3.
Correlation coefficients between ECB feeding and nitrogen concentrations and C/N ratio among tested years and treatments.

may have an impact on larval feeding such as phenols, carbohydrates, and other components [46]. The highest nitrogen concentrations on average were determined in 2012, while the lowest was found in 2014 (**Figure 9**). In both years, a statistically significant difference occurred between C2 and C4 hybrid. On average, hybrid C4 had the highest nitrogen concentration, and it was higher from 2.75 to 9.45% than observed in other hybrids.

The C/N ratio was found to be significantly different among several hybrids only in 2012. The hybrid C1 had significantly wider value than the hybrid C4 (**Figure 10**). On average, no significant difference occurred between the hybrids; however, the widest C/N ratio was measured for hybrid C2 (18:1).

The relationship between nitrogen concentration and C/N ratio was strong negative in all years and all treatments. The relationship between tunnel length in stalks caused by the ECB larvae and nitrogen concentrations was weak or moderately strong but inconsistent over the years of investigation. Plants require carbon and nutrients for growth. If nutrients are limited, plants tend to accumulate more carbohydrates that can be immediately used. When the ratio of carbon is increased to nutrients, some carbohydrates can be incorporated into secondary metabolism of plant. Secondary metabolites have a defensive role in plants [47]. Carbon, water, and mineral nutrient allocation in a plant depend on genotype and plant environment [48]. The concentration of secondary metabolites increases with drought stress [49]. Nitrogen fertilization leads to a high concentration of nitrogen in plant tissue and a lower concentration of secondary metabolites, but drought stress limits nitrogen adsorption, and such plants are not attractive to herbivores. Our investigation did not give strong evidence that nitrogen concentration and the C/N ratio impact the feeding behavior of ECB larvae in maize stalks (**Table 3**).

6. Conclusion

Nitrogen uptake was increased in irrigation treatments in drought year characterized by high temperatures and a small amount of rainfall. Decreased plant nitrogen concentrations were observed in optimal or extremely wet years with a large amount of rainfall. By increasing the level of nitrogen fertilization, the C/N ratio was significantly reduced. The highest nitrogen concentration in this research and the lowest damage from ECB larvae were observed in maize hybrid C4. The relationship between nitrogen concentration and C/N ratio was strongly negative. We found a weak or moderately strong relationship between damage caused by the ECB larva and nitrogen concentration. Our results indicate that maize damage caused by ECB is negatively affected by plant nitrogen concentrations only when plants are under drought stress. However, the relationship between ECB larval damage and plant nitrogen concentration depends on the nitrogen fertilization rates. We did not find strong evidence for this hypothesis and did not prove that plant nitrogen concentration or more quality plants would be more damaged by European corn borer. Further studies, in controlled environments, are needed since our results were inconsistent over the years and indicate the great impact of agroclimatic conditions (drought) on the potential of ECB to create damage.

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Conflict of interest

All authors saw and approved this book chapter. We warrant that the chapter is the authors' original work and is not under consideration for publication elsewhere. All coauthors agree that the corresponding author will be responsible for the submission. We warrant that all authors have contributed significantly to the work.

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