Control of western corn rootworm damage by application of soil insecticides at different maize planting times

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Abstract

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (WCR), is one of the most damaging maize crop pests. Damage to maize is primarily caused by the larvae feeding on roots, which results in a reduction of water and nutrient uptake and may cause lodging as well as a substantial reduction in grain yield.

The effects of soil insecticides, applied at different planting times, on controlling WCR damage were investigated in 76 naturally infested fields in northern Italy over a 4-year period.

Without furrow insecticides, plant density was reduced at all of the considered planting times (+5.3%). Insecticide application let to a significant reduction in the WCR larval density (-36%). As a consequence, the root injury measured by nodal injury scale, was reduced (-75%) as was the incidence of plants with "gooseneck" symptoms (-71%). Furthermore, the soil-applied insecticide plots showed a significant increase in plant biomass yield at the dough stage (+4.9%) and in grain yield at physiological maturity (+8.3). The application of soil insecticides showed a positive yield increase in 95% of the compared production situations. The yield increase was higher than 5% in 70% of considered cases. The effect of the soil-applied insecticides on root and plant damage symptoms, and the consequent biomass and grain advantage were steady over the different intervals between planting time and WCR egg hatching.

The results of this study have underlined that the application of a soil insecticide to the furrows at planting leads to a clear control of injuries from insects and a consequent maize yield advantage.

Key words

maize, grain yield, Western Corn Rootworm, insecticide, planting time

Abbreviations: DM, dry matter; GDD, growing degree days; GS, growth stage; IPM, Integrated Pest Management; NIS, node injury scale; PS, production situation; RR, relative ratio; WCR, Western Corn Rootworm.

1. Introduction

The Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is a dangerous maize (*Zea mays* L.) pest in several countries. It is native to Central America, and it has been recognized as one of the most destructive pests in the Midwestern United States since the middle of the last century (Park and Tollefson, 2006). In Europe, WCR is considered an invasive species, which was accidentally introduced through multiple events from early 1980s till 2000s (Lemic et al., 2015). WCR was reported for the first time in 1992 in Serbia. After introduction and establishment period, WCR rapidly spread to other European maize growing areas and it has currently reached high population levels in Central-Southern Europe, included northern Italy (Kiss et al., 2005). Economic damage is more common in areas in which maize is grown as a continuous crop and where the environmental conditions are favourable to the building of high WCR populations.

According to Boriani et al. (2006) an action threshold of about 5 adults/trap/day is used to evaluate captures with 6–8 Pherocon® AM traps/field (depending on field size), over a six/eight-week period. Economic damage has been reported in several growing seasons and areas of northern Italy, when the adults captures exceed this threshold, without insecticide application (Blandino et al., 2014).

Since WCR larvae only feed on maize roots and can only survive in small numbers on a limited number of non-maize grasses (Oyediran et al., 2004), crop rotation has proved to be an effective pest management strategy when the action threshold is exceeded. However, the intense use of a simple crop rotation, such as the maize-soybean rotation adopted in the Midwest in the USA, has selected for an insect strain that can lay down eggs in soybean fields. The larvae hatching from these eggs emerge into maize fields the following spring (Gray et al., 2009).

Crop rotation is also the main effective integrated pest management (IPM) control option adopted in Europe. However, in the more profitable maize growing areas where continuous maize is preferred, if the pest is widespread and reaches high population levels, soil and/or foliar insecticides are frequently applied. Insecticide sprays used to control the adults can be applied at the beginning of female egg laying to reduce oviposition or, in case of extremely high infestation, before silking to reduce silk feeding by WCR adults. In this case, the timing of the insecticide application is critical, and needs precise knowledge on the phenology of the WCR population in the field.

The most common strategies used to protect maize roots from WCR are the application of a soil insecticide at planting, and the use of an insecticide seed dressing (Sutter et al., 1990; van Rozen and Ester, 2010) when available. Numerous conventional insecticides, in granular and liquid formulations (pyrethroid, organophosphate, neonicotinoid, and phenyl pyrazole classes), are registered for WCR larval control in Europe. These active ingredients have also proved to control other soil insects that could attack maize seedlings, and thus reduce plant density, such as *Agriotes* spp. wireworms (Ritter and Richter, 2013).

In recent years, the use of maize seeds treated with insecticides belonging to the neonicotinoid class has been restricted in several European countries because of their adverse effects on honeybees (Girolami et al., 2012). This restriction has determined a notable increase in soil insecticide applications for maize crops, thus raising concerns about their undesired side effects on the agroecosystem and non-target organisms, and about their effective benefit to the crop (Furlan and Kreutzweiser, 2015).

The effectiveness of soil-applied insecticides and seed dressings is variable and still debated (Cox et al., 2007), and there are different opinions concerning the effect of planting time applications. Some authors claim that they are ineffective in WCR control and eradication programs (Furlan et al., 2006), but their efficacy in containing root damage has been confirmed. The effects on yield of soil insecticides, such as tefluthrin, thiamethoxam, tebupirimphos and cyfluthrin, are reported quite inconstant, varying from none to more than 60% (Cox et al., 2007, 2008; Ma et al., 2009; Dun et al., 2009; Petzold-Maxwell et al., 2013). Many factors can affect the efficacy of soil treatments: the interactions between planting time and soil texture, application depth, organic matter, pH and weather conditions (rainfall), with consequent variable effects on its persistence. In addition to leaching, insecticide persistence could be affected by volatilization and chemical degradation with higher temperatures and lower soil moisture levels (van Rozen and Ester, 2010; Furlan and Kreutzweiser, 2015).

In order to correctly address the control strategies for this pest in Europe, there is an urgent need to assess the impact of soil-applied insecticides to control WCR and develop integrated strategies to minimize yield loss in different environments. While the effect of selected insecticides on the control of WCR has often been mentioned under experimental conditions at a single field scale, or in semi-field conditions with artificial rootworm infestations or in small-plot studies (Sutter et al., 1990; Magalhaes et al., 2007), its evaluation under natural infestation conditions at a regional scale has been investigated less (Kuhar et al., 1997; Fuller et al., 1997), especially in Europe where this invasive species is still spreading.

The aim of this study was to verify the effect of soil insecticides on the control of WCR damage and quantify the related yield advantage in naturally infested fields. The effect of the insecticides applied at planting has been investigated in different production situations for full-season maize hybrids in northern Italy, but considering different intervals between the application times (planting) and the occurrence of the WCR larvae in the field, or different agronomic conditions for soil tillage and irrigation.

2. Materials and Methods

2.1. Experimental set up

The effect of soil insecticide application in seed furrows at planting on the control of WCR larval damage and the consequent maize yield was investigated in 76 production situations (PS) over a 4-year period (2010-2013) in northern Italy. In each location the soil insecticide application at planting time was compared to an untreated control using a completely randomized block design with 3 replicates per treatment. The plots were all 20 m long and 8 rows wide and they were staked out side by side in a fully planted field. Row distance was 0.75 m, while plant distance per row was 0.17-0.20 according to maize hybrid maturity group. All the measurements were conducted in the two middle rows.

A list of the compared PS and their main geographic and agronomic information is reported in Table 1.

The choice of the experimental sites was made considering fields with a high WCR infestation recorded in the previous year (above the threshold of 5 adults/trap/day with Pherocon® AM traps), according to the information obtained from the adults territorial monitoring and for which a high presence of the pest might be expected during the survey. Moreover, the previous crop was always continuous maize cultivated without any former foliar insecticide application to control WCR populations or other maize pest.

The effect of soil insecticide application was evaluated considering different intervals between planting time and expected egg hatching. The compared PS were subdivided into 3 groups on the basis of the interval of cumulated growing degree days (GDD) from the maize planting date to the end of egg hatching, expressed as the 90% of 1st instar WCR larvae cumulative occurrence (WCR90%). The logistic equation developed by Davis et al. (1996) with a minimum and maximum developmental threshold for the WCR larvae of respectively 11°C and 18°C was used to calculate the cumulated GDD from January 1st to WCR90% and to the maize planting date of each PS. The quantification of the cumulated GDD interval between maize planting date and the end of egg hatching (MP-WCR90%) was performed using the maximum and minimum air temperatures recorded by weather stations located next to each experimental site.

In order to obtain an equal distribution of recorded cases, the considered PS were subdivided into the following MP-WCR90% classes: GDD < 150 °C d^{-1}(23 cases), 150-230 °C d^{-1} (28 cases), and > 230 °C d^{-1}(25 cases).

The soil insecticide was distributed using a calibrated granular dispenser or sprayer applied to the planter. The insecticide was distributed in seed furrows at a depth of 5-10 cm from the soil surface at the recommended rate for each product according to the equipment setting and the desired seeding depth. No insecticide was applied as a seed dressing in any of the tested PS. The applied soil insecticides belong to the pyrethroid, neonicotinoid or organophosphate classes, and they are listed in Table 1. The formulation and the application rate are reported in the table footnotes.

The considered PS included the use of full season maize for both grain and whole plants as silage. The maturity class (FAO 500-700) of the tested hybrids was selected according to the characteristics of the growing area and the adopted planting time. Different irrigation systems were adopted, according to the typical farm management practices used in the area; 11 of the considered trials were performed under non-irrigated conditions. In most of the cases, the maize was planted after conventional soil tillage practices, which included a 0.25-0.40 m depth ploughing, followed by disk harrowing; in 9 cases out of 76 trials, minimum tillage techniques were applied instead of ploughing. Other agronomical practices, such as fertilization and weed control, were conducted according to the typical management system of each farm and to the ordinary agronomic techniques of the area.

In order to evaluate the interaction of soil insecticide with planting times and in agreement with the common full season maize planting time in northern Italy, the planting dates were comprised between mid-March and late May over the four-year period. Very late planting times, after most of the WCR larvae had hatched, were excluded because of the possibility of the negative impact of the lack of maize roots on larval survival.

2.2. Entomological analyses

The WCR larval infestation was recorded in 19 PS, which were representative of the surveyed growing seasons and areas. A 25 cm cube of soil core containing the root system and the surrounding soil was collected and individually washed inside a plastic container (40x53x25 cm) at the time of the maximum larval occurrence, according to the Davis et al. (1996) model. The soil was repeatedly moved to allow all the larvae floating on the water surface to be collected with a thin brush and counted. After being washed, the roots were immediately transferred to the laboratory and individually placed inside a modified Berlese funnel kept at room temperature (23±1°C T, 65±2% RH). All the larvae that emerged from the drying roots were collected in a plastic cup filled with tap water which had been placed at the bottom of each funnel. The funnels were checked daily for at least three weeks.

In all the PS, the WCR damage to the root system was quantified at the beginning of adult emergence, using the nodal injury score (NIS) developed by Oleson et al. (2005).

Larval infestation and NIS were recorded on 60 plants (20 plants per plot, randomly selected in the two rows adjacent to the two middle ones) for each soil insecticide treatment and the untreated control in each surveyed PS.

2.3. Crop measurements

After plant emergence, the homogeneity of crop density in the insecticide-treated and untreated plots of each PS was verified at 2-4 leaf stage (growth stage - GS 12-14, according to BBCH scale).

In all the PS, the crop density and the percentage of plants with "gooseneck" symptoms were quantified at the dough stage by counting the number of plants in the two middle rows of each plot along the whole plot length (20 m).

Whole plants were collected manually at the dough stage (GS 85) in a representative PS sub-sample (48 cases over 4 growing seasons) from a 3 m^2 area (two rows 2 m long) randomly selected in the middle of each plot. Plant samples were weighed in order to establish the biomass yield and then passed through a field chopper. About 3 kg of chopped subsample was weighed before and after being dried at 105°C for 48 hours in order to establish the dry matter (DM) content.

Ears were collected manually at physiological maturity (moisture content between 22 – 30%), in all the PS, with the exception of fields fully harvested at the dough stage for silage (16 cases), from a 4.5 m^2 area (two rows 3 m long) randomly selected in the middle of in each plot and were then passed through an electric sheller. The grain yield was corrected to a commercial moisture level of 14%.

2.4. Statistical analysis

The relative ratio (RR= soil-applied insecticide/untreated control) between the soil-applied insecticide in the seed furrows at maize planting and the untreated control was calculated for each measured parameter for all the PS for each MP-WCR90% class and for PS that differ for soil tillage and irrigation. The cases with no root injury and plants with no "gooseneck" symptoms in both the untreated control and insecticide-treated plots were excluded for the calculation of the RR, in order to not consider undefined RR values for these parameters. The average RR and the confidence interval were calculated at significance levels of 99.9% ($P<0.001$), 99% ($P<0.01$) and 95% ($P<0.05$) for crop density, WCR larval density, root injury, plants with "gooseneck" symptoms, plant biomass at the dough stage and grain yield.

The analysis of variance (ANOVA) was used for each recorded parameter to compare the RR obtained in the different MP-WCR90% intervals or for the different soil tillage or irrigation. The residual normal distribution was verified using the Kolmogorov-Smirnov test, while the homogeneity of variances was verified using the Levene test. Multiple comparison tests were performed, according to the Tukey test, on the treatment means. Within the same PS, all the environmental and agronomic conditions were the same for the soil insecticide treated and the untreated control, allowing a direct comparison of soil-insecticides application in different conditions.

SPSS for Windows statistical package, Version 21.0 (SPSS Inc., Chicago) was used for the statistical analysis.

3. Results

The results of the application of soil-insecticides to the maize seed furrows are summarized in Table 2 for the different planting times and compared to the untreated control.

Without furrow insecticides, plant density was reduced at all of the considered planting times ($P<0.001$). On average, the untreated control resulted in a 5.3% lower plant density compared to the soil-applied insecticide treatment. Moreover, the advantage of insecticide application was significantly affected by the planting time: in the early sowings, which were conducted between mid-March and mid-April (MP-WCR90% > 230 GDD), the RR of the soil-applied insecticide/untreated control was significantly higher than that of the later planting times ($P=0.007$), and the difference in plant density between the insecticide treatments and the control was 7.3%. Conversely, the plant density at the 150-230 planting time and <150 °C d^{-1} increased to 4.8% and 3.7%, respectively. Although no specific measurements were conducted to quantify the possible damage to seedlings caused by other soil insects, the field observations highlighted the presence of *Agriotes* spp. wireworms in several production situations, particularly in the early sowings.

On average in the considered PS, the application of insecticides significantly reduced ($P<0.001$) the WCR larval density, that is by 36%, compared to the untreated control. A significant effect of soil-applied insecticide on controlling WCR larvae was observed at all the planting times. Although no significant differences ($P=0.145$) were reported between the RR for the different MP-WCR90% intervals, the effectiveness of soil-applied insecticides on reducing the larval population slightly decreased as the intervals between the maize planting and the occurrence of larvae in the soil increased. The average larval reduction obtained by means of the soil-insecticide treatments, compared to the untreated control, was 45%, 40% and 22%, with MP-WCR90% intervals of < 150, 150-230 and > 230 °C d^{-1}, respectively.

Root injuries attributable to WCR larvae were detected in 63 PS (83% of the cases); among these, root damage resulted in visible symptoms in the aerial part of the crop in 36 cases, with the occurrence of "gooseneck" plants. This data confirm that the relationship between adult infestation levels and the impact of WCR on maize damage is highly variable, as a consequence of the interaction with the environmental and agronomic conditions.

The soil-insecticides applied to the maize seed furrow resulted in a significant reduction ($P<0.001$) of root injury (-75%) and in the occurrence of plants with "gooseneck" symptoms (-71%), compared to the untreated control (Table 2). Moreover, the reduction was similar in all the considered intervals between maize planting and larval occurrence.

On average, the soil-applied insecticide significantly increased the plant biomass at the dough stage (+4.9%) and the grain yield at the end of maturity (+8.3%), compared to the untreated control ($P<0.001$) (Table 2). The insecticide application at planting significantly affected the grain yield at all of the considered intervals between maize planting and larval occurrence, while a not significant increase in plant biomass was only reported for MP- WCR90% intervals < 150 and > 230 of °C d^{-1} ($P<0.05$). The RR of the soil-applied insecticide/untreated control for the grain yield increased slightly from late to early planting time, although no significant differences were observed between the different MP-WCR90% intervals.

No evident adult feeding activity on silk has been observed in any of the compared PSs. The results of the application of soil-insecticides to the maize seed furrows compared to the untreated control in different agronomic conditions (soil tillage or irrigation) are summarized in Table 3. The application of soil insecticide confirms a significant effect on the recorded parameters also in these considered conditions. The RR of the soil-applied insecticide/untreated control for all the parameters was not significantly different between the compared tillage practices. Otherwise, a significant increase of RR for grain yield was observed in non-irrigated fields compared to irrigated ones.

Figure 1 represents the percentage of grain yield variations obtained applying the soil insecticides, sorted from the lowest to the highest, in all of the 60 PS considered for this parameter. In 95% of the cases, a positive yield increase was shown, with 70% of the cases resulting in a 5% higher yield increase than the untreated control.

4. Discussion

This study has proved that conventional soil-applied insecticides into seed furrows in growing areas with high *D. v. virgifera* infestations, where maize is grown as a continuous crop, lead to a reduction in the WCR larval presence and in root injury, which in turn contributes to an overall significant grain and biomass yield advantage.

The data collected as part of an extensive comparison of different PS, in intensive maize farming systems and under natural WCR infestation, have confirmed the positive role of soil-applied insecticides on maize yield, as already reported in other specific field experiments (Ma et al., 2009) and in surveys conducted in the USA (Fuller et al., 1997; Kuhar et al., 1997).

Under artificial and controlled infestation, Sutter et al. (1990) reported that yield protection by means of insecticides was effective and significantly higher at greater WCR infestation levels, and the compared insecticides (carbofuran, terbufos, isofenphos active ingredients) did not differ in their crop protection ability. Furthermore, the application of soil insecticide treatments significantly reduced lodging, although with differences according to the active ingredients.

Dillen et al. (2010) developed a bio-economic simulation model to assess the absolute and relative competitiveness of alternative WCR larval damage abatement strategies (no control, crop rotation, Bt maize, seed treatment, soil insecticide). The authors reported that, in a Hungarian grain maize monoculture, in growing areas where Bt maize was not deregulated, the application of soil insecticides led to an average economic advantage of 113 € ha^{-1}, and it creates benefits in 54% of the cases. By considering a maize grain price of 170 € t^{-1} and an average cost of soil insecticide of 80 € ha^{-1}, an economically profitability of soil insecticide application was observed in 85% of the production situations analyzed in the present study. On average, the economic advantage of using soil insecticide was 125 € ha^{-1}. Conversely, in other environmental, agronomic and insect pressure conditions, some authors have reported insecticide treatments against soil maize pests as not being justified, both in the presence (Kuhar et al., 1997; Furlan et al., 2006; Cox et al., 2007; Petzold-Maxwell et al., 2013) and absence of *Diabrotica* populations (Piqué et al., 1998).

In the present study, although a general higher effect has been observed concerning the control of the WCR larvae when a short interval elapses between planting time and egg hatching (later planting), the effect of soil-applied insecticide on root damage and gooseneck plant incidence and the consequent biomass and grain advantage were steady for the different planting times. It is important to highlight that the study did not consider very late planting times, after most of the WCR larvae had hatched. Conversely, in experiments that also took into account plantings after the maximum larval occurrence (Hoffmann et al., 2000; Obopile et al., 2013), both root damage and plant lodging were shown to be more reduced by applying soil insecticides at early planting time. The authors attributed the reduction in gain in the late plantings to lower infestations, due to the mortality by starvation of the WCR larvae.

The present study, which was conducted in a large number of different production situations, suggests that the effectiveness of conventionally applied soil insecticides is hardly influenced by the maize planting date. However, in specific production situations, a variable response could be expected. The efficacy of this control strategy depends on a number of environmental and application-related factors, such as the level of pest population, the applied active ingredient and its rate, the mechanical and operational aspects, the interaction with pedo-climatic conditions and the insecticide leaching or degradation (van Rozen and Ester, 2010).

Although several in-furrow soil insecticide products have been considered, the present study was not designed to compare the efficacy of different active ingredients. Since these direct control products could differ according to formulation, level of solubility and method of placement as well, an *ad hoc* investigation on the impact of the different available solutions is needed. In particular, it is necessary to determine whether a different efficacy on WCR is obtained through the application of systemic or non-systemic soil insecticides, considering both applications to seed furrows or as seed dressings, where available.

The application of insecticides to seed furrows also plays an important role in controlling other soil insects, such as *Agriotes* spp. wireworms (Ritter and Richter, 2013). Although these larvae are polyphagous, they can feed on maize roots in the same way as *D. v. virgifera* larvae, and can cause comparable economic damage. Moreover, wireworm feeding may prevent germination and increase seedling mortality, which implies a reduction in plant density (Barsics et al., 2013).

The data recorded in the present work confirm the higher positive effect of soil-applied insecticides in preserving plant density, especially in early planted maize compared to later planted maize, thus highlighting that the wireworm activity on seedlings had been reduced. These insects, in fact, overwinter in the soil at the larval stage and they are already active at the beginning of spring, and the risk of economic damage is therefore higher for early plantings, which are characterized by a poor early vigor and slow seedling development (Richards, 2000). Conversely, later sowings are characterized by a more rapid development, thus reducing the critical phase in which the crop is grown, and are thus less susceptible to wireworm larval attack. These greater effects of controlling the damage by other insect species and of preserving plant density could explain the increasing trend in grain yield observed from the late planting time to the earlier one.

The collected data underline that planting-time soil insecticides can be used as an effective tool to control the damage caused by soil pests. Nevertheless, their application needs to be evaluated for each production situation and they need to be included in an IPM strategy that is adapted to the local conditions (Vasileiadis et al., 2011). The insecticide control activity needs to be combined with the application of other good agronomic practices, in order to guarantee potential compensatory mechanisms that could permit a rapid re-growth of damaged root systems, such as the hybrid choice, irrigation, N fertilization and tillage (Riedell et al., 1992; 1996; Urías-López and Meinke, 2001). The data collected clearly underline the different productive benefits of the soil-insecticide application in irrigated and non-irrigated fields.

Moreover, considering the greater attention paid to IPM in the EU, through a higher sustainable use of pesticides (Directive 2009/128/EC), the insecticide application needs to be based on an effective pest monitoring programme, in order to identify population thresholds (Lemic et al., 2016). A proper WCR adult monitoring through traps could help growers to avoid the unnecessary use of soil insecticide and to make decisions regarding appropriate management strategies (i.e. crop rotation or application of foliar insecticides). Thresholds (5-6 WCR adults trap^{-1} day^{-1}, with 6-8 traps per field depending on the field size) have been established and introduced in several growing areas to trigger the control of this insect before economic injury levels are reached (Boriani et al., 2006; Kos et al., 2014). Monitoring tools for *Agriotes* wireworms are at a good stage of development and they need to be considered when deciding on the use of soil insecticides within rational IPM strategies (Furlan, 2014).

In conclusion, taking into account the comparison of different PS in continuous maize growing areas with a high occurrence of WCR, the soil insecticide conventionally applied to seed furrows has been shown to lead to a good control of insect injuries and to a clear maize yield advantage. However, maize growers, before deciding whether to adopt soil insecticide measures, should consider the crop and pest history, the insect pressure in relation to economic thresholds and the cost-to-benefit ratio, the damage risk related to other pests and the whole IPM strategy applied to the cropping system. Further studies are still necessary to compare all the available direct control strategies (e.g. different active ingredients, timing and application methods), targeted to control soil pests, and specifically WCR.

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