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# Chemical-based strategies to control the western corn rootworm, *Diabrotica virgifera virgifera* LeConte

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## Abstract

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the most severe pests of cultivated maize, *Zea mays* L. Most of the damage to this crop is caused by larvae feeding on the root system, causing important economic costs in terms of yield losses and management efforts.

This research was carried out to evaluate the effect of different chemical control strategies to minimize larval damage in maize fields under natural infestation of the pest. Field-based research was performed in a two-year period (2011-2012) in five locations of Northern Italy. Different insecticide strategies (belonging to the pyrethroid, neonicotinoid or organophosphate classes) were compared to an untreated control. The effects on larval infestation, root damage, silage and grain yield were assessed.

Our data highlighted that insecticide application at sowing led to a significant reduction in the WCR larval density, both considering insecticide seed treatments and in-furrow soil applications. In particular, seed-applied clothianidin (systemic) and tefluthrin (no-systemic) applied at sowing led to a maximum increase in grain yield of 18% and 19% respectively, when compared to the untreated control.

In all the surveyed plots, limited plant lodging was observed. Furthermore, while the silage yield did not significantly differ among untreated and treated plots, significant differences were recorded with regard to grain yield. No significant results occurred with the liquid insecticide applied in the intra-row space at ridging, with regard to reduction in WCR larval density and grain yield.

**Keywords**

western corn rootworm, soil insecticide, insecticide seed treatments, root injury, grain yield

## Introduction

*Diabrotica virgifera virgifera* LeConte, commonly known as the western corn rootworm (WCR), is the most important pest of maize (*Zea mays* L.). This species probably originated in Central America, and rapidly spread over the United States Corn Belt. Following its first detection in Europe in Serbia in 1992, it has become the most important pest for this crop in several countries of Central and Eastern Europe (Boriani et al. 2006), being reported in 28 countries (EPPO 2016). In Italy, it was first reported in 1998 (Furlan et al. 1998), and then rapidly spread to other regions. *D. v. virgifera* has one generation per year and overwinters at the egg stage in the soil (Toepfer et al. 2008). In Northern Italy, eggs hatch in mid-late May and three larval instars feed almost exclusively on maize roots (Boriani et al. 2006; Moeser and Hibbard 2005). Larval feeding reduces the capacity of crop to uptake water and nutrients by disrupting root system structure and function, resulting in significant yield losses (Wesseler and Fall 2010; Schumann and Vidal 2012). Moreover, extensive root injury makes the plant more susceptible to lodging and additional yield losses could result from mechanical difficulty at harvest time. If environmental conditions allow, in terms of water availability especially, the plants can grow upright again showing the characteristic shape known as "gooseneck" (Sivčev et al. 2012). Pupation occurs in soil chambers in the root zone and lasts between 5 and 10 days (Fisher 1986). The first adults emerge from the soil at mid-late June and they are normally present in the field until first frosts (Toepfer and Kuhlmann 2006; Dunbar, 2011). WCR adults feed on the leaves, silks, pollen and young kernels of maize, but only high density population of adults may reduce yields by interfering with pollination when feeding on silks (Gray et al. 2009). Oviposition takes place almost exclusively in maize fields from July to mid-September. The total area of maize production in Italy is actually 600,000 hectares, often grown as continuous crop especially for grain and less frequently for silage, seed and sweet maize. Economic thresholds for *Diabrotica* spp. focus on estimating the adult population density (Hein and Tollefson, 1985). Visual counts or sticky traps are commonly used to predict the severity of larval injury the following year. In the USA, WCR management involves the use of transgenic maize, application of soil insecticide to control larval root feeding, insecticidal seed treatments, crop rotation, adult management with aerial applications of insecticides targeted to reduce oviposition, and baits to control adults (Gray et al. 2009; Levine and Oloumi-Sadeghi 1991; Van Rozen and Ester 2010; Wright et al. 2000). The strict use of a maize-soybean rotation is considered responsible for the development of rotation-resistance, and failures in the protection from the larval infestations have been often recorded (O'Neal et al. 2002). Genetically modified maize resistant to WCR damage was commercially introduced in 2003 in eastern of U.S. Corn Belt. The adoption of these *Bt* varieties was initially successful, mainly at the expense of soil insecticides, even if resistance to transgenic corn was later reported (Gassmann et al. 2011). Conversely, this control option is not feasible for European farmers, because currently the use of transgenic *Bt* maize hybrids targeting rootworms is not authorized in Europe.

To allow the practice of a continuous maize growing in Europe, chemical-based control measures (e.g., granular soil insecticides or insecticide-coated seeds against the larvae) are commonly adopted to minimize root injury and prevent yield losses, particularly in continuous crop conditions. Foliar insecticides against the adults are also occasionally applied at the beginning of female egg laying to reduce oviposition or, in case of extremely high infestation, to reduce silk feeding. Furthermore, in the literature biological control with entomopathogenic fungi (such as *Beauveria* spp. and *Metarhizium* spp.) and entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) is reported, but some critical aspects due to the highly variable efficacy, higher costs when compared to commonly used insecticides, and non-target effects for native fauna have been raised (Lynch et al. 2001; Toepfer et al. 2007).

Thus, the most common control strategy used to protect maize roots from WCR in Europe is the application of soil insecticides at planting. This application can be performed in seed furrow during planting operation or through seed treatment, using pyrethroids or a systemic insecticide, such as those of neonicotinoid class (Sutter et al. 1990; van Rozen and Ester 2010). However, over the years, the use of maize seeds treated with neonicotinoid (imidacloprid, clothianidin and thiamethoxam) has been restricted in several European countries because of their adverse effects on non-target organisms, particularly honeybees (Cressey 2013). 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-targeted organisms, and about their effective benefit to the crop (Furlan and Kreutzweiser 2015).

Moreover, since many factors can affect the efficacy of soil treatments, the effectiveness of soil-applied insecticides at planting could be variable and it is still debated. In particular, an interval elapses between insecticide application (maize in North Italy is commonly planted from late March to mid-April) and WCR egg hatching (mid-late May). Weather conditions (rainfall, temperatures), in relation to the soil characteristics, could lead to insecticide leaching, volatilization and chemical degradation (van Rozen and Ester 2010), reducing insecticide persistence and ultimately impacting the ability to control WCR larvae. Insecticide applications at maize ridging (6-8 leaf stages) could be an option in order to apply insecticide at a timing closer to the larval occurrence. To the best authors' knowledge, no data have been reported still now on the efficacy of this control strategy.

Overall, few field-based studies have compared the impact of the different available solutions for the direct control of WCR larvae.

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 treatment and different application timings.

Since the overall cost for the 'no control' option has been estimated in the range of several hundred million euros per year in Europe (Wesseler and Fall 2010), it is necessary to evaluate the effect of different available chemical control strategies to minimize larval damage in maize fields under natural infestation of the pest. Specifically, the effect of two granular insecticides at planting, one liquid insecticide at ridging and one insecticidal seed treatment have been evaluated for the impact on crop density, larval infestation, root injury, plant lodging, crop biomass and grain yield. The aim of the study was to compare the available direct control strategies (e.g. different active ingredients, timing and application methods), targeted to WCR larvae and to correctly address the control strategies for this pest in Europe.

## Materials and methods

### *Experimental set-up*

Field experiments were conducted in a two-year period (2011-2012) in five locations of Northern Italy in order to quantify the effect of soil applied insecticide on the control of WCR larvae and maize silage and grain yield. In each location, different insecticide strategies were compared to an untreated control (UC):

- SF1, non-systemic granular insecticide applied at sowing in seed furrow;
- SF2, systemic granular insecticide applied at sowing in seed furrow;
- ST, systemic insecticide applied at sowing as seed treatment;
- R, non-systemic liquid insecticide applied in the intra-row space at ridging.

The applied soil insecticides belong to the pyrethroid, neonicotinoid or organophosphate classes, and additional information such as formulation and application rate are listed in Table 1.

For SF1 and SF2 treatments, the soil insecticides were distributed in seed furrow at 5-10 cm depth from soil surface at the recommended doses for each insecticide using a calibrated granular dispenser attached to the planter. All seeds were treated with fungicide fludioxonil and metalaxil-m (Celest XL®, Syngenta Crop Protection S.p.A., Milan, Italy). Only treatment ST was treated also with insecticide (Clothianidin, Poncho® 600 FS, Bayer S.r.l., Milan, Italy).

The insecticide distribution in treatment R was carried out by spraying a liquid insecticide in the middle of intra-row space using a single nozzle precision sprayer (T-Jeet 110/04) just before the ridging operation, performed at V7 stage (GS 17; Lancashire et al., 1991) with ordinary farm machine. No other insecticides were applied in the experimental fields.

Table 1. Insecticide treatment compared in the study and dose of application.

| Treatment | Application timing and method | Insecticide class | Active ingredients | Formulation | Application rate (g AI ha$^{-1}$) | Commercial product |
|---|---|---|---|---|---|---|
| UC | untreated control | - | - | - | - | - |
| SF1 | in seed furrow at sowing | pyrethroid, non-systemic | Tefluthrin | granular | 100 | Force®, 0.5% Syngenta Crop Protection |
| SF2 | in seed furrow at sowing | neonicotinoid, systemic | Clothianidin | granular | 77 | Santana®, 0.7%, Sumitomo Chemical |
| ST | seed treatment at sowing | neonicotinoid, systemic | Clothianidin | - | 94 | Poncho® 600 FS, 48%, Bayer |
| R | in intrarow space at ridging | organophosphate, non-systemic | Chlorpyrifos ethyl | liquid emulsifiable concentrate | 668 | Alisè EC®, 44.5%, Dow AgroSciences |

The experimental design at each location was a randomized complete block with four replications. The plots were all 20 m long and 8 rows wide and they were staked out side by side in a fully planted field. Two middle rows were used for the measurements. Row spacing was 0.75 m, while plant spacing per row was 0.18 - 0.22 m according to production system.

The main geographic, soil and agronomic information of the experimental fields is reported in Table 2. The maize hybrid used for the experiment was Pioneer P1758, FAO maturity class 700 and 132 days to maturity. Planting was carried out after a proper setting of the seedbed, which consisted of 30 cm deep ploughing and disk harrowing, according to the typical farm management system place in the area. With the exception of Binago trial, performed in a non-irrigated area, irrigation was used in furrow surface method in the other location, in order to prevent drought stress until the physiological maturity stage (GS87). Other agronomical practices, such as fertilization and weed control, were conducted according to the typical farm management system and the ordinary agronomic techniques of the cultivation area and they were the same for all compared insecticide application treatments.

All the experimental locations were naturally infested. 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. Moreover, the previous crop was always continuous maize cultivated without any former foliar insecticide application to control WCR or other maize pests.

Table 2. Main agronomic and phenological information of the field experiments conducted in the 2011-2012 period in North Italy.

| | | | | | | | | date | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | **Site** | **Geographic Coordinates** | **Altitude (m)** | **Soil texture (%)** | | | **Soil pH** | **Planting** | **3 leaves GS[a] 13** | **7 leaves[b] GS 17** | **Flowering GS 65** | **Dough stage[c] GS 85** | **Grain harvest** |
| | | | | **Sand** | **Silt** | **Clay** | | | | | | | |
| 2011 | Binago (CO) | 45°46'N, 8°54'E | 402 | 23.2 | 67.4 | 9.4 | 6 | 14-Apr-11 | 09-May-11 | 30-May-11 | 10-Jul-11 | 31-Aug-11 | 10-Oct-11 |
| 2011 | Pombia (NO) | 45°39'N, 8°38'E | 286 | 13.9 | 74.7 | 11.4 | 6.7 | 05-Apr-11 | 06-May-11 | 30-May-11 | 28-Jun-11 | 09-Aug-11 | 04-Oct-11 |
| 2012 | Orzinuovi (BS) | 45°24'N, 9°54'E | 78 | 35.4 | 55.0 | 9.6 | 7.6 | 23-Mar-12 | 25-Apr-12 | 23-May-12 | 27-Jun-12 | 06-Aug-12 | 10-Sep-12 |
| 2012 | Villareggia (TO) | 45°18'N, 7°58'E | 237 | 39.8 | 47.6 | 12.6 | 5.9 | 23-Mar-12 | 26-Apr-12 | 28-May-12 | 24-Jun-12 | 21-Aug-12 | 02-Oct-12 |
| 2012 | Savigliano (CN) | 44°38'N, 7°40'E | 321 | 34.0 | 53.1 | 12.9 | 6.4 | 22-Mar-12 | 02-May-12 | 30-May-12 | 28-Jun-12 | 22-Aug-12 | 11-Sep-12 |

[a] Growth stage (BBCH, Lancashire et al., 1991)

[b] Insecticide application at maize ridging

[c] Crop biomass harvest (whole plant) for silage

*Entomological analyses*

To evaluate the WCR larval infestation, a 25 $cm^3$ of soil containing the root system was collected. The soil cores were collected and individually washed in a plastic tray containing water at the time of maximum larval occurrence, then the roots were transferred to the laboratory, and individually placed inside a modified Berlese funnel kept at room temperature (23±1°C T, 65±2% RH) according to the method described by Blandino et al. (2017).

All the larvae floating on the water surface and inside the funnels were collected, counted, and preserved in a 70% alcohol in plastic vials (50 mm in height by 10 mm in diameter).

WCR damage to the root system was quantified at the beginning of adult emergence, in accordance with the node injury scale (NIS) developed by Oleson et al. (2005). Larval infestation and NIS were recorded on 60 plants (15 plants for each plot) for each treatment at each experimental field.

*Crop measurements*

In all the surveyed fields, the crop density and the percentage of lodged plants and with gooseneck symptoms were quantified at flowering and dough stage (GS 85) by counting the number of plant in the two middle rows of each plot for the plot length (20 m).

Whole plants were collected manually at the dough stage from an area of 3 $m^2$ on each plot. Plant samples were weighed in order to establish the silage yield and then passed through a knife chopper set at a 2-cm theoretical length of cut. A subsample of about 3 kg of chopped fresh sample from each plot was weighed before and after being dried at 120 °C until constant weight to assess the dry matter (DM) content.

At the end of maturity (moisture content between 22–30%), ears were collected manually from an area of 4.5 $m^2$ (two rows 3-m long) randomly selected in the middle of each plot and were then passed through an electric sheller in order to obtain the grain weight. The grain yield was then corrected to the commercial moisture level of 14%, by using a Dickey-John GAC2000 grain analysis meter.

*Statistical analysis*

Experimental data were evaluated utilizing analyses of variance (ANOVA). When F-values were significant (P>0.05), Tukey's mean separation test was performed.

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

**Results**

*WCR damage*

The effect of insecticide strategies on the control of WCR larval infestation and damage on maize plants are reported in Table 3.

The average number of WCR larvae recorded in the untreated control varied from 1.8 (Pombia, 2011) to 7.2 (Villareggia, 2012). For larval infestation, there were statistical differences between UC and at least one of the treatments in 4 out of 5 test sites, with ST and SF2 having significantly lower infestation when compared to UC where applied (Table 3).

For NIS, there were significant differences between UC and at least one treatment for Savigliano and Villareggia. Furthermore, clear evidence of negative impact of insect activity on crop stability (lodged and gooseneck plants) was observed in 2 out of 5 test sites (Savigliano and Villareggia), with 23% and 9% of plants with lodging/gooseneck symptoms in UC (Table 3).

Table 3. Effect of soil insecticide application to control WCR on larval infestation, root injury (NIS), lodged and "gooseneck" plants at flowering stage. Field experiments were carried out at 5 sites of Northern Italy in 2011-2012.

| **Trial** | **Treatment** | **Larval infestation** (average no. larvae plant$^{-1}$) | | **NIS** | | **Lodged and "gooseneck" plants (%)** | |
|---|---|---|---|---|---|---|---|
| Binago 2011 | UC | 3.90 | b | 0.49 | ab | 0.0 | |
| | SF1 | 7.80 | a | 0.22 | b | 0.0 | |
| | ST | 3.70 | b | 0.17 | b | 0.0 | |
| | R | 6.70 | ab | 0.78 | a | 0.0 | |
| | *P* | 0.017 | | 0.008 | | - | |
| | F | 7.77 | | 3.55 | | | |
| | df | 3 | | 3 | | | |
| | SEM | 0.78 | | 0.10 | | | |
| Pombia 2011 | UC | 1.80 | a | 0.02 | a | 0.0 | |
| | SF1 | 1.10 | b | 0.03 | a | 0.0 | |
| | ST | 1.11 | b | 0.04 | a | 0.0 | |
| | R | 2.02 | a | 0.01 | a | 0.0 | |
| | *P* | < 0.001 | | 0.356 | | - | |
| | F | 1.98 | | 2.77 | | | |
| | df | 3 | | 3 | | | |
| | SEM | 0.75 | | 1.09 | | | |
| Orzinuovi 2012 | UC | 3.90 | a | 0.45 | ab | 0.23 | a |
| | SF1 | 3.20 | a | 0.31 | b | 0.0 | a |
| | SF2 | 1.60 | b | 0.31 | b | 0.0 | a |
| | ST | 2.00 | b | 0.33 | b | 0.0 | a |
| | R | 2.10 | b | 0.56 | a | 0.0 | a |
| | *P* | 0.002 | | 0.006 | | 0.577 | |
| | F | 5.53 | | 3.20 | | 3.47 | |
| | df | 4 | | 4 | | 4 | |
| | SEM | 0.34 | | 0.05 | | 0.05 | |
| Savigliano 2012 | UC | 5.60 | a | 0.37 | a | 20.70 | a |
| | SF1 | 3.63 | b | 0.06 | b | 5.77 | c |
| | SF2 | 1.20 | c | 0.02 | b | 8.13 | bc |
| | ST | 2.81 | b | 0.02 | b | 7.69 | c |
| | R | 3.31 | b | 0.32 | a | 11.13 | b |
| | *P* | < 0.001 | | 0.011 | | < 0.001 | |
| | F | 2.37 | | 4.21 | | 6.38 | |
| | df | 4 | | 4 | | 4 | |
| | SEM | 0.84 | | 1.35 | | 1.27 | |
| Villareggia 2012 | UC | 7.24 | a | 0.50 | a | 9.26 | a |
| | SF1 | 6.22 | b | 0.03 | b | 0.0 | b |
| | SF2 | 4.52 | b | 0.04 | b | 0.35 | b |

| | | | | | | |
|---|---|---|---|---|---|---|
| ST | 6.71 | b | 0.06 | b | 0.12 | b |
| R | 7.80 | a | 0.37 | a | 3.06 | b |
| *P* | < 0.001 | | < 0.001 | | < 0.001 | |
| F | 3.67 | | 2.48 | | 3.56 | |
| df | 4 | | 4 | | 4 | |
| SEM | 3.39 | | 0.73 | | 0.63 | |

*Plant density, silage and grain yield*

The results of the application of soil-insecticide compared to the untreated control for plant density, silage and grain yield are summarized in the Table 4.

Significantly higher crop density was found in treatment ST when compared to the other treatments in Villareggia and Binago (Table 4). For silage yield, no significant differences were found among treatments for silage yield (Table 4). For grain yield, significant differences were found between UC and other treatments in 4 out of the 5 test sites, with insecticide treatments exhibiting higher yield, and ST having higher yield compared to UC in all significant sites (Table 4).

Table 4. Effect of insecticide application to control WCR on crop density at flowering, silage and grain yield. Field experiments were carried out at 5 sites of Northern Italy in 2011-2012.

| **Trial** | **Treatment** | **Crop density (plant m$^{-2}$)** | | **Silage yield (t ha$^{-1}$ DM)** | | **Grain yield (t ha$^{-1}$)** | |
|---|---|---|---|---|---|---|---|
| Binago 2011 | UC | 5.33 | b | 26.20 | a | 14.65 | b |
| | SF1 | 5.34 | b | 25.50 | a | 14.24 | b |
| | ST | 5.94 | a | 22.10 | a | 15.50 | a |
| | R | 5.26 | b | 20.50 | a | 14.28 | b |
| | *P* | 0.003 | | 0.165 | | < 0.001 | |
| | F | 6.90 | | | | 10.08 | |
| | df | 3 | | 3 | | 3 | |
| | SEM | 0.10 | | 1.42 | | 0.30 | |
| Pombia 2011 | UC | 6.77 | a | 18.85 | a | 13.74 | a |
| | SF1 | 6.60 | a | 19.39 | a | 14.23 | a |
| | ST | 6.46 | a | 19.84 | a | 14.16 | a |
| | R | 6.72 | a | 20.25 | a | 13.99 | a |
| | *P* | 0.121 | | 0.275 | | 0.730 | |
| | F | 2.48 | | 1.50 | | 0.44 | |
| | df | 3 | | 3 | | 3 | |
| | SEM | 0.27 | | 1.36 | | 1.04 | |
| Orzinuovi 2012 | UC | 7.30 | a | 23.60 | a | 14.20 | c |
| | SF1 | 7.90 | a | 23.10 | a | 16.90 | a |
| | SF2 | 7.80 | a | 22.90 | a | 15.30 | bc |
| | ST | 7.50 | a | 26.40 | a | 15.90 | ab |
| | R | 7.30 | a | 24.20 | a | 14.40 | c |
| | *P* | 0.123 | | 0.482 | | 0.002 | |
| | F | 5.53 | | 1.99 | | 7.77 | |
| | df | 4 | | 4 | | 4 | |
| | SEM | 0.34 | | 1.35 | | 1.38 | |
| Savigliano 2012 | UC | 7.31 | a | 25.82 | a | 15.25 | b |
| | SF1 | 7.26 | a | 27.29 | a | 17.55 | a |
| | SF2 | 7.31 | a | 26.39 | a | 17.93 | a |
| | ST | 7.36 | a | 27.21 | a | 18.13 | a |
| | R | 7.32 | a | 26.22 | a | 16.15 | b |
| | *P* | 0.909 | | 0.457 | | < 0.001 | |
| | F | 0.24 | | 0.96 | | 14.56 | |
| | df | 4 | | 4 | | 4 | |
| | SEM | 0.28 | | 2.54 | | 1.27 | |
| Villareggia | UC | 6.63 | b | 25.65 | a | 14.88 | b |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2012 | SF1 | 6.90 | b | 26.12 | a | 15.75 | a |
| | SF2 | 6.83 | b | 26.25 | a | 15.43 | ab |
| | ST | 7.20 | a | 26.07 | a | 15.65 | a |
| | R | 6.83 | b | 26.02 | a | 14.98 | b |
| | *P* | 0.005 | | 0.994 | | 0.007 | |
| | F | 5.67 | | 0.05 | | 5.37 | |
| | df | 4 | | 4 | | 4 | |
| | SEM | 0.34 | | 3.60 | | 0.67 | |

Means followed by different letters are significantly different (the level of significance is shown in the table)

## Discussion

The invasion of Europe by the WCR, one of the most destructive maize pest, has represented a serious threat to European maize production. Several different control strategies for WCR management have been explored so far, and crop rotation is considered as the primary non chemical control option currently available. Nevertheless, in the eastern of U.S. Corn Belt, crop rotation is considered to have limited value for WCR control because it has been proved that the insect can lay eggs also in secondary hosts as soybean, and it can also feed on roots of other grasses besides maize (Sivčev et al. 2012). Since 2003, *Diabrotica*-resistant transgenic maize (*Bt* maize) has been grown commercially in the USA. In Europe, there are countries, such as Italy, with well-established WCR populations in growing areas dedicated to maize crop, where high specialization of cropping system and market demands make it difficult to interrupt continuous maize production, and thus monoculture represents the main economic solution. However, genetically modified crops are still an ethical issue in political and society discussions in most parts of Europe.

Wesseler and Fall (2010) assessed the potential damage costs in Europe under a ‘no control’ scenario, resulting in an average annual damage cost expected to range between €143 and €1700 million a year. In the actual situation where *Bt* maize to control WCR is not deregulated, crop rotation and broad-spectrum soil insecticides are most significant and will remain in the near future in the EU (Dillen et al. 2010; van Rozen and Ester 2010; Niu et al. 2017).

In the present paper, the results obtained in the different experimental fields revealed some inconsistencies in the effect of the insecticides on the number of WCR larvae and on the damage caused by the insect. Insecticide treatments at sowing were generally effective, and in particular both seed- and soil-applied insecticides provided acceptable corn rootworm control under natural infestation of the pest. Besides slight differences among locations and years, both tefluthrin- and clothianidin-based treatments resulted in a significant reduction of the larval infestation. In all the experimental fields, a moderate rootworm feeding pressure was observed, leading to limited plant lodging. Except for Pombia where only minor feeding and scarring were detected, in the other fields about half a node of roots was pruned, according to the NIS scale. Generally, even when low levels of WCR larval infestation and feeding damage were recorded, both tefluthrin and clothianidin in seed furrow and clothianidin as seed treatment significantly increase grain yield, with no impact on plant biomass for silage.

In all cases where insecticides were applied at sowing, root node injury levels were less than 0.40, corresponding to commercially acceptable control of corn rootworm in an environment with moderate environmental stress as highlighted by Oleson et al. (2005). In particular, considering the untreated control, the extent of root injury was significantly more severe than that occurring in tefluthrin-treated plots, conversely to Cox et al. (2007). Considering all the untreated plots, grain yield losses attested between 3.5 and 16% compared to treated ones, confirming results obtained by Blandino et al. (2017) where the insecticide application at sowing under 60 different production situations led to an increase of 8% in grain yield. Seed-applied clothianidin (systemic) and tefluthrin (no-systemic) applied in sowing furrow led to a maximum increase of 18% and 19% in grain yield respectively, when compared to the control. Chemical properties of insecticides, microbial activity, and abiotic factors may play a significant role in influencing the effectiveness of treatments. Our data underline that although a large interval elapses between planting time (late March – early April) and egg hatching (late May), the anticipated application of insecticide at sowing is able to guarantee adequate control of WCR larvae and increase yield as consequence. These data are in line with Toth et al. (2020), suggesting that commonly used pesticides can, in general, control WCR larvae over their relatively long presence in the soil. In the study of Blandino et al. (2017) the effect of insecticides applied in seed furrow on root damage and the incidence of gooseneck plants and the consequent biomass and grain advantage were steady for the different planting times.

The insecticide chrolpyriphos ethyl applied at ridging did not provide good larval control nor increased yield when compared to control, in three of the experimental fields. The results of our study suggest that the insecticide applied close to the roots (i.e. at sowing or seed treatment) seem more efficient in reducing larval infestation, as opposed to application in center row (ridging) which may be due to limited mechanical distribution of the insecticide.

Seed treatment with systemic insecticide exhibited better plant stand when compared to control. Even if in this study the presence of wireworms (*Agriotes* spp.) was not specifically investigated, in some experimental fields (Binago and Villareggia) several individuals were recorded and they could be responsible for the plant density reduction observed in all treatments with the exception of systemic seed treatment.

The findings of this research corroborate with previous reports investigating application and efficacy of different insecticides (Sivčev et al. 2012; Ma et al. 2009; Blandino et al. 2017). Although in the literature the efficacy of insecticides applied in seed treatment is not always satisfactory (Sivčev et al. 2012; Obermeyer et al. 2006), our data highlighted that insecticide application at sowing led to a significant reduction in the WCR larval. Moreover, systemic insecticide application may help prevent against secondary pests, such as aphids, wireworms, and leafhoppers (Pons and Albajes 2002; Liu et al. 2009).

In spite of their effectiveness, availability of active ingredients against WCR has decreased in recent years due to environmental concerns and resistance evolution (Vasileiadis et al. 2011; Souza et al. 2019). In particular, in 2008 the Italian Government banned all three neonicotinoid and phenylpyrazole compounds registered for seed treatment, namely imidacloprid, thiamethoxam, clothianidin and fipronil, due to their involvement in the colony collapse disorder of honeybees and the reports of bee mortality in spring during maize sowing (Porrini et al. 2016).

Due to the limited supply of active ingredients, chemical control of WCR is becoming a difficult choice for maize farmers. Thus, a system approach is needed to provide a reliable, sustainable and durable WCR management, also considering the recent advances concerning the effects on insect pollinators.

Seed treatment has advantages when compared to application of pesticides by different methods, such as reduced insecticide doses when compared to soil and foliar applications, with potentially less harmful side effects.

Disadvantages related to seed treatment are the seed particles and insecticide dust that can drift in the environment (Nuyttens et al 2013), making seed treatment quality, seed drill technology and environmental conditions critical factors affecting the risk of dust drift. Moreover, since unprotected pneumatic seeders has been identified since early 2000 to endanger bees, devices equipped with modifications designed to reduce the amount of insecticide drift in the environment are thus needed to balance the benefit of controlling the pest *versus* the need to preserve useful insects (Biocca et al., 2019; Nuyttens et al., 2013).

Due to the need of providing practical information to European farmers, more research is required to determine if the results of this study are consistent across different production situations, with higher levels of larval infestation and node injury, and variable environmental and application-related factors.

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