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Diabrotica virgifera virgifera LECONTE LARVAL SIZE MAY BE INFLUENCED BY ENVIRONMENTAL CONDITIONS IN IRRIGATED MAIZE FIELDS IN NORTHWESTERN ITALY

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Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, larvae were collected in different irrigated maize fields in Brescia Province in Northwestern Italy in 2006, 2007 and 2008. Head capsule widths, considered a valid indicator of larval instar stage, were measured for 2063 specimens and data for the three years were combined and grouped in increments of 20 μm width sizes. Frequency of larvae within each grouping was graphed as a histogram and three distinct peaks, interpreted to represent the peak frequency of the head capsule widths of the three larval instars, were observed. Assuming a normal distribution of head capsule widths, a multiple nonlinear Gaussian curve regression was applied to the frequency histogram. The peaks of the fitted normal curves were 227 ± 3 μm, 353 ± 6 μm, and 519 ± 3 μm for the first, second and third instars, respectively. These values are higher than those reported by other authors in similar studies. The possible impact of irrigation and other environmental factors is discussed.

Western Corn Rootworm, head capsule width, environmental conditions, irrigation, maize, soil moisture, nonlinear Gaussian curve regression, larval size, larval instars, *Diabrotica*

M. AGOSTI, L. MICHELON i C. R. EDWARDS: *Diabrotica virgifera virgifera* LeConte, mogući utjecaj okolišnih čimbenika i navodnjavanja kukuruza na veličinu ličinki u sjeverozapadnoj Italiji

Ličinke kukuruzne zlatice, *Diabrotica virgifera virgifera* LeConte skupljane su u različito navodnjavanim poljima kukuruza provincije Brescia (SZ Italija) 2006., 2007. i 2008. godine. Širina glave smatra se valjanim pokazateljem razvojnih faza ličinki, stoga su obavljena mjerenja širine glave 2063 jedinki te vrste, a podatci trogodišnjih istraživanja spojeni su i rangirani u veličine od 20 μm širine. Frekvencija ličinki unutar istih rangiranja prikazana je grafički histogramom s tri vrha koji interpretiraju vrhove u frekvenciji širine glava ličinki triju različitih stadija razvoja. Za prikaz histograma frekvencija, uz pretpostavku normalne distribucije širine glave ličinki, korištena je višestruka nelinearna Gausova krivulja.

Vrhovi normalnih krivulja bili su 227 ± 3 μm, 353 ± 6 μm i 519 ± 3 μm za prvu, drugu i treću fazu razvoja. Vrijednosti su veće od vrijednosti koje su opazili autori u sličnim studijama. Rad raspravlja o mogućem utjecaju navodnjavanja i ostalih čimbenika okoliša.

***Diabrotica virgifera virgifera*, kukuruzna zlatica, širina glave ličinki, okolišni čimbenici, navodnjavanje, kukuruz, vlaga u tlu, nelinearna Gausova krivulja regresije, veličina ličinke, razvojna faza ličinke**

Introduction

The Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte, is becoming one of the most important pests of maize in the Po Valley, Lombardy Region, Northwestern Italy, especially where maize is grown in monoculture. *Diabrotica virgifera virgifera* population levels are variable among the different administrative districts (Furlan, 2009), and in some areas, economic populations are present.

In the central Po Valley, most of the *Diabrotica* damage to maize is caused by larval feeding on the roots. In highly infested fields, larval feeding can cause plant lodging, reduction of plant fitness and economic losses (Agosti et al., in press).

In this area, most maize is regularly irrigated during the growing season. Water is provided every 10-12 days by flooding or overhead sprinklers. Adequate soil moisture for optimal plant development is an important factor in the significantly high yields of 14-15 metric tons/hectare which have been recorded in a number of fields.

Soil moisture provided by irrigation is important for root growth and regrowth during and at the end of *Diabrotica virgifera virgifera* larval feeding activity. Plant standability and fitness in infested irrigated fields are better than in non-irrigated fields, and the economic impact in these irrigated fields is usually low, even when significant larval-caused root damage occurs (Dun et al., in press).

The economic impact of adult activity on silks is usually less important. Peak of adult presence in fields often does not coincide with pollination. Moreover, pollen shed and silk elongation are usually more than adequate even if clipping occurs, thus reducing the risk of fertilization failure.

During our routine sampling activities, we collected larvae of *Diabrotica virgifera virgifera* from the soil over a three-year period (2006-2008). Considering that larval body size can be influenced by environmental factors, we checked larval body size in irrigated maize fields in a typical maize growing area of Central Po Valley and compared the results with other published data. Head capsule width, considered a valid body size indicator, was measured to determine the average size of the three larval instars.

Materials and Methods

The *Diabrotica virgifera virgifera* larvae were collected in 2006, 2007 and 2008 in three different irrigated maize fields of Brescia Province of the central Po Valley. In the sampled fields, high populations of adults were observed during the previous year.

The sampled fields were regularly irrigated every 10-12 days during the season to provide adequate water for optimal plant development and to prevent drought stress. The beginning of the irrigation season depended on the amount of spring rainfall, and usually ended at the beginning of September, which was about two to three weeks before harvest. All fields received water by flooding. On the basis of what was observed in the Po Valley in 2004 (AA.VV., 2004), we calculated that the equivalent of 35-45mm of rain was distributed during each irrigation treatment.

Each year, the beginning of the larval sampling period was determined by following the data output of a provisional degree day model previously validated for the area and routinely used in our research studies and outreach activities. *Diabrotica virgifera virgifera* development was followed by calculating Accumulated Degree Days (DD°) based on air temperature data obtained from the weather station located in Chiari (BS) and maintained by the Office of Agriculture of the Brescia Province. Cumulative Degree Days (CDD°) were calculated using the DEGDAY 1.01 program (Snyder, 2005). According to Davis et al. (1996), air temperature data with a minimum threshold of 11°C and maximum threshold of 18°C were used.

Each week, two groupings of five adjacent plants each were randomly selected in the study fields. The root system and surrounding soil for each plant were dug up and transported to the laboratory for processing. *Diabrotica virgifera*

virgifera larvae were collected from roots using modified Berlese funnels and by sieving the soil collected from around the roots. The first instar larvae were also detected by direct examination of root apexes. The root systems were retained in the Berlese funnels for at least three weeks to allow all the larvae to escape from the roots. The larvae were collected in a plastic cup filled with tap water that was attached to the bottom of each funnel.

The first larvae were found on May 21, April 29 and May 11 in 2006, 2007 and 2008, respectively, and the collecting ended when no larvae were could be collected from the sampled soil and roots. The collected larvae were preserved in a 70% alcohol:30% water solution for several months and examined during the fall of each study-year. Head capsule width was measured using a dissecting scope (80X magnification, 20 µm of precision) outfitted with a measuring grid. Data for the 2063 specimens for the three years were combined and grouped in increments of 20 µm width sizes and the frequency of larvae within each grouping was graphed as a histogram.

Assuming a normal distribution of head capsule widths, multiple nonlinear Gaussian curve regressions were applied to the frequency histogram as described in Hammack et al. (2003), using the Origin 8.0 software.

To better understand the effects of varying soil moisture levels in irrigated fields due to natural rainfall on larval body size, head capsule widths of larvae in 2007 and 2008, dry and wet early growing seasons respectively, were examined. The means of the three larval instars calculated using the same procedure as described above were compared using the Student t test.

Results and Discussion

The frequency distribution of head capsule widths showed three distinct groupings that were interpreted to represent the frequency of the head capsule widths of the three *Diabrotica virgifera virgifera* larval instars (Figure 1). Multiple normal Gaussian nonlinear regressions of the frequencies showed a good fit of the data, as reported also by Hammack et al. (2003), for all the three larval instars (Table 1).

Peaks and Standard Error (SE) of the calculated fitted curves were 227 ± 3 µm, 353 ± 6 µm, and 519 ± 3 µm, compared to the grouping mean values of 225, 350 and 524 µm for the first, second and third instars, respectively.

A comparison of these data (Table 2) with those reported in a similar study by Hammack et al. (2003) for larvae collected from a field in South Dakota, USA,

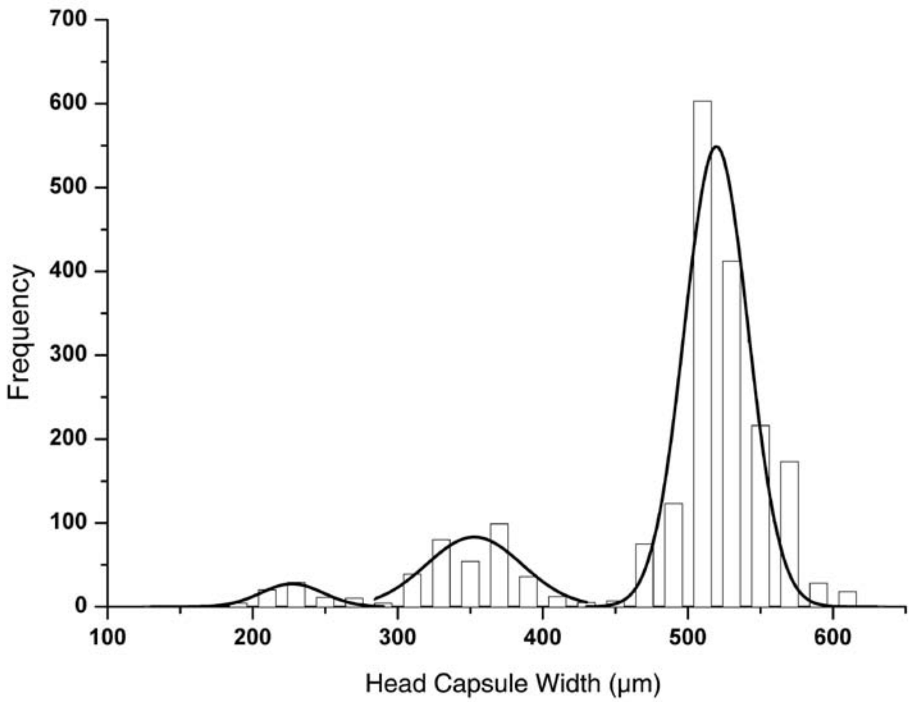

Figure 1: Frequencies of head capsule widths observed for *Diabrotica virgifera virgifera* larvae collected from irrigated maize fields in Northwestern Italy in 2006, 2007 and 2008 (n= 2063).

Table 1: Parameters of the three fitted normal Gaussian curves for *Diabrotica virgifera virgifera* larval instars.

	Peak±SE µm	R^2	Lower limit (µm) 95%	Upper limit (µm) 95%
First instar	227.14±3.01	0.88	219.77	234.51
Second instar	353.73±5.98	0.79	338.09	367.35
Third instar	519.51±3.09	0.88	512.39	526.64

showed larger head capsule widths of the larvae from the irrigated fields from Northwestern Italy.

The Northwestern Italy head capsule widths were also larger when compared to the results published by George & Hintz (1966) from *Diabrotica virgifera vir-*

Table 2: Mean of *Diabrotica virgifera virgifera* larval head capsule widths observed in Northwestern Italy compared to other published data.

	Northwestern Italy (2006, 2007 & 2008)	George and Hintz (1966)	Hammack *et al.* (2003)
First instar	225.05±3.02	200	216±1
Second instar	350.36±1.49	325	332±1
Third instar	524.11±0.74	500	501±1

gifera larvae reared on a squash diet in the laboratory. Their results, nevertheless, were very similar to the results reported by Hammack and colleagues.

Larval size depends on genetics, but can also be influenced by environmental conditions. For example, size can be related to the quality of root tissue consumed (Moeser & Vidal, 2004) while soil texture and moisture are important for larval survival (Turpin & Peters, 1971) and movement (MacDonald & Ellsbury 1990). Our results suggest that excellent soil moisture provided by regular irrigation created an environment that was optimal for plant and larval development and thus could have had a positive influence on increasing *Diabrotica virgifera virgifera* larval size. Moreover, in South Dakota, maize is grown mostly without irrigation (USDA-NASS), and Hammack and colleagues did not indicate that their larvae were collected from irrigated fields, so we assumed that their observations were from non-irrigated fields.

To try to understand this hypothesis better, we compared the first, second and third *Diabrotica virgifera virgifera* larval head capsule widths from years 2007 and 2008. Spring rainfall amounts for the two years were quite different, with 2007 being relatively dry (14 rainy days) and 2008 being wet (26 rainy days) in the early growing seasons prior to the irrigation period. For both the second and third instars, the mean value for larval head capsule width was significantly higher in 2008 than in 2007 (Table 3), thus supporting the hypothesis of the positive effect of soil moisture on plant development and *Diabrotica virgifera virgifera*

Table 3: Mean comparison of *Diabrotica virgifera virgifera* larval head capsule widths (µm) observed in 2007 and 2008.

	2007 (dry spring)	2008 (wet spring)	Sig. (t-test)
First-instar	210.00±5.93	227.30±0.46	n.s.
Second-instar	340.68±5.07	372.12±4.09	**
Third-instar	514.28±1.54	536.62±3.20	**

larval size. There were no significant differences between 2007 and 2008 for the first instar larval size, but numerically, the 2008 larval size was greater.

Based on the results of this study, it appears that irrigation has a positive effect on increasing larval size. These findings could have an impact not only on root damage level and maize fitness, but also on the level of larval management achieved with standard control measures. Most of the chemicals used for larval control, as well as *Diabrotica*-specific Bt proteins in transgenic maize, act through insect ingestion and their effectiveness is often related to larval age and size and the amount of toxicant ingested. Thus, larger larvae, as observed in the irrigated fields in Northwestern Italy, could have a greater rate of survival and a subsequently different impact on control efficacy and resistance development and management.

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