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ENVIRONMENTAL AND GENOTYPIC EFFECTS FOR WESTERN CORN ROOTWORM TOLERANCE TRAITS IN AMERICAN AND EUROPEAN MAIZE TRIALS

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ABSTRACT - The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (WCR) is the most destructive pest of maize in North America currently causing considerable economic losses also in Central and Southeast Europe. Developing and releasing of commercial hybrids with higher level of native (host-plant) resistance to WCR could be a sustainable alternative to transgenic approaches. For WCR native resistance breeding strategies, it is important to examine size and patterns of genotype by environment interaction (GEI). The objectives of the study were to determine i) environmental and genotypic effects of host-plants and ii) patterns of GEI for maize root traits associated with WCR resistance (damage, size, regrowth) at two distinct locations. Field experiments were conducted in 2001-2003 in Missouri (manual infestation) and from 2001 to 2006 in Croatia (natural infestation, continuous growing in 2004-2006). Environmental variances were much greater than respective genotypic variances for root damage and root size resulting in low repeatability estimates for both traits, especially in Croatia. The high repeatabilities for root regrowth under both natural and manual infestation indicated that susceptible and/or tolerant maize genotypes to WCR can be reliably identified under both infestation treatments. No specific interaction between host genotypes and putative WCR populations for a given geographic region, nor the threefold interaction maize genotypes × putative WCR population × edaphic/climatic factors were identified for root regrowth.

KEY WORDS: *Diabrotica virgifera virgifera*; Maize genotypes; Genotype by environment interaction; Root damage; Root regrowth; Root size; Western corn rootworm.

INTRODUCTION

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is the most destructive pest of maize (*Zea mays* L.) in North America (Krysan and Miller, 1986), which began its successful invasion of Central Europe in 1992 (Baca, 1994). The pest was identified in Croatia in 1995 and today is infested in approximately 23.500 ha (Igrc-Barcic and Bazok, 2004). Currently, the main areas of economic damage from the western corn rootworm (WCR) invasion are in Hungary, Serbia and Croatia (Kiss *et al.*, 2001). It has been predicted that WCR will spread to all maize production areas within the European Union (Baufeld and Enzian, 2001) potentially causing considerable losses.

Investigations on WCR host-plant resistance started 60 years ago in the U.S. Corn Belt (Bigger *et al.*, 1941) as an alternative to insecticides. It was found that certain maize inbred lines had resistance (mostly tolerance) to rootworms. Owens *et al.* (1974) found that the host-plant (native) resistance is associated with larger root system and greater secondary root development. A tolerant plant sustains as much feeding damage as a susceptible plant, but is able to develop and produce high grain yield regardless of the injury (Riedell and Evenson, 1993). Studies in Missouri in 1997 and 1998 identified several crosses that had significantly less damage caused by corn rootworm larvae (Hibbard *et al.*, 1999). However, no maize cultivars with high levels of native resistance under moderate to high insect pressure were yet released. Developing and releasing of commercial hybrids with higher level of native resistance to WCR would reduce the amount of insecticides and could be a sustainable alternative to transgenic approaches.

In contrast to the U.S. Corn Belt, only a few studies are published on the WCR native resistance in Europe (e.g. Ivezic *et al.*, 2006). Subsequently, there is no report comparing two major regions of WCR invasion, which could indicate specific interactions between maize genotypes and WCR infested

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environments for native resistance traits. In field trials planted under WCR infestation across diverse geographic regions, the total genotype by environment interaction (GEI) variance includes interaction effects between i) genotypes and specific climatic and edaphic factors differing at the locations, ii) between host genotypes and putative *Diabrotica* races and biotypes, and iii) the threefold interaction genotypes × putative *Diabrotica* races or biotypes × edaphic/climatic factors.

Size and pattern of the GEI could have important implications for the management of WCR in North America and Europe as well as for maize resistance breeding strategies. The study of GEI can help the breeder to identify distinct regions with specific GEI, to detect most representative and reliable native resistance traits and thereby to develop more efficient testing procedures (BROWN *et al.*, 1983). Pattern analysis as one variant of cluster analysis consisting of joint and complementary application of classification and ordination techniques can be used in order to simplify the data set by grouping individuals with similar response for certain attributes. In the case of GEI tables of native resistance traits, clustering can be used to simplify the data set by grouping the genotypes over all environments with similar response patterns for WCR traits, then grouping the environments over all genotypes with similar response patterns for all yields (BYTH *et al.*, 1976). The theory underlying these procedures and reasons for their use are discussed in WISHART (1969) and DELACY *et al.* (1996). The objectives of the study were to examine i) environmental and genotypic effects of host-plants and ii) patterns of GEI for maize root traits associated with WCR resistance at two locations in USA and Europe.

MATERIALS AND METHODS

Field experiments were conducted in 2001, 2002 and 2003 at two geographically distinct locations: Columbia in Missouri, USA (38.9°N, 92.2°W) and Osijek in Croatia, Europe (45.3°N, 18.4°E), a major maize production area with natural WCR occurrence. The experiments are denoted as MO01, MO02, MO03 in Missouri and HR01, HR02, HR03 in Croatia. Additionally, the experiments were planted in Croatia in 2004, 2005, and 2006 (HR04, HR05, HR06) under continuous maize growing conditions. The genetic material comprised variable number of maize experimental and commercial hybrids as well as inbred lines across the locations and years. Germplasm included was developed either in U.S. Corn Belt or in Europe possessing different level of native WCR resistance. In the U.S., there were total of 15, 15, and 17 genotypes in trials planted in 2001, 2002 and 2003, respectively. The numbers of maize genotypes in Europe were 12 in 2001 and 14 in 2002, while 16 genotypes were evaluated from 2003 to 2006. The experimental design at each location was a randomized complete block with four replications. In Croatia, genotypes were grown in plots 6 m long with 2 seeds per hill, 25 cm between hills, and 0.70 m row spacing under natural WCR infestation conditions. In the USA, the plots were machine planted with a 0.91 m row spacing with plants spaced approximately 17 cm apart. In Missouri, the plots were one row wide and 1.83 m long. Eggs of the WCR were provided by the USDA-ARS Northern Grain Insects Research Laboratory, Brookings, SD. Eggs were suspended in 0.15% agar solution, and were used to mechanically infest plots (MOELLENBECK *et al.*, 1994) at the V2 stage of plant development, with approximately 1000 eggs per 30.5 cm of row. Usual soil and crop management practice for high yield maize production was applied in both locations across the years. Although set of genotypes varied across the nine environments (year × location combinations), the following seven maize hybrids which had been developed in Europe were available for all environments: Os444, Os499, Os552, Os596, Os602, Os617, and Os644.

Methods used for evaluating three maize root traits associated with WCR native resistance were described by IVEZIÇ *et al.* (2006) in detail. Briefly, root damage was rated on the Iowa State University 0-3 Node-Injury Scale, (OLESON *et al.*, 2005), while the reversed Eiben 1-6 Scale (ROGERS, 1975) was used to rate root size and regrowth. Data from individual plants of each plot were averaged to obtain plot mean for every maize genotype in each replication.

Initially, data from each environment was statistically analyzed separately. Entry means and error mean squares were used for further combined analyses of variance (COCHRAN and COX, 1957). Each of the nine location/year combinations was considered as an environment, where the genotypic effects were assumed as fixed, and the other effects (environments, genotype × environment interaction, and error) as random variables. Estimates of the pooled error variance and estimates of variance components were computed as described by SEARLE (1971). Direct F tests were available for all sources of variation in the analysis of variance. In order to avoid analysis of unbalanced data sets, only seven maize hybrids included at all nine environments were considered in the combined analysis using pooled error variance.

Repeatability estimates were calculated in order to show how much is to be gained by the repetition of measurements and to set upper limits to the ratio of variance components, throwing light on the nature of the environmental variance (FALCONER and MACKAY, 1996). Repeatability was calculated as percentage presenting relation of genotypic variance component (σ^2_g) and error mean square (σ^2) divided by the number of replications (R): $\sigma^2_g / (\sigma^2_g + \sigma^2/R)$. Repeatability estimates in the combined analysis were also calculated, based on entry means which corresponds to the heritability estimate (HALLAUER and MIRANDA, 1981) taking into account also variance component due to genotype by environment interaction, (σ^2_{ge}):

$$r = \frac{\sigma^2_g}{\sigma^2_g + \frac{1}{E}\sigma^2_{ge} + \frac{1}{ER}\sigma^2}$$

where σ^2 the pooled error variance, and E and R the number of environments and replications, respectively. Confidence intervals at the 95% probability level were calculated for the repeatability estimates by the method proposed by KNAPP and BRIDGES (1987). The PLABSTAT program package (UTZ, 1995) was used for these statistical procedures.

Raw data for the pattern analysis of the seven genotypes were entry means at each environment resulting in entry × environment means. Pattern analysis was applied to the environment-standardized matrix of the entry × environment means (FOX and ROSIELLE, 1982) leading to a grouping environments of those environments that are most similar in the way they rank genotypes. Similarly, the genotypes groupings obtained with this standardization are such that genotypes showing similar performance levels are placed in a group. An agglomerative hierarchical procedure with an incremental sum of squares grouping strategy (WARD, 1963) was employed for the purpose of classification. The squared Euclidean distance was used as a dissimilarity measure required by Ward's method. Performance plots are used to illustrate each genotype group's performance in a series of environment groups. They contain more than one genotype group on each plot and it was used to compare the genotype group performances across environment groups. The performance of a genotype group is the mean of the individuals in the genotype group for the specific environment group. The pattern analysis was performed using GEBEI program (WATSON *et al.*, 1996).

RESULTS

Significance levels of the effects of replications and maize genotypes varied across the environments for root damage and root size (Table 1). The effect of genotypes was significant at all three U.S. environments, while it was not significant at most instances in Europe for both traits. In contrast, consistently non significant effect of replication and significant effect of genotype across all nine environments were found for root regrowth.

Estimates of repeatability in individual experiments were the highest for root regrowth at six environments, exceeding notably the respective estimates of other two traits (Fig. 1). It occurred particularly in European environments in 2001 and 2003. The differences among repeatability estimates for the three root traits were less variable across environments in Missouri. At five European environments there was the same ranking of repeatability estimates for the three traits: the highest repeatabilities had root regrowth, the second highest had root size, whereas the root damage had the lowest repeatabilities including no estimated repeatabilities for two environments due to negative estimates of genotypic variance component.

TABLE 1 - *Significance levels of the effects of replications (R) and genotypes (G) from the analysis of variance (ANOVA) of individual trials for three root traits associated with* Diabrotica *tolerance.*

Environment	Damage		Size		Regrowth	
	R	G	R	G	R	G
USA (Missouri)						
MO01	ns	**	**	*	ns	**
MO02	*	*	ns	+	ns	*
MO03	*	*	ns	**	ns	**
Europe (Croatia)						
HR01	ns	ns	ns	ns	ns	**
HR02	ns	+	+	*	ns	**
HR03	**	ns	**	ns	ns	**
HR04	**	ns	**	*	ns	**
HR05	ns	ns	*	ns	ns	**
HR06	+	**	**	ns	ns	**

ns - not significant; +, *, ** - significant at 0.1, 0.05, 0.01 probability levels, respectively, according to the F-test.

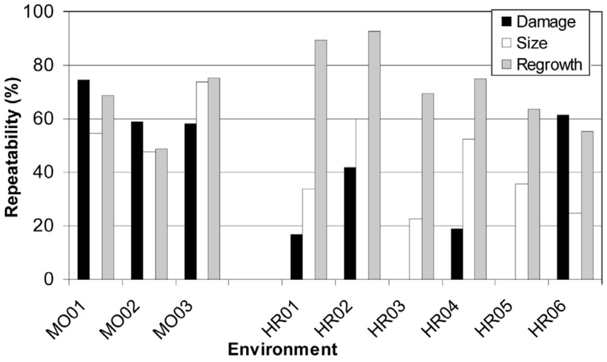

FIGURE 1 - Repeatability (%) estimates for three root traits associated with WCR tolerance in nine maize trials at three U.S. (Missouri, MO) and six European (Croatia, HR) environments in 2001-2006.

Due to consistently high environmental and low genotypic variance components associated with great standard errors of mean at Croatian environments, root damage was excluded from the average rating comparison among environments (Fig. 2). Across the US environments, there were constant average ratings for root size of about 3, while average ratings for root regrowth varied from 1.5 to 4.7. There was a continuous increase of average ratings for root size in Croatia from 2001 to 2006, whereas average ratings for root regrowth had a significant increase only from 2005. Nevertheless, there was a clear increasing trend for the root ratings in Croatia under continuous growing and natural infestation conditions exceeding the scores rated in the USA in 2006.

TABLE 2 - *Significance levels of the effects of maize genotypes (G), environments (E), and their interaction (GEI) in six maize genotypes from the combined analysis of variance (ANOVA) across nine environments for three root traits associated with* Diabrotica *tolerance.*

Root trait	Effects			Repeatability	
	Genotype (G)	Environment (E)	GEI	Estimate (%)	95% Confid. Int.
Damage	ns	**	ns	16.6	-316.6 – 68.9
Size	ns	**	ns	20.5	-297.4 – 70.4
Regrowth	**	**	**	89.3	46.3 – 96.0

ns - not significant; ** - significant at 0.01 probability level according to the F-test.

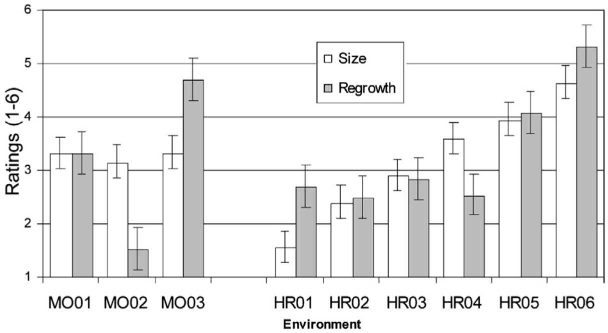

FIGURE 2 - Average ratings for root size and root regrowth in maize trials at three U.S. (Missouri, MO) and six European (Croatia, HR) environments in 2001-2006. Vertical bars mark the respective standard error of the mean.

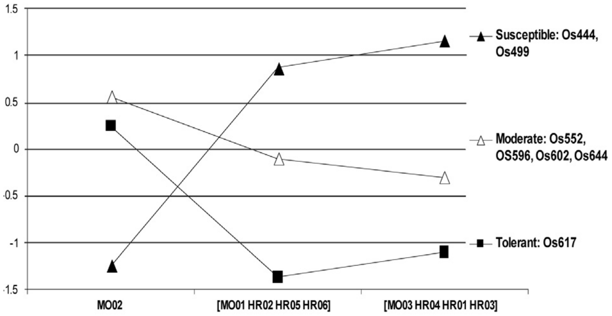

FIGURE 3 - Performance plots of three entry groups (susceptible, moderate and tolerant maize genotypes) for root regrowth at three groups of environments according to pattern analysis.

Combined analysis indicated no significant effects of genotypes and GEI for root damage and root size in six maize genotypes across nine environments (Table 2). In contrast, all three effects were significant for root regrowth which had notable higher repeatability estimates with reasonable 95% confidence intervals. Thus, the pattern analysis was done only for the root regrowth.

The Ward's grouping method in the pattern analysis clearly revealed three respective groups of environments and maize genotypes for root regrowth (data not shown). In the first environment group there is only one environment (MO02), the second group includes the environments MO01, HR02, HR05, while the environments MO03, HR01, HR03, HR04 and HR06 belong to the third group (Fig. 3). Maize genotype groups consist of 2, 4 and 1 genotypes denoting as susceptible (Os444, Os499), moderate (Os552, Os596, Os602, Os644), and tolerant (Os617). Excluding the susceptible group at the environment MO02, performance plots of the three genotype groups showed consistent trend to different environment groups (Fig. 3). For instance, the tolerant group with the genotype Os617 had consistent lower performance scores across the environments than the moderate group.

DISCUSSION

Environmental variance has often a wide range of causes and its nature depends mostly on the trait examined. As a source of error, it decreases precision of genetic studies. Thus, the aim of the experimenter or breeder is to reduce this variance, or if it is feasible, to examine another trait which could reliably replace the original trait. A trait measured in units of length or weight usually is more reliable than those traits graded by judgment into classes in which the variance due to measurement might be considerable (Falconer and Mackay, 1996).

In our study, it was demonstrated that the most reliable (heritable) trait for WCR resistance breeding in maize seems to be root regrowth, due to small environmental variance. This was the case because it was the only trait for which variability actually existed among the germplasm evaluated. The exception was the experiment in Missouri in 2002 (MO02) where genotypic variation was somewhat smaller and ratings were notably lower compared to all other environments (Table 1, Fig. 2) which could generate inconsistent performance scores of entry groups at this environment (Fig. 3).

Ivezic *et al.* (2006) presented comparable results for the same three root traits in similar maize genetic material evaluated in Iowa and Croatia. In addition, they compared in Iowa root regrowth ratings by visual scale (as in our study) and weighing measured quantitatively in grams. Strong positive and highly significant correlation (r = 0.85) was found between visual scale and weighing the roots in grams during three years of investigations. These results demonstrated that visual scale is good enough to serve for root regrowth evaluation and it can be replaced by labor intensive root weighing.

Root damage and root size had notably greater environmental variances than root regrowth according to significant environmental effects (replications) and lower repeatabilities estimated in individual trials. This is also true for combined analysis of variance where environmental variances were much greater than respective genotypic variances resulting in low combined repeatability estimates for both traits. However, repeatabilites for all three traits were similar across the US environments indicating that ratings in Missouri were more repeatable than in Europe. In contrast, low repeatabilities for root size and root damage in Europe implied great environmental variances suggesting that more measurements (replications) had been needed to obtain a worthwhile gain in precision.

There was no relation between repeatabilities and insect pressure. The undeniably high insect pressure in 2006 in Europe had no impact on higher repeatabilites. In contrary, the repeatability estimates for root regrowth decreased along with higher insect pressure due to smaller genotypic variances. Smaller variation among maize genotypes for WCR resistance traits at high infestation environments was expected, but the pattern of genotype response did not change. Although the differences among maize hybrids got smaller, the tolerant maize hybrid Os617 remained the most tolerant genotype across all environments. It indicates that genetic variability among maize hybrids exists for root regrowth to some extent under moderate to high WCR pressure, corroborating herewith the results from Hibbard *et al.* (1999) for root damage.

The high repeatabilities for root regrowth under both natural and manual infestation indicate that susceptible and/or tolerant maize genotypes to WCR can be reliably identified under both infestation treatments. Comparable findings were presented by Bohn *et al.* (1999) for damage caused by European corn borer in European maize hybrids. This is important, since the high cost of insect rearing for manual infestation along with inevitable time and labor consuming evaluation of roots are still the main constraints for conventional WCR resistance breeding.

Pattern analysis of genotype × environment interaction for root regrowth in maize trials did not reveal distinct groups of environments according to geographic regions, since some environments in USA and Europe showed similar response patterns of host plants to diverse WCR infested environments. It suggests that our study did not identify specific interaction between host genotypes and putative WCR populations for a given geographic region, nor the threefold interaction maize genotypes × putative WCR population × edaphic/climatic factors. Kim and Sappington (2005) found little genetic differentiation of WCR populations across the wide geographic range sampled in USA, implying that there should be no significant genetic structuring of WCR population within Europe either, and between Europe and USA. Further, Miller *et al.* (2006) found no evidence for general genetic differentiation between WCR samples collected in cornfields under both rotation and continuous growing conditions.

Nevertheless, given data from wider range of environments and maize genotypes, it should be possible to obtain better estimates of environmental and genotypic variances for all WCR resistance associated traits. A more extensive breeding study would be required to clearly separate maize genotype × location from maize genotype × WCR population interactions defining target environments to ensure developing and releasing of commercial maize hybrids with higher level of native resistance.

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