Conventional breeding and biotechnological methods for the control of Western corn rootworm (*Diabrotica virgifera virgifera*)

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Summary

The presentation covers the impact of western Corn Rootworm on the Hungarian maize production, and deals with the possible protection means, like crop rotation, usage of insecticide and breeding with conventional methods and transgenic solution.

Key words

Resistance breeding, Diabrotica virgifera virgivera

Introduction

The Western corn rootworm (*Diabrotica virgifera virgifera*), first described almost 100 years ago (GILLETTE 1912), appeared in Europe 15 years ago (BAČA 1993) and has already become a major maize pest. Specimens were first caught in Hungary in 1995 and the pest took only eight years to spread to all major maize-growing regions of the country, in many of which it causes serious economic losses. According to surveys made in the USA, the economic losses caused by the Western corn rootworm amount to an annual 800 million dollars, with another 200 million dollars being spent on pest control (KRYSAN and MILLER 1986). Investigations made by SIVCEV and TOMASEV (2002) showed that in Southern Europe yield losses due to Western corn rootworm damage averaged 30% on infected fields, with a range of 1-70%. In Hungary, more than 105,000 hectares are estimated to be affected, with damage due to plant lodging on approximately a third of this area (RIPKA 2004).

The Western corn rootworm has a single generation a year and overwinters in egg form in the soil. Under Hungarian conditions the larvae emerge between mid-May and mid-July (HATALÁNÉ and RIPKA 2001). The major damage is caused by feeding on the roots, leading to reduced plant uptake of nutrients and water and causing the plants to lodge when exposed to strong winds. Secondary damage is caused by the imagos, which feed on leaves, pollen and silks, resulting in poorer fertilisation and consequent losses both for commercial maize production and seed production. In addition, injuries to the ears lead to a loss of quality, especially in the case of sweetcorn, as they facilitate attacks by secondary pests such as *Fusarium* sp. and *Ustilago maydis*.

Control measures against Western corn rootworm are essential to prevent damage and eliminate or mitigate economic losses.

A. Control measures against Western corn rootworm

Crop rotation is one of the most important and efficient methods for the agronomic control of the Western corn rootworm. Regulations in Hungary ban the sowing of maize on fields where Diabrotica larvae were found in the previous year. Small-scale farmers in Hungary tend to be reluctant to change to crops less profitable than maize, but crop rotation has the advantage that it does not pollute the environment and is at present the cheapest and most efficient way of controlling the pest.

Experiments are underway to develop other forms of biological control, involving the use of entomopathogenic fungi or predatory spiders, but so far these methods do not provide satisfactory control of the corn rootworm.

Among the various forms of chemical control, seed dressing or soil disinfection can be used to prevent larval damage, while field or aerial spraying can be employed against the imagos. Seed dressing does not provide satisfactory protection against the pest, while soil disinfection is only effective if the application is correctly timed, to coincide as closely as possible with the emergence of the larvae. The insecticides currently available are able to destroy 70-80% of the imagos, thus reducing the insect population in the following year. The disadvantage of this means of control is that imagos may recolonise the fields from untreated neighbouring areas.

The aim of control is to prevent or limit contact between the corn rootworm and the maize plant. This can best be achieved by integrated plant protection, which takes into consideration the whole of the given agro-ecosystem and employs various techniques to protect crops without damaging useful organisms and without polluting the biological environment. One important component of integrated plant protection is resistance breeding

B. Resistance breeding

The importance of resistance breeding lies in the fact that resistant plants have genetically determined traits that enable them to defend themselves against pests. Resistant varieties have complete resistance to the given pest, while tolerant plants have only partial resistance. PAINTER (1951) reported three basic mechanisms of host-plant resistance: non-preference, antibiosis and tolerance. Non-preference is based on plant traits which lead to the insect rejecting the plant as a habitat, feed plant or refuge, i.e. an allelopathic relationship develops due to perception by the herbivorous insect. When we speak of resistance as a plant trait, rather than as a response by the pest, Kogan and Ortman suggested using the term antixenosis, meaning the ability of plants to repel insects. Antibiosis is the sum of factors that have a damaging effect on the life-cycle and metabolism of insects feeding on resis-

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tant host-plants, ranging from retarded development to mortality. Tolerance is the ability of the plant to develop and reproduce despite the damage caused by the pest, and in some cases to repair the damage caused.

Among these three mechanisms, conventional breeding is currently only able to exploit tolerance, as no germplasm with natural resistance to the Western corn rootworm has yet been found in general cultivation or in gene banks. Differences between hybrids tend to be based on plant characteristics such as stronger stalks or greater root mass.

Various selection methods can be applied in resistance breeding, one of which involves selecting for reduced root lodging. This method concentrates on a trait important for growers, as root lodging results in physiological losses due to reduced photosynthesis and to harvesting losses. In addition, a significant correlation has been demonstrated between root lodging and yield losses. The method suffers from the disadvantage that it is greatly dependent on environmental effects. A further possibility is the direct evaluation of root damage by scoring using the Iowa scale (1 = healthy roots, 6 = major root damage), but this is complicated by the fact that the data are not always correlated with yield levels and variation between the genotypes tends to be small.

In Martonvásár the main method used to select for tolerance is the measurement of root resistance, supplemented by root size measurements, the evaluation of root damage and the calculation of the extent of regeneration. Root resistance is the force (kp) required to pull the roots out of the soil. This parameter facilitates efficient genotype selection, as there are considerable differences between the values obtained for this genetically variable trait, while the method can be rapidly and efficiently employed for the testing of large quantities of breeding materials. Root size measurements and the calculation of regeneration are useful in selection, as these traits are closely correlated to the degree of tolerance.

Material and Methods

Experiments were set up in 2007 at four locations with 79 hybrids in order to determine the level of tolerance to Western corn rootworm.

The locations were chosen on the basis of the level of natural infection with corn rootworm in the previous year. The soil in Kőszárhegy was heavy in texture and was severely infected, while Mezőfalva had a loose soil with moderate infection. Of the two locations in Martonvásár, both had heavy texture, but Lászlópuszta was severely infected, while no infection was recorded in Martonvásár. At each location root resistance measurements were carried out on two occasions (June 22 and Sept. 15), the 79 genotypes were scored for visual root damage using the Iowa scale, and the root diameter was recorded.

Results

The most severe infection was recorded in Kőszárhegy at the first sampling date, followed by Lászlópuszta, Mezőfalva and Martonvásár, while at the second sampling date the order was Lászlópuszta, Kőszárhegy, Mezőfalva and Martonvásár (*Figure 1*). A substantial difference was observed between the values recorded on the Iowa scale in Kőszárhegy at the first and second sampling dates. This could be attributed chiefly to the high rate of root regeneration at this location, partly due to the timely arrival of rainfall, which allowed the damaged plants to develop new roots and thus reduced the size of the yield loss. Considerable variation was observed between the genotypes for root

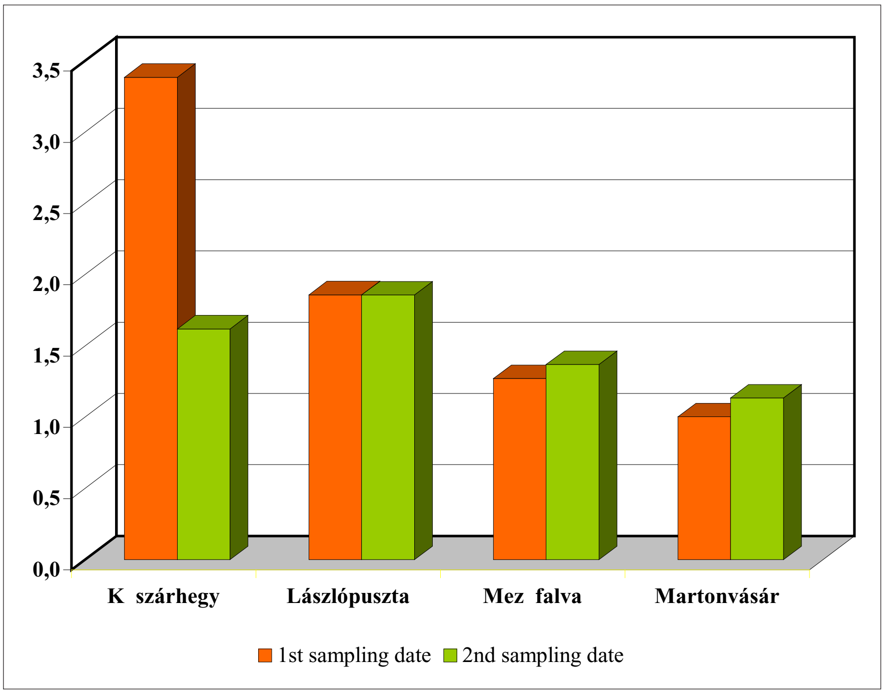

Figure 1: **Level of root damage on the IOWA scale, averaged over the hybrids**

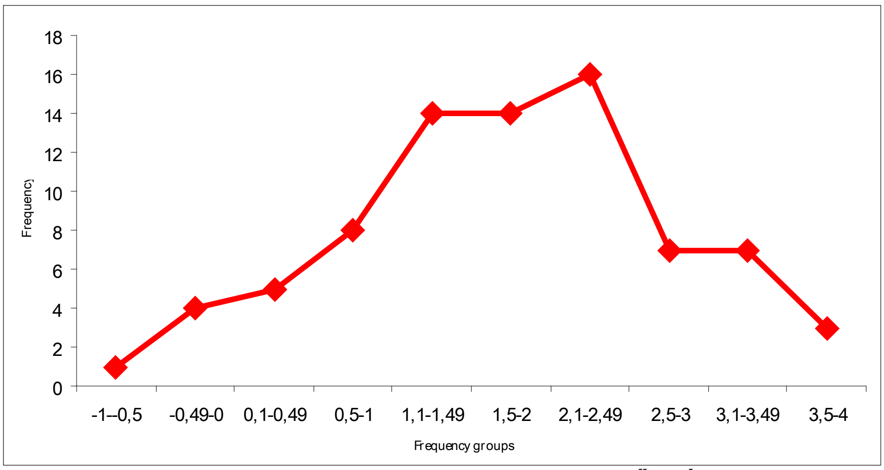

Figure 2: **Frequency distribution of root regeneration (KŐSZÁRHEGY 2007)**

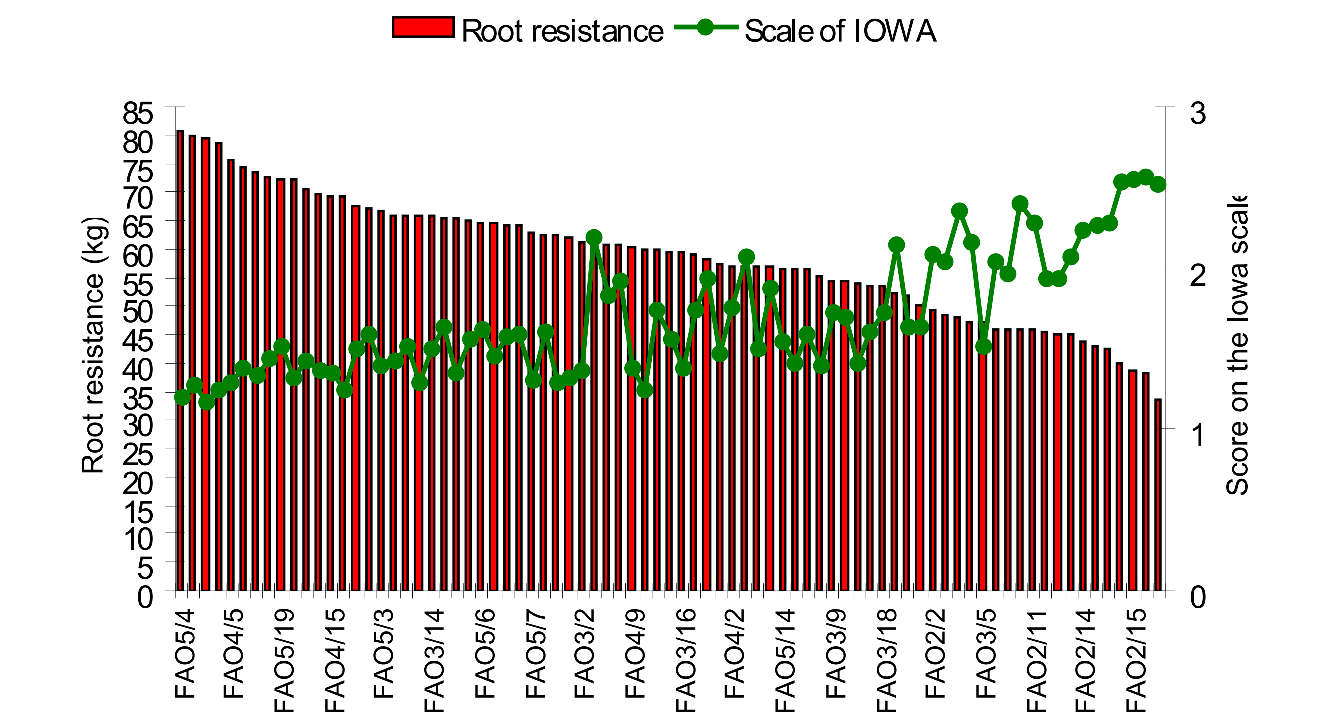

Figure 3: **Root resistance values and infection levels (Iowa scale) for the maize hybrids, averaged over four locations in 2007**

regeneration ability *(Figure 2*), suggesting that this trait could be improved by selection.

Substantial differences were observed between the genotypes for root resistance (34-82 kg) and for the scores recorded using the Iowa scale (1.1-2.5) (*Figure 3*). The greatest force was required to pull up the hybrids with the strongest root system and the least larval damage. It is clear from *Figure 3* that the greater the root damage (Iowa scale) the less force was required to remove the plants from the soil. These data confirm the fact that tolerant genotypes could be developed through breeding.

Another way to breed for resistance is to develop transgenic plants, which are completely resistant to the Western corn rootworm. This method exploits a trait based on antibiosis. Maize plants transformed with a gene originating from the bacterium *Bacillus thuringiensis* produce a Bt protein which is toxic to corn rootworm larvae, causing the cessation of the peristaltic motion of the pest and resulting in approximately 100% mortality. The incorporation of this gene into inbred maize lines bred in Martonvásár is currently underway using in vivo gene transfer techniques and conventional breeding tools (backcross). A single cross with the donor is followed by 5-6 generations of backcrossing to the recurrent line. *In vivo* gene transfer is required because the desired traits from the transgenic maize line (donor) cannot be transferred into the parental components of the hybrids by means of conventional breeding techniques. Maize hybrids carrying resistance to Western corn rootworm will be subjected to agronomic evaluation, their environmental effects will be studied, selection will be made based on genotypic differences and the seed of transgenic lines will be multiplied.

Summary

The control of the Western corn rootworm will only be successful if the most appropriate methods are applied in the right place at the right time. One of the most effective defences against the pest is crop rotation. If for any reason this cannot be achieved, chemical plant protection (soil disinfection) is an essential component of the control technology. The cultivation of tolerant maize genotypes also has a place in the control of the corn rootworm, as it can be incorporated into an integrated control system very simply and at no extra cost to the grower. The cultivation of tolerant genotypes is cheaper, as no chemical plant protection is required in the case of weak or medium infection, which also makes the method environmentally sound and human-oriented, as it does not contaminate the biological environment and no residues toxic to humans will be accumulated in the grain.

Data in the literature indicate that the production of transgenic maize provides more efficient control, so the potential of this technique should be investigated.

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