



**Assessment of adult western corn rootworm  
(*Diabrotica virgifera virgifera* LeConte, Coleoptera:  
Chrysomelidae) silk damage in sweet maize**

**Thesis of PhD Dissertation**

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# 1. INTRODUCTION AND OBJECTIVES

Sweet corn/maize (used later on as synonyms) is the largest field crop (vegetable one, according to commodity group categorisation) grown in Hungary, making Hungary the largest producer of this type of commodity in Europe. The sweet corn is an input intensive crop that requires a deep cultivation experiences, knowledge: For its successful production it is essential to be supported with science based, professional plant protection solutions in line with the 8 principles of the Integrated Pest Management Strategy (IPM) (Barzman et al., 2015).

One of the key issues in farmers decision-making along plant protection interventions is to highly consider the economic thresholds of pests, taking into account the pest monitoring, quality requirements for the product, be aware of detection threshold first and then the economic/damage thresholds followed by the intervention/action threshold. The economic threshold for silk damage by WCR (*Diabrotica virgifera virgifera* LeConte) (hereinafter referred to as D.v.v., or WCR) adults, known pest of maize, depends on the type of maize hybrid and the purpose of cultivation (Tuska et al., 2002). With this information, the farmer will be able to take decisions for plant protection management interventions taking into account the principles of Integrated Pest Management (IPM) (Kiss, Zanker, & Eke, 2017). WCR is known in USA and in Europe as key pest of maize due to feeding of its larvae on maize root system. Less attention has been paid to feeding activity of its adults, specifically that on silks of maize cobs). In order to support IPM development in sweet maize, research was needed on activity and consequences of WCR adult feeding in a value added crop, namely sweet maize. The aim of my three-year study was to determine the economic threshold of WCR adult silk feeding damage in sweet corn.

## **Objectives were to:**

- measure the silk feeding damage by varying WCR adult numbers,
- measure and analyse the impact of WCR adult density on yield parameters,
- determine the economic damage threshold for a given hybrid,
- compare of the above with artificial silk cutting as a proxy method,
- analyse the silk damage response of various sweet corn hybrids,
- analyse the stressor (WCR adults) and year effect on yield parameters,
- recommend an intervention threshold,
- place the practical intervention in integrated pest management system context,
- develop IPM recommendations for sweet corn.

## 2. MATERIAL AND METHODS

### 2.1 Field experimental setup

Field experiments were carried out in 2016, 2017 and 2018 in non-irrigated areas of Plasmoprotect Kft., near Martonvásár, in a typical field crop production region in Central Transdanubia, Hungary. The agronomic and crop production practices were similar every year and followed the usual practices in the region.

The experimental areas were located in the same block a few hundred meters apart in each year. Planting was carried out in the experimental plots with a seed rifle, with 55,000 seeds per hectare, with 75-centimeter row spacing of maize. In the experimental field, the soil was prepared for sowing (after autumnal deep plowing) by tillage in the spring. Plant protection products used in the experiment were as follows: Prior to sowing, Force 1.5G insecticide soil treatment was applied at a rate of 10 kg product / ha (tefluthrin 15g / kg), after which no insecticide treatment was applied. Post-emergence herbicide treatment was performed as weed control. No fungicide treatment was applied in the maize growing period.

### 2.2 Western corn rootworm adult silk clipping experiments

In 2016, 2017, and 2018, I set up tests for artificial WCR adult infestation on Suregold sweet corn hybrid. The experiment consisted of a plot of 6 meters (8 rows) wide and 9.2 meters long in an area of 55.2 m<sup>2</sup>, with 38 seeds per row. Each row randomly included the five treatments (WCR adult density) in 2016 and the six treatments and untreated control plants in 2017 and 2018, selecting 5 consecutive plants per treatment in 4 replicates, thus examining 20 plants, silk in the 8 rows. This plot arrangement is in line with EFSA Guidance for measuring agronomic and phenotypic parameters of crops). As isolation at the edges, 4 rows of sweet corn were sown 3 meters wide to protect our experimental plots from wildlife and wind.

Because we worked with individual isolated plants in our studies, the plot size (as opposed to the “usual” arrangement for plot treatments of non-isolated plants) is not a relevant approach. Our requirement was the homogeneity of the plant stand and of the soil, agronomic methods for the individual plants.

For our experiment, WCR adults were collected from the surrounding, insecticide-free corn plants 1-2 days before placement of the cages. Until the placement, they were stored in a box covered with a specially designed bridal veil, ensuring their proper living conditions with fresh corn tassel, silk and leaves.

The studied plants were monitored daily from end of June, at tasseling stage (VT, Iowa System, Abendroth et al., 2011, that is when the tassel is completely visible when the plant has reached its full height and will begin to shed its pollen). Development of individual ears was followed, and caging was done when the silk appearance was likely the next day (R1 stage, Iowa System, Abendroth et al., 2011, that occurs 3 days after the tasseling stage) to ensure that experiment started before the first silks emerged from the husk leaves, i.e. right before the beginning of R1 stage of each studied plant. Therefore, the first day of the WCR adult infestation varied slightly among plants. Bridal veil cages were used in the experiment to isolate the ears and WCR adults. The size was 400 × 250 mm with 1 mm webbing holes. The cages were placed over the ears with 0, 1, 2, 4 or 8 WCR adults inside in 2016 and with 0, 1, 2, 4, 8 or 12 adults in 2017 and 2018. Moreover, 20 uncaged ears of control plants were also marked as untreated each year. In order to assess the natural WCR adult infestation in the experimental plots (since this may have affected uncaged silk length), three Pherocon AM yellow sticky traps (Trece Inc, USA) were placed in the research area and number of captured adults were recorded weekly. The traps were replaced weekly. At the end of the bridal veil, elastic bands were placed to fasten the cages and to provide access for measuring silk length and for counting adults within the cage. The number of adults were adjusted daily to maintain the target infestation level, if dead adults were needed to be replaced. The cages were used to ensure that the adults remained confined on the ear, as well as to isolate the silks and cob from pests other than WCR adults present in the study area. The length of silk was measured daily for 9–11 days from the start of artificial WCR adult infestation to the end of flowering and drying of silks.

The cobs of the experimental plants were harvested by hand on 17 August 2016, on 28-29 July 2017, from 24 July to 1 August 2018 on the basis of the parameters defined by the production companies, and then after harvesting the weed leaves were measured. After removal, the weight of the cobs, the length of the cobs and their circumference 50 mm far from the cob ends and their circumference at the center of the cob. During the laboratory measurements, the number of grain rows and the number of grains in each cob were also counted. From the obtained data, the fertility index was determined: the number of grains was divided by the circumference of the average cob cross-section (average of the three circumferential values) and then multiplied by the length of the cob. This index thus reflects the number of grains per unit area of cob surface, assuming that the cobs within the hybrid under study have a similar shape.

The weather conditions varied from year to year, which had a significant effect on the yields, the regeneration of the studied silk length, and thus the extent of the damage. In 2016, the period of silking period and fertilization was favourable with high humidity and rainy weather, and there was no outstanding maximum daily temperature, when the viability of pollens could have been significantly reduced “they could have lost their fertility”. In 2017, this period was drier; moreover, during the period of pollination and grain filling, higher temperatures did not favor the edge of the pollen viability, grain saturation. In 2018, the studied period was still dry, but with a more modest daily maximum temperature, the rainfall supply was adequate during the grain filling period, which significantly contributed to the achievement of adequate yields.

### **2.3 Artificial silk cutting setup**

In 2017 and 2018, we expanded our WCR adult experiment with a so-called artificial silk cutting experiment, the essence of which was to recapture the silk by simulating the WCR adult silk feeding damage. Treatments were tested on 7 sweet corn hybrids Suregold, Kinze, MV July, GSS 5649, GSS 8529, Moreland, GH 11754, as we hypothesized that sweet corn hybrids with different genetic backgrounds respond differently to different degrees of silk damage under different weather conditions in each year. . Each hybrid was examined in one plot (7 plots in total), each 3 m wide (4 rows) and 9.2 m long, with a total area of 27.6 m<sup>2</sup> and 38 seeds per row. Each row was randomized to include the three treatments and untreated control plants, selecting 5 consecutive plants per treatment in 4 replicates, so that 20 plants per treatment in 4 rows were tested (according to EFSA Guidance requirements). Here again, the experimental area was surrounded by 4 rows of 3-meter isolation area.

Plants were monitored daily from mid-June from the tasseling stage (VT Iowa System, Abendroth et al., 2011, the stage when the tassel becomes fully visible, maize has reached full height, and pollen dispersal begins). The development of the examined silks was monitored, and with the appearance of the silks, the daily silk removal work began in stage R1 (stage R1, Iowa System, Abendroth et al., 2011), which occurs 3 days after tasseling. In our experiment, the cut-off time was 25 June to 23 July 2017 and 21 June to 19 July 2018 in the 7 sweet corn hybrids tested.

Cobs were not isolated in the artificial silk cutting experiment because we also wanted to monitor the “natural or background” WCR adult infection. The silks above the apex of the husky leaves were cut back to 0, 1, and 2 cm from the apex of the husky leaves, respectively, to simulate different WCR levels of infection and silk damage. In the case of different sweet corn hybrids, 20 control plants were marked

in each experimental plot, the silk length of which was measured on a daily basis. Three Pherocon AM yellow sticky traps (Trécé Inc, USA) were placed in the experimental plots to observe the infectious background infestation of the natural WCR adult, and the number of corn catches of the WCR adult was recorded weekly, as this could affect the length of the cobs without isolation mesh. Traps were changed weekly.

All sweet corn cobs tested were harvested manually at their appropriate ripening stage (according to the parameters prescribed by the processing companies) from 24 July to 3 August 2017 and from 18 July to 1 August 2018. The cobs were separated, stored in crates, then transported to the laboratory for measurements. The measurement endpoints were the same as described in the WCR adult infestation experiment. In addition, *Helicoverpa* damage was recorded on the cobs examined.

The weather conditions were similar to those described in the WCR adult experiment, however, the testing period for the 7 sweet corn hybrids included in the silk damage experiment spanned a broader period in 2017 and 2018 as well.

## **2.4 Data analysis and statistical inference**

In the first experiment with artificial WCR adult infestation, silk length data from day 1 to day 9 were used, as data from this period were available for all investigated maize cobs. Data were also standardised in accordance with first day of silk emergence also the starting point of artificial WCR adult infestation. Factors influencing silk length were analysed using general additive models (GAMs), where days after silk appearance was the smoothed non-linear explanatory variable (using penalized regression splines), and WCR adult density, year and veil cover were linear predictors. The possible effect of WCR adult density at silking, year and their interaction on fertility index and cob weight were analysed with linear mixed models (LMMs) with plot as the random variable. Multiple comparison technique with false discovery rate correction was used to compare the three years pairwise when year was found to be an influential explanatory variable. In addition, the possible effect of bridal veil on fertility index and cob weight was investigated with Welch t-tests comparing data of covered ears without WCR adults and control ears (uncaged and caged with 0 adults).

In the second experiment with artificial silk cutting, the *Helicoverpa* natural infestation levels in the investigated hybrids and years were compared with binomial GLMs. Then the possible effect of hybrid, year and their interaction on fertility index and cob weight was analysed with linear models as described in the previous

experiment. Moreover, the reduction of cob weight and fertility index caused by the artificial silk cutting (compared to uncut controls) was analysed to determine the sensitivity of different hybrids to silk cutting as an estimate to silk feeding damage.

Using data of Suregold hybrid both experiments, the linkage of silk cut and WCR adult number per ear was estimated. We fitted linear regression models of artificial WCR adult infestation data, i.e. the pest silk clipping – weight and fertility index reduction relationship for 2017 and 2018. Then, we calculated the reduction of the clipped cobs vs the corresponding uncut control cobs and these reduction values were used to insert in the above-mentioned regression models to estimate the hypothetical WCR adult densities for silk cut. Using both cob weight and fertility index reduction data resulted in 12 data points being sufficient for the estimations. Moreover, these estimations were used to calculate the mentioned coefficients for the other hybrids and visualization of hybrid sensitivity to WCR adult was possible. Data manipulation, -visualization and statistical inference were performed using R.



### 3. RESULTS AND DISCUSSION

#### 3.1. Results of western corn rootworm adult silk clipping experiments

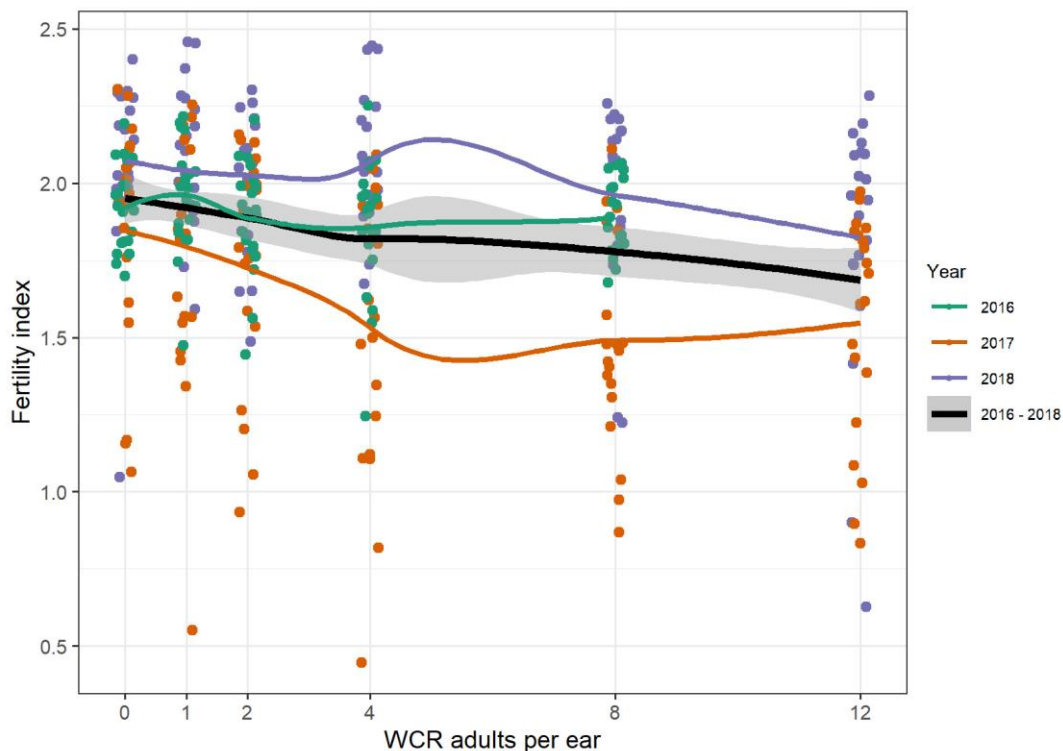
In the three years of the experiment, the natural infection level of the adults was low on the experimental plots during the period of silking. In the first week of silking, WCR captures averaged  $14.0 \pm 4.4$  adults per Pherocon AM traps;  $11.3 \pm 9.5$  and  $7.0 \pm 2.0$  adults in 2016, 2017, and 2018, respectively. Two weeks after silking started, captures averaged at of  $22.3 \pm 6.1$  per trap in 2016, 2017, and 2018; there were  $15.0 \pm 1.7$  and  $3.7 \pm 1.5$  adults per week.

The Suregold sweet corn hybrid could be characterized by a rich, dense silk in all three study years. On the third day after the appearance of the silks, they reached their maximum length of  $95.0 \text{ mm} \pm 29.3 \text{ mm}$  (mean  $\pm$  SD),  $78.2 \text{ mm} \pm 13.7 \text{ mm}$  and  $88.0 \text{ mm} \pm 18.4 \text{ mm}$  in the untreated control in 2016, 2017 and 2018, respectively. The highest maximum silk length was recorded on bridal veil covered cobs without WCR adults in each study year. The bridal veil covers increased the silk length by  $20.3 \pm 3.6 \text{ mm}$  at peak length. The dynamics of the silk length were similar, consisting of a shorter increasing period and a longer decreasing. This pattern was analysed by an additive model (GAM) over the 9-day study period ( $F = 254.3$ ;  $df = 2.90$ ;  $p < 0.001$ ). The length of the silks also varied from year to year: in 2016 it was significantly longer than in 2017 (mean difference:  $19.2 \text{ mm}$ ;  $t = 21.36$ ;  $df = 3053$ ;  $p < 0.001$ ) and in 2018 (mean difference:  $16.2 \text{ mm}$ ;  $t = 18.11$ ;  $df = 3053$ ;  $p < 0.001$ ) and in 2017 were significantly shorter than in 2018 (mean difference:  $2.9 \text{ mm}$ ;  $t = 3.45$ ;  $df = 3053$ ;  $p = 0.002$ ).

Damage caused by the WCR adults was observed on bridal veil covered cobs. The adults had a significant reduction on silk length, with an average decrease of  $1.7 \text{ mm}$  per adult ( $t = -18.42$ ;  $df = 3053$ ;  $p < 0.001$ ; corrected  $R^2$  of the model:  $0.37$ ).

The use of bridal veil cages had no effect on the fertility index in any of the study years when we compared the 20–20 uncaged control cobs and the cob covered with the bridal veil but not infected with WCR adult. (2016:  $t = -0.22$ ;  $df = 38.0$ ;  $p = 0.827$ ; 2017:  $t = -1.25$ ;  $df = 24.9$ ;  $p = 0.224$ ; and 2018:  $t = -0.66$ ;  $df = 33.1$ ;  $p = 0.513$ ). However, this index differed significantly among study years, and significantly decreased with increasing number of WCR adults (Fig. 1). The interaction of WCR adult number and year had no effect on the fertilization of the maize cobs ( $F = 1.31$ ; numerator  $df = 2$ , denominator  $df = 325.26$ ;  $p = 0.272$ ). We obtained lower index values in 2017 than in 2016 ( $t = 3.20$ ;  $df = 9.64$ ;  $p = 0.004$ ) and in 2018 ( $t = 5.15$ ;  $df = 9.64$ ;  $p < 0.001$ ), while in 2016 and 2018, fertility indexes did not differ ( $t = 1.844$ ;

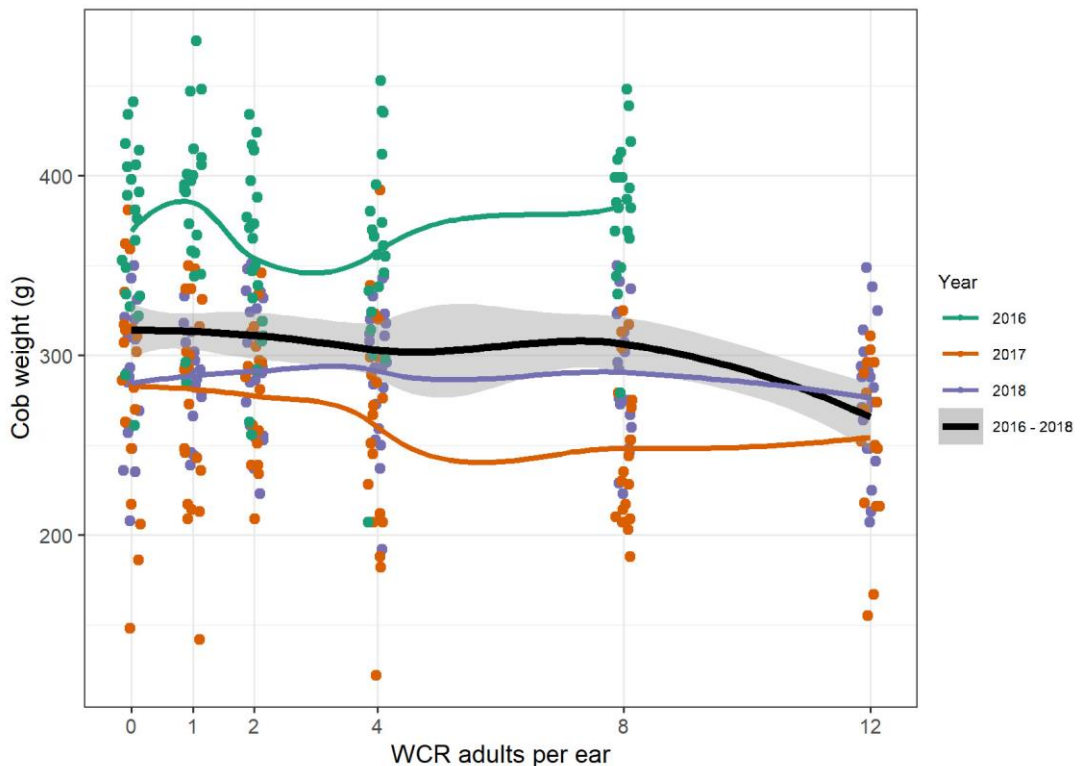
df = 9.64; p = 0.156). A slight decrease in cob fertilization was observed, 1.04% per WCR adult (CI95%: 0.62% - 1.45%; t = 4.91; df = 327.26; p <0.001; Figure 1). However, the corrected R<sup>2</sup> value of the fitted model was 0.29, and the number of WCR adults per cob was responsible for only 6% of the variability in the fertility index.



**Fig. 1.** Fertility index of harvested maize cobs infested with 0–12 WCR adults at silking when ears were covered with bridal veil. Smoothed trend lines are presented for individual study years (green, orange and purple lines), as well as for all data points with confidence intervals (black line with grey shaded area), Martonvásár, Hungary, 2016–2018.

Bridal veil had no effect on cob weight in any of the years when compared 20-20 uncaged control cobs and cobs covered with bridal veil but not infested with WCR adults (2016: t = 0.28, df = 37.94). p = 0.781, 2017: t = -1.18, df = 31.29, p = 0.249, and 2018: t = -1.98, df = 35.32, p = 0.055). When a mixed linear model was fitted to the bridal veil covered cobs with the explanatory variables of the year, the number of WCR adult, and their interaction, the WCR adult number (F = 1.48, numerator df = 1, denominator df = 325.01, p = 0.225) and the said interaction (F = 2.73, numerator df = 2, denominator df = 325.01, p = 0.067) had no significant effect on cob weight. However, the average weight of the cobs varied significantly among years (F =

17.98; numerator df = 2; denominator df = 12.49;  $p < 0.001$ ; Figure 2). The highest weight cobs were measured in 2016 (compared to 2017:  $t = -5.51$ ;  $df = 12.72$ ;  $p < 0.001$ ; and 2018:  $t = -4.87$ ;  $df = 12, 72$ ;  $p < 0.001$ ), whereas maize cobs had similar weights in 2017 and 2018 ( $t = 0.65$ ;  $df = 12.72$ ;  $p = 0.792$ ). However, the  $R^2$  value of the fitted model was 0.55, and the number of WCR adults per cob was responsible for only 4% of the variability in weight.



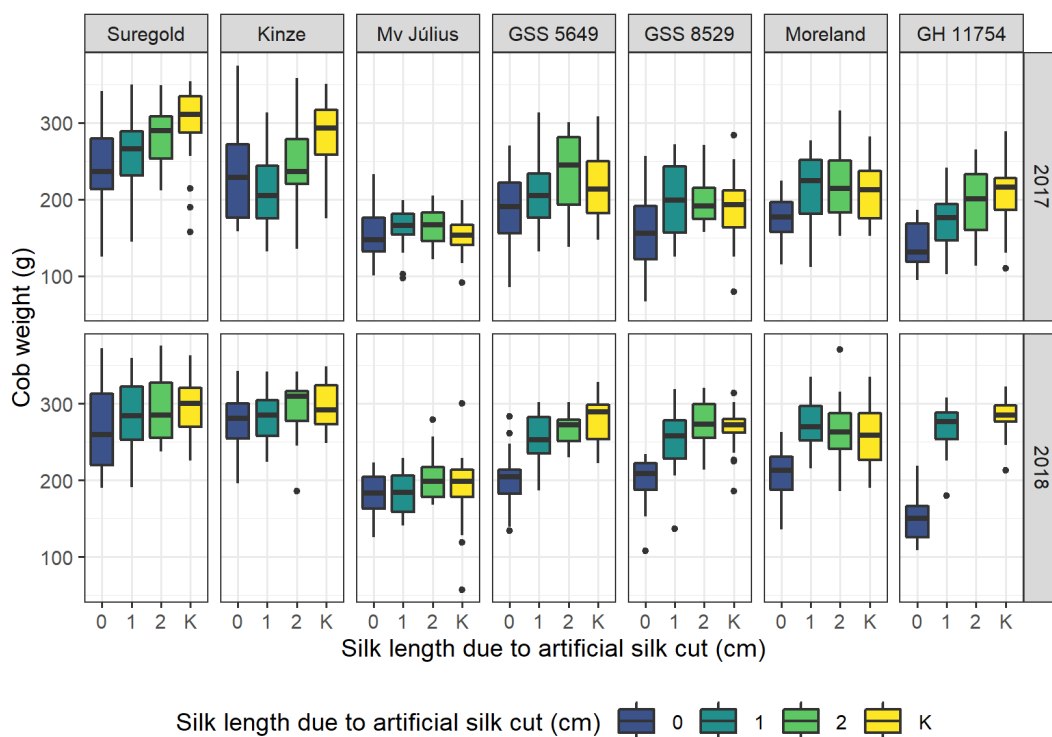
**Fig. 2.** Weight of harvested maize cobs infested with 0–12 WCR adults at silking when ears were covered with bridal veil. Smoothed trend lines are presented for individual study years (green, orange and purple lines), as well as for all data points with confidence intervals (black line with grey shaded area), Martonvásár, Hungary, 2016–2018

### 3.2. Results of artificial silk cutting experiment

The natural WCR adult infestation was low in the experimental plots during the period of silking: Practically low natural infestation 3–23 WCR adults / trap / week on Pherocon AM yellow sticky trap captures corresponds to 0.25–0.5 WCR adult / maize plant infection (Bažok et al., 2011), thus it is unlikely to bias the results of our experiment. In addition to the natural WCR adult infestation, damage of *Helicoverpa*

*armigera* was observed on 183 cobs (16% of the examined cobs). The presence of *Helicoverpa* differed significantly between the two years studied (was more frequent in 2018;  $p < 0.001$ ) and between hybrids ( $p < 0.001$ ).

A total of 1,096 cob weights were measured in the two years, with the lowest value of 56 g (MV July, 2018, uncut control) and the highest being six times heavier of 376 g (Suregold, 2018, cut to 2 cm) (Fig. 3).



**Fig. 3.** Cob weight as affected by 0, 1 and 2 cm of persistent artificial silk cutting in seven sweet maize hybrids, K: control with uncut silk. Martonvásár, Hungary, 2017-2018

The cut of the silks ( $p < 0.001$ ), the hybrid ( $p < 0.001$ ), the year ( $p < 0.001$ ) and the pairwise interaction of these three variables all had significant effect on the husked cob weight ( $p = 0.006$  for the cut and the year interaction, in the other cases:  $p < 0.001$ ). The value of  $R^2$  in the regression model was 0.55. In 2018, husked cobs were on average 50 grammes heavier (CI95%: 43 to 58g) than in 2017. The silks cut to 0 cm ( $p < 0.001$ ) and 1 cm ( $p < 0.001$ ) resulted in cobs with lower weights than the uncut control. However, the weight of cobs with 2cm silk did not differ from the weight of the control cobs ( $p = 0.688$ ). Compared cobs with 0 cm silk to cobs with 1 cm and 2 cm silk, there were significant difference in cob weight ( $p < 0.001$  in both cases), while cobs with 1 cm and 2 cm silk did not differ ( $p = 0.187$ ). Silk cutting to

0 cm resulted in a significant decrease in cob weight under adverse (dry) weather conditions.

The husked cob weight varied among the seven investigated hybrids ( $p < 0.001$ ). The pairwise comparisons of hybrids mostly resulted in significant difference with  $p < 0.001$ , except for GH 11754 - Moreland ( $p = 0.004$ ); only Kinze - Suregold ( $p = 0.184$ ), GSS 8529 - GSS 5649 ( $p = 0.050$ ), Moreland - GSS 5649 ( $p = 0.986$ ), Moreland - GSS 8529 ( $p = 0.310$ ) and GH 11754 - GSS 8529 ( $p = 0.587$ ) did not differ.

### **Fertility index:**

Artificial silk cutting, the hybrid, the year and the pairwise interaction of these three variables all had a significant effect on the fertility index ( $p < 0.001$  in each case). The  $R^2$  value of the fitted model was 0.582. This index was higher in 2018 than in 2017 (CI95%: 0.32 to 0.45). The fertility index was lower for both the 0 cm ( $p < 0.001$ ) and 1 cm cut silk ( $p < 0.001$ ) cobs compared to the uncut control. However, the fertility index of cobs with 2 cm silk did not differ from the control cobs ( $p = 0.300$ ). Compared cobs with 0 cm silk to cobs with 1 cm and 2 cm silk, significant index reduction was detected ( $p < 0.001$  in both cases), while cobs with 1 cm and 2 cm silk did not differ ( $p = 0.367$ ).

When comparing the hybrids pairwise, the fertility index did not differ for GSS 8529 - Suregold ( $p = 0.948$ ), GSS 5649 - Kinze ( $p = 0.884$ ), GSS 8529 - Kinze ( $p = 0.423$ ) and GH 11754 - Mv July ( $p = 0.936$ ). All the other cases the hybrids differed ( $p < 0.001$ , except for Kinze - Suregold ( $p = 0.042$ ) and SS 8529 - GSS 5649 ( $p = 0.023$ )).

Suregold: In 2017 (dry vintage, less precipitation) there was no significant yield difference between uncut (untreated control) cob and cobs with 1 and 2 cm silk. However, silk cutting to 0 cm resulted in similar yield reductions as at 8-12 WCR adult / cob infestation levels. In 2018, due to more favorable weather conditions, there was no significant difference between treatments (cutting or WCR adults larvae density).

Kinze: In 2017, silk cutting to 0 and 1 cm resulted in a significant yield reduction, while in 2018 (more favorable weather conditions) there was no yield difference between treatments.

MV July: There was no significant difference in the yields of the MV July sweet corn cob for the different treatments in any of the experimental years.

Moreland: Silk cutting to 0 centimeters resulted in a significant drop in yield in both years.

The same response was observed for the GSS5649, GSS8629, and GH11754 sweet corn hybrids. In the case of the latter, due to the early drought, poor germination did not allow data collection in 2018 for 2 cm silk treatment.

It is important to note that weather conditions during the growing season had a significant effect on yields, which is particularly important for all hybrids during the silking period.

Linear models were also fitted to the cob weight loss and fertility index relative to the control to compare the different responses of each hybrid to silk cut.

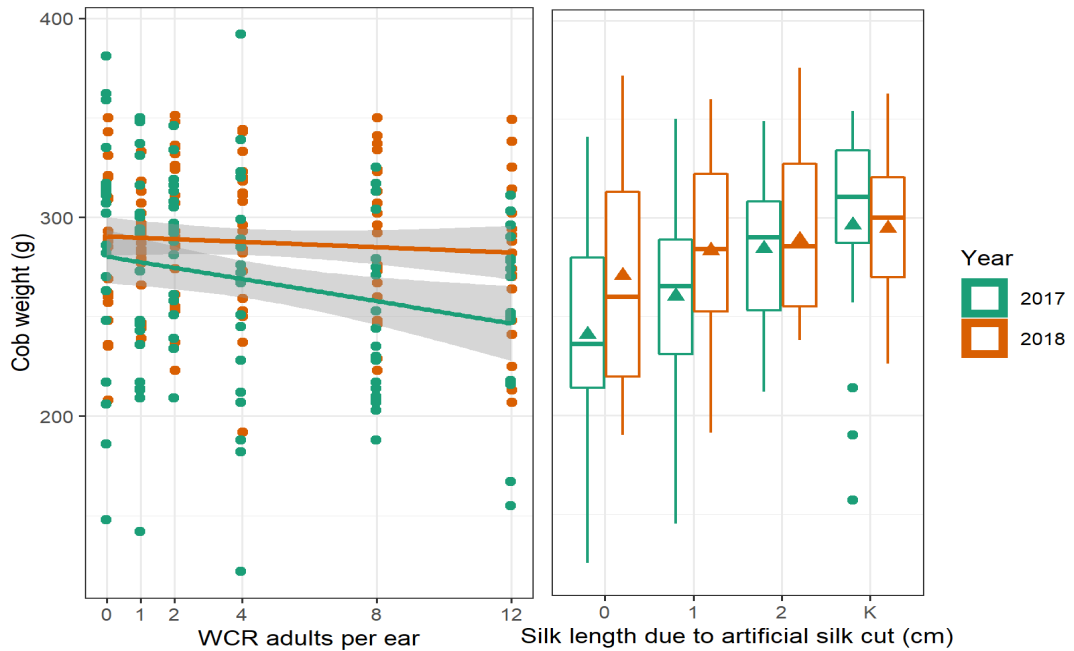
In the case of the husked cob weight, the cutting of the silks to 0 cm caused a smaller decrease in the Mv July hybrid than in the other investigated hybrids, and in the GH 11745 hybrid it caused a larger decrease than in the others. As for silk cutting to 1cm, Moreland cob weight decreased less than Suregold, Kinze, and GH 11745 cob weights. While the silk cutting to 2cm resulted a slightly different pattern: the Kinze differed from the Mv July, GSS 5649, GSS 8529 and Moreland hybrids.

The silks cutting to 0 cm caused a smaller decrease of the fertility index in the Mv July hybrid than in the other investigated hybrids, and a greater decrease in the GH 11745 hybrid than in the others. The fertility index of the Suregold, Kinze, and GH 11745 hybrids decreased more than the other four hybrids when the silks were cut to 1 cm. As for silk cutting to 2cm, GH 11745 differed from Mv July, GSS 5649 and GSS 8529 hybrids.

### **3.3 Correlation of western corn rootworm adult silk clipping to artificial silk cutting**

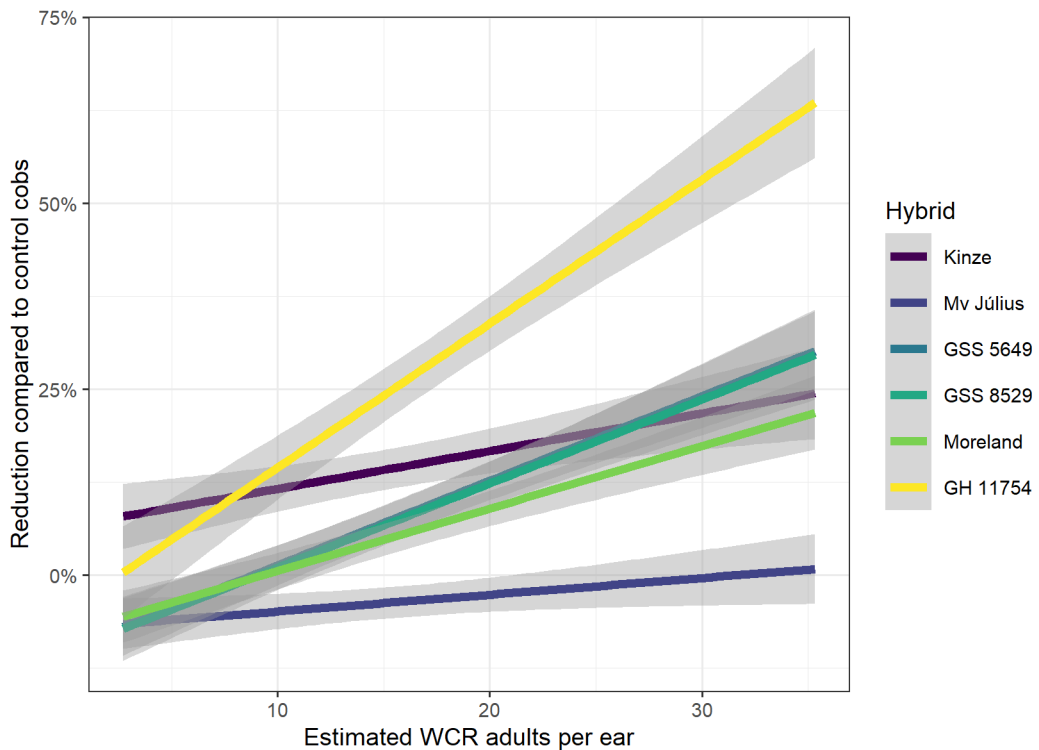
The yield response of the Suregold sweet corn suggested that the hybrid even tolerated the infestation of 8-12 WCR adults per cob.

In the Suregold hybrid, both the artificial WCR adult infestation and artificial silk cutting experiment were performed (see Chapters 3.1 and 3.2). Comparing the results of the two studies allowed us to estimate the damage-based theoretical adult density (adult / cob) for silk length of 0, 1, and 2 cm (Figure 4). For simplicity, the relationship between the number of adults per cob and the reduction of cob weight or fertility index was assumed to be linear, and the number of adults for each silk length level was extrapolated. Based on husked cob weight, the silk cut to 0 cm corresponds to an average of 27.6 adults per cob, the silk cut to 1 cm corresponds to 14.8 adults, and the silk cut to 2 cm corresponds to 6.4 adults. Based on the fertility index, these values were similar: 26.2 adults for 0 cm silk, 12.8 adults for 1 cm silk, and 3.8 adults per cob for 2 cm silk.



**Fig. 4.** Correlation of cob weight in Suregold sweet maize hybrid by WCR adult damage (left panel) and artificial silk cutting (right panel) to 0, 1 and 2 cm, as well as data of cobs with uncut silk (K), Martonvásár, Hungary, 2017-2018

Using all twelve estimated WCR adult densities, the overall susceptibility of hybrids to WCR adult damage can be examined. The more sensitive a hybrid is, the steeper the fitted line is (Figure 5), i.e. the least sensitive hybrid is estimated to be the Mv July followed by the Kinze hybrid. Moreland, GSS 5649 and GSS 8529, show a very similar susceptibility, while the GH 11754 hybrid is estimated to be the most sensitive to WCR adult damage.



**Fig. 5.** Reduction of cob weight and fertility index compared to control cobs at the estimated WCR densities. Estimation is based on data of Suregold hybrid Martonvásár, Hungary, 2017-2018

### 3.4. Discussion of western corn rootworm silk clipping results

In our WCR adult infestation experiment, we examined the possible silk damage caused by WCR adults on the Suregold sweet corn hybrid for three years. In these experiments, it was crucial to cover the cobs with an isolator bridal veil before silking and then placing WCR adults determined for each treatment under the bridal veil covered cobs (Culy, Edwards, & Cornelius, 1992; Tuska et al., 2002), since the WCR adult, pollination before damage may impact experimental effects. In this study, WCR adults were placed on from the V stage of maize to the VT stage, i.e., before silking. Thus, we were able to successfully avoid the possible effects of early pollination.

The maximum silking varied from year to year due to different weather effects. Fuad-Hassan, Tardieu, and Turc (2008) examined the silk growth of well-irrigated plants exposed to mild to moderate groundwater shortages. Mild to moderate groundwater scarcity was found to reduce the size of the silk and increase the duration of the silking, which did not fully compensate for the silk feeding.



Isolator bridal veil placed on corn cobs also affected the length of the silk, consistent with the results of Tuska et al., (2002) who found that isolation of the silk may have been an obstacle to pollination and led to an increase in the length of the silk. However, the cover of the maize cobs with the isolator bridal veil did not affect the calculated fertility index or cob weight. Based on these, it can be stated that bridal veil isolator is suitable for the study of damage to the consumption of WCR silks, even if they affect the microclimatic conditions and the growth of the silks.

In our study, the calculated fertility index decreased slightly at higher levels of WCR adult infestation. This overall decrease is consistent with the results of Tuska et al., (2002). In the case of seed maize and non-irrigated commodity maize, the specified damage thresholds for WCR adult are much lower than the damage levels for WCR adults in sweet corn, which caused a slight decrease in fertility in sweetcorn (2002). The silk feeding tested did not lead to a significant reduction in cob weight even in 12 WCR adults / maize cobs, while in some circumstances the number of WCR adults was much lower (1-3 WCR adult per cob in seed corn production (Tuska, Edwards and Kiss, 2003) can affect crop quantity and quality. Most often, the pest density that causes economic damage (per m<sup>2</sup> or per hectare) is lower than for higher-yielding, intensively grown crops.

However, our results show the opposite in the case of sweet corn, as in the case of grain corn, due to the intensive silk growth of sweet corn and the increased probability of fertilization. Among the studied years, the dry conditions in 2017 had a negative effect on silk regeneration after WCR adult damage, as well as a negative effect on fertility and yield. This is consistent with the results of Schoper et al., (1986). Although dry conditions have had less of an effect on fertility in seed and grain maize, genetic differences may lead to different reactions between maize hybrids (Abel et al., 2000; Ivezic et al., 2006). Since only one sweet corn hybrid was tested in the experiment, the effect of genetic differences on the responses of plants to different populations of WCR adults has yet to be investigated.

### **3.5. Discussion of artificial silk cutting results**

The two-year study was performed on a non-irrigated sweet corn field in seven different sweet corn hybrids. There was a low population of WCR adult in the study area during the period of silking in both years. After the appearance of the silks, the silk was cut back at 0, 1, 2 cm above the apex of the cotyledon to simulate the different levels of infection of the WCR adult and the silk shrinkage they caused. The silk length of untreated control plants was measured daily until the silks dried.

As the quality requirements for sweetcorn at harvest are much higher than for maize, it is necessary to set action thresholds for the various pests that directly or indirectly affect quality.

The WCR damage thresholds determined in seed maize and non-irrigated grain maize were much lower (Tuska et al., 2002; Tuska, Edwards, & Kiss, 2003) than in moderate WCR adult treatments in sweet corn. Furthermore, the observed silk damage did not lead to a significant reduction in cob weight in our sweet corn experiment even for 12 WCR adults / cob. Most often, pest control thresholds and farmers risk tolerance levels are lower in more valuable, intensive crops. However, our experimental results for sweet corn show the opposite of the damage threshold observed for grain maize, probably due to the silk regeneration of sweetcorn and better fertility.

Of the years studied, the dry weather conditions in 2017 had a negative effect on silk regeneration after maize root rot damage and had a negative effect on fertility and yield. These observations are consistent with the research findings of Schoper et al., (1986). However, in the case of sweet corn, dry, suboptimal weather conditions had less of an effect on fertility than some previous experimental results showed in the case of seed corn and grain corn, respectively. Since sweet corn is mostly grown by irrigation, dry weather conditions are compensated by irrigation. Our results suggest that the timing of irrigation may contribute to reducing the consequences of silk damage and thus reduce the application of insecticides during fertilization. In addition, when biological control solutions are used during the silking period, knowledge of the WCR adult damage threshold associated with hybrid tolerance to WCR adult infestation helps farmers reduce their use of chemical insecticides.

However, genetic differences (hybrids) can lead to different plant response, so maize hybrids may tolerate WCR adult damage differently. (Abel et al., 2000; Ivezic et al., 2006). Therefore, information on the yield loss response of different hybrids may need to be performed in conjunction with variety trials or demonstration experiments under regional and / or local conditions for the WCR adult.

### **3.6. Economic threshold estimation**

In the case of the Suregold sweet corn hybrid, even a high (8 adults / cob) WCR adult density did not cause a decrease in silk length that would result in a significant decrease in fertility and cob weight. Therefore, this sweet corn hybrid does not require interventions, such as insecticide treatment at a density of less than 8 WCR

adults per cob, unless the presence of other pests threatens the crop or we want to reduce the number of egg laying females of the WCR in the corn field for the next year, though this decision may differ from the date of silking. In addition, our results indicate the development of economic damage thresholds for WCR adult plants in maize should be placed into an integrated pest management context.

The simulation of WCR adult silk clipping (silk cutting) resulted in different responses among sweet corn hybrids studied. Surprisingly, the 1 cm silk cutting resulted in a moderate yield reduction. Significant yield loss was measured only at the 0 cm cutoff.

#### **4. NEW SCIENTIFIC RESULTS**

Based on the results obtained from our three-year-long WCR adult silk clipping experiments and two-years artificial silk cutting experiments, I came to the following findings for the seven sweet corn hybrids examined in Hungary:

- I found that under 8 WCR adult / cob infection levels in sweet corn there is no justification for insecticide treatment against WCR adults, which was confirmed by silk examination, silk length measurement, cob weight measurement and fertility index calculations.
- Sweet corn hybrids are able to compensate for the infestation of low (8 adults / cob) WCR adults silk clipping without loss of yield quantity and quality.
- Different weather conditions (extreme drought, lack of irrigation or rainy weather) in each year affect the extent of WCR adult damage in sweet corn.
- The examined sweet corn hybrids responded differently to the WCR adult infestation simulated by the silk cuttings. In the case of each hybrid, their silk regeneration and yield response was different, and the quantity and quality of the yield were also affected differently by the different degrees of damage to the silks.
- My experiments contributed to the development of integrated pest management of sweet maize and to reduce the burden by unjustified insecticide applications on the environment.

## 5. CONCLUSIONS AND SUGGESTIONS

### **Based on 3 years field experiments,**

- Relevant fertility and cob weight decrease were not detected in Suregold sweet maize hybrid even at rather high (8 adults/ear) WCR adult densities.
- Therefore, for this sweet maize hybrid, insecticide control measures at WCR densities below eight adults per ear are not necessary unless the presence of other pests threaten the crop or an attempt is being made to reduce WCR female egg laying for the following year's maize crop.
- In addition, our results support the development of economic thresholds for WCR adults in maize within the framework of integrated pest management.
- Artificial WCR silk clipping (silk cutting) showed different responses based on maize hybrid. Surprisingly, silk cutting to 1 cm resulted in moderate yield reduction. Significant yield reduction was measured only at 0 cm cutting.
- Thus, the action threshold, in general, for adult control only is justified at high adult population levels for sweet maize hybrids. Response of individual sweet maize hybrids to WCR adult damage may be slightly different, thus this should be considered according to local conditions (multiple abiotic and biotic stressors, e.g. other pests), but usually there is no need for WCR adult control treatments.
- These favours reduced insecticides application, environmental load and increased consumers safety.

### **Reducing the number of WCR population (area-wide IPM approach):**

When making our decision, we need to take great care of what we want to produce next year. If maize does not follow maize in our crop rotation, however, a reduction in the number of WCR adult may have an impact on the broader "area wide" IPM approach. Some of the adults do not stay on the given field in the given year but migrate to other corn areas where they lay their eggs, as a result of which the following year's infections can be observed in an even larger area in many cases. This means that the adult population in a given year may have a wider impact on a

given field, requiring an IPM defense decision to be made in a broader “area wide” context.

### **WCR adult reduction strategy for the next year:**

There are several options based on IPM principles. In the case of chemical pesticides, the use of various pyrethroids may be an obvious plant protection solution, which is by far the most common method of control in sweet corn. If we intend to reduce the number of WCR adults, *H. armigera* larvae, or other herbivore populations in sweet corn, appropriate pesticides can provide a good solution, while respecting the re-entry or pre-harvest intervals. *Helicoverpa* and / or *Ostrinia* of biological control e.g. Bt preparation will not affect WCR adults. Trichogramma egg parasitoids can also be used to control *Helicoverpa* and *Ostrinia* larvae.

### **Informed decision:**

Full monitoring of pest population on the farm is essential, generating data at field level, preferably within the field. It is important to know whether there is already a need for intervention against pests in the area, whether the infection is already economically justified or has not yet reached the economic threshold. If intervention is necessary, it is worthwhile to use decision support systems as to whether it is necessary to treat the entire of the field or even use partial treatments only.

### **Integrated plant protection intervention decision:**

In plant protection planning and interventions, we have to take into account the detection threshold, then the intervention threshold and the economic threshold. Based on all above we should make the decision on the necessity and type of treatment in order to avoid qualitative and quantitative losses of the crop. With continuously changing unpredictable economic conditions (costs, market prices), these thresholds are valid as criteria, but we should adopt them to specific actual conditions.

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#### *Peer reviewed scientific papers (English)*

**A. Gyeraj**, M. Szalai, Z. Pálincás, C. R. Edwards, J. Kiss (2022): Sweet maize hybrids' yield response to adult Western corn rootworm (*Diabrotica virgifera virgifera* LeConte, col.: Chrysomelidae) silk feeding and IPM implications thereof. *North – Western Journal of Zoology*. **IF 0,969** (in press).

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