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# Coincidence of Silking Time of Corn, *Zea mays* and Flight Period of Cotton Bollworm, *Helicoverpa armigera* Hbn.: How Does It Affect Follow-Up Abundancy of Larvae on Cobs and Grain Damage in Various Corn Hybrids?

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In order to investigate whether some corn *(Zea mays)* hybrids of various seasonal growing rates (FAO numbers) are damaged by larvae of cotton bollworm, *Helicoverpa armigera* (Lepidoptera, Noctuidae) in different levels, a set of field experiments was conducted at Mezőhegyes, Southeast Hungary, in course of 2003–2004. Phenological stages of corn hybrids and pattern of captures of *H. armigera* moths in pheromone traps were monitored in course of the season. Abundances of larvae of the next generation on developing cobs were also recorded. At harvest, the weight of grains damaged by *H. armigera* larvae, as well as the total weight of grains taken off from cobs were measured for each corn hybrids.

At the time of peak flight in 2003, freshly hatched larvae appeared in the cobs of those hybrids which were already flowering (female flower), i.e. reached the silking stage, as in case of DK 391, DK 443 and Maraton (FAO numbers: 300, 380 and 450, respectively), while $L_1$ larvae were found in the cobs of Maxima (FAO number: 580), only considerably later, when it reached silking stage. In 2004, however, all hybrids, including Maxima, flowered by the time of peak flight, and $L_1$ larvae appeared on cobs of each hybrids without delay.

The mean number of larvae was the highest in 2003 in DK 391 (141.5 ± 4.5 larvae / 50 cobs), while DK 443, Maraton and Vilma did not differ significantly from each other (92.0 ± 10.0, 88.5 ± 3.0 and 78.8 ± 3.4 larvae / 50 cobs, respectively). The lowest number of larvae was found in Maxima (34.0 ± 3.0 larvae / 50 cobs). In contrast to this, the highest number of larvae were found on Maxima in 2004, differring significantly from that of DK 391, DK 391 and Maraton, these latter three hybrids housing only negligible low numbers of larvae.

The mean weight of damaged grains per cob was the highest in DK 391 (3.5 ± 0.6 gram) and lowest in Maxima (1.1 ± 0.2 gram), differring from each other significantly, in 2003. A reverse result was obtained in 2004, when far the highest weight of damaged grains per cob was found in Maxima (112.2 ± 0.7 gram), while pratically no damage occurred at DK 331, DK 443 and Maraton.

The mean weight of all grains per cob was the highest in 2003 in Maraton (111.5 ± 5.3 gram) and the lowest for DK 391 (79.2 ± 4.3 gram), the difference being statistically significant. In 2004, however, no significant differences were found in this respect (values ranged from 162.9 ± 2.8 gram to 192.4 ± 21.0 gram).

These results indicate that the abundace of *H. armigera* larvae on cobs and subsequent damage depends on the coincidence of silking of corn and peak capture of moths in pheromone traps, and not on features of the corn hybrid. Differences found in this study among hybrids barely reflect to the above-mentioned coincidence, therefore the level of infestation of the studied hybrids may vary by years and localities, and does not imply an inherent tolerant feature of the hybrids concerned. It is discussed that control measures should be considered to perform without delay in cases whenever such a coincidence is observed.

Keywords: silking stage, larval stage, seasonal flight pattern, monitoring, pheromone traps, phenology, timing of control measures.

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Many corn (*Zea mays* L.) hybrids are grown worldwide in large areas. There are several comparative field studies which reported on susceptibility of some corn hybrids, grown in North- or South-America, to damage of various key pests, such as *Helicoverpa zea* Boddie and/or *Spodoptera frugiperda* J. E. Smith (Lepidoptera, Noctuidae) ( Wiseman et al., 1973; Scott and White, 1985; Nagai et al., 1986; Sawazaki et al., 1990; Widstrom et al., 1992; Wiseman and Isenhour, 1994; Silveira et al., 1998; Butron et al., 2002; Williams et al., 2006; Ni et al., 2007). However, similar studies for corn hybrids, widely grown in Europe, are still awaited. For example, the infestation of cobs of DK 391, DK 443, Maraton, Vilma and Maxima hybrids by larvae of the cotton bollworm, *Helicoverpa armigera* Hbn. (Lepidopteara, Noctuidae) have to our best knowledge never been studied. As the abovementioned corn hybrids are cultivated in large areas in Central-Europe, and *H. armigera* has become since its invasion in 1993 (Szeőke, 1994) a permanent key pest of corn in this region (Szeőke, 2007; Keszthelyi, 2008), this knowledge would be of interest.

In order to study whether there are differences in the level of infestation of developing cobs of DK 391, DK 443, Maraton, Vilma, Káma and Maxima corn hybrids, caused by *H. armigera* larvae, series of field experiments were conducted at Mezőhegyes, Southeast Hungary, in course of 2003–2004. As we hypothetized that the phonology of corn plays an important role, corn hybrids of various seasonal growing rates, as expressed in corresponding FAO numbers, were selected. Results of this study related to the synchrony between peak flight, as monitored by pheromone traps, appearance of $L_1$ larvae on cobs and phenology of corn hybrids have already been reported (Dömötör et al., 2007). Here we report the abundace of *H. armigera* larvae on cobs by larval stages, as well as weight of damaged seeds on these corn hybrids.

## Materials and Methods

*Experimental plots of corn hybrids*

Experimental design of field trial was reported in Dömötör et al. (2007). Corn hybrids were grown in adjacent plots (12 rows, length: 800 m, area: ca. 0.8 hectare) at Mezőhegyes, South-East Hungary, in 2003 and in 2004. Plots were surrounded by a puffer zone, consisting of 60 rows. No pesticides were used in these plots and in the puffer zone, in course of the experiment. The following corn hybrids were grown: “DK 391” (FAO number: 300), “DK 443” (FAO number: 380), “Maraton” (FAO number: 450), “Vilma” (FAO number: 510) (only in 2003), “Káma” (FAO number: 510) (only in 2004) and “Maxima” (FAO number: 580).

*Meteorological data*

Minimum-, maximum- and average daily temperatures, as well as daily average relative humidities at Pitvaros (situated ca. 10 km from the site of the field trials), were obtained from the Institute of Hungarian Meteorological Service (Budapest, Hungary).

*Monitoring of corn phenology, moths' flight and larval population*

Phenological stages of the development of corn hybrids were recorded, using the Iowa State University Scale (1996) ($R_1$ stage: silking, $R_2$: blistering, $R_3$: milking, $R_4$: doughing, $R_5$: denting, $R_6$: physiological maturity). The seasonal flight pattern of *H. armigera* was monitored by pheromone trapping, using four funnel type of pheromone traps (Csalomon® VARL + ) (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary). *H. armigera* larvae were sampled in cobs, by checking 200 randomly chosen cobs (4 times 50 cobs) of each corn hybrid. Cobs checked at an occasion were excluded from later inspections. Collected larvae were identified to species, and the larval stage of *H. armigera* larvae was determined, based on the width of their head capsule (Scsegolev, 1951). In course of the relavant part of the season, all of these recordings were made twice a week.

*Measuring weight of damaged and undamaged grains*

At harvest, grains from fourty randomly chosen undamaged cobs of each hybrids were taken off, and their water content set to 14%, using NVM4 desiccator. The total weights of grains, taken off from a cob, were measured by a FD 8405 balance (Bosch, Germany) (i.e. 40 samples from each hybrids). Similarly, the total weight of those grains, taken off from a cob, which were infested by *H. armigera* larvae, were also measured.

*Statistical analysis*

Data were transformed by $\sqrt{(x + 0.5)}$ and subjected to ANOVA. If the F-value was significant, differences between means were tested for significance by Games-Howell post-hoc test, using the statistic package of SuperANOVA® (Abacus Concepts Inc., Berkeley, CA, U.S.A.). Zero means were compared to non-zero means by Bonferroni/Dunn (Control) test of the same statistic package.

# Results

*Effect of coincidence of silking stage of corn hybrids with moths' flight on follow-up abundance of larvae*

Seasonal pattern of development of subsequent (from $L_1$ to $L_6$) larval stages of *H. armigera* showed a parallel trend to the phenological stages (corresponding to FAO number) of corn hybrids. Reaching the silking stage ($R_1$) was decisive for the appearance of freshly hatched larvae, provided that the peak flight, as measured by pheromone traps, preceded silking stage. In 2003, peak flight just preceded silking stage (female flower) ($R_1$) of DK 391, DK 441 and Maraton corn hybrids (FAO numbers: 300, 380 and 450, respectively). Freshly hatched larvae appeared in the cobs of these corn hybrids right at the time of peak flight. Vilma corn hybrid (FAO number: 510) entered silking stage at peak flight and $L_1$ larvae were first noticed just a the time of next recordings. In contrast to this, $L_1$ larvae were found in the cobs of Maxima (FAO number: 580), only considerably later, when it reached silking stage. In 2004, all hybrids, including Maxima, flowered by the time of peak flight, and $L_1$ larvae appeared on cobs of each hybrids without delay. These data, as well as of cummulative number of larvae (including all larval stages) for DK 391 and Maxima (hybrids of the smallest and the largest FAO numbers, respectively) for 2003, and for 2004 are shown in *Figs 1, 2*. Average daily temperature and relative humidity for the flight periods in 2003 and 2004, respectively, are also shown on *(Figs 1, 2)*.

The mean number of larvae (including all larval stages) was highest in 2003 in DK 391 (141.5 ± 4.5 larvae in 50 cobs), while DK 443, Maraton and Vilma significantly did not differ from each other (92.0 ± 10.0, 88.5 ± 3.0 and 78.8 ± 3.4, respectively). The lowest number of larvae was found in Maxima (34.0 ± 3.0) *(Table 1)*. Means sorted by larval stages show a similar trend *(Table 1)*. In 2004, highest number of larvae were observed on Maxima (45.5 ± 2.2), followed by Káma *(Table 2)*. Hardly any larvae were recorded on Maraton, DK 443 and on DK 391.

**Table 1**

Mean number (± S. E.) of *Helicoverpa armigera* larvae in 50 cobs, in Mezőhegyes, Hungary, in 2003

| Corn hybrid | $L_1$ larval stage | $L_2$ larval stage | $L_3$ larval stage | $L_4$ larval stage | $L_5$ larval stage | $L_6$ larval stage | **All larvae** |
|---|---|---|---|---|---|---|---|
| DK 391 | 14.8 ± 1.3 c | 20.0 ± 1.8 c | 32.0 ± 3.7 c | 26.0 ± 5.8 b | 26.3 ± 3.7 b | 22.5 ± 2.6 b | **141.5 ± 4.5 c** |
| DK 443 | 7.2 ± 1.1 b | 13.8 ± 1.9 bc | 15.3 ± 1.8 b | 11.3 ± 2.8 ab | 20.3 ± 2.5 b | 24.3 ± 4.7 b | **92.0 ± 10.0 b** |
| Maraton | 5.0 ± 1.0 b | 11.3 ± 1.3 ab | 18.0 ± 1.7 b | 11.0 ± 0.8 b | 20.8 ± 1.5 b | 22.5 ± 2.3 b | **88.5 ± 3.0 b** |
| Vilma | 5.5 ± 0.2 b | 10.3 ± 1.1 ab | 12.0 ± 0.0 b | 15.8 ± 0.6 b | 17.8 ± 2.8 b | 17.5 ± 0.9 b | **78.8 ± 3.4 b** |
| Maxima | 1.0 ± 0.5 a | 6.5 ± 0.8 a | 8.3 ± 0.4 a | 6.5 ± 0.8 a | 6.3 ± 0.6 a | 5.5 ± 1.5 a | **34.0 ± 3.0 a** |

Means followed by the same letter are not significantly different at P = 5% probability level (ANOVA followed by Games-Howell post-hoc test)

**Table 2**

Mean number (± S. E.) of *Helicoverpa armigera* larvae in 50 cobs, in Mezőhegyes, Hungary, in 2004

| Corn hybrid | $L_1$ larval stage | $L_2$ larval stage | $L_3$ larval stage | $L_4$ larval stage | $L_5$ larval stage | $L_6$ larval stage | **All larvae** |
|---|---|---|---|---|---|---|---|
| DK 391 | 0.0 ± 0.0 a | 0.2 ± 0.2 a | 0.0 ± 0.0 a | 0.0 ± 0.0 a | 0.7 ± 0.2 a | 0.2 ± 0.2 a | **1.2 ± 0.4 a** |
| DK 443 | 0.0 ± 0.0 a | 0.5 ± 0.5 a | 0.5 ± 0.2 ab | 0.0 ± 0.0 a | 0.2 ± 0.2 a | 0.2 ± 0.2 a | **1.5 ± 0.2 a** |
| Maraton | 0.0 ± 0.0 a | 0.7 ± 0.2 a | 0.7 ± 0.2 b | 0.5 ± 0.2 b | 0.7 ± 0.4 a | 0.5 ± 0.5 a | **3.0 ± 0.4 a** |
| Káma | 2.7 ± 0.8 a | 2.0 ± 0.5 a | 6.2 ± 0.9 bc | 6.2 ± 0.8 c | 6.2 ± 0.4 ab | 5.0 ± 1.1 ab | **29.0 ± 1.2 ab** |
| Maxima | 4.5 ± 1.3 a | 4.2 ± 1.6 a | 9.2 ± 1.0 c | 8.5 ± 1.3 abc | 10.2 ± 0.8 b | 8.7 ± 1.2 b | **45.5 ± 2.2 b** |

Means followed by the same letter are not significantly different at P = 5% probability level (ANOVA followed by Games-Howell post-hoc test), or not significantly different from zero (Bonferroni-Dunn (control) test)

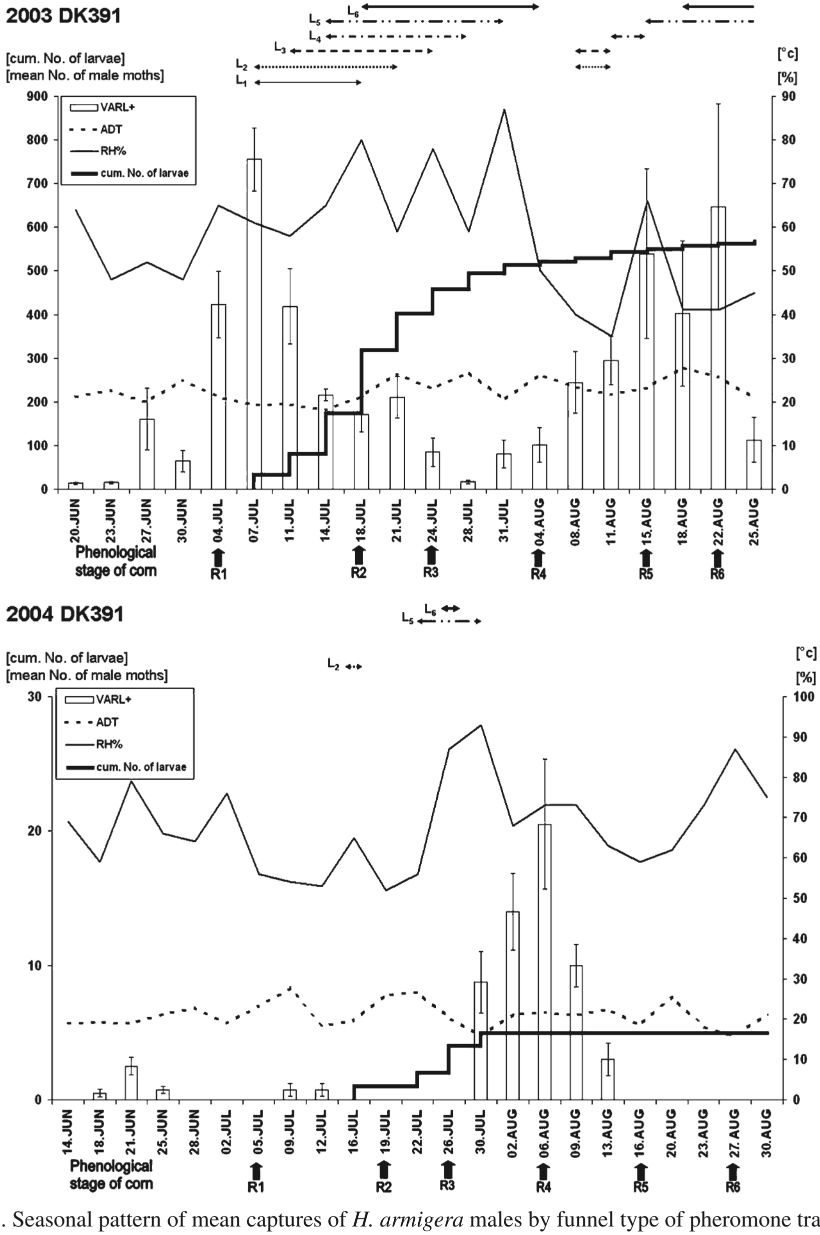


Fig. 1. Seasonal pattern of mean captures of *H. armigera* males by funnel type of pheromone traps, by inspection (± S. E.), presence of *H. armigera* larvae of $L_1$, $L_2$, $L_3$, $L_4$, $L_5$ and $L_6$ stages in cobs, as well as cummulative average of larvae (all stages) in DK 391 and in Maxima corn hybrids, respectively, in 2003, at Mezőhegyes, Hungary. Average number of larvae refer to 50 cobs in case of each corn hybrids. Phenological stage of corn hybrids ($R_1$, $R_2$, $R_3$, $R_4$, $R_5$ and $R_6$), are indicated by arrows. Average daily temperature and relative humidity are plotted

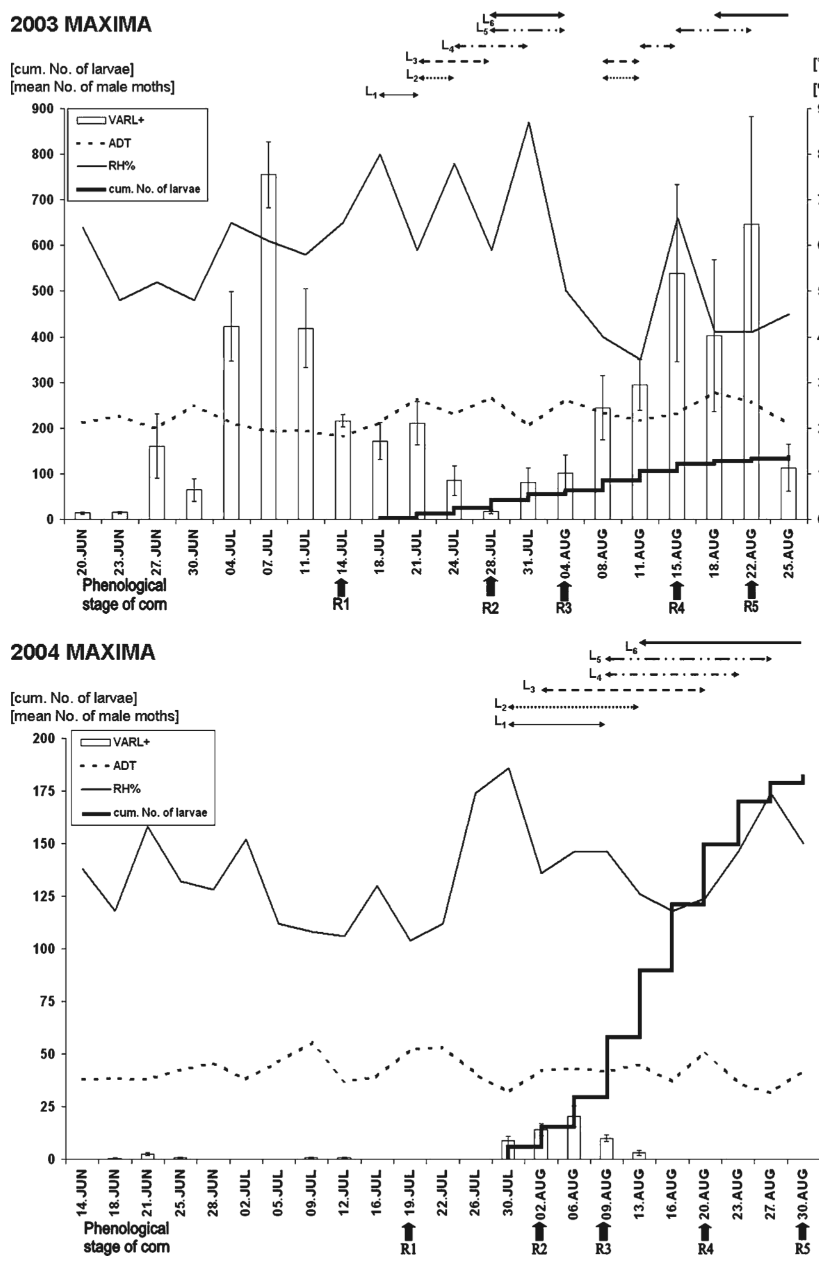


Fig. 2. Seasonal pattern of mean captures of *H. armigera* males by funnel type of pheromone traps, by inspection (± S. E.), presence of *H. armigera* larvae of $L_1$, $L_2$, $L_3$, $L_4$, $L_5$ and $L_6$ stages in cobs, as well as cummulative average of larvae (all stages) in DK 391 and in Maxima corn hybrids, respectively, in 2004, at Mezőhegyes, Hungary. For more details see legend of Fig. 1

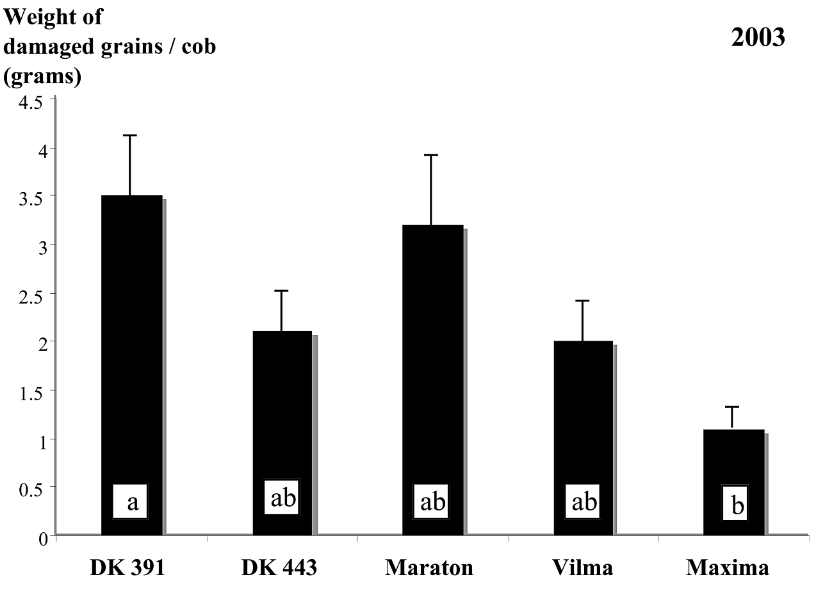


Fig. 3. Weight of damaged grains per cob of various corn hybrids, caused by *H. armigera* larvae, in 2003, in Mezőhegyes, Hungary. Means labeled by the same letter do not differ significantly from each other at P = 5% probability level (ANOVA, followed by Games-Howell post-hoc test). Bars denote standard error

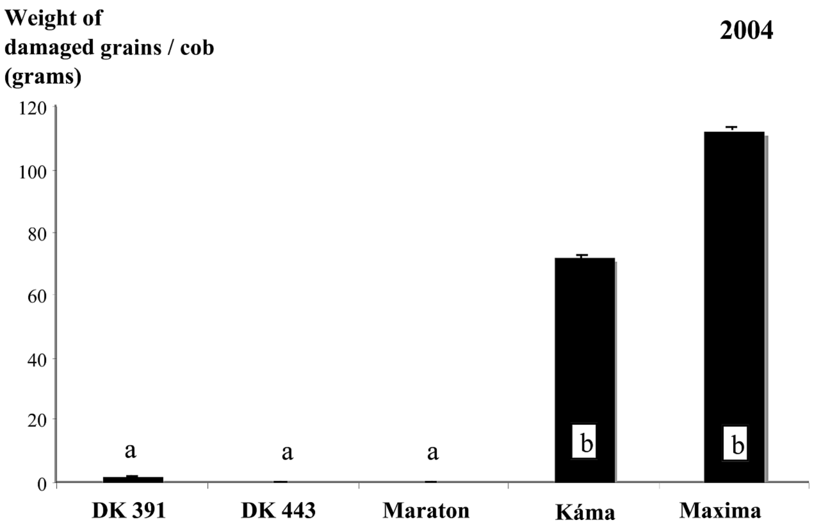


Fig. 4. Weight of damaged grains per cob of various corn hybrids, caused by *H. armigera* larvae, in 2004, in Mezőhegyes, Hungary. Means labeled by the same letter do not differ significantly from each other at P = 5% probability level (ANOVA, followed by Games-Howell post-hoc test, or, in case of zero vesus non-zero values, Bonferroni/Dunn (control) test). Bars denote standard error

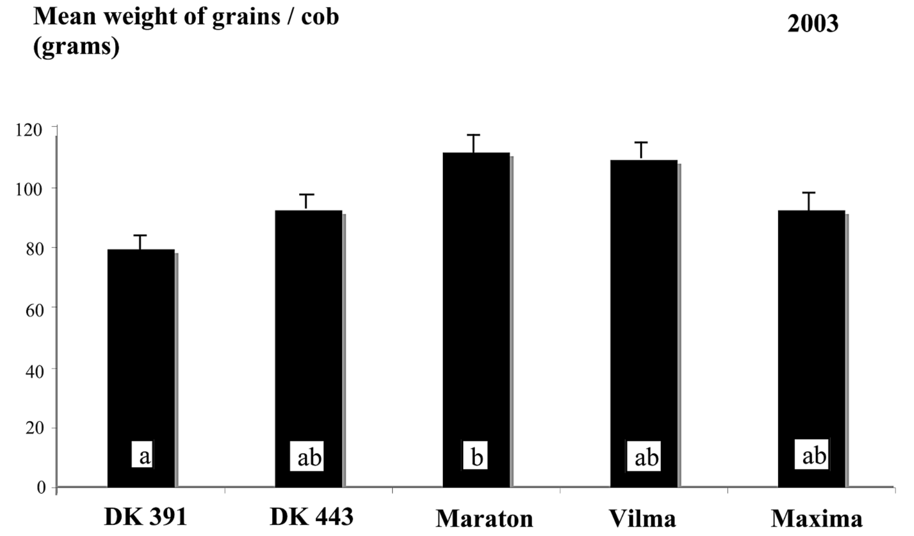


Fig. 5. Mean weight of grains per cob, taken off from cobs of various corn hybrids, in 2003, in Mezőhegyes, Hungary. Means labeled by the same letter do not differ significantly from each other at P = 5% probability level (ANOVA, followed by Games-Howell post-hoc test). Bars denote standard error

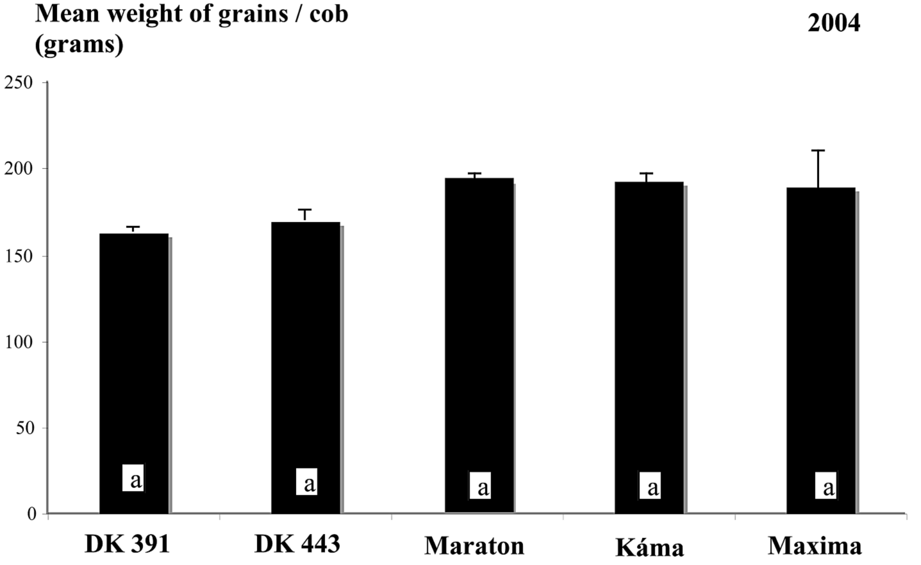


Fig. 6. Mean weight of grains per cob, taken off from cobs of various corn hybrids, in 2004, in Mezőhegyes, Hungary. Means labeled by the same letter do not differ significantly from each other at P = 5% probability level (ANOVA, followed by Games-Howell post-hoc test). Bars denote standard error

*Weight of damaged grains per cob*

In 2003, the weight of damaged grains per cob was the highest on DK 391 (3.5 ± 0.6 gram) and the lowest on Maxima (1.1 ± 0.2 gram), differring significatly from each other *(Fig. 3)*. Intermedium values were obtained at DK 443, Maraton and Vilma, not differring significantly from each other and that of DK 391 and Maxima, respectively. In 2004, the weight of damaged grains were outstandingly high for Maxima (112.2 ± 0.7 gram) and for Káma (71.7 ± 0.6 gram), not differing significantly from each other, however, sharply differring from that of DK 391, DK 443 and Maraton, where there was either no damaged grains found, or if any, then only so extremely rarely that they weight was negligible *(Fig 4)*.

*Weight of all grais per cob*

In 2003, the weight of all grains per cob showed modest differences, as ranged from 79.2 ± 4.3 gram (DK 391) to 111.5 ± 5.3 gram (Maraton) *(Fig. 5)*. In 2004, values were higher than in 2003, while variation was even less pronounced, ranging from 162.9 ± 2.8 gram (DK 391) to 192.4 ± 4.3 gram (Káma) *(Fig. 6)*. None of the differences were statistically significant.

# Discussion

Comparing result from 2003 and 2004 on the respective hybrids, it is indicative that it is the coincidence of the appearance of silking stage of the corn with the flight of *H. armigera* moths, which is decisive in respect of the follow-up abundance of larvae on developing cobs, and not an inherent feature of the hybrids concerned. In 2003, DK 391, for example, was already in sliking stage ($R_1$ stage on IOWA scale) at peak flight. $L_1$ larvae appeared on cobs without delay, resulting to the development of the significantly largest larval population, compared to all other hybrids. This was associated to the highest level of infested seeds by harvest. In 2004, however, DK 391 was already far more developed (reaching $R_4$ stage) at the time of peak flight. Then hardly any larvae were observed on cobs, and the weight of damaged seeds was also negligibly low. A completely reverse tendency was observed in these two years for Maxima. In 2003, Maxima has not reached silking stage by peak flight. The follop up abundance of larvae on cobs, as well as the weight of damaged grains at harvest was far the lowest, as compared to the other hybrids tested. In 2004, however, Maxima passed silking stage and reached only $R_2$ stage by the flight period of the moths. The abundance of the follow up larval populations was then here by far the highest, which was followed by far the highest level of grain damage by harvest.

The aboves indicate a tolerance based on asynchrony, a shift in time between silking phenological stage of corn and peak flight of moth, and does not support that any inherent resistance mechanism would be responsible for this. It is likely that if the peak flight happens at another time in course of the time-scale of silking stages of these corn hybrids, the order of infestation levels of the hybrids concerned would be different. Therefore, no direct risk-assessment in this respect can be predicted for the tested hybrids before the season, as this coincidence of silking stage and moths' flight may or may not happen in a year. Rather, the practical recommendation can be made that each year and each locality both the phenologtical stage of the corn hybrid and the flight of *H. armigera* should be monitored in course of the season. Control measures, either chemical, or biological, should be performed without delay at recording mass-captures of moths in pheromone traps, in those corn hybrids, which are around the silking stage, while in case of either much earlier, or much later flowering hybrids, despite of the fact that the flight had peaked, $L_1$ larvae can be expected cobs only in low numbers (in case of later flowering hybrids, only from the time onwards of silking).

Infestations of cobs by *H. armigera* larvae were measured in this study on corn hybrids, which were planted at the same date, however, their growing rates were different. Therefore, various hybrids were at different phenological stage at the time when developing cobs were exposed to *H. armigera* females, looking for oviposition sites. An analogues situation could be achieved by planting the same corn hybrid at different dates. In this respect our results seem to be in a line of that of published by Wiatrak et al. (2004), reporting that fall armyworm, *S. frugiperda,* and *H. zea* (the sibling species of *H. armigera*) infestation depended on corn hybrids and planting dates, in a field trail conducted at Quincy, FL., U.S.A. To reduce infestation of non-Bt-corn, Wiatrak et al. (2004) recommended to plant until April (cf. climate conditions at that place). However, no significant interaction between planting dates and resistance parameters were found in relation of two corn hybrids and injury caused by *H. zea,* by Wiseman and Isenhour (1992), using corn hybrids different from that of used either by Wiatrak et al. (2004), or in our present study.

Athough direct crop loss caused by *H. armigera* larvae were found to be relatively low in the present study in case of each hybrids, these damages were accompanied by high percentages of infested cobs. The latter phenomenon points to high level of economic risk and vetenary (fodder) as well as of human heath problem (sweet corn for human consumption), because of secondary damages, caused by mycotoxin-producing fungi, can be expected (see e.g. Dowd, 2003).

Meteorological conditions around the peak flight (egglaying period) may influence the length of egg stage, while these conditions during the entire season influence silking time. Therefore the influence of meteorological data should be considered when these findings are attempted to be applied to other geographical regions or to other years.

In this study we reported on relationships between phenological stage of corn, timing of peak flight of *H. armigera,* infestation of cobs, and weight loss of cobs. It was beyond the objective of the present study to investigate in details the reasons, or mechanisms in behind the observed phenomena. We emphesize that the differences in infestetion levels between hybrids depended partly on the experimental setup (e.g. sawing time), and partly on external factors, such as meteorological parameters and flight period of the pest, therefore no risk assessment can be associated in general to the tested corn hybrids, as an attribute of the hybrids concerned. For this, further studies would be needed. To exclude, for example, the factor of dependance on various phenological stages of the respective corn hybrids, resulting from the fact that different corn hybrids were in various phenological stages at the time of peak flight of *H. armigera,* an experimental design of planting the set of corn hybrids at different dates could be developed. By a properly planned stepwise planting, the development of various corn hybrids of different growing rates could be synchronized so that at least some of them were in the same phenological stage at the time of peak flight of *H. armigera*. This would allow to exclude the effect of phenological stages, as a possibile factor for tolerance against *H. armigera,* and hence measured differences in infestation levels would then more directly relate to the inherent characteristic of the corn hybrids concerned. When, as a results of such experiments, evidence on resistance (in contrast to tolerance based on phenology) were obtained, then the mechanism of that resistance could be further studied. In case of other corn hybrids such mechanisms of resistance (e.g. antibiotic compounds) have already been reported (Wiseman and Snook, 1996; Widstrom and Snook, 1998).

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