ORIGINAL ARTICLE

Evaluation of influencing factors on the location and displacement of *Ostrinia nubilalis* larvae in maize stalks measured by computed tomography

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Abstract

Ecology and life characteristics of overwintering larvae of the European corn borer (*Ostrinia nubilalis* Hbn.) (Lep.: Crambidea) are partly unexplored due to their hidden lifestyle. In plant protection research the best way to study these phenomena is to apply less used, non-destructive, *in vivo* methods. The objective of our CT survey was to examine the factors influencing the location of the overwintering *O. nubilalis* larvae in maize stalks. The findings obtained by CT-analysis can be used for monitoring the presence and location of *O. nubilalis* larvae in the stalk, as well as both their displacement and movement. Our results showed that both the location and the distance from the brace root of *O. nubilalis* larvae were significantly influenced by the sampling time, the number of larvae per plant, the stalk diameter and finally the prevailing temperature. The location of the larvae situated nearest to the brace roots (first larvae) was significantly lower in stalks containing several larvae, than those where only a single larva was found in the stalk. The thickness of stalks was related to the simultaneous presence of more larvae, and to the ground level position of the first larvae. These overwintering larvae were located closer to the brace root (and to the soil), possibly because of having moved downwards inside the stalk, where the temperature is slightly milder than in the upper part of the stalk.

Keywords: CT, location, non-destructive method, *Ostrinia nubilalis*, overwintering larvae

Introduction

The European corn borer (*Ostrinia nubilalis* Hbn.) (Lepidoptera: Crambidae) is one of the most important pests of corn both in American and European corn cultivations. Its economic importance has increased with the spread of monocultures and the expansion of the sowing area and large agricultural concerns (Keszthelyi 2010a). In the infested areas, *O. nubilalis* occurs in a large per cent of fields, ranging from 20% in Hungary to 60% in Spain and estimated yield losses between 5% and 30% are typical without control measures (Meissle *et al.* 2009).

This pest overwinters as fully developed larva inside the plant. Its diapause is controlled by photoperiod (day length), temperature, and the genetic makeup of the population (Sáringer 1976). Diapause ensures survival through the fall and winter; the diapaused fifth instars remain in suspended development throughout winter until spring when diapause ends and the larvae resume development and pupate. Larvae spend the winter in maize stalks, cobs, or weed stems. Specimens that survive the winter in the climate of central Europe terminate diapause around May and then continue their development to the adult stage (Svobodová *et al.* 1998). *Ostrinia nubilalis* develops in different generation numbers depending on its distribution territory. According to the annual number of generations, uni-, bi- and multivoltine ecotypes have been defined (Keszthelyi 2010b).

Agrotechnical practices are the most important factors in the destruction of larvae developing diapauses (Keszthelyi 2010a). The most determining factor of this protection method is crop rotation where this pest cannot accumulate. The entire destruction of stubble and stem remains should be achieved by ploughing before stalk crushing at the end of autumn (Roger-Estrade *et al*. 2010). Therefore, the distance of the place of overwintering from the soil surface is a very important factor in the case of the survival of diapausing larvae (Hsu *et al*. 2009).

In plant science, studies making use of computed tomography have remained comparatively scarce. Its first use can be linked to forestry testing (Wei *et al*. 2011). The number of studies in the domain of "plant sciences which comprises the topic "computed tomography" is approximately 6× less than in "zoology" and 27× less than in an average ISI-referenced publication (Stadler *et al*. 2013). Thus, computed tomography is comparatively underused in plant sciences, and there is no clear sign towards change. The goal of the present investigation was the assessment of the potential usability of CT imaging for the study of overwintering *O. nubilalis* larvae. We were particularly interested in the influencing effects (seasonal pattern, temperature, larvae number) of larvae location and displacement, which could be observed by this non-destructive method. Therefore, our results may give impetus to efforts aimed at implementing *in vivo* observations in order to better understand the hidden lifestyle of arthropod pests.

Materials and Methods

Sampling and experimental setting

Entire maize plants (with roots) damaged by *O. nubilalis* were collected at two different times at the end of the vegetation cycle. The sample collecting times were as follows: T1 (before maize harvesting) – September 5, 2017; T2 (after maize harvesting, on the stubble) – November 8, 2017. These samples were derived from first year maize acreage in the periphery of Kaposvár (Somogy county, Hungary; geographical coordinates: 46°22'58.96"N, 17°44'50.43"E). In the field the seeds of the SY Photon hybrid (FAO 350) were sown on May 3, 2017. Chemical fertilizers were spread on the basis of soil analysis following adequate ground-clearance. Spraying of stands with insecticide did not take place in 2017.

Two subgroups were formed after the first sampling (T1). The subgroups were composed of 25 maize plants, which were placed under controlled abiotic conditions (relative humidity: 65%). The first subgroup (T1a) was kept at a continuous temperature (10°C) in a climate chamber, in which the relationship between the larvae numbers and their locations was analysed by means of computer tomography (CT). The plants of another subgroup (n = 5) (T1b) were stepwise cooled in five 48-hour sections (+25, +15, +5, −5, −15 and −5°C). Samples kept at every temperature range were analysed by CT, to ascertain whether the displacement tendencies of larvae depended on temperature change.

CT imaging and processing

The CT measurements were performed covering whole maize stalks using a Siemens Somatom Definition Flash CT scanner (Siemens Ltd., Erlangen, Germany) at the Institute of Diagnostic Imaging and Radiation Oncology of Kaposvár University (Hungary). The following scanning parameters were used for data collection purposes: 100 kV, 300 mAs and spiral data collection with pitch 0.6. Axial scans were reconstructed every 0.1 mm with 0.6 mm slice thickness and 55 mm field of view using medium soft convolution kernel: B30s and abdomen window.

The imaging numbers of one maize stalk were 931-2614 depending on the length of stalk, on average: 2,100. The images were archived in Dicomworks 1.3.5 software. At first, those stalks which contained larvae were used. The stalk of the collected maize plants was arbitrarily divided into different sections for the exact determination of *O. nubilalis* larvae location (Fig. 1).

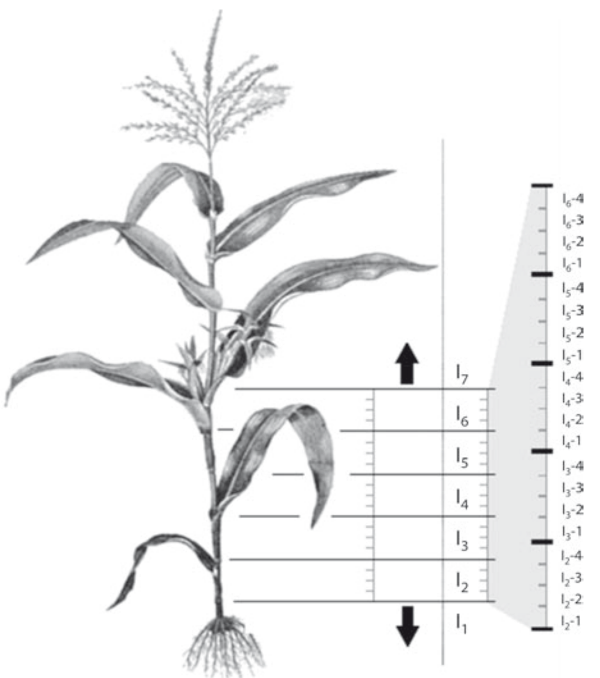

Fig. 1. Theoretical subdivision of the examined maize stalks (I – internode)

Subsequently, the locations of the larvae were recorded based on these displayed codes. The numbering of larvae was begun from the brace root, and the first larva residing nearest to the brace roots (at the same time the lowest level of larvae). The distance measured between the larvae and the brace root was expressed in mm. The diameters (mm) of the internodes of brace roots of damaged stalks were measured, and were automatically calculated by Mipav 8.0.2 software upon delineating the area concerned (Fig. 2). Three-dimensional recordings were done by means of Mango 4.0.1 software.

Statistical analysis

The examined variables (larvae position, data of stalk size) were arranged by Microsoft Excel 2013 software and statistical analysis was done by the IBM SPSS 20.0 software package. The effects of temperature change, stalk diameter and larvae numbers (in the case of more larvae per plant) on larvae positions were examined by (one-way ANOVA) variance-analysis. Post-hoc tests were performed using the Tukey (HSD) test, with the significance level set to $p < 0.05$. The means, standard deviations, standard errors and maximum and minimum values of confidence intervals were displayed. The relationships of examined variables (between larvae number per plant and the place; between stalk diameter as well as the larvae number and location) were examined by the Pearson correlation.

Results

Seasonal patterns of the vertical distribution of larvae inside the stalks

Larvae positions and their distances from brace roots of samples collected at two different times are shown in Table 1. The average numbers of larvae were 2.75 in the

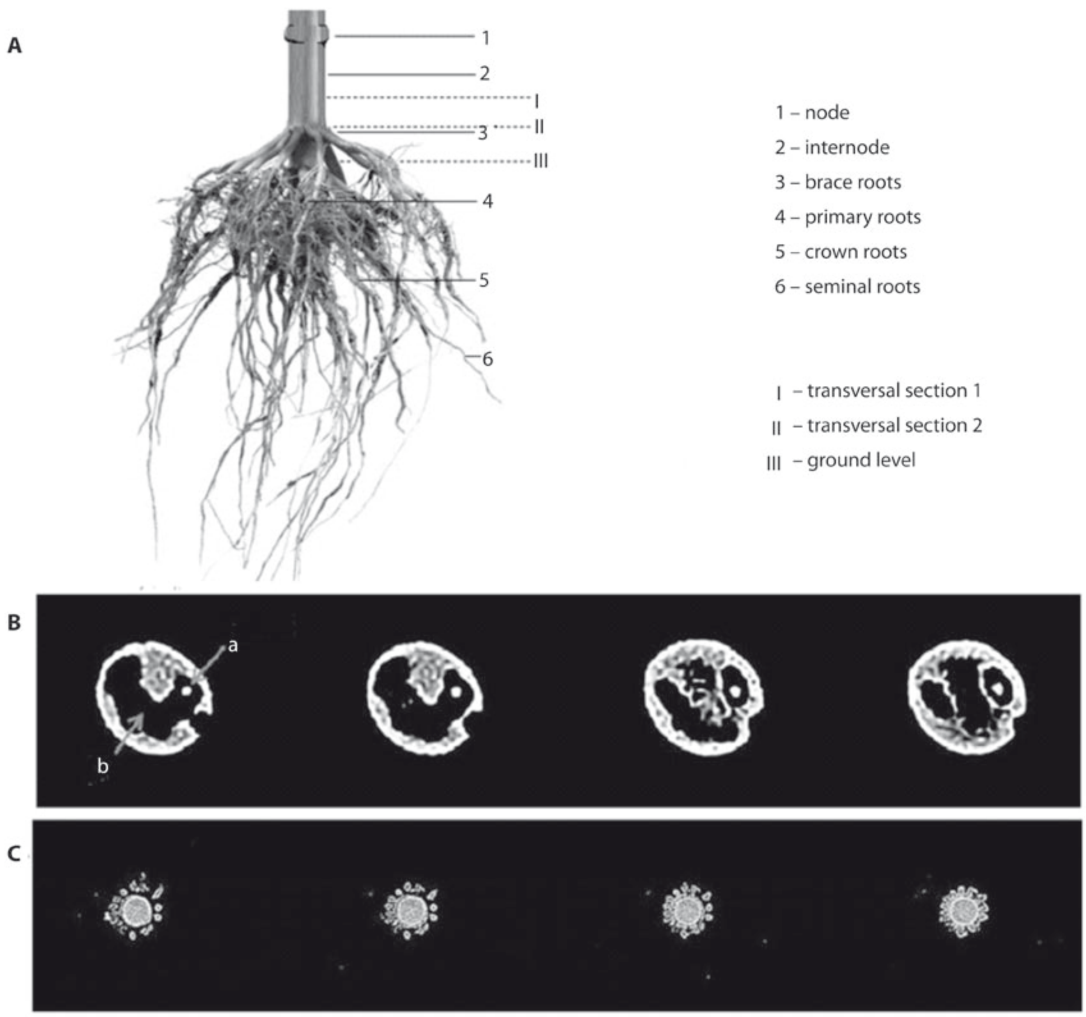

Fig. 2. Transversal computer tomography (CT) recordings of examined maize stalk containing *Ostrinia nubilalis* larvae. A – main parts of examined maize stalk; B – transversal section 1 of the first node, which represented by directly consecutive four recordings (reconstructed by Siemens Syngo fastView); C – transversal section 2 of the node situated under the brace root, which represented by directly consecutive four recordings (reconstructed by Siemens Syngo fastView); a – larva, b – cavity

Table 1. Larvae positions measured from the brace root

Sample collecting times		N	Distance from the brace root of maize [mm]				
			mean	SD	SE	min.	max.
T1a	1st larva	24	224.13 a	108.31	22.11	53.40	361.20
	2nd larva	24	593.50 b	194.05	39.61	336.00	854.40
	3rd larva	6	465.40 a	44.53	18.18	406.08	496.20
	4th larva	2	495.60 a	4.24	3.00	492.60	498.60
	5th larva	1	925.00		–	–	–
T2	1st larva	17	139.91 a	21.80	89.86	30.60	378.60
	2nd larva	4	225.96 b	69.50	155.41	102.00	457.20

T1a – first sample collecting time (September 5, 2017), was kept at a continuous temperature (10°C); T2 – second sample collecting time (November 8, 2017); *N* – number of units; a, b, c at $p < 0.01$

samples taken in September (T1a). The proportions of the position of the larva located nearest to the ground (in the following: first larva) were similar in some parts of ground level internodes (I_3-4 and I_4-1) (33.3%). The second larvae, counted from the ground (in following: second larva, and so on), were most often found (37.5%) in internode part I_6-1. In the presence of three larvae per plant, the third larva could be mostly observed (50.0%) in I_5-3. Rarely occurring fourth and fifth larvae were usually found in higher internode parts (I_5-1–4) of the examined stalks. Significant differences were shown in the case of the position of the first, third and fourth larvae.

In samples collected in November (T2), the average number of larvae per plant was 1.29. This value is approximately half of those registered in September. The majority of larvae (82%) in these samples occurred in the third internode (I_3-1–4) as opposed to samples collected in September for which we did not find larvae up to the third internode (I_3-4). The largest number of the first larvae was detected in I_3-1 and 3. Both second and third larvae counted from the brace root were mostly in the third internode (I_3). The average distance of the first larvae from the brace root was 14 cm, while the location of second larvae was 23 cm. Larvae collected in November were located statistically significantly closer to the soil than larvae in the samples gathered in September. The average distance from the brace root was 33 cm less in the case of the first larvae, while the location of the second larvae was 39 cm closer to the brace root. Interestingly, these late autumn overwintering caterpillars were observed to be spherical in shape, which can be seen well in the 3D reconstruction.

Relationships of larvae location between larvae number and stalk thickness

To determine if the location of the larvae depends on the number of larvae per plant was investigated in the samples taken in September (Fig. 3). The location of the first larvae was placed significantly lower in stalks containing more larvae, especially if there were three larvae in the same plant. In parallel, single larva could be observed in higher stalk portions (Fig. 4). The same tendency was also evinced in the case of second larvae, i.e. the second larva was located on average 67 cm from the brace root in the presence of two larvae, while this value was only 42 cm in the case of the simultaneous presence of three larvae.

The correlation coefficient was negative between larvae numbers per plant and the place of the 1st larva ($r = -0.685$). A somewhat weaker, but similar direction and a significant relationship were detected when the 2nd larvae were examined ($r = -0.562$). We registered very close, positive correlations in the examination of larvae location ($p < 0.001$) (place of the 1st larva–place of the: 2nd larva: $r = 0.711$; 3rd larva: $r = 0.953$; 4th larva: $r = 0.999$; place of 2nd larva–place of 3rd larva: $r = 0.918$; 4th larva: $r = 0.970$; place of the 3rd larva and place of the 4th larva: $r = 0.999$).

Significant correlations between stalk diameter size as well as the larvae number and location were evinced by the results of larvae number influenced by maize stalk thickness ($p < 0.001$). The thicker stalks had more larvae ($r = 0.390$), which was coupled with the first larvae being closer to the soil. The 3rd and 4th larval places were found to be higher than the location of the 1st larvae in thicker maize stalks.

Effects of temperature on larvae location and its displacement

The effect of temperature on the movement of larvae in the stalk was surveyed with CT imaging. The results showed the distribution of groups without tracking the movement of individual larvae (Fig. 5). Twenty-seven per cent of the larvae remained in the internode part, where we our observations were first recorded. Most

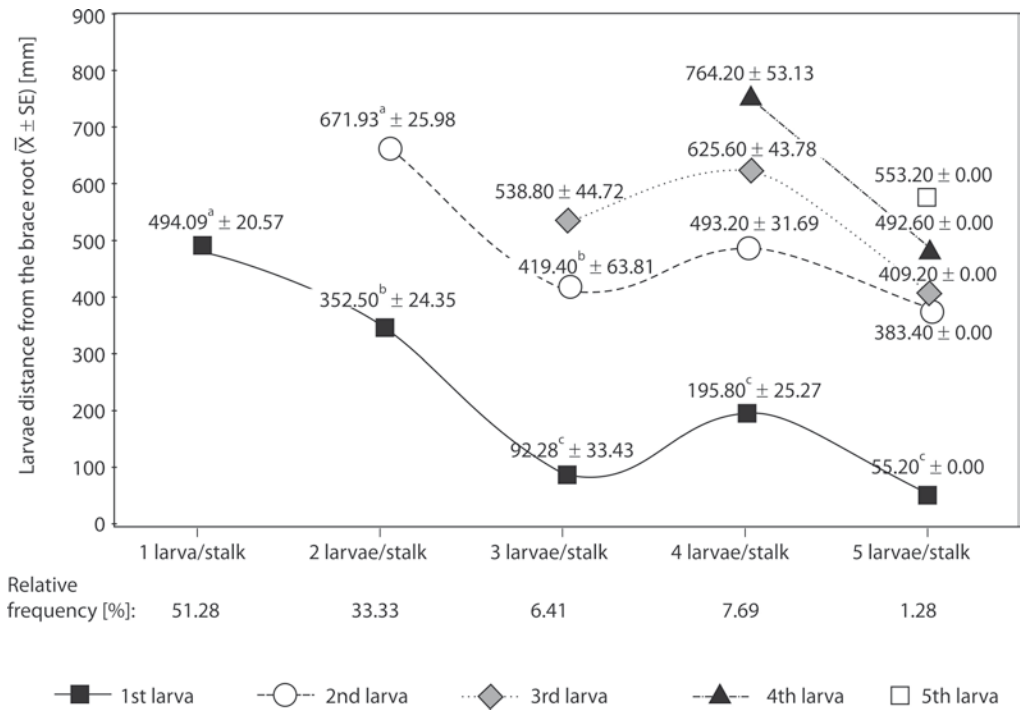

Fig. 3. Larva distance from the brace root (mean ± SE) as a function of *Ostrinia nubilalis* larvae number per maize stalk. a, b, c at $p < 0.01$

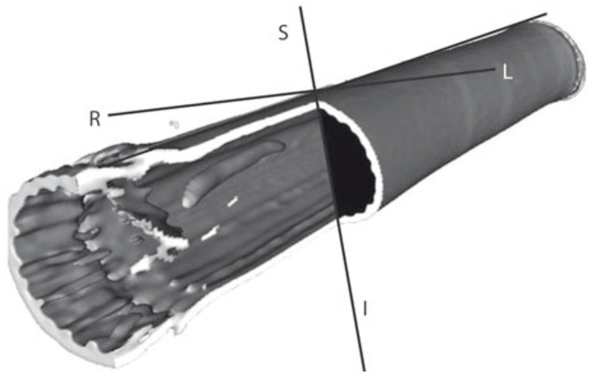

Fig. 4. Computer-based three-dimensional reconstructions of damaged maize stalk and *Ostrinia nubilalis* larva. L – left; R – right; I – interior; S – superior

Table 2. Larvae positions measured from the brace root as a function of different temperature

Temperature [°C]	*N*	Larvae positions [mm]			
		1st larva		2nd larva	
		mean	SD	mean	SD
+25		221.55 a	123.21	1002.75	347.47
+15		227.55 b	125.74	960.50	396.88
+5	5	237.90 a	121.56	989.75	377.46
–25		224.70 a	126.00	994.75	358.36
–15		217.80 a	118.46	992.50	353.39
–5		215.25 b	117.48	994.75	355.58

N – number of units; a, b at $p < 0.01$

larvae (45.45%) changed internodes, and a smaller group of larvae (27.27%) moved to another internode quarter. The highest rate of larvae changing internodes was registered upon cooling from +15°C to +5°C, followed by cooling periods from +25°C to +15°C and from –5°C to –15°C (uniformly 18.18%). The larvae moved upward in positive temperature ranges, which can be seen well by the distance of their location from the brace root (Table 2). By contrast, the distance of larvae from the brace root decreased in negative temperature ranges (–5°C, –15°C, –5°C). The same tendency was observed in the case of the second larva position.

Discussion

The presented findings acquired by CT can be used for detecting the presence and location of *O. nubilalis* larvae in the stalk. Furthermore, both their displacement and movement can be displayed. This method provides additional data for biological and ecological studies aimed at disentangling the hidden lifestyles of covertly developing insects (Keszthelyi *et al.* 2016; 2018). Therefore, it can greatly contribute to the realisation of Integrated Pest Management (IPM) criteria in stored product protection.

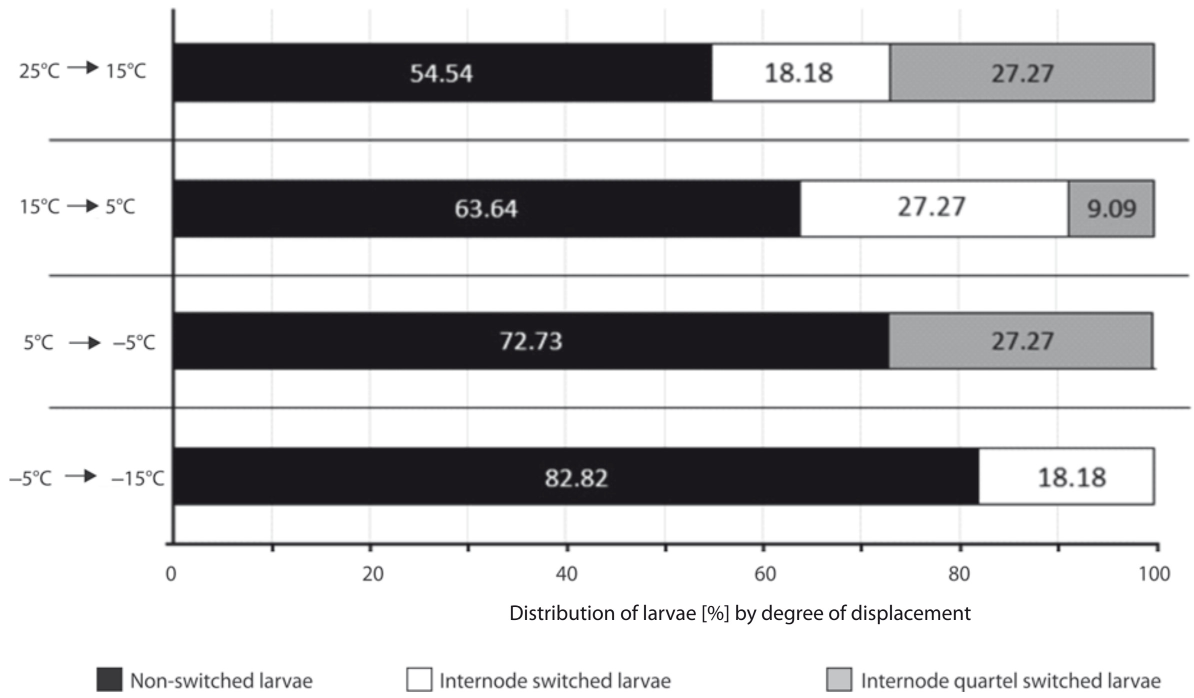

Fig. 5. Percentage of internodes changing of *Ostrinia nubilalis* larvae as function of different temperature ranges

Significant differences could be found pertaining to the location of larvae from the brace root. According to earlier studies (Bohnenblust *et al*. 2014; Oyediran *et al*. 2016), more larvae can develop in a given stalk. Camerini *et al*. (2016) focused on the influence of ECB larval density on the position in maize plant organs. According to their results *O. nubilalis* density ranged from 0.2 to 3.2 larvae/plant, and disparities of larvae per plant cannot be evinced in samples originating from the edge and centre of the acreage. Georgescu *et al*. (2015) pointed out that yearly climate has a significant impact on the number *of O. nubilalis* larvae per plant. The distribution and number of larvae were significantly affected by the weight of maize ears and the quantity of the basic nutrient components (raw protein, fat and starch) incorporated in the kernel. The plants appeared to be more vulnerable to damage caused by larvae situated above the cob (Pál-Fám *et al.* 2010).

Our results indicate that the location and the distance from the brace root of *O. nubilalis* larvae were significantly influenced by the sampling time, the larvae number per plant, the stalk diameter and the prevailing temperature. Furthermore, it has been verified that the ground level position of the larvae at the same sampling time and temperature was triggered by higher larvae number per plant. Thicker stalks corresponded to the simultaneous presence of more larvae, and the ground level position of the first larvae. We observed the displacing of larvae caused by temperature decrease, but the proportion of non-switched stage was significantly increased at colder temperatures. In total, these overwintering developing stages were located closer to the brace root (and the soil) due to the effect of the temperature decrease. This phenomenon was confirmed by the lower location in the late autumn samples.

Cold-hardy winter larvae survived up to 3 months at –20°C in the absence of contact moisture (Andreadis *et al.* 2008). Besides, several studies (Kojić *et al*. 2010; Bohnenblust *et al*. 2014) dealt with the effects of temperate alteration on the physiological features of *Ostrinia* larvae, but its consequences on the location and displacement are largely unknown.

Ostrinia nubilalis overwinters in the full-developed larval stage, which is triggered by the decrease in daytime length (Sáringer 1976) and temperature drop (Andreadis *et al.* 2008). Diapause apparently is induced by the exposure of last instar larvae to long days, but there is also a genetic component. So, the most critical point of the larval stage is the overwintering (Kutinkova *et al.* 2008). Survival conditions can be further deteriorated by the autumn cultivation (Roger-Estrade *et al*. 2010). According to Calcagno *et al*. (2010), *O. nubilalis* larvae exhibit positive geotaxis when they are about to enter diapause, whereas *O. scapulalis* larvae do not. This research has shown that this genetically determined behaviour is strongly adaptive on maize: by increasing the chance of being below the harvest cut-off line, it confers on *O. nubilalis* a survival advantage of about

50% over *O. scapulalis* in harvested fields. Therefore, low-cut maize stubble is recommended in the case of fast autumn cooling or colder than an average autumn based on our results, because most larvae preparing for overwintering can be destroyed by this cheap agronomic mode.

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Sándor Keszthelyi conceived and designed the research project. Gabriella Holló conducted the experiments and analysed the data. Sándor Keszthelyi wrote the manuscript. Both authors read and approved the manuscript.

References

Andreadis S.S, Vryzas Z., Papadopoulou-Mourkidou E., Savopoulou-Soultani M. 2008. Age-dependent changes in tolerance to cold and accumulation of cryoprotectants in overwintering and non-overwintering larvae of European corn borer *Ostrinia nubilalis*. Physiological Entomology 33 (4): 365–371. DOI: https://doi.org/10.1111/j.1365-3032.2008.00642.x

Bohnenblust E.W., Breining B.A., Shaffer J.A., Fleischer S.J., Roth G.W., Tooker J.F. 2014. Current European corn borer, *Ostrinia nubilalis*, injury levels in the northeastern United States and the value of *Bt* field corn. Pest Management Science 70: 1711–1719. DOI: https://doi.org/10.1002/ps.3712

Calcagno V., Bonhomme V., Thomas Y., Singer M.C., Bourguet D. 2010. Divergence in behaviour between the European corn borer, *Ostrinia nubilalis*, and its sibling species *Ostrinia scapulalis*: adaptation to human harvesting? Proceedings of the Royal Society B: Biological Sciences 277: 2703–2709. DOI: 10.1098/rspb.2010.0433

Camerini G., Groppali R., Tschorsnig H.P., Maini S. 2016. Influence of *Ostrinia nubilalis* larval density and location in the maize plant on the tachinid fly *Lydella thompsoni*. Bulletin of Insectology 69: 301–306.

Georgescu E., Burcea M., Cana L., Rasnoveanu L. 2015. Technology of the European corn borer (*Ostrinia nubilalis* Hbn) mass rearing, successive generations, in controlled conditions, at Nardi Fundulea. Bulletin UASVM Agriculture 72: 113–121. DOI: http://dx.doi.org.10.15835/buasvmcn-agr:11186

Hsu Y.T., Shen T.C., Hwang S.H. 2009. Soil fertility management and pest responses: a comparison of organic and synthetic fertilization. Journal of Economic Entomology 102 (1): 160–169. DOI: https//doi.org/10.1603/029.102.0123

Keszthelyi S. 2010a. An important corn enemy: The European corn borer in the new approximation. Hungarian Agricultural Research 5: 20–23.

Keszthelyi S. 2010b. Flight dynamics analysis of the European corn borer (*Ostrinia nubilalis* Hbn.) populations in Hungary from the second part of the twentieth century until the present. Archives of Phytopathology and Plant Protection 43: 1286–1294. DOI: 10.1080/03235400802425713

Keszthelyi S., Bosnyákné Egri H., Horváth D., Csóka Á., Kovács Gy., Donkó T. 2018. Nutrient content restructuring and CT-measured density, volume attritions on damaged beans caused by *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae). Journal of Plant Protection Research 58 (1): 91–95. DOI: 10.24425/119123

Keszthelyi S., Kovács Gy., Donkó T. 2016. Computer tomography-assisted imaging analysis in damaged maize grain caused by *Sitotroga cerealella*. Journal of Plant Diseases and Protection 123 (2): 89–92. DOI: 10.1007/s41348-016-0009-0

Kojić D., Purać J., Popović Ž.D., Vukašinović E., Grubor-Lajšić G. 2010. Importance of the body water management for winter cold survival of the European corn borer *Ostrinia nubilalis* Hübn. (Lepidoptera: Pyralidae). Biotechnology and Biotechnological Equipment 24 (Sup. 1): 648–654. DOI: https://doi.org/10.1080/13102818.2010.10817915

Kutinkova H., Samietz J., Dzhuvinov V. 2008. Combination of mating disruption and granulosis virus for control of codling moth in Bulgaria. Journal of Plant Protection Research 48 (4): 509–513. DOI: 10.24425/119123

Meissle M., Mouron P., Musa T., Bigler F., Pons X., Vasileiadis V.P., Otto S., Antichi D., Kiss J., Palinka Z., Dorner Z., van der Weide R., Groten J., Czembor E., Adamczyk J., Thibord J.B., Melander B., Cordsen Nielsen G., Poulsen R.T., Zimmermann O., Verschwele A., Oldenburg E. 2009. Pests, pesticide use and alternative options in European maize production: current status and future prospects. Journal of Applied Entomology 134 (5): 357–375. DOI: https://doi.org/10.1111/j.1439-0418.2009.01491.x

Oyediran I., Dively G., Huang F., Burd T. 2016. Evaluation of European corn borer O*strinia nubilalis* (Lepidoptera: Crambidae) larval movement and survival in structured and seed blend refuge plantings. Crop Protection 81: 145–153. DOI: https://doi.org/10.1016/j.cropro.2015.12.008

Pál-Fám F., Varga Zs., Keszthelyi S. 2010. Appearance of the microfungi into corn stalk as a function of the injury of the European corn borer (*Ostrinia nubilalis* Hbn.). Acta Agronomica. Hungarica 58 (1): 73–79. DOI: 10.1556/AAgr.58.2010.1.9

Roger-Estrade J., Anger C., Bertrand M., Richard G. 2010. Tillage and soil ecology: Partners for sustainable agriculture. Soil and Tillage Research 111 (1): 33–40. DOI: https://doi.org/10.1016/j.still.2010.08.010

Sáringer Gy. 1976. Diapause experiments with a population of *Ostrinia nubilalis* Hbn. (Lepid.: Pyraustidae) in Hungary. Journal Applied Entomology 80 (1–4): 426–434. DOI: https://doi.org/10.1111/j.1439-0418.1976.tb03345.x

Staedler Y.M., Masson D., Schönenberger J. 2013. Plant tissues in 3D via X-ray tomography: simple contrasting methods allow high resolution imaging. PLoS One 8: e75295. 1–9. DOI: https://doi.org/10.1371/journal.pone.0075295

Svodobová E., Trnka M., Dubrovsky M., Semerádová D., Eitzinger J., Štěpánek P., Žalud Z. 2013. Determination of areas with the most significant shift in persistence of pests in Europe under climate change. Pest Management Science 70 (5): 708–715. DOI: https://doi.org/10.1002/ps.3622

Wei Q., Leblon B., La Rocque A. 2011. On the use of X-ray computed tomography for determining wood properties: a review. Canadian Journal of Forest Research 41 (11): 2120–2140. DOI: https://doi.org/10.1139/x11-111