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AKADÉMIAI KIADÓ

Evaluation of two artificial defoliation methods to simulate damage by the cereal leaf beetle (*Oulema melanopus*) larvae in winter wheat

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RESEARCH ARTICLE

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ABSTRACT

Defoliation reduces photosynthetic area, negatively effecting overall plant vitality, which at the end, severely impacts seed quality and production. The economic importance of the loss in winter wheat (*Triticum aestivum* L.) due to larvae of the cereal leaf beetle (*Oulema melanopus*, CLB) generated studies investigating the significance of the flag leaf. Simultaneously, the role of other leaves remains rather undiscovered. We simulated herbivory caused by CLB larvae in a two-year study between 2017 and 2018. We removed different amounts of leaf material from two winter wheat cultivars, either from the flag leaves only, or from all leaves. The impact of artificial defoliation was measured in grain production per ear, and related to natural CLB larval herbivory. Removing all leaves simulated CLB larval herbivory more closely than the artificial defoliation of flag leaves only. Our results suggest that the relative importance of flag leaves in seed production may be lower than previously assumed. Further studies involving various cultivars are invited to enhance the knowledge on the significance of the damage done by CLB larvae.

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KEYWORDS

pest damage evaluation, compensation, flag leaf, leaf surface loss, *Oulema melanopus*

INTRODUCTION

Cereal production in Hungary positions the country the eighth within the European Union (Strategie Grains, 2018). Due to its climate and soil quality, Hungary is self-sufficient in grains, and is able to export them. In terms of cultivated area, cereals, with winter wheat (*Triticum aestivum* L.) outstanding among them, are the most important crops both in conventional and organic production (Földi et al., 2021).

The cereal leaf beetle (CLB, *Oulema melanopus* L.) has long been recognized as one of the most important insect pests of winter wheat (Haynes and Gage, 1981). CLB overwinters as adult in ruderal or wooded areas in the surroundings of the previous seasons' cereal fields and invade cereal fields during spring (Casagrande et al., 1977; Philips et al., 2012; Lajos et al., 2020). After mating, females lay eggs on the freshest leaves of wheat. The damage is caused by the larvae, which move upwards as the plant grows, and consume the upper epidermis of flag leaves and other leaves. The removal of mesophyll cells seriously impairs the photosynthetic ability of the plant, and this damage is aggravated by the mucus and faeces left on the surface (Buntin et al., 2004; Würschum et al., 2020; Mazurkiewicz et al., 2021). Feeding results in scars, enabling pathogens to enter more easily. It is also assumed that herbivory may make the plant more susceptible to water stress (Steinger et al., 2020). The economic loss caused by CLB has been expressed in the reduction of yield, and the observed figures may vary between 5.4% to reaching a maximum of 40%, with only one larva per stem (Jossi and Bigler, 1996; Buntin et al., 2004; Herbert et al., 2007).

When a winter wheat plant reaches its generative life stage, most assimilates produced by photosynthesising tissues, mainly leaves, are used for seed production. When a wheat plant is defoliated, it usually lags in its development and has a decreased seed production, leading to a reduction in yield (Macedo et al., 2006; Ahmadi and Joudi, 2007; Steinger et al., 2020). Not all leaves contribute to seed development equally, though. The last leaf emerging before the plant enters its reproductive phase is also called the 'flag leaf'. It is usually larger, its tissue is denser, and its photosynthetic rate is usually also higher than those of any previous leaves (Araus and Taipa, 1987). Most of the carbohydrates allocated to the development of grains originate from the flag leaf. The artificial removal of this leaf and the penultimate one reduces grain yield and may result in a yield loss up to 50% in the temperate zone (Füzi and Kövics, 2002; Ali et al., 2010).

Several studies tested the effects of artificial defoliation on the reproductive traits of winter wheat (Buntin, 1994; Zhu et al., 2004, 2006; Macedo et al., 2006, 2007; Shao et al., 2010; Bijanzadeh and Emam, 2010; Steinger et al., 2020). Some of these studies simulate the impact of leaf damage caused by insect pests like the larvae of the fall armyworm (*Spodoptera frugiperda* J. E. Smith; Macedo et al., 2007) or the cereal leaf beetle (Steinger et al., 2020) on the yield of winter wheat. However, the main issue of artificial defoliation is how well this method is suitable to simulate the damage caused by a pest species, and its effects on the yield of winter wheat. Therefore, in our study, which was a part of a more complex study presented in Császár et al.

(2021), we intended to examine how artificial defoliation of flag leaves only or all leaves affects the yield of winter wheat. Our goal was to test which of the two defoliation methods was more suitable to simulate the negative effects of natural defoliation caused by CLB larvae on the yield. We also wished to examine the role of other leaves, and the two cultivars, 'Altigo' and 'Alcantara' on the expression of CLB damage.

MATERIAL AND METHODS

Field conditions

The effects of artificial defoliation and natural leaf damage caused by CLB larvae were examined on two different winter wheat (*T. aestivum* L.) cultivars 'Alcantara' and 'Altigo'. Field experiments were carried out on the experimental area of the Department of Integrated Plant Protection of the Hungarian University of Agriculture and Life Sciences (Gödöllő, Hungary), in 2017 and 2018. Both cultivars were sown in the preceding years, on 27 October 2016 and 31 October 2017, corresponding to ca. 167 seeds per m^2. Experimental wheat plots received no irrigation or pesticides and were carefully hand-weeded when needed. Our experimental fields were placed on different locations. In 2017, water permeability of the soil was good, and no shade was cast over the plots, whereas in 2018, the area suffered a constant high level of ground water, and partially shaded by surrounding trees.

Weather conditions during the experiment

There were notable differences recorded in the weather between the two study years. April and May were warmer and drier in 2018 than in 2017, while the total precipitation during June 2018 was much higher than in 2017 (Table 1, abridged from Császár et al., 2021).

Treatments

There were three different treatments: (1) artificial all-leaf defoliation; (2) artificial flag-leaf defoliation; and (3) natural CLB larval defoliation. Besides this, there was also one control group for the two cultivars and study years, respectively. These untreated control groups consisted of 10 plants per each cultivar and year. Artificial (1) all-leaf and (2) flag leaf defoliation treatments were carried out with (1) all leaves and (2) only the flag leaves cut on 12 plants per year for both cultivars.

Table 1. Meteorological conditions during pre- and postanthesis (April–June) for both study years in the small region of Gödöllő. Data were obtained from the nearest meteorological station located in Aszód (OMSZ, 2020)

Climatic parameter	April	May	June
2017			
Mean temperature (°C)	10.4	16.2	21.4
Total precipitation (mm)	67	57	20
2018			
Mean temperature (°C)	15.4	19.2	21
Total precipitation (mm)	34	26	116

This means that a total of 24 plants per cultivar and year were artificially defoliated. The extent of artificial defoliation was set in an increasing degree, with 25, 50, 75 or 100% of the leaf area removed, with three plants in each degree. Leaf tissues were removed on 7 June 2017 and 10 May 2018, when most plants were at the beginning of the 'inflorescence emergence' development stage, which corresponds to Zadoks 50–51 (Zadoks et al., 1974). Artificial defoliation was also synchronized to the observation of CLB-damage on adjacent winter wheat fields. This, and the unfavourable weather conditions in April 2017 that delayed the development of the studied plants resulted in the difference in the timing of treatments. For natural defoliation (3), CLB adults were collected from the winter wheat fields nearby our experimental site on 16 May 2017 and 19 April 2018. We selected 30 and 50 wheat plants from our study area in 2017 and 2018, respectively, caged them, and introduced three adult beetles to each confinement. We made sure that the three individuals were of both sexes. Once the presence of eggs was confirmed, adults were removed. Once the larvae hatched, on 1 July 2017 and 5 May 2018, larvae were collected and reallocated to have 10 treatments per cultivar with an increasing number of larvae per plant from 2 to 20, in increments of 2. Plants were monitored every second day and once no larvae were found, isolation cages were removed to be able to inspect leaf damage. For more details of this treatment, please refer to Császár et al. (2021).

Data collection

To quantify seed production, every grain from every ear of treated and control plants was hand-harvested on 10 July 2017 and 17 July 2018. Otherwise, the procedure was the same as described in Császár et al. (2021): ears were cut with scissors and put into separate paper bags, which were left drying for one month. Afterwards the number of grain kernels was counted and the weight of grains per ears was determined with an analytical balance (Ohaus AS 200S), from which the average yield per ear per plant was calculated. To quantify leaf loss in the natural defoliation treatment, plant leaves were carefully photographed with a digital camera. These digital pictures were used to determine the total leaf area and the area of the damaged leaf surface in Adobe Photoshop CS3 version 10.0 based on pixel numbers. CLB defoliation was calculated as percentage of leaf surface loss in relation to the total leaf area. For further details, please refer to Császár et al. (2021).

Statistical analyses

All statistical analyses were carried out in R 3.6.3 (R Core Team, 2020). All graphs were created with the R package 'ggplot2' (Wickham, 2016), and Welch Two Sample *t*-tests were applied to evaluate the differences in yield between the two cultivars and study years.

We tested several issues regarding the effects of artificial defoliation on the yield. First, we evaluated if there were significant differences between the yield at 25% artificial defoliation and the yields measured at the three other levels of defoliation for each of the two cultivar types, treatments and study years applying linear models. These linear models were checked for uniformity, dispersion and outliers using functions from the R package 'DHARMa' (Hartig, 2020), which did not detect any significant deviations of the residuals. This relationship was tested to check whether an increasing extent of artificial defoliation did indeed lead to a decreasing yield or not. Except for the yields measured at the 100% 'flag-leaves' and the 75% 'all-leaves' treatment for the 'Alcantara' cultivar type in 2018, the yields of most treated plants were not significantly different from the yields at 25% artificial defoliation (Table A1). Therefore, the yields of all defoliated plants, which were not significantly different from the yields at 25% artificial defoliation, were combined into one common group per treatment. As a next step, Wilcoxon signed-rank tests (R-package 'ggpubr' by Kassambara, 2020) for each of the two treatments, study years and cultivar types were used to test if there was a significant difference between the combined yields of the treated plants and the yields of winter wheat plants defoliated by CLB larvae as well as the yields of the untreated control plants. For the CLB-defoliated wheat plants, we took the yields measured for 30 (in 2017) or 50 (in 2018) plants per cultivar type as reported in Császár et al. (2021). The average CLB larval defoliation of all (= both flag and other) leaves ranged between 11.64 and 23.27% for the two cultivar types and study years (Table A2).

RESULTS

The Welch Two Sample *t*-test showed that there was a significant difference in yield between the two years ($t = 6.26$, df = 161.42, *P*-value < 0.001). Additionally, the numbers of ears produced by the two cultivars were considerably lower in 2018, with 5.18 ± 0.46 ears produced by 'Alcantara' and 4.91 ± 0.46 by 'Altigo', than in 2017, with 9.5 ± 0.83 for 'Alcantara' and 9.71 ± 0.91 for 'Altigo'. There was a significant difference between the yield of the two cultivars in 2018 ($t = 3.57$, df = 65.72, *P*-value < 0.001), but not in 2017 ($t = -1.94$, df = 85.90, *P*-value = 0.06).

The effects of defoliation caused by CLB larvae on the yield was best simulated by the 'all-leaves' and 'flag-leaves' artificial defoliation for 'Alcantara' in 2017 (Fig. 1A), as well as by the

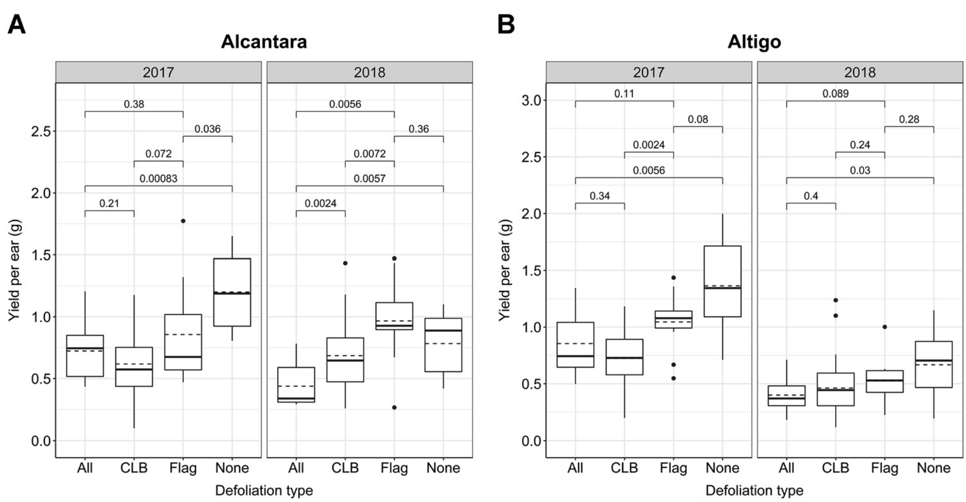

Fig. 1. Boxplot diagrams of the yields per ear for the two winter wheat cultivars **A)** 'Alcantara' and **B)** 'Altigo'. Four different defoliation treatments were applied on these plants: Artificial defoliation of all leaves or flag leaves, natural defoliation of all leaves by CLB larvae, and control plants, which were left untreated. Wilcoxon signed-rank tests from the R-package 'ggpubr' (Kassambara, 2020) were used to test if there were significant differences between the yields

'all-leaves' artificial defoliation for 'Altigo' in both study years (Fig. 1B), as the yields of artificially defoliated plants did not significantly differ from the yields of CLB-defoliated plants, but were significantly lower than the yields of untreated control plants. In all other cases, artificial defoliation did not simulate the effect of CLB defoliation on the yield so well. In these set ups, cultivars or years, neither the yields of the artificially defoliated plants significantly differed from the yields of CLB-defoliated plants, nor were significantly lower than the yields of untreated control plants, or none. Significant differences between the yields of the 'all-leaves' and 'flag-leaves' treatments were only detected for 'Alcantara' in 2018, where none of the two treatments provided a good simulation of CLB defoliation.

DISCUSSION

The economic importance of *O. melanopus*, a major pest of cereals is considered high, yet yield loss figures to winter wheat with the same number of CLB larvae per plant is highly variable, and the explanation is conflicting (Steinger et al., 2020). To understand yield fluctuations, we have to consider other influential factors besides pest pressure, with weather, site and cultivar as the most important ones among them. In a 7-year study on organic wheat production for example, climatic conditions (= year) had the greatest impact on yield. This was followed by soil conditions, and finally, cultivar seemed to have the lowest influence on the variability of yield (Földi et al., 2021).

In our experiment we approached the significance of the pest from a methodology point of view. Previous studies have investigated the impact of defoliation patterns, where only the flag leaf and the penultimate leaf, or all leaves, or all leaves except some specific leaves were removed or in any other combinations (Buntin, 1994; Füzi and Kövics, 2002; Zhu et al., 2004, 2006; Macedo et al., 2006, 2007; Ali et al., 2010; Shao et al., 2010; Bijanzadeh and Emam, 2010; Steinger et al., 2020). We chose two patterns: the removal of the flag leaves only and the removal of all leaves and wanted to find out which of the two artificial defoliation methods simulated the natural damage done by CLB better and examine what factors influence the compensation ability of winter wheat.

Our results indicated that the removal of all leaves simulated the effects of CLB larval herbivory on yield more precisely than cutting off the flag leaves only for both wheat cultivars.

At the same time, despite for losing all their leaves, winter wheat plants still produced some grains, suggesting that to some degree, non-foliar tissues were able to compensate for the loss, but we have to mention that compensation was higher when defoliation was less drastic and only affected the flag leaves. It concurs with the findings of Gavloski and Lamb (2000), who investigated the biomass and seed production of two cruciferous species, and found that the less severe the extent of defoliation the higher the compensation capability of the plants.

Compensation means that other, photosynthetically active plant tissues including the stem, sheath, chaff, or other leaves, seem to provide enough assimilates for seed production, despite the partial or total absence of flag leaves (Biswal and Kohli, 2013). In durum wheat (*Triticum turgidum* L. var. *durum*) for example, the transportation of assimilates to the ears from the stem and the chaff increased once the flag leaf was cut off (Álvaro et al., 2008).

Factors influencing compensation include stress and phenological phase. When the flag leaves of wheat were stressed and unable to operate at their full potential, Vicente et al. (2018)

observed an upregulation of genes responsible for CO_2-fixation, nitrogen assimilation and respiration in the ears. In their experiment, the improved metabolism of the ears compensated, therefore heading and early grain filling suffered no loss. Simkin et al. (2020) appraise the knowledge on the photosynthetic activity of stem, ear, embryo, and other, non-foliar tissues in various crops including wheat and conclude that these tissues assist plant development, growth, and yield. Macedo et al. (2007) however, reported a slightly contrasting finding: a simulated fall armyworm (*S. frugiperda*) defoliation of winter wheat had no effect on the photosynthesis rate of the remaining, injured leaves, although these remaining tissues still had their stomas activated at higher conductivity figures than before the defoliation.

However, it is important to note that these observations cannot be generalized. The reaction of the photosynthetic rate of the remaining, unaffected leaves to injuries or defoliation often depends on their developmental stage and also of the whole affected plant itself. For example, in birch (*Betula pendula* L.), younger plants usually showed an increased photosynthesis rate, while older ones rather had a delayed photosynthetic senescence, thus serving as a compensation to the lost leaves or leaf parts (Ovaska et al., 1992). On the other hand, an early season leaf damage was found to have caused significant reductions to both vegetative growth and grain production (Webster et al., 1982; Buntin et al., 2004). We may speculate that the effect of herbivory depends on timing in terms of the ratio between assimilation and dissimilation processes within the plant. If the plant is in the assimilation phase during herbivory, the effects will be more pronounced.

The role of abiotic and biotic conditions including climate and soil on seed production must not be overlooked either. In our experiment, climate, and precipitation in particular, in 2018 seemed to negatively affect seed production for treated and control plants alike, and simulation was better in the climatically more favourable year of 2017. The effect of site, with the water-logged area and a partial shade, was also unfavourable in 2018. This suggests that compensation was hindered both by the unfavourable weather and site conditions. While we found no difference between the compensation abilities of the cultivars we tested, because the effect of year and site were more evident, experimenting with corn (*Zea mays*, L.) highlighted the importance of cultivars, too (Keszthelyi et al., 2009; Zheng et al., 2021).

The influence of biotic and abiotic factors on the manifestation of damage due to herbivory was also confirmed by other authors, who reported that plant response to actual or simulated insect damage largely depends on (a) the development stage of the plant during herbivory, (b) the extent of the damage, (c) the role of the damaged plant tissue, (d) the damage type and also strongly on (e) abiotic factors like weather conditions or soil quality (Pedigo et al., 1986; Macedo et al., 2007; Shao et al., 2010).

On the other hand, special, stressful circumstances may result in defoliation being beneficial as Iqbal et al. (2012) observed an increased metabolism and photosynthetic activity in the remaining tissues. The positive effects of defoliation in corn have also been demonstrated in some of the tested cultivars in terms of protein content (Keszthelyi et al., 2009) and yield (Zheng et al., 2021).

In agreement with the earlier studies of Biswal and Kohli (2013), we suggest that the role of compensating tissues shall be subjected for further studies to understand their function, that is, to still be able to supply assimilates to the procedure of grain production, when the plant is under stressful conditions such as herbivory or draught. We have to define the circumstances when wheat is able to compensate for the loss of leaves, or in other words, we have to define the circumstances that necessitate immediate pest control measures against CLB, and perhaps, as Steinger et al. (2020) pointed out, the economic significance of the pest may need a revision, too.

We also have to emphasize that based on the degree of contribution of other plant tissues to grain production, the relative importance of flag leaves in seed production may seem exaggerated and therefore, the role of the flag leaf should be re-evaluated.

Conflict of interest: The authors declare no conflicts of interest.

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REFERENCES

Ahmadi, A. and Joudi, M. (2007). Effects of timing and defoliation intensity on growth, yield and gas exchange rate of wheat grown under well-watered and drought conditions. *Pakistan Journal of Biological Sciences,* 10: 3794–3800.

Ali, M., Hussain, M., Khan, M., Ali, Z., Zulkiffal, M., Anwar, J., Sabir, W., and Zeeshan, M. (2010). Source-sink relationship between photosynthetic organs and grain yield attributes during grain filling stage in spring wheat (*Triticum aestivum*). *International Journal of Agriculture and Biology,* 12: 509–515.

Álvaro, F., Royo, C., García del Moral, L.F., and Villegas, D. (2008). Grain filling and dry matter translocation responses to source-sink modifications in a historical series of durum wheat. *Crop Science,* 48: 1523–1531.

Araus, J.L. and Tapia, L. (1987). Photosynthetic gas exchange characteristics of wheat flag leaf blades and sheaths during grain filling: the case of a spring crop grown under mediterranean climate conditions. *Plant Physiology,* 85: 667–673.

Bijanzadeh, E. and Emam, Y. (2010). Effect of defoliation and drought stress on yield components and chlorophyll content of wheat. *Pakistan Journal of Biological Sciences*, 13(14): 699.

Buntin, G.D. (1994). Simulated insect defoliation of seedlings and productivity of winter small-grain crops. *Journal of Entomological Science*, 29(4): 534–542.

Buntin, G.D., Flanders, K.L., Slaughter, R.W., and DeLamar, Z.D. (2004). Damage loss assessment and control of the cereal leaf beetle (Coleoptera: Chrysomelidae) in winter wheat. *Journal of Economic Entomology*, 97: 374–382.

Biswal, A.K. and Kohli, A. (2013). Cereal flag leaf adaptations for grain yield under drought: knowledge status and gaps. *Molecular Breeding,* 31: 749–766.

Casagrande, R.A., Ruesink, W.G., and Haynes, D.L. (1977). The behavior and survival of adult cereal leaf beetles. *Annals of the Entomological Society of America*, 70(1): 19–30.

Császár, O., Tóth, F., and Lajos, K. (2021). Estimation of the expected maximal defoliation and yield loss caused by cereal leaf beetle (*Oulema melanopus* L.) larvae in winter wheat (*Triticum aestivum* L.). *Crop Protection*, 145: 105644.

Földi, M., Bencze, S., Hertelendy, P., Veszter, S., Kovács, T., and Drexler, D. (2021). Farmer involvement in agro-ecological research: organic on-farm wheat variety trials in Hungary and the Slovakian upland. *Organic Agriculture*. https://doi.org/10.1007/s13165-020-00335-x.

Füzi, I. and Kövics, G. (2002). A gombás betegségek és a terjedésüket szimuláló mesterséges levéleltávolítás hatása az őszi búza terméshozamára. [The impact of fungal diseases and their simulation by artificial defoliation on winter wheat yield, in Hungarian]. *Növényvédelem,* 38: 194–198.

Gavloski, J.E. and Lamb, R.J. (2000). Compensation for herbivory in cruciferous plants: specific responses to three defoliating insects. *Environmental Entomology,* 29: 1258–1267.

Hartig, F. (2020). DHARMa: residual diagnostics for hierarchical (Multi-level/Mixed) regression models. R package version 0.3.3.0. Retrieved from https://CRAN.R-project.org/package=DHARMa.

Haynes, D.L. and Gage, S.H. (1981). The cereal leaf beetle in North America. *Annual Review of Entomology,* 26: 259–287.

Herbert, D.A., Jr., Van Duyn, J.W., Bryan, M.D., and Karren, J.B. (2007). Cereal leaf beetle. In: Buntin, G.D., Pike, K.S., Weiss, M.J., and Webster, J.A. (Eds.), *Handbook of small grain insects*. Entomological Society of America, Lanham, MD, USA, p. 120.

Iqbal, N., Masood, A., and Khan, N.A. (2012). Analyzing the significance of defoliation in growth, photosynthetic compensation and source-sink relations. *Photosynthetica,* 50: 161–170.

Jossi, W. and Bigler, F. (1996). Getreidehähnchen: ertragsverluste bei Winterweizen. *Agrarforschung*, 3(3): 120–123.

Kassambara, A. (2020). ggpubr: 'ggplot2' based publication ready plots. R package version 0.4.0. https://CRAN.R-project.org/package=ggpubr.

Keszthelyi, S., Pál-Fám, F., and Pozsgai, J. (2009). Reactions of the different breeding season corns as a function of injury of cotton bollworm (*Helicoverpa armigera* Hbn.). *Cereal Research Communications*, 37(2): 321–326.

Lajos, K., Császár, O., Sárospataki, M., Samu, F., and Tóth, F. (2020). Linear woody landscape elements may help to mitigate leaf surface loss caused by the cereal leaf beetle. *Landscape Ecology*, 35: 2225–2238. https://doi.org/10.1007/s10980-020-01097-3.

Macedo, T.B., Peterson, R.K.D, Dausz, C.L., and Weaver, D.K. (2007). Photosynthetic responses of wheat, *Triticum aestivum* L., to defoliation patterns on individual leaves. *Environmental Entomology*, 36: 602–608.

Macedo, T.B., Peterson, R.K.D., and Weaver, D.K. (2006). Photosynthetic responses of wheat, *Triticum aestivum* L., plants to simulated insect defoliation during vegetative growth and at grain fill. *Environmental Entomology*, 35: 1702–1709.

Mazurkiewicz, A., Jakubowska, M., Tumialis, D., Bocianowski, J., and Roik, K. (2021). Foliar application of entomopathogenic nematodes against cereal leaf beetle *Oulema melanopus* L. (Coleoptera: Chrysomelidae) on wheat. *Agronomy*, 11: 1662. https://doi.org/10.3390/agronomy11081662.

OMSZ – Országos Meteorológiai Szolgálat. [Hungarian Meteorological Service] Database. https://www.met.hu/idojaras/aktualis_idojaras/napijelentes_2005-2019/ (Accessed: 22 November 2020).

Ovaska, J., Walls, M., and Mutikainen, P.I.A. (1992). Changes in leaf gas exchange properties of cloned *Betula pendula* saplings after partial defoliation. *Journal of Experimental Botany,* 43: 1301–1307.

Pedigo, L.P., Hutchins, S.H., and Higley, L.G. (1986). Economic injury levels in theory and practice. *Annual Review of Entomology,* 31: 341–368.

Philips, C.R., Herbert, D.A., Kuhar, T.P., Reisig, D.D., and Roberts, E.A. (2012). Using degree-days to predict cereal leaf beetle (Coleoptera: Chrysomelidae) egg and larval population peaks. *Environmental entomology*, 41(4): 761–767.

R Core Team. (2020). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

Shao, L., Zhang, X., Hideki, A., Tsuji, W., and Chen, S. (2010). Effects of defoliation on grain yield and water use of winter wheat. *The Journal Agricultural Science*, 148: 191–204.

Simkin, A.J., Faralli, M., Ramamoorthy, S., and Lawson, T. (2020). Photosynthesis in non-foliar tissues: implications for yield. *The Plant Journal*, 101: 1001–1015.

Steinger, T., Klötzli, F., and Ramseier, H. (2020). Experimental assessment of the economic injury level of the cereal leaf beetle (Coleoptera: Chrysomelidae) in winter wheat. *Journal of Economic Entomology*, 113(4): 1823–1830.

Strategie Grains. (2018). EU grain report No.312, tallage SAS, 13/12/18, 4–5.

Vicente, R., Vergara-Dıaz, O., Medina, S., Chairi, F., Kefauver, S.C., Bort, J., Serret, M.D., Aparicio, N., and Araus, J.L. (2018). Durum wheat ears perform better than the flag leaves under water stress: gene expression and physiological evidence. *Environmental and Experimental Botany*, 153: 271–285.

Wickham, H. (2016). ggplot2: elegant graphics for data analysis. Use R!, 2nd ed. Springer International Publishing, New York.

Webster, J.A., Smith, D.H., Jr., and Hoxie, R.P. (1982). Effect of cereal leaf beetle on the yields of resistant and susceptible winter wheat. *Crop Sciences*, 22: 836–840.

Würschum, T., Leiser, W.L., Langer, S.M., Tucker, M.R., and Miedaner, T. (2020). Genetic architecture of cereal leaf beetle resistance in wheat. *Plants*, 9: 1117. https://doi.org/10.3390/plants9091117.

Zadoks, J.C., Chang, T.T., and Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, 14(6): 415–421.

Zheng, Z., Powell, J.J., Ye, X., Liu, X., Yuan, Z., and Liu, C. (2021). Overcompensation can be an ideal breeding target. *Agronomy*, 11: 1376.

Zhu, G.X., Midmore, D.J., Radford, B.J., and Yule, D.F. (2004). Effect of timing of defoliation on wheat (*Triticum aestivum*) in central Queensland 1. Crop response and yield. *Field Crops Research*, 88(2–3): 211–226.

Zhu, G.X., Midmore, D.J., Yule, D.F., and Radford, B.J. (2006). Effect of timing of defoliation on wheat (*Triticum aestivum*) in central Queensland: 2. N uptake and relative N use efficiency. *Field Crops Research*, 96(1): 160–167.

APPENDIX

Table A1. Results of linear models testing for significant differences between the yield at 25% artificial defoliation of all leaves and flag leaves only, serving as a reference, and the yields at all other extents of artificial defoliation for the cultivar types A) 'Alcantara' and B) 'Altigo' in the two study years 2017 and 2018. Significant differences are marked bold

A) Alcantara				
All leaves				
Study year: 2017				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	0.735	0.152	4.823	0.001
50	0.084	0.216	0.390	0.707
75	−0.125	0.216	−0.578	0.579
100	−0.006	0.216	−0.029	0.978
Study year: 2018				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	0.341	0.174	1.960	0.086
50	0.229	0.246	0.931	0.379
75	0.780	0.246	**3.167**	**0.013**
100	0.062	0.246	0.252	0.807
Flag leaves				
Study year: 2017				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	0.857	0.241	3.565	0.007
50	−0.110	0.340	−0.324	0.754
75	0.237	0.340	0.697	0.505
100	−0.131	0.340	−0.385	0.711
Study year: 2018				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	1.278	0.170	7.518	0.000
50	−0.514	0.240	−2.137	0.065
75	−0.420	0.240	−1.748	0.119
100	−0.631	0.240	**−2.624**	**0.030**
B) Altigo				
All leaves				
Study year: 2017				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	0.781	0.176	4.446	0.002
50	−0.056	0.248	−0.225	0.828

(continued)

Table A1. Continued

B) Altigo				
All leaves				
Study year: 2017				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
75	0.285	0.248	1.147	0.284
100	0.066	0.248	0.266	0.797
Study year: 2018				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	0.327	0.069	4.715	0.002
50	0.146	0.098	1.491	0.174
75	0.191	0.098	1.944	0.088
100	−0.047	0.098	−0.476	0.647
Flag leaves				
Study year: 2017				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	1.176	0.140	8.395	0.000
50	−0.262	0.198	−1.324	0.222
75	0.001	0.198	0.006	0.996
100	−0.261	0.198	−1.316	0.225
Study year: 2018				
Defoliation (%)	Estimate	Std. Error	*t* value	Pr(>|*t*|)
(Intercept)	0.456	0.121	3.780	0.005
50	−0.020	0.171	−0.119	0.908
75	0.138	0.171	0.812	0.440
100	0.166	0.171	0.972	0.360

Table A2. Average CLB larval defoliation per plant on all leaves (= both flag and other leaves) in percent (±SD) for the cultivar types 'Alcantara' and 'Altigo' in the two study years 2017 and 2018

Cultivar	Year	Defoliation (%) ± SD
Alcantara	2017	15.15 ± 7.03
	2018	13.19 ± 9.20
Altigo	2017	11.64 ± 7.84
	2018	23.27 ± 12.07