https://doi.org/10.17221/159/2021-PPS

Measurement of post-dispersal invertebrate seed predation of some relevant weed species in maize fields in Hungary: An ecosystem service provided in crop fields contributing to weed management

Mohammed Gaafer Abdelgfar Osman[1,2]*, *Mark Szalai*[3], *Mihaly Zalai*[1], *Zita Dorner*[1], *Jozsef Kiss*[1]

[1]*Department of Integrated Plant Protection, Plant Protection Institute, Hungarian University of Agriculture and Life Science (MATE)Volt (SZIE), Gödöllő, Hungary*
[2]*Weed Research Department, Integrated Agricultural Pests Research Centre, Agricultural Research Corporation (ARC), Wad Medani, Sudan*
[3]*Institute of Agricultural Economics, Budapest, Hungary*

**Corresponding author: gafero20@gmail.com*

Citation: Osman M.G.A., Szalai M., Zalai M., Dorner Z., Kiss J. (2022): Measurement of post-dispersal invertebrate seed predation of some relevant weed species in maize fields in Hungary: An ecosystem service provided in crop fields contributing to weed management. Plant Protect. Sci., 58: 351–359.

Abstract: Invertebrate seed predation is a potential ecosystem service that substantially reduces weed seeds in crop fields, decreasing the seedling emergence and, thus, limiting the weed competition next season. It may, thus, be considered as a natural component of the long-term weed management toolbox. This study aimed to measure the post-dispersal invertebrate seed predation levels of the following relevant weed species in Hungarian maize fields: *Ambrosia artemisiifolia*, *Datura stramonium, Chenopodium album,* and *Echinochloa crus-galli*, and to compare the predation levels among them. We hypothesised that invertebrate seed predators will predate weed seeds, but the predation levels may vary with the weed species. Two sampling rounds were performed, in November 2019 and October 2020, in Gödöllő, Hungary. A total of 100 seed cards/round were placed on the soil surface inside a maize field prior to harvest, 10 m from the field's edge, along 25 transects, with four cards/transect. A distance of 10 m was set between the transects and 1 m between the cards. Twenty seeds of each weed species were glued onto sandpaper (25 × 10 cm, P-60), and a wire mesh was used to exclude vertebrate predators. The seed removal was calculated every 24 h, for seven days in 2019 and for five days in 2020, and then the seed predation was measured using the number of removed seeds on each card. The results showed high seed predation levels on all the seed cards, with an overall average of 85.9 ± 13.7%. Besides, there was a decrease in the % of remaining seeds on the cards starting from the first day after exposure due to seed predation in both years. The optimum exposure period for measuring the seed predation was found to be three to four days, though the number of predated seeds on days 3 and 4 significantly differed between years ($P < 0.001$), with higher predation rates in 2020 than in 2019. However, no differences were detected in the predation rates among the weed species ($P = 0.962$, 0.079). These findings indicate the potential contribution of seed predation by invertebrates to weed management in Hungarian maize fields.

Keywords: invertebrate seed predators; ecosystem services; key weed; maize

Supported by the Ministry for Innovation and Technology within the framework of the Thematic Excellence Program 2020-Institutional Excellence Subprogram (No. TKP2020-IKA-12) for research on plant breeding and plant protection, and the remarked financial fund for the Tempus Public Foundation, Government of Hungary, for the doctoral scholarship (Stipendium Hungaricum Scholarship Program Registration No. SHE-19332-002/2018-2022).

https://doi.org/10.17221/159/2021-PPS

Weed species impose an abiotic constraint on most cropping systems, as they compete for resources with crops (Oerke 2006) and, thus, reduce the crop yield and quality (Barzman et al. 2015). A preliminary investigation indicated that weed infestations in the early growing stage of a maize crop cause major effects in the absence of herbicide applications (Lehoczky et al. 2013). For example, in Hungary, maize (*Zea mays* L.), is an important field crop, planted on about 1.3 million ha, approximately 25% of the total arable area. Weed-crop competition, causes significant grain yield losses due to the dense weed infestation (Rajcan & Swanton 2001; Lehoczky et al. 2005; Yeganehpoor et al. 2015), counting for up to 34% of the global crop losses (Abouziena et al. 2015), and decreases the crop biomass by up to 64% compared to a weed-free maize field (Lehoczky et al. 2013). Overall, weeds are responsible for similar proportions of production loss as animal pests and pathogens combined (Oerke 2006), as well as playing a major role in preserving ecosystem services by supporting a higher density and diversity of invertebrates (Navntoft et al. 2007). Weed management, thus, offers a significant challenge to farmers who rely on herbicides alone (Ghersa et al. 2000; Oerke 2006), as this negatively affects the farm biodiversity and may lead to the development of resistant weed populations (Délye et al. 2013; Annett et al. 2014). This has engendered a need to consider more environmentally friendly measures (Bohan et al. 2011), and agro-ecological alternatives for weed control to reduce the herbicide usage (Petit et al. 2015). For example, adopting broader weed management strategies, such as instituting biological controls, and promoting ecosystem services such as weed seed predation.

Weed regulation by means of the weeds' natural enemies arises from an important ecosystem service provided in agricultural fields (Begg et al. 2017). Among these, weed seed predation, is a potential biological control process which causes a substantial decrease in the germinating weed seeds and, thus, contributes to the weed management (Westerman et al. 2008; Navntoft et al. 2009; Baraibar et al. 2011). It was noted by Westerman et al. (2003a), O'Rourke et al. (2006), and Baraibar et al. (2009) that seed predation contributes significantly to the seed mortality, with 90% predation rates observed after a few days of seed exposure. However, the adoption of this service as a standard strategy in weed management programmes remains challenging due to the high variability and unpredictability in terms of the time and place (Westerman et al. 2003b; Saska et al. 2008; Petit et al. 2011; Kulkarni et al. 2015a). Seed predation was firstly used by (Janzen 1971; Zhang et al. 1997) to differentiate between animals that consume and destroy seeds and those that ingest seeds, leaving their fate undecided. It relates to the capture of prey, describes the viable seed removal from the seed bank, and is thought to be responsible for a considerable amount of seed loss, for both after and prior to the seeds shedding. Seed predation is generally categorised into pre-dispersal seed predation, which describes attacks on seeds before they are shed by their parent plants, and post-dispersal seed predation, which occurs once seeds are readily available on the soil surface, where they act as food for seed predators (Janzen 1971).

All weed seed predation reduces and regulates the weed population density (Petit et al. 2018; Sarabi 2019) and influences the weed population demography (Kauffman & Maron 2006). Blubaugh and Kaplan (2016) noted that seedling emergence of weed species (*Chenopodium album*) and its biomass were decreased by 38% and 81%, respectively, in cover crops due to seed predation, while Firbank and Watkinson (1985) reported that annual weed seed losses of 25% to 50% are sufficient to substantially decrease the weed population growth. Therefore, seed predation may be encouraged to reduce the use of herbicides, especially if combined with other non-chemical control methods (Shields et al. 2019). However, its efficacy depends on the relevant predators' abilities to respond in a directly density dependent way to the increasing weed seed densities (Westerman et al. 2008).

Invertebrate seed predators, such as carabid beetles (Coleoptera: Carabidae), a major driver of weed seed predation (Westerman et al. 2003b; Kulkarni et al. 2015b), were found to consume weed seeds both in the laboratory (Honěk et al. 2007; Petit et al. 2014; Saska et al. 2019) and under field conditions (Kromp 1999; Honěk et al. 2003; Kulkarni et al. 2015b; Petit et al. 2017). Seed predators were also found to consume from 53% to 95% of the annual seed production of some evaluated weed species (Harrison et al. 2003; Westerman et al. 2003b; Honek et al. 2005; Westerman et al. 2011; Davis et al. 2013). For instance, Holland (2002) reported six species of such beetles performing this service, *Harpalus pensylvanicus*, *Bembidion quadrimacu-*

https://doi.org/10.17221/159/2021-PPS

latum oppositum, *Pterostichus melanarius*, *Chlaenius tricolor*, *Harpalus herbivagus*, and *Bembidion rapidum*. In Hungary, invertebrate seed predators, carabid beetles (Coleoptera: Carabidae) are widespread predators in agricultural fields, where they play an important role in reducing animal pest populations in many crop ecosystems (Lövei & Sunderland 1996). They are widely used indicators for measuring ecological impacts because the family has a high number of species, are taxonomically well recognised, and are abundant in arable crops and sensitive to habitat changes (Lövei & Sunderland 1996; Rainio & Niemela 2003).

However, quantification of the consumed seeds has varied among studies due to classification differences: Honěk et al. (2007) considered a seed consumed when > 50% had been destroyed, whereas other researchers considered a seed consumed when the seed coat was cracked and part of the endosperm was damaged (Carmona & Landis 1999). Previous studies in arable fields utilised weed seeds in dishes sunken into the soil (Diaz 1992; Cardina et al. 1996; Cromar et al. 1999; Tooley et al. 1999a, b) or glued to cards placed on the soil surface (Hurst & Doberski 2003; Westerman et al. 2003b), with any removed seeds considered to be predated in both approaches.

Our study adopted the ground-based seed removal approach in order to measure the invertebrate post-dispersal seed predation levels of some relevant weed species in Hungarian maize fields, identified as *Ambrosia artemisiifolia, Datura stramonium, C. album* and *Echinochloa crus-galli*, and to compare predation levels among them during exposure periods of five and seven days in 2019 and 2020. Specifically, to test the hypotheses that (1) invertebrate seed predators will predate these weed seeds and (2) seed predation levels may vary among the weed species.

MATERIAL AND METHODS

Site description, ecological and metrological features, and crop management. Field measurements for the invertebrate weed seed predation were performed in maize fields at the Hungarian University of Agriculture and Life Science (MATE) research farm (Szárítópuszta), Gödöllő, Hungary (47°34'49.1"N 19°24'05.0"E). The crop production demonstration centre operates over 140 hectares, of which 12 hectares are irrigated. The soil type is rust-brown forest soil (Chromic Luvisol), with other parameters at the 0 to 40 cm depths including a pH (H_2O) of 6.76; an organic matter (OM) content of 1.32%; a P content of 371.1 mg/kg, and a K content of 184.0 mg/kg. Both the humus content and N-supply in the topsoil are, thus, deemed poor, whereas the K and P supplies are sufficient. The upper 40 cm layer of the soil consists of 53% sand, 26% loam, and 20% clay fractions. The climate is continental, with regular weather extremes. The mean annual temperature is 9.7 °C, and the average annual precipitation is 550 mm, two-thirds of which falls between April and September.

Preparation of the seed predation cards. The seed card method was followed, as it is generally considered the standard for such type of research which provides short-term estimates of invertebrate seed predation measured directly in the field (Westerman et al. 2003a, b). This is because it inhibits those processes related to the long-term cumulative weed seed burial, such as seeds washing into the soil cracks and gradual seed burial by plant residue (Westerman et al. 2009). The seed cards were prepared by gluing [glue spray adhesive 3Ml (400 mL/282 g)] 20 fresh seeds of each weed species [*A. artemisiifolia, D. stramonium, C. album* and *E. crus-galli,* obtained from Herbiseed® (Twyford, UK)], onto sandpaper [25 × 10 cm, P = 60 (kL361 J-Flex Klingspor)], thus affixing the seeds onto the cards and preventing them from being removed by rain and wind. Wire meshes (hole size 25 mm) were used as a technique to permit entry and easy access only to small invertebrates, such as ground beetles, and to protect the seed cards from large vertebrate predators, such as rodents or birds.

Quantification of the seed predation. The seed predation was measured based on short-term point estimates of the seed removal rates of the examined weed species. Two sampling rounds of exposure field trials were performed in the maize field in November 2019 and October 2020, prior to the harvest and after the assumed natural seed ripening of the tested weed species. A total of 100 seed cards per year/round were placed on the ground inside the maize field, 10 m from the field edge, along 25 transects, at a rate of four cards per transect, with 10 m between transects and 1 m between cards. There were initially 20 seeds of each weed species per card. The exposure periods lasted for seven days in November 2019 and for five days in October 2020, with the latter reduction being due to unfavourable climatic conditions. The num-

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ber of seeds remaining on each card was counted every day in the field, and the proportion of the seed predation was estimated based on the removal rate of the weed seeds starting 24 h after the first field exposure. The number of seeds remaining on the cards was converted into a proportion representing the seed predation relative to the total number of glued-on seeds as follows:

$$Mi = (Ci - Ri)/Ci \times 100 \quad (1)$$

where: Mi – proportion of the seed predation during exposure; Ri – number of the remaining seeds on the cards; Ci – number of the total glued seeds.

The data collection included the number of seeds predated and the seeds remaining after five and seven days in the field. The count of the number of remaining seeds on the cards was, however, influenced by human bias and error factors during sampling. This is evidenced by the fact that within the data, 3.2% of the total records were higher than those recorded on the previous day, representing 38 cases of negative predation relative to 100% predation on day 5 and 6. However, those cards were still fully included in the statistical analysis. The statistical analysis was performed based on an assessment of the seed consumption rates on the selected day/s during the exposure time in the field. The seed predation data for day 0 to day 3 and day 0 to day 4 were analysed to better estimate the seed predation levels, as fewer remaining seeds were available on the latter days of the field exposure. This fact led to a prediction that a suitable exposure period for estimating the weed seed predation in maize fields could be between three and four days. Statistical data analyses were performed using R statistical software (version 4.1.1., R Development Core Team 2021), and these included the fit of linear models and a single-factor analysis of variance (ANOVA). The binomial models were also fitted and validated on the seed and card levels, comparing the seed predation between the weed species and across the years. Diagnostic plots were also investigated to ensure the model fit assumptions.

RESULTS

The results revealed seed predation on all the seed cards placed inside the maize fields during the exposure periods in both years. The weed seeds suffered an overall predation average of 85.9 ± 13.7% (SD), ranging from 71.60 ± 12.96% in *E. crus-galli* in 2019 to 96.80 ± 2.84% in *A. artemisiifolia* in 2020 (see Table 1 and Figure 1). In addition, the results showed a decrease in the % of remaining seeds on the cards starting from the first day after exposure in both years due to seed predation. While the predation levels and their temporal pattern seemed different in the two years, a similar pattern was observed for the four weed species. This pattern, therefore, was used to select the 3- and 4-day long exposure time, from day 0 to day 3 and day 4, 72 and 96 h, respectively, for further analysis. The results also showed significant differences in the seed predation levels across between the two assessed years, with the predation levels significantly higher in 2020 than in 2019, whereas, the differences in the seed predation were not significant between the weed species. All the weed seeds were predated at similar rates, with no large differences in the numbers of consumed seeds (Figure 1).

The statistical analysis was performed based on an assessment of the seed predation rates on the selected day/s during the exposure time in the field. There was a rapid decrease in the seed consumption over day 3 and day 4, and due to the low numbers of remaining seeds in the last days of the field exposure, the data for days 3 and 4 were, thus, analysed separately (Figure 2). The data analysis of the weed seed consumption from day 0 to day 3 showed no differences (P = 0.962 5) in the seed consumption across the weed species in both years (Figure 2), whereas significant differences emerged

Table 1. Averages (and standard deviation) of the invertebrate seed predation of the weed species inside the maize fields during the 7- and 5-day exposure periods in 2019 and 2020, respectively, Gödöllő, Hungary

Weed species	Year	Seed predation (%, mean ± SD)
Ambrosia artemisiifolia	2019	82.40 ± 15.28
Datura stramonium	2019	77.80 ± 14.29
Chenopodium album	2019	76.40 ± 13.50
Echinochloa crus-galli	2019	71.60 ± 12.96
Ambrosia artemisiifolia	2020	96.80 ± 2.84
Datura stramonium	2020	95.40 ± 2.46
Echinochloa crus-galli	2020	94.60 ± 3.51
Chenopodium album	2020	92.80 ± 3.55

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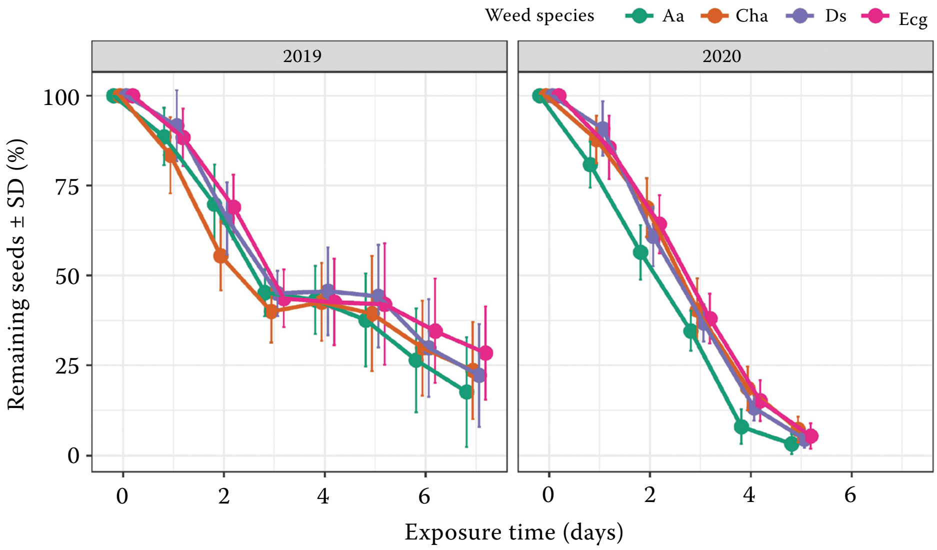

Figure 1. Temporal pattern of the seed predation of the weed species by the invertebrate seed predators, exposed inside the maize fields in 2019 and 2020, Gödöllő, Hungary
Aa – *Ambrosia artemisiifolia*; Cha – *Chenopodium album*; Ds – *Datura stramonium*; Ecg – *Echinochloa crus-galli*
To enhance readability of the plots, the horizontal position of the data points and error bars were adjusted to avoid overlapping, i.e., all the points around a certain day had the same exposure time

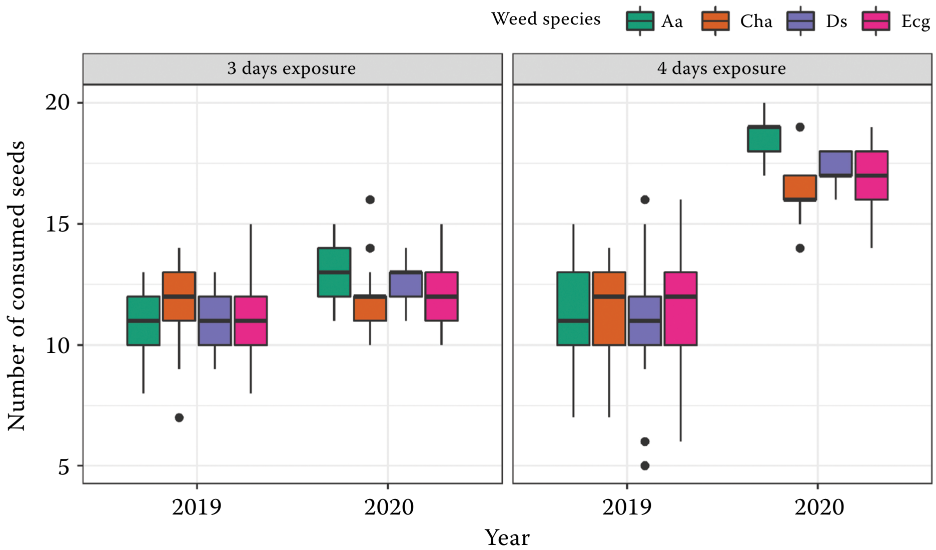

Figure 2. Invertebrate seed predation from day 0 to day 3 (left panel) and day 4 (right panel) of the weed species
Aa – *Ambrosia artemisiifolia*; Cha – *Chenopodium album*; Ds – *Datura stramonium*; Ecg – *Echinochloa crus-galli*
Seed cards (n = 25 for each species in each year) with 20 seeds per card were exposed inside the maize fields in 2019 and 2020 in Gödöllő, Hungary

between the years (P < 0.001). Figure 2 also shows the differences in the seed consumption between 2019 and 2020 on day 4. The statistical analysis of the weed seed consumption from day 0 to day 4 similarly showed no differences (P = 0.079) in the number of consumed seeds across the weed species in both years, yet it showed significant differences between the years (P < 0.001).

DISCUSSION

Seed predation is becoming recognised as an increasingly important factor in the functioning of an ecosystem by ecologists, agroecologists, and plant population ecologists (Westermann et al. 2003a; Kulkarni et al. 2015b; Blubaugh et al. 2016; Petit et al. 2017). However, its incorporation into weed

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control programmes requires further clarification of the relevant temporal and spatial patterns, additional information on seed predators and their responses to increasing seed densities, feeding preferences, and mechanisms. In Hungary, ground beetles (Coleoptera: Carabidae) are frequent seed predators in agricultural fields, where they serve an essential role in lowering pest populations in many crop ecosystems (Lövei & Sunderland 1996). A total of 55 558 specimens of 141 species of carabid beetles (Coleoptera, Carabidae) were collected from eight locations (apple orchards and maize stands) using light traps (Kádár & Szél 1989). Grain maize fields were sampled for ground dwelling arthropods during the maize growing seasons between 2001–2003 in Hungary, where 44 103 individuals of 58 species were collected using pitfall traps. The most common species were *Calathus ambiguus, Dolichus halensis, Harpalus distinguendus, H. rufipes, Poecilus sericeus* and *Trechus quadristriatus* (Szekeres et al. 2006).

Here, we performed exclosure field trials in November 2019 and October 2020 in Gödöllő, Hungary, to measure the invertebrate seed predation levels of some relevant weed species in the maize fields, based on measuring the seed removal rates in artificially exposed seeds over short periods (Westerman et al. 2003a, b) in a similar manner to the work of Brust and House (1988), Honek et al. (2003), and Davis et al. (2011). Despite that no original data were collected on the abundant seed predator populations, an exclusion technique was applied to successfully prevent the vertebrate predators from removing the seeds, evidenced by there being no destroyed or missing seed cards, which indicates that the recorded estimates of the seed predation under the wire meshes were representative of the invertebrate seed predation. Moreover, Gallandt et al. (2005) highlighted that the superior importance of invertebrate seed predators in seed predation compared to vertebrates has been confirmed by many studies; Cromar et al. (1999) and Westerman et al. (2003b), reported, for example, that invertebrates account for 80% to 90% of seed predation in maize, soybean, and wheat fields. The current findings could, therefore, be considered as a close approximation of the total seed predation ecosystem service.

Our results showed a pattern of weed seed predation on the soil surface supported by similar local-scale studies performed across Europe (Westerman et al. 2005; Trichard et al. 2014; Carbonne et al. 2020). High seed predation levels were observed (100% of the seed cards were affected, where 86% of the weed seeds were predated), confirming the initial hypothesis that the weed seeds will be predated when exposed to seed predators. This agreed with the findings of several studies on agricultural fields, where seed predation has been described as a major cause of seed losses on the soil surface (Brust & House, 1988; Swanton et al. 1999; Menalled et al. 2000). In addition, this finding is consistent with those reported by Jonason et al. (2013), who found high seed predation rates in *Stellaria media* (L.) Vill. and other weed species in cereal fields on conventional and organic farms in Sweden. Mauchline et al. (2005) similarly observed more than 70% seed predation of the weed species *S. media* L., *Polygonum aviculare* L., *C. album* L., and *Sinapis arvensis* L. in spring barley fields (*Hordeum vulgare* L.) (cv. Chariot) at Reading University's Farm at Sonning, Berkshire, UK. These high predation levels may be due to a positive relationship between ecosystem services and the invertebrate predators' activity-density; however, they may also be considered as a direct response to the high food resource availability according to Frank et al. (2011). Some other research findings showed that seed predators selectively predate specific seed species; however, such selectivity is not always a persistent pattern.

In accordance with Westerman et al. (2006), Petit et al. (2018), and Sarabi (2019), who stated that seed predation is a potential biological control process that limits weed population densities and growth, the hypothesis, in this study, was that the population densities and growth of the assessed weed species might be decreased due to the high seed predation levels. However, Westerman et al. (2008) reported that the seed predation efficacy partially depends on the predator's response, in a direct density dependent way, to an increase in the weed seed densities. According to Blubaugh and Kaplan (2016), the seedling emergence of weed species (*C. album*) and the resulting biomass were reduced by 38% and 81%, respectively, due to seed predation; thus, the prediction here was that the seedling emergence and the biomass of the weed species *C. album* might be decreased. The findings, in this case, disagreed with those of Moles et al. (2003) and Gaba et al. (2019), who found that seed predation levels varied with the weed species. The current results showed that the seeds of all the weed species were similarly predated, with significant differences in the predation rates seen only between years. This is in agree-

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ment with Zhang et al. (1997), who stated that seed losses caused by predators are often substantial, but that they tend to vary between years (Cardina et al. 1996; Tooley et al. 1999a, b). Our results also found that the period of time from day 3 to day 4 is the optimum exposure period in which to measure the weed seed predation in maize fields. To upgrade these findings, both studies of insects behaviour and entomology studies are required to determine the exact potential of insects involved in seed predation in maize crops in Hungary and, information on identifying the seed predators' groups and the individuals involved and their responses to increasing seed densities, feeding preferences, and mechanisms need to be elucidated upon. Moreover, the factors that influence and enhance the weed seed predation inside crop fields need to be analysed.

CONCLUSION

Our results revealed significant levels of invertebrate seed predation of the weed species *A. artemisiifolia, D. stramonium, C. album* and *E. crus-galli* in Hungarian maize fields in November 2019 and October 2020. In both years, the seed predation resulted in a drop in the percentage of the remaining seeds on the seed cards beginning on the first day following exposure. This signifies that this ecosystem service has the potential to be useful in weed management. The seed predation levels varied over years rather than between the weed species (with greater predation levels in 2020 than in 2019). Furthermore, the optimal exposure period for measuring the weed seed predation was discovered to be between days 3 and 4. Overall, encouraging weed seed predation contributes to weed management and reduced the herbicide use. Future research may focus on placing this ecosystem service into spatial (crops, non-cropped habitats, e.g., seminatural habitats as overwintering, surviving ones) and temporal patterns (crop sequence/cropping system) to identify the best options for integrated pest management and farming.

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Received: November 12, 2021
Accepted: April 28, 2022
Published online: July 29, 2022