THE IMPORTANCE OF NATURAL REGULATING MECHANISMS AS ECOSYSTEM SERVICES IN WEED MANAGEMENT: MEASUREMENT OF INVERTEBRATE WEED SEED PREDATION INSIDE FIELD CROPS AND IN ADJACENT SEMI-NATURAL HABITATS IN HUNGARY

DOI: 10.54598/003310

Thesis of the PhD dissertation

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Gödöllő

2023

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1. BACKGROUND AND AIMS

Weed infestation substantially reduce crop yields, while contribute in preserving ecosystem services by supporting higher density and diversity of natural enemies e.g., invertebrate pests (Navntoft et al. 2007). Weeds are widely managed with herbicides, that lead to negative consequences on human health (Zhang et al. 2019), beneficial organisms and the environment (Straw et al. 2021) and development of herbicides resistance (Powles and Yu, 2010). In the European Union (EU), herbicides account for above 40% of the overall pesticides' consumption (average of 179,798 tonnes/year (FAO, 2021). Thus, eliminating the risk of synthetic pesticides to human health and environment was phrased under the Integrated Pest Management (IPM) approach in a Framework Directive on the Sustainable Use of Pesticides in the EU (Directive 2009/128/EC). The Integrated Weed Management (IWM) programs became essential to reduce the adverse effects of the herbicides and mitigate the rising prevalence of herbicide resistance (Norsworthy et al. 2012). Besides it creates a balance of weed control while preserving the botanical diversity of weed species and weed seed predators.

Weed seed predation is an important ecosystem service provided in agricultural fields (Begg et al. 2017), causes substantial seed losses of weed species, decreasing weed populations in agroecosystems and in SNHs (Garren and Strauss, 2009). Weed seeds act as a major food for many animals, including vertebrates and invertebrates (Kolb et al. 2007). Some carabid species consume weed seeds under laboratory conditions (Honek et al. 2003), while others showed responses to weed seed patches in the fields. Seed losses caused by seed predators reported to be substantial, vary over years and across fields (Menalled et al. 2000). Recently, in Hungary Osman et al. (2022), reported seeds of weed species: *Ambrosia artemisiifolia, Datura stramonium, Chenopodium album*, and *Echinochloa crus-galli*, were similarly consumed in maize fields, but consumption levels significantly differed between years.

The potential of invertebrate seed predators in agricultural systems has been highlighted e.g., Gallandt et al. (2005) reported their contribution to be 80 to 90 % of the total seed predation (Westerman et al. 2003). In Hungary, the invertebrate seed predators, carabid beetles are widespread in the agricultural fields. They have been used for measuring ecological impacts because of their high number of species, and taxonomically well recognized. Kiss et al. (1993, 1994) confirmed the occurrence of arthropods seed predator mainly Carabids, e.g., the peak of mixed feeder individuals of *Harpalus* and *Amara* species were sampled in a winter wheat field and in its borders in Kartal, Northern Hungary. Semi-natural habitats adjacent to the crop fields found to be advantageous for crop protection and sustainability (Hatvani et al. 2001). Their presence is also crucial for biological weed control as they host many potential weeds seed predators and natural enemies.

Although, the potentiality of weed seed predation on weed management was addressed in the literature (Westerman et al. 2003, 2005; Bohan et al. 2011), weed seed predation research appears still in the early stages as compared to other regulating ecosystem services. In Hungary, there is a dearth of research observations on weed seed predation in agricultural fields. The Department of Integrated Plant Protection at MATE University carried out the first surveys in winter wheat fields in Hungary within the (QUESSA) EU project (2013 - 2017), which become the ground for subsequent research in this field.

This study used the ground-based seed removal approach (seed cards method) to assess the importance of natural regulating mechanisms in weed management by quantifying the delivery of ecosystem services "weed seed predation" provided in arable fields and adjacent SNHs, and to test whether: predation could be observed on weed seeds; is different due to weed species, and if shows sensitivity to the presence of adjacent SNHs (as general aims), while the specific study objectives were to:

(1) Measure of post-dispersal invertebrate seed predation patterns (as % /day) during different exposure periods on the most important weed species in a maize field: *Ambrosia artemisiifolia, Datura stramonium, Chenopodium album and Echinochloa crus-galli*, and on other weed species in a winter wheat field: *Galium aparine, Papaver rhoeas, and Apera spica-venti,* and in the adjacent SNHs, in Hungary.

We hypothesized that weed seeds are to be predated by seed predators, but predation levels may differ by weed species and habitat types.

(2) Investigate the importance of the invertebrate weed seed predation as an IWM tactic: the case of seed predation on *A. artemisiifolia* inside crop (winter wheat and maize) fields, and in the adjacent SNHs.

2. MATERIALS AND METHODS

2.1 Study site description

Field experiments of invertebrate weed seed predation were performed in a maize and winter wheat field and the adjacent SNHs at the Hungarian University of Agriculture and Life Science (MATE) research farm (Szárítópuszta), near Gödöllő, Hungary (47.5803° N, 19.4014° E). The crop rotation in the study area usually includes winter wheat, maize, barley, oil seed rape, pea, sunflower. The field edge is undisturbed and consisted of small forest patches and herbaceous undergrowth with grasses.

2.2 Description of crops, fields and the adjacent SNHs

This study was conducted in two arable fields (winter wheat and maize) surrounded by SNHs at the fields borders. Winter wheat (*Triticum aestivum*) is a the second most cultivated crop in Hungary (FAO, 2020) grown on a million hectares. This large area constitutes a wide spread of artificial habitats related to arthropod seed predators' populations (Kiss et al. 1994). Weeds causes greatest yield losses on wheat production up to 18-29% (Oerke, 2006). This situation besides changing policies on herbicide use forced the farmers to change their strategies for weed management of wheat production systems. Maize crop (*Zea mays* L.), is an important field crop in Hungary, planted on about 1.3 million ha, approximately 25% of the total arable area. Weed competition causes significant yield losses up to 34 % of the global crop losses and decreases crop biomass by 64% compared to weed-free maize (Lehoczky et al. 2013). This requires to consider environmentally friendly measures for weed control to reduce herbicide usage. For example, adopting biological weed control and promoting ecosystem services e.g., weed seed predation.

The existence of SNHs in the field edges affects seed predation on soil surface, as seed predators need non-agricultural habitats at some stages of their life cycles (Menalled et al. 2000). Many studies have confirmed the importance of SNHs as they provide valuable ecosystem services such as food and shelter for natural seed predators. In Hungary, SNHs have been shown as a favourable for beneficial invertebrates seeking to overwinter (Kiss et al. 1994).

2.3 Assessment of weed flora inside crop fields and in the adjacent SNHs

Sampling of vegetation cover (weed cover %) was performed once in summer of 2019, by assessing the weed flora and identify of typical weed species inside crop field e.g., winter wheat and in the adjacent SNHs at the field border. The method of evaluating of weed covering percentage was followed which has the advantage of being simple, easy, and fast (Zalai et al., 2012). An area of 1 m^2 square was assigned randomly, the sampling quadrates of 1x1 meter area were randomly placed at least 2 meters distance from the field edges to avoid the edge effects. Weed cover percentages were recorded inside the sampling quadrates for each weed species.

2.4 Experimental design, and seed predation assessment methods

Seed card method was used as standard for such types of research of seed predation (Daedlow et al. 2014) to provide estimates of short-term (7 days) seed predation rates evaluated directly in the field. Besides, it is time and cost-effective seed predation measurement tools.

In this study, seed cards were prepared by gluing (glue spray amount adhesive 3Ml (400 ml/282 g ordered from KLINGSPOR, POLAND), 20 fresh seeds (ordered from Herbiseed® (Twyford, UK), of 4 relevant weed species in maize fields: *A. artemisiifolia*, *D. stramonium*, *C. album* and *E. crus-galli*; and different 3 weed species relevant in winter wheat field: *G. aparine* L., *P. rhoeas* L., and *A. spica-venti* L, to sandpaper cards; the glue was applied first and then 20 seeds of each weed species were fixed on the sticky sandpaper surface (25/10 cm, P=60 (kL361 J-Flex ordered from KLINGSPOR, POLAND). The glue was agreed to be used in some EU partner countries under research project: QUESSA (Quantification of Ecological Services for Sustainable Agriculture) (2013 to 2017). The P=60 roughness was chosen to resemble the soil surface of the experimental site in both colour and roughness, while the adhesive ensured the seeds would not be displaced under normal weather conditions (wind, rainfall) or during the placement of the cards.

An exclosure treatment of wire mesh (hexagonal mesh, holes size 25 mm diameter) was used to ease access of small invertebrates while preventing entry to and securing the seed cards against larger vertebrate predators. Our study stands on previous studies by Kiss et al. (1993 and 1994) on the activity density, key mixed feeder species, and their phenology in a winter wheat field and in the adjacent SNHs as they confirmed the occurrence of arthropods invertebrate seed predator individuals, mainly Carabids.

Although, we did not collect data on seed predators during this study, but we thought the exclusion approach as a confirmation step to prevent vertebrate predators from consuming the seeds, which was ensured by no damaged or missing seed cards. This would indicate the estimations of seed predation obtained through the mesh were indicative of invertebrate seed predation. Further, the literature showed invertebrate seed predators, e.g., carabid beetles known as a main cause of weed seed predation (Westerman et al. 2003), consume weed seeds in the laboratory (Saska et al. 2019). Also, many studies confirmed the superior importance of invertebrate seed predators in seed predation compared to vertebrates. Thus, its justifiable to consider the presented results as a participation of invertebrates' predators in the observed seed predation levels.

2.5 Assessment of weed seed predation

2.5.1 Inside maize field

Using of 4 relevant weed species in maize fields: *A. artemisiifolia, D. stramonium, C. album and E. crus-galli*, two sampling rounds of seed exposure to invertebrate seed predation were performed in a maize field in autumn November 2019 and October 2020, before harvest. A total of 100 seed cards were placed horizontally on the ground surface inside the maize field, at 10 m from the field edge, along 25 transects, with 4 cards/transect (1 card for each species), 10 m between transects, 20 seeds/card, and 1 m between cards. The exposure periods lasted for 7 days (1-7 November) in 2019 and for 5 days (22-26 October) in 2020, because of unfavourable climatic conditions (continuous rain fall) in the last days of field exposure which made the sampling process difficult, that resulted in different exposure lengths.

2.5.2. Inside winter wheat field

For winter wheat trail, different 3 weed species were evaluated: *G. aparine* L., *P. rhoeas* L., *and A. spica-venti* L. The sampling rounds were performed twice in a winter wheat field and the adjacent SNH, in June of 2019 and 2021, prior to crop harvest and after the seed ripening of the assessed weed species. The experiment was design as follows: a total of 240 seed cards (120 per round) with 60 seed cards were placed horizontally on the soil surface both inside the wheat field, the 60 seed cards were arranged along 20 transects, and 20 seed cards in the adjacent SNHs, with 3 cards per transect (1 card for each weed species), distanced by 10 m between transects, and 1 m between cards. The sampled SNH was deemed to be that habitat wider than one meter adjacent to the crop field (small forest patches). the exposure lengths differed, lasting for 5 days (23-27 June) in 2019, and 6 days (25-30 June) in 2021.The number of seeds remaining on each card was recorded every 24 hours, beginning 24 hours after the first day of field exposure. Small size weed seeds e.g., *P. rhoeas* were counted by using a manual magnifier.

Identifying the optimum exposure period for estimating weed seed predation levels was challenging, as most of the relevant previous studies assessed seed predation during long term exposure periods, ranging from a couple of weeks to several months (Ichihara et al. 2021; Deroulers et al. 2019). In maize study, the seed consumption estimations of day0-day3 and day0-day4 were analysed as an example to estimate seed predation levels, because there were fewer remaining seeds on the last days of field exposure. That led us to predict that the suitable exposure period for estimating weed seed predation in maize fields could be between day 3 and 4. Whereas in winter wheat study, the data on seed predation from day0 to day2 (48-hour exposure period) were used to achieve accurate estimates of seed predation levels. This has supported the finding that a suitable exposure time for estimating weed seed predation in winter fields could be as 2 days (48 hours) after first field exposure.

2.5.3. Seed predation of weed species in-fields vs. semi-natural habitats

As seed predation is thought to vary due to weed species and crop type. We thus aimed to compare seed predation levels between weed species inside field crops in maize and winter wheat fields and in the adjacent SNHs. For this comparison three seed cards were placed at 20 transects in winter wheat field and the adjacent SNHs (total 60 seed card), each seed card containing of 20 seeds of each of the following weed species: *A. spica-venti* L., *G. aparine* L., *P. rhoeas* L. The same method was applied in maize field and in adjacent SNHs, but for different weed species: *A. artemisiifolia* L., *C. album* L., *D. stramonium* L., *E. crus-galli* L. The data of both in-fields and in the adjacent SNHs were combined and analyzed to investigate if seed predation will differ base on habitat types or not.

2.5.4. Assessment of seed predation on A. artemisiifolia weed species

Considering the importance of the common ragweed species as a serious weed difficult to control by the common weed control methods, we thus intended to examine whether the ecosystem service of weed seed predation of *A. artemisiifolia* can be a viable alternative for regulation of *Ambrosia artemisiifolia* weed species. Seed predation patterns on *A. artemisiifolia* were estimated inside a winter wheat field in summer (23-27 June 2019, and 25-30 June 2021), and in a maize field in autumn (1-7 November 2019 and, 22-26 October 2020), and in the adjacent SNHs before crop harvest. Using a total of 160 seed cards, 4 sampling rounds of *A. artemisiifolia* weed seeds exposure to invertebrate seed predators were performed. Per sampling round/year, a total of 40 seed cards were placed on the soil surface, with 20 seed cards inside the crop field and 20 in SNHs, 10 m from the field border. Twenty fresh seeds of *A. artemisiifolia* were attached to the sandpaper with the adhesive glue, then covered by the metal wire meshes as a vertebrate exclusion strategy.

For all seed predation estimations including *Ambrosia* measurements, the number of remaining seeds on each card was counted every day directly in the field. The proportion of seed predation was estimated by measuring removal rate of weed seeds starting at 24 hours after field exposure.

The number of seeds remaining on the cards was converted into a proportion of seed predation relative to the total number of glued seeds using Abbott's correction formula (Abbott, 1925):

$$Mi=(Ci-Ri)/Ci * 100$$

Mi = proportion of seed predation during exposure

Ri = number of remaining seeds on the cards

Ci = number of total glued seeds

2.6 Data collection and statistical analysis procedure

Data collection includes the number of seeds consumed and seeds remaining after 5 and 7 days in the maize field and the Adjacent SNHs. Number of remaining seeds on cards is mostly influenced by human bias and error factors during sampling. For example, in our data, 3.2% of the total records were higher than those recorded on the previous day. Specifically, we observed number of 38 cases of negative consumption on day 5 and 6. However, those cards were included in the statistical analysis, because the consumption data of day0-day3 and day0-day4 were analysed to estimate seed predation levels because there were fewer remaining seeds on the last days of field exposure. The same data were collected for both; winter wheat fields and in the adjacent SNHs after 5 and 6 days of exposure in the field, and in the case of *Ambrosia,* the exposure period lasted for 5, 6 and 7 days. The explanatory variables were integrated into linear models together and separately. The dependent variable was the percentage of seed loss (seed predation rate %), while the independent variables were: season, exposure time, habitat type (crop, SNH).

The statistical data analyses were performed using R statistical software (version: 4.1.1, R Development Core Team 2021), including Wilcoxon test, linear models, and single-factor analysis of variance (ANOVA). Tukey test was performed for comparison between groups. Binomial models were fitted and validated at seed and card levels to compare seed predation between weed species and years (full model: comparing seed consumption across weed species and years). Diagnostic plots were plotted to investigate the interactions between weed species and years and to ensure model fit assumptions (Faraway, 2016).

To compare weed seed predation levels between the two habitat types (crop field vs SNHs), we analysed seed predation 72 h after exposure, as seed loss rates reached 80% during this period, which allowed us to study all seed loss in a uniform way. For this comparison, binomial generalized linear models (GLM) were fitted. We then calculated the mean 72-h seed loss for each weed species at each habitat for each of the crop and SNHs, paired them by habitat (n = 14 pairs), and used a paired t-test to examine the potential effect of semi-natural habitats at the 95% confidence level.

3. RESULTS

3.1 Assessment of weed flora inside crop fields and in the adjacent SNHs

The percentages of weed cover was determined by visual estimation (using sampling quadrate method) for each weed species frequently occurred in all studied quadrates inside winter wheat field. Weed species *Polygonum aviculare*, resulted in highest cover (59%), followed by *Cirsium arvense* with (40%), then *Ambrosia artemisiifolia* (33%), *Convolvulus arvensis* (26%), *Stachys annua* (13%), and the lowest cover percentages was reported by *Chenopodium album* (9%) *Consolida regalis* (8%).
Weed species were also recorded in all quadrates in the adjacent SNHs of winter wheat field. Weed species *Avena fatua, Fallpia convolvulus,* and *Polygonum aviculare, Solidago gigantea* were the most frequent species in the adjacent SNHs, followed by *Ambrosia artemisiifolia, Conyza. canadensis, Tripleurospermum inodorum* and *Stenactis annua.* While the lowest frequency was recorded by *Setarai pumila and Geum urbanum.*

3.2. Weed seed predation in maize field in 2019 and 2020

The results revealed seed predation on all seed cards placed inside maize field during the exposure periods in both years. 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. Also, there was 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 seemed different in the two years, similar pattern was observed for the four weed species. This pattern, therefore, was used to select 3- and 4-days long exposure time, from day 0 to day 3 and day 4, 72 and 96 hours, for further analysis.

Furthermore, there were significant differences ($p < 0.001$) in seed predation levels across the years, with predation levels significantly higher in 2020 than in 2019, while the differences in seed predation levels were not significant ($P = 0.962, 0.079$) in 2019 and 2020, between weed species. Where, all weed seeds were predated at similar rates, with no large differences in the numbers of predated seeds.

3.3 Weed seed predation in wheat field and in SNHs in 2019 and 2021

Seed consumption was observed in each seed card (100% of the cards) placed inside winter wheat field and in adjacent SNH in both years: 100% consumption was observed in 70% -100% and 30%-58% of cards during the exposure periods of 2019 and 2021. There were significant differences on seed predation levels between the two years ($p<0.001$) narrowing the options of the exposure period of joint statistical analysis. Therefore, we used data from day 2 (48 hours of exposure) to quantify and compare seed predation among weed species and habitat types with statistical model fitting.

The binomial model of the weed seed consumption data showed significant differences among weed species, studied years, and their interactions ($p < 0.001$). There were no differences between the habitat types ($p=0.802$), interaction of weed species and habitats ($p=0.353$), or the interaction of habitats and years ($p=0.842$). Seed consumption was more intensive in 2019 than in 2021 in both habitats. *Papaver rhoeas* had the highest consumption levels ($p<0.001$ compared to the two other species), followed by *Apera spica-venti* ($p<0.001$), and the lowest consumption rate was observed in *Galium aparine* in SNH after 48h consumption.

3.4. Compare weed seed predation inside crop fields versus SNHs

To compare seed predation patterns among habitats, we analysed seed predation after 72 h exposure of all sampling rounds. The tested weed seeds resulted in a variable rate of predation during the 72-h exposure, with an average seed consumption of 83% (min: 60% / max: 100%) in winter wheat field, 60% (min: 35% / max: 80%) in maize field, and 71% (min: 45% / max: 100%) in the SNHs.

For winter wheat trial, there was significant difference ($p < 0.01$) in seed predation of each weed species inside the field and in the adjacent SNHs, more obvious in 2019 than 2020, seed consumption was higher on *Papaver rhoeas*, followed by of *Apera spica-venti*, and then *Galium aparine*.

While, there was no significant difference ($p = 0.23$) between seed predation level inside winter wheat field and in adjacent SNHs. While, in maize, there was no significant difference ($p = 0.22$) between weed species in the number of weed seeds remaining on seed cards. Seeds of weed species *Datura stramonium* showed similar levels of seed loss compared to seeds of *Chenopodium album*, *Echinochloa crus-galli*, and *Ambrosia artemisiifolia*.

When comparing the results obtained in maize field in autumn with those of winter wheat in summer, it was found that seed predation was higher in summer than in autumn. Moreover, seed cards placed in the adjacent SNHs had nearly the same level of seed consumption with no significant difference (p= 0.22) compared to those inside maize field. When the results of seed consumption in wheat and maize fields and in adjacent SNHs were combined, no difference (p = 0.14) was detected in weed seed predation levels between both. Thus, the variability in seed predation was not explained by habitat type.

3.5 Seed predation of *Ambrosia artemisiifolia* in wheat and maize fields and in SNHs

The results revealed that there was high seed predation in all seed cards placed inside crop fields and in SNHs, during the exposure periods with an overall consumption average of 95.2% (± 8.5%). Seed consumption was higher inside fields in wheat field in summer (2019: 99 ± 2%), than in maize field in autumn (2019: 81.7±16%), with slight difference between SNHs and inside fields. Further, there was high increase in the number of consumed seeds from the first day of exposure periods. Then decreased over days 3, 4, and 5 due to the low number of remaining seeds at the end of field exposure. Seed consumption on *Ambrosia* seeds was observed inside crop fields and in SNHs during 4 sampling rounds in both seasons, after 72 hours (3 days) after field exposure. Around 50% or more of the remaining seeds were consumed in the first day of field exposure and the rest were consumed consequently until the end of the exposure period where all seeds were consumed.

The consumption rate of *Ambrosia* seeds was highest (80-100%) in summer of 2019 in winter wheat in both habitats, higher (60-75%) in summer 2021, while the lowest seed consumption was obtained in maize field and in SNHs in autumn of 2019 and 2020, only higher in SNHs than in maize field in 2019. The results further showed that seed consumption slightly higher but not significantly different in SNH habitats than in crop filed habitats during the 4 rounds, despite the exposure periods were different in each round. years.

4. DISCUSSION

4.1 Weed seed predation in maize field in 2019 and 2020

Field experiments were performed in a maize field to measure the invertebrate seed predation levels of relevant weed species in maize fields, by estimate seed removal of artificially exposed weed seeds for short periods similarly as reported by Davis et al. (2011).

First: our results showed high patterns of weed seed predation on the soil surface due to seed consumption by seed predators. This result could be supported by similar local scale studies performed across Europe showed actual levels of weed seed predation on the soil surface (Carbonne et al. 2020). The high seed predation levels confirm our initial hypothesis that the examined weed seeds might be consumed by the relevant seed predators. Further, these results agreed with the findings of (Menalled et al. 2000) where seed predation described as a major cause for seed losses on the soil surface. Besides, this finding is concurred with those reported by Jonason et al. (2013) who found high seed predation rates on weed species (*Stellaria media* (L.) and other weed species in cereal fields in Sweden. These high levels of seed predation could be attributed to the positive relations between the ecosystem service seed predation, and the invertebrate predators' activity density. According to Sarabi (2019) who stated that seed predation is a potential biological control process limits weed population densities and growth, here, although no data were collected on weed populations, but we expect that the population densities and growth of the assessed weed species might be decreased due to the high estimated levels of seed predation by our study.

Secondly, our results showed that seeds of all tested weed species were similarly consumed with no significant difference in the number of consumed seeds inside maize filed and in SNHs. Our finding is in line with those of Gaba et al. 2019 who mentioned that seed predation levels vary due to weed species considering the morphological and physiological characteristics and the nutrient value of the seeds. The results further shows that there were significant differences in seed consumption rates between the studied years.

4.2 Weed seed predation in wheat field and in SNHs in 2019 and 2021

In winter wheat we investigated seed predation levels following the same methodology as in maize case, but on three different weed species including: *G. aparine*, *P. rhoeas*, and *A. spica-venti*. Results showed the potential of weed seed predation on soil surface inside the field and in the adjacent SNHs in both years, where soil dwelling arthropods may have played a significant role by consuming weed seeds. This result is consistent with the findings of Carbonne et al. (2020) which showed relevant patterns of weed seed predation on soil surface. We certainly found high intensity (100%) of seed predation on almost all the seed cards placed in both habitats: this indicates the potential contribution of invertebrate seed predators on consuming the assessed weed seeds. This result agreed with those reporting that invertebrates were described as the most important seed predators' group (Mauchline et al. 2005), while vertebrates were significant in others.

As seed predation is an important depletion factor for weed seedbanks, reporting the total seed loss from 32-70% on weed species *Lolium multiflorum* in organic wheat fields, in Netherlands. In this context, and although we have not evaluated the impact of seed predation on the seed bank of the examined weed species, we expect that the seed banks of the tested weed species will be reduced due to the high levels of seed predation rates observed in wheat filed.

Seed predation levels were expected to differ due to weed species and during time; our study confirmed this by showing significant differences in seed predation levels among weed species across years. This finding agreed with those of Gaba et al. (2019) who reported that seed predation levels vary due to weed species. Meiss et al. (2010) also found different seed predation rates between different weed species; highest on *Viola arvensis*, then *Alopecurus myosuroides*, and lowest on *Sinapis arvensis*.

Our results showed that habitat type, SNHs vs in-field, did not influenced seed predation levels; seed cards placed in the adjacent SNHs have received nearly similar levels of seed predation with no significant difference compared to those inside maize field. When the results of seed consumption in wheat and maize fields and SNHs were combined, no difference was found between the two habitats. Thus, the variability in seed predation was not explained by habitat type, agreeing with that reported by Ichihara et al. (2011) who found similar seed predation patterns at field edges to those in the field interior areas.

Observing similar patterns of seed predation in both habitats was contrary to our expectations as we assumed seed predation will vary due to habitat types. That could be due to the presence of same individuals/communities of seed predators in both habitats, or both habitats were comfortable for seed predators which resulted in similar rates of seed consumption. Whereas, Navntoft et al. (2009) reported that vegetation cover at the field edges did not affect seed consumption, as seed predation was decreased when moving from field edge to the interior. Jacob et al. (2006) also found higher seed predation rates in the field borders near to the adjacent habitats than those in the field centre. However, our results are inconsistent with the findings of González et al. (2020), who observed different levels of seed predation across habitat types.
This study although measured seed predation in wheat field and SNHs in a narrow period (48 h after field exposure), in each of the two years, the over all findings indicated the potentiality of seed predation on the examined weed species in both habitat types, and showed acceptable level of validity, and consistency with the previous findings. Therefore, our results could act as a new finding of short-term estimations on weed seed predation in winter wheat fields and adjacent SNHs in Hungary, as well as a base for future research on this viable ecosystem service of weed seed predation.

4.3 Weed seed predation inside crop fields versus SNHs

When comparing the results obtained on maize and winter wheat field, and in SNHs, there was a difference in seed predation levels among weed species only inside wheat field, while no significant difference inside maize field nor in the adjacent SNHs. That means crop type may influence seed predation levels. These results are in line with those of Fox et al. (2013) who reported that seed predation levels are differed due to crop type. This difference could be due to the differences in crop structure and management practices which directly affected invertebrate seed predators' activity and density, and seed predation process accordingly. For instance, each arable crop has specific agronomic characteristics and cultural and management practices that influence seed predators' activity and later seed predation.
Our study further showed crop seasonality plays an important role in seed predation; e.g., seed predation rates were higher in summer rather than in autumn, when weed seed predators were more active, this like the case of the Italian ryegrass where seed losses found to be 32-70% in summer in organic wheat fields in the Netherlands due to the predation process.

4.4. Seed predation of *A. artemisiifolia* in wheat and maize fields and SNHs

Observed of high patterns of seed predation on *A. artemisiifolia* on the soil surface, support the findings reported across Europe on seed predation (Carbonne et al. 2020), and confirm our hypothesis that *A. artemisiifolia* seeds might be consumed by the abundant seed predators. Our results showed high seed predation rates in summer season in consistence with those found by González et al. (2020), as they mentioned seed predation may increase in summer season, but differ between habitats types. That agrees with our study which found seed predation levels were a bit different between habitat types, being slightly higher in SNHs, but not significantly different from those observed inside crop fields. This is possibly because the field boundaries constitute important and safe habitat for seed predators. However, Ichihara et al. (2011) found that the degree of seed predation in the field edges is like that in the field interior areas. Yet, these findings are contrasting with a previous result by (Saska et al. 2008), showed less levels of seed predation near to the field edges. That because seed density can be high at field boundaries, that makes seed consumers less hungry and not attracted to the exposed seeds.

Overall, our current results could be counted as a new reported case of invertebrate seed predation on *A. artemisiifolia* and the other serious weed species assessed in maize and winter wheat and in adjacent SNHs in Hungary. These findings confirm the potential of weed seed predation contributing to sustainable weed control. Support of weed seed predators and enhance weed seed predation requires minimize the intensive field management practices, design more diversified cropping systems at both field and landscapes and availability of weed seeds as a food source for seed predators. From the farmer's point of view, it is important to explain how weed seed predation can reduce weed-related crop losses and protect crop yield, while would fill the research gab on describing the relationship with crop yield.

5. CONCLUSIONS AND RECOMMENDATIONS

- This study presents promising results on the potential of weed seed predation inside the fields and in adjacent SNHs, to achieve sustainable weed control.
- **In maize field**, results found significant levels of seed predation (100% of the cards were affected, where 86% of seeds were predated), on the evaluated weed species *A. artemisiifolia, D. stramonium, C. album* and *E. crus-galli*.
- The observed seed predation levels were varied over years (with higher rates in 2020 than in 2019) rather than between the weed species. The optimal exposure period for measuring the weed seed predation in maize field was identified to be between 3 and 4 days after field exposure.
- **In winter wheat field** the results reported weed seed predation in all investigated seed cards inside wheat field and in the adjacent SNHs. However, there was a difference in seed predation levels among weed species only inside the crop field. Despite, relatively short period of time (48 h of exposure) as one measurement for every year was identified to measure seed predation levels, the findings showed seed predation reducing the number of exposed seeds on the soil surface, thus decreasing the soil weed seed bank, and the number of germinated weed seedlings next cropping season.
- Comparing the data from maize field to those of winter wheat field, differences in seed predation levels were observed between weed species only in wheat field, while no differences were found in maize field or in the adjacent SNHs. Thus, the variability in weed seed predation levels was not explained by habitat type, since the combined assessment of seed consumption data from maize and winter wheat fields, and the adjacent SNHs, showed no difference between the two habitats in seed predation%.
- **In *Ambrosia* case**, the study found high seed predation rates in all seed cards inside crop fields and in the adjacent SNHs, during the exposure periods of 5, 6 and 7 days in 2019, 2020 and 2021, in both seasons autumn and summer. However, consumption levels were largely higher in summer season in wheat fields rather than autumn in maize fields, and slightly higher but not significantly different in SNHs more than in crop filed habitats.
- It worth that future research should study the impact of weed seed predation on crops yield, and design of cropping systems that enhance weed seed predation while maintaining crop yields.
- Studies should also place this ecosystem service into temporal scales (crop sequence/cropping system) to identify the best options for IWM.

- Further studies are required on the conservation and sustaining of seed predation and to confirm the identity of the seed predators involved.
- Biological weed management studies should focus of combined effects of pre and post dispersal weed seed predation to investigate the influence on weeds population dynamics relevant to the different predator groups.

6. NEW SCIENTIFIC RESULTS

1. **In maize field:** high weed seed predation rates were observed on all seed cards in both years with an overall seed predation average of 85.9%.
2. The optimum exposure period to measure weed seed predation in maize field was identified 3 or 4 days after exposure (first record in Hungary).
3. Seed predation levels observed in maize field did not differ between weed species in both years, while significant differences in seed predation levels were observed over the years.
4. **In winter wheat field** and adjacent SNHs: high intensity (100% consumption) of seed predation was observed in (70% -100% and 30%-58%) of the cards during both years (2019 and 2021). However, seed predation rates were significantly higher in 2019 than in 2021.
5. The period 48 h after field exposure was identified to measure weed seed predation levels in winter wheat field (first report in Hungary).
6. Significant differences in seed predation were observed in wheat field among weed species, years, and their interactions. While, no differences were found between habitat types, interaction of weed species and habitats.
7. The variability in seed predation rates was not explained by habitat type, as no differences were found between the fields and the adjacent SNHs.
8. **In *Ambrosia* case**: there was high seed predation rates in all seed cards placed inside crop fields and SNHs, during 5, 6 and 7 days, each year, and seasons, where all *Ambrosia* seeds were consumed with an overall consumption average of 95.2% (±SD 8.5%).
9. Seed consumption was largely higher in winter wheat fields in summer rather than maize fields in autumn, and slightly higher but not significantly different in SNHs more than in crop field.

PUBLICATIONS RELATED TO THE DISSERTATION

Articles in an English peer-reviewed journals

- **Osman, M.G.A.**, Szalai, M., Zalai, M., Dorner, Z., Kiss, J. (2022) Assessing the Importance of Natural Regulating Mechanisms in Weed Management: The Case of Weed Seed Predation in a Winter Wheat Field and in Adjacent Semi-Natural Habitat in Northern Hungary. Agronomy 2022, 12, 2666. https://doi.org/10.3390/agronomy12112666

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

Peer reviewed article in Hungarian language

- Dorner Z., **Osman M.G.A**., Szalai M., Zalai M. (2022) Gyommag-predáció, mint ökoszisztéma-szolgáltatás felmérése őszi búza- és kukorica táblákban, valamint a szomszédos féltermészetes élőhelyeken. NÖVÉNYVÉDELEM (0133-0829): **83** 58 pp 358-363.

Other publication

- Dorner Z., **Osman M.G.A.**, Zalai M. (2022) Gyomirtás helyett gyomszabályozás: alig ismert segítőink a gyommagfogyasztók AGROFÓRUM EXTRA 94 pp. 62-63., 2 p. (2022).

International/national conference proceedings

- **Osman M.G.A.,** Szalai M, Zalai M., Dorner Z., Kiss J. (2022) *Ambrosia artemisiifolia* seed predation levels in Hungarian arable fields and adjacent semi-natural habitats: a key ecosystem service for weed management In: M. Thibaudon, D. Magyar, T. Szigeti, G. Kazinczi, T. Kőmíves, Z. Botta-Dukát, L. Orlóci, L. Makra (eds.) RAGWEED: A SUCCESSFUL STORY: Tackling Ragweed: a multidisciplinary and international approach Conference: Budapest, Hungary 2022.09.08. - 2022.09.09.: pp 33-33 (2022) | Conference paper (Abstract) Published by Ecocycles, Vol. 8, No. 3. Special issue, pp. 6-68. final program and book of abstracts of the conference of the international ragweed society 8-9 September, 2022, Budapest, Hungary DOI https://doi.org/10.19040/ecocycles.v8i3.242

- **Osman M.G.A.**, M. Szalai, M. Zalai, Z. Dorner (2021) Assessment of post-dispersal seed predation of *Ambrosia artemisiifolia* as an ecosystem service contributing to integrated weed management in Hungary Goran Malidza (eds.) 11th Weed science congress and symposium of herbicides and growth regulators Conference: Palić, Palics, Srbija 2021.09.20. - 2021.09.23: Herbolosko Društvo, pp 59-60 (2021) Book link (s): ISBN: 9788691196554 Publication: 32660076 Published Book: 32290286 Core Chapter in Book (Abstract)

- **Osman M.G.A.**, Pintér O., Zalai M., Dorner Z. (2021) Assessment of weed flora in organic and conventional sunflower (*Helianthus annuus* L.) fields in Jászság region in Hungary. Poster abstract published 67. Növényvédelmi Tudományos Napok, Budapest, február 16-17, 2021. ISSN 0231 2956 page 48

7. REFERENCES

ABBOTT, W. S. (1925): A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, *18* (2), 265-267.

BEGG, G.S., COOK, S.M., DYE, R., FERRANTE, M., FRANCK, P., LAVIGNE, C., LÖVEI, G.L., MANSION VAQUIE, A., PELL, J.K., PETIT, S., QUESADA, N., RICCI, B., WRATTEN, S.D., BIRCH, A.N.E. (2017): A functional overview of conservation biological control. *Crop Protection*. (97), 145 –158. https://doi.org/10.1016/j.cropro.2016.11.008.

BOHAN, D.A., BOURSAULT, A., BROOKS, D.R., PETIT, S. (2011): National-scale regulation of the weed seedbank by carabid predators. *Journal of Applied Ecology*, 48, 888 –898. https: //doi.org/10. 1111/j.1365-2664.2011.02008. x.

CARBONNE, B., BOHAN, D. A. & PETIT, S (2020): Key carabid species drive spring weed seed predation of *Viola arvensis*. *Biological Control* 141, 104148.

DAEDLOW, D., WESTERMAN, P.R., BARAIBAR, B., ROUPHAEL, S. & GEROWITT, B. (2014): Weed seed predation rate in cereals as a function of seed density and patch size, under high predation pressure by rodents. *Weed Research*, 54 (2), 186– 195. https://doi.org/10.1111/wre.12066.

DAVIS, A. S., DAEDLOW, D., SCHUTTE, B. J., & WESTERMAN, P. R. (2011): Temporal scaling of episodic point estimates of seed predation to long-term predation rates. *Methods in Ecology and Evolution*, 2 (6), 682–890.

DEROULERS, P., & BRETAGNOLLE, V. (2019): The consumption pattern of 28 species of carabid beetles (Carabidae) to a weed seed, *Viola arvensis*. *Bulletin of entomological research*, 109 (2), 229-235.

FAO, (2021): Food and Agriculture Organization of the United Nations Statistics Division. *Rome.*
FAO, (2020): Food and Agricultural Organization. FAOSTAT. http/www.faostat.fao.org/

FARAWAY, J. J. (2016): Extending the linear model with R: generalized linear, mixed effects and nonparametric regression models. *Chapman and Hall/CRC.*

FOX AF, REBERG-HORTON SC, ORR DB, MOORMAN CE, FRANK SD. (2013): Crop and field border effects on weed seed predation in the south eastern US coastal plain. *Agriculture, Ecosystem, Environment* 177:58–62.

GABA, S., DEROULERS P., BRETAGNOLLE F., BRETAGNOLLE V. (2019): Lipid content drives weed seed consumption by ground beetles (Coleopterea, Carabidae) within the smallest seeds. *Weed Research*, 59: 170–179. doi:10.1111/wre.12354.

GALLANDT, E.R., MOLLOY T., LYNCH R.P. AND DRUMMOND F. A. (2005): Effect of cover-cropping systems on invertebrate seed predation. *Weed Science*. 53, 69–76.

GARREN, J. M., & STRAUSS, S. Y. (2009): Population-level compensation by an invasive thistle thwarts biological control from seed predators. *Ecological Applications*, 19 (3), 709-721.

GONZÁLEZ, E., SEIDL, M., KADLEC, T., FERRANTE, M., & KNAPP, M. (2020): Distribution of ecosystem services within oilseed rape fields: Effects of field defects on pest and weed seed predation rates. *Agriculture, Ecosystems & Environment*, 295, 106894.

HATVANI, A., KÁDÁR, F., KISS, J., PÉTER, G. (2001): Habitat preference of carabids (Coleoptera: Carabidae) in Central Hungary in winter wheat field and adjacent habitats, *IOBC/wprs Bulletin*, 24 (6): 87-90.

HONĚK, A., MARTINKOVA Z., JAROSIK V. (2003): Ground beetles (Carabidae) as seed predators. *European Journal of Entomology*, (4) 100: 531–544.

ICHIHARA, M., MARUYAMA, K., YAMASHITA, M., SAWADA, H., INAGAKI, H., & ASAI, M. (2021): Quantifying the ecosystem service of non-native weed seed predation in traditional terraced paddy fields. *Weed Biology and Management*, 21(4), 192– 201. https://doi.org/10.1111/wbm.12238.

ICHIHARA, M., MARUYAMA, K., YAMASHITA, M., SAWADA, H., INAGAKI, H., ISHIDA, Y., & ASAI, M. (2011): Quantifying the ecosystem service of non-native weed seed predation provided by invertebrates and vertebrates in upland wheat fields converted from paddy fields. *Agriculture, ecosystems & environment*, 140 (1-2), 191-198.

JACOB, H., MINKEY, D., GALLAGHER, R., & BORGER, C. (2006): Variation in post-dispersal weed seed predation in a crop field. *Weed Science*, 54 (1), 148-155. doi:10.1614/WS-05-075R.1.

JONASON, D., SMITH H.G., BENGTSSON J. AND BIRKHOFER K. (2013): Landscape simplification promotes weed seed predation by carabid beetles (Coleoptera: Carabidae). *Landscape and Ecology*. 28, 487–494.

KISS, J., KÁDÁR, F., KOZMA, E. & TÓTH, I. (1993): Importance of various habitats in agricultural landscape related to integrated pest management: a preliminary study. *Landscape and Urban Planning* 27: 191-198.

KISS, J., KÁDÁR, F., TÓTH, I., KOZMA, E., TÓTH, F. (1994): Occurrence of predatory arthropods in winter wheat and in the field edge. *Ecologie*, 25 (2), 127-132.

KOLB, A., EHRLEN, J., & ERIKSSON, O. (2007): Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspectives in Plant Ecology, Evolution and Systematics,* 9 (2), 79-100.

LEHOCZKY, É., MÁRTON, L., & NAGY, P. (2013): Competition for nutrients between cold-tolerant maize and weeds. *Communications in soil science and plant analysis*, 44 (1-4), 526-534.

MAUCHLINE, A. L., WATSON, S. J., BROWN, V. K., & FROUD-WILLIAMS, R. J. (2005): Post-dispersal seed predation of non-target weeds in arable crops. *Weed Research*, 45 (2), 157-164.

MEISS, H., LE LAGADEC, L., MUNIER-JOLAIN, N., WALDHARDT, R., & PETIT, S. (2010): Weed seed predation increases with vegetation cover in perennial forage crops. *Agriculture, ecosystems & environment*, 138 (1-2), 10-16. https://doi.org/10.1016/j.agee.2010.03.009.

MENALLED, F. D., MARINO P.C., RENNER K. A., & LANDIS D.A. (2000): Post dispersal weed seed predation in Michigan crop fields as a function of agricultural landscape structure. *Agriculture, Ecosystems and Environment*, 77, 193–202.

NAVNTOFT, S., PETERSEN, B.S., ESBJERG, P., JENSEN, A.M., JOHNSEN, I., KRISTENSEN, K., PETERSEN, P.H., ØRUM, J.E., (2007): Effects of mechanical weed control in spring cereals-flora, fauna and economy.

NORSWORTHY, J. K., WARD S. M., SHAW D. R., LLEWELLYN R. S., NICHOLS R. L., WEBSTER T. M., & BARRETT. M. (2012): Reducing the risks of herbicide resistance: best management practices and recommendations. *Weed science*, 60 (SP1), 31-62.

OERKE, E. (2006): Crop losses to pests. *The Journal of Agricultural Science*, 144: 31–43, DOI 10.1017/S0021859605005708.

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 Protection Science*, 58: 351–359.

POWLES, S. B., & YU, Q. (2010): Evolution in action: plants resistant to herbicides. *Annual review of plant biology*, 61, 317-347.

SASKA, P., HONĚK, A. & MARTINKOVÁ, Z. (2019): Preferences of carabid beetles (Coleoptera: Carabidae) for herbaceous seeds. *Acta Zoology. Academic. Science. Hungary*. 65, 57–76

SARABI, V. (2019): Factors that influence the level of weed seed predation: A review. *Weed Biology and Management*, 19: 61-74. https://doi.org/10.1111/wbm.12186.

SASKA, P. (2008): Effect of diet on the fecundity of three carabid beetles. *Physiological Entomology*, 33 (3), 188–192. https://doi.org/10.1111/j.1365-3032.2008.00618.x.

STRAW, E.A., CARPENTIER, E.N. & BROWN, M.J.F. (2021): Roundup causes high levels of mortality following contact exposure in bumble bees. *Journal of Applied Ecology*, 58 (6), 1167–1176. https://doi.org/10.1111/1365-2664.13867.

WESTERMAN, P. R., BORZA, J. K., ANDJELKOVIC, J., LIEBMAN, M., & DANIELSON, B. (2008): Density-dependent predation of weed seeds in maize fields. *Journal of Applied Ecology*, 45 (6), 1612-1620.

WESTERMAN, P. R., WES J. S., KROPFF M.J., VAN DER WERF W. (2003): Annual losses of weed seeds due to predation in organic cereal fields. *Journal of Applied Ecology*, 40, 824-836.

WESTERMAN, P.R. (2005): Are many little hammers effective? Velvetleaf (*Abutilon theophrasti*) population dynamics in two- and four-year crop rotation systems. *Weed Science*, 53: 382–392.

ZALAI, M., Z. DORNER, L. KOLOZSVARI, ZS. KERESZTEZ AND M. SZALAI (2012): What does the precision of weed sampling of maize filed depends on? *Novenyvedelem*, 48, 451-456. (In Hungarian with English abstract).

ZHANG, L., RANA, I., SHAFFER, R.M., TAIOLI, E. & SHEPPARD, L. (2019): Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A metanalysis and supporting evidence. *Mutation Research/Reviews in Mutation Research*, 781, 186–206. https://doi.org/10.1016/j.mrrev.2019.02.001.