HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Doctoral School of Plant Sciences

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

Doctoral (Ph.D.) Dissertation

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LIST OF ABBREVIATIONS

SNHs	Semi-Natural Habitats
IWM	Integrated Weed Management
IOBC	International Organization for Biological and Integrated Control
IPM	Integrated Pest Management
QUESSA	Quantification of Ecological Services for Sustainable Agriculture
MEA	The Millennium Ecosystems Assessment
GLM	Generalized Linear Models
SD	Standard Deviation
ANOVA	Analysis of Variance

1. INTRODUCTION AND OBJECTIVES

The interference of biodiversity losses caused by the continues change in agricultural practice with ecosystem services minimized their contribution on agricultural pests e.g., weed suppression by ecosystem services. Weeds species substantially reduce crop yields, yet, they play a major role in preserving ecosystem services by supporting higher density and diversity of natural enemies such as invertebrate pests. Despite, weed control has been based on wide range of tools (mechanical, physical, agronomic, and biological), weeds are widely controlled with herbicides, that resulted in negative consequences on human health, beneficial organisms, and environment besides increase cases of herbicides resistance. In the European Union (EU), herbicides account for more than 40% of the overall consumption of an average of 179,798 tonnes/year of pesticides. Thus, eliminating the risk of synthetic pesticides 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). In line with this approach, Integrated Weed Management (IWM) programs are essential to eliminate the adverse effects of the herbicides and to create a balance of weed control, while preserving the botanical diversity of weed species and weed seed predators.

Weed seed predation is a key ecosystem service provided in agricultural fields. It is a potential biological control process causes substantial seed losses of weed species, decreasing weed populations in agroecosystems and in the adjacent semi-natural habitats (SNHs). Some invertebrates e.g., carabid species found to consume weed seeds as a major food under laboratory conditions, while others showed responses to weed seed patches in the fields. Such feeding behaviour is considered as a regulatory ecosystem service for weed management. Seed predation occurs in two forms at different times: pre-dispersal seed predation, which occurs when the seeds are still on the crop and not yet ripe, and post dispersal seed predation, which occurs on or in the soil surface or on another substrate after seed shedding. Many studies indicated the potential of invertebrate seed predators in agricultural systems, reporting their contribution to be 80 to 90 % of the total seed predation. In Hungary, the invertebrate seed predators, carabid beetles are widespread in arable fields. In addition, the occurrence of arthropod individuals, mainly Carabids, was confirmed in winter wheat fields and in the field margins (Kiss et al., 1993). Also, the peak of mixed feeder individuals of *Harpalus* and *Amara* species reported in a winter wheat field and in its borders in Northern Hungary by Kiss et al. (1994).

Weed seed losses caused by weed seed predators are substantial; however, they vary over years and across fields. Recent results in Hungary (Osman et al., 2022) showed seeds of weed species: *Ambrosia artemisiifolia, Datura stramonium, Chenopodium album,* and *Echinochloa crus-galli*, similarly consumed in maize fields, but seed consumption levels were significantly varied between years. Weed seed predation, despite causes serious seed losses of weed species contributing to weed management, but many factors affect its effectiveness. For example: the presence of semi-natural habitats (SNHs) adjacent to crop fields found to be favourable for beneficial invertebrate species seeking to overwinter and crucial for biological weed control as they host many potential weeds seed predators. Although, the importance of weed seed predation has been addressed during the last two decades, yet weed seed predation research is still in the early stages as compared to other regulating ecosystem services.

In Hungary, there is a dearth of literature and research observations on invertebrate weed seed predation in arable fields. The Department of Integrated Plant Protection at MATE University in Gödöllő, Hungary (former SZIU) performed the first surveys in winter wheat fields within the EU project Quantification of Ecological Services for Sustainable Agriculture (QUESSA) (2013 to 2017), which become the ground for subsequent research work in this field. The present study was designed based on the contribution of invertebrates' seed predators specifically activity density, key mixed feeder species, and their phenology from previous study by Kiss et al. (1993), on weed seed predation in a maize and in winter wheat field and in the adjacent SNHs, including *A. artemisiifolia* weed species. We hypothesized that weed seeds are to be predated by seed predators, but predation levels may differ by weed species and habitat types. 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.

(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. LITERATURE REVIEW

2.1. Importance of some arable crops in Hungary

Winter wheat (*Triticum aestivum*) is one of the most valuable crops and a major source of human diet worldwide (Mondal et al., 2016). The global wheat production increased from 10 889 (hg/ha) in 1961 to 35 468 (hg/ha) in 2019 (FAOSTAT, 2021). Yet, biotic, and abiotic stressors, e.g., climate change and pests, constrains the ability of global agriculture to meet wheat production needs (Iizumi et al., 2021). In Hungary, winter wheat is the second most cultivated crop, (FAO, 2020) grown on a million hectares with wide spread of artificial habitats related to the arthropod seed predator's populations (Kiss et al.,1994). Among all agricultural pests, weeds cause the greatest yield losses of 18-29% (Oerke, 2006). Also, the extensive use of herbicides reduced weeds diversity (Storkey and Neve, 2018) and imposed great selection pressure on remaining weed species to develop resistance. This situation besides changing policies on herbicide use (Kudsk and Mathiassen, 2020) forced the farmers to change their weed management strategies and integrate other nonchemical methods for weed management into wheat production systems.

Similarly, maize crop (*Zea mays* L.), is an important arable crop in Hungary, planted on about 1.3 million ha, approximately 25% of the total arable area. Weed competition, causes significant yield losses in the absence of herbicides use (Yeganehpoor et al., 2015), counting for up to 34 % of the global crop losses (Abouziena et al., 2016), and decreases crop biomass by 64% compared to weed-free maize field (Lehoczky et al., 2013). This requires to consider environmentally friendly measures and agro-ecological alternatives for weed control to reduce herbicide usage (Petit et al., 2015). Two examples are adopting weed management strategies such as biological controls and promoting ecosystem services such as weed seed predation. Accordingly, wheat and maize were selected as appropriate crops to assess the importance of weed seed predation as a tool for sustainable weed management.

2.2 Weeds of arable fields in Hungary

During the past 70 years, a huge change of weed flora was happened in arable fields, in Hungary (Novák et al., 2009). At that period there was a spread of tolerant/resistant weed biotypes. Therefore, an extensive weed surveys were performed to characterize the weed flora in some arable crops such as winter wheat and maize (**Table 1**), and to provide an answer to farmers questions e.g., which are important weed species and how to manage them? Besides, to investigate the presence of poisonous (*Datura stramonium* and *Solanum nigrum*) and allergenic weed species in those fields. The results of these surveys indicated that both crop production systems and chemical weed control have resulted in significant changes of weed flora in arable crops in Hungary. Besides, the suppression of *A. artemisiifolia*, which causes economic losses and harm effect to human health, should have the research priority by herbologist while, the prevention of further spread of other invasive species is also necessary.

Table 1. Dates of the national weed surveys in arable crops (maize and winter wheat) in Hungary (Source:https://www.yumpu.com/en/document/read/19615391/national-weed-surveys-in-hungary-r-novak-i-dancza-l-szentey).

National weed survey	Period
First	1947-53
Second	1969-71
Third	1987-88
Fourth	1996-97
Fifth	2007-2008
Sixth	2018-2019

In Hungary the information on weed flora of Maize crop are important especially with wide use of herbicides. Chemical weed control e.g., seed dressing is important for maize production in Hungary, thus play major role in change of weed flora in maize fields. According to the 5th National Weed Survey in Hungary 2007-2008 (Novák et al., 2009) the most important weed species in maize fields were: common ragweed (*Ambrosia artemisiifolia* L.), jimson weed *(Datura stramonium* L.), lamb quarters (*Chenopodium album* L.) and barnyarsd grass (*Echinochloa crus-galli* L.). Weed-crop competition especially during the early growing crop stage causes significant grain yield losses (Rajcan and Swanton, 2001; Lehoczky et al., 2005; and Yeganehpoor et al., 2015), counting to 34 % of the global crop losses (Abouziena et al., 2016), and decreases crop biomass by 64% compared to weed-free maize (Lehoczky et al., 2013). The same economic value is also applicable to winter wheat (*Triticum aestivum* L.), which is the second most cultivated crop in Hungary (FAO, 2020). The most important weed species of winter wheat fields were the followings: common ragweed (*A. artemisiifolia*), loose silky bent (*Apera spica-venti* L.), field poppy (*Papaver rhoeas* L.), cleavers (*Galium aparine* L.), and lambs quarters (*Chenopodium album*), according to the 6th National Weed Survey in Hungary (Novák et al., 2020).

2.3. Botany and importance of serious arable weed species in Hungary

2.3.1 Common ragweed (*Ambrosia artemisiifolia* L.)

Origin and distribution in Europe and in Hungary

Ragweed is native to the semi-desert areas of Arizona (USA) in the southern part of North America (Bassett and Crompton, 1975). It is distribution in Europe started after the First World War (Comtois, 1998) with grain shipments, then gradually spread out due to favourable environmental conditions. There are three main regions invaded by Ambrosia in Europe: the valley of the Rhone (France), Northern Italy and the Carpathian Basin (Juhász,1998, Rybníček and Jäger, 2001). In Hungary, Ambrosia ''Serbian grass'' as a popular name, was observed at the beginning of the 20th century at Orsova (Javorka, 1910), near the southern border of the country along the banks of the river Danube, and within 30 years it occupied the whole region and became most common weed in Hungary. It is considered as the most noxious invasive weed species in Hungary (Novák et al., 2009, 2020).

Among approximately 50 ragweed species, the three most common species in Europe are *A. artemisiifolia*, *A. psylostachia*, and *A. trifida*. In the network mapping of the plant flora atlases have been developed for Europe (Bullock et al., 2010) and for Hungary (Bartha et al., 2019; 2022). Based on the data of these atlases, *A. artemisiifoliai* found the most distributed ragweed species vs. other ragweed species in Europe (Makra et al., 2005; Bullock et al., 2010; Vinogradova et al., 2010; Páldy et al., 2006) with estimated distribution rate of approx. 90:10, while in Hungary approx. 95:5. Besides, ragweed has a higher spread potential than other indigenous annual dicotyledonous and grass-weeds species in Central Europe according to the population genetic data of Mátyás and Vignesh (2012). This situation requires high plant monitoring and implementation of an accurate control strategy. But, in many countries, the legal situation is not sufficient to monitor and manage the spread of ragweed.

According to the 5th Hungarian national weed survey (2007-2008), *A. artemisiifolia* positioned among species of agricultural weeds and increasing in abundance and spreading into the north-western parts of the country (Novák et al., 2009). The infestation was not homogeneous; as some regions were highly infested (mean cover above 10%), while others are less (mean cover under 0.5%) infested, indicating that specific environmental and land use factors might influence the abundance of this weed species in arable fields. The 6th national weed survey (2018-2019) in Hungary was carried out in winter wheat and maize crops at the end of spring and early summer. *Ambrosia artemisiifolia* was one of the most important weeds of maize fields in Hungary. While in winter wheat field it is the dominant weed in the rank of order, however it only significantly damage incomplete or underdeveloped wheat.

The invasiveness of *A. artemisiifolia* is attributed to several features of the plant. It has a wide ecological tolerance and can colonize a large range of disturbed habitats (Fumanal et al., 2008a). Its invasion is also facilitated by its large persistent seed bank (Fumanal et al., 2008b), huge plasticity in seed mass (Fumanal et al., 2007), resistance to certain herbicides (Kazinczi et al., 2008c), allelopathic effect (Kazinczi et al., 2008a,b), arbuscular mycorrhizal fungal symbiotic interactions (Fumanal et al., 2006), the lack of natural enemies (MacKay and Kotanen, 2008), the high genetic variability of invasive populations (Genton et al., 2005, Chun et al., 2010) and the high incidence of out-crossing in colonizing populations (Friedman and Barrett, 2008). *A. artemisiifolia* is dispersed predominantly by human activities, agricultural machines, soil or seed transport, water dispersal along river corridors and by birds are also documented (Bohren et al., 2006, Lavoie et al., 2007).

Botanical description

A. artemisiifolia L. **(Figure 1)**, is an annual weed species, belongs to the family Asteraceae. It is challenging, as it is a fast-propagating weed, can quickly re-grow after cutting, and occurs in many different habitats, including crop fields, road margins and natural riparian ecosystems. Seed production depends on the plant size, where small sizes produce few hundred seeds, medium size produce 3000 seed/plant, while large ones provide 6000 seed per plant (Béres and Hunyadi, 1980, Kömives et al., 2006). Seed or fruit types are Achene or a bur. The shape of both Bur and achene is obovate. The seed size is varied as follows: Bur length: 1.9 - 3.7 mm, width is 1.5 - 2.8 mm, while for Achene length: 1.5 - 3.0 mm, and width is 1.5 mm. Seeds colour is brown for achene, while bur is dark grey with purple streaks. Seed weight ranged between 2.4 mg (without fruit coat) according to (Shergill et al., 2020), and 4.4 mg (with fruit coat), or 1.7-3.7 mg according to (Buttenschøn et al., 2010). The stems are erect. Leaves opposite (proximal) and alternate, with blades lanceolate. Flowers arranged in capitula, the male capitula (5-20 flowers per capitulum, the involucre being cup-shaped) forming a terminal spike-like inflorescence, the female capitula proximal to the male ones. Fruit globose to pyriform, 2-3 mm long.

Figure 1. Plant seedling (Photo: https://extension.umass.edu/landscape/weeds/ambrosia-artemisiifolia) and seeds (Photo: Richter and Robert 2015) of *Ambrosia artemisiifolia*

Effect and importance

Hungary is one of the most highly infested country with the ragweed in central Europe (Kazinczi et al., 2008a), and considered to be the centre of ragweed invasion and a major ragweed-source for neighbouring countries (Fenesi and Botta-Dukát, 2011). The ragweed is considered as a major natural, economic, human, and environmental health problem in highly exposed countries. It is a very serious and harmful weed species, has the potential to supress other weeds due to the allelopathy and competitiveness. Furthermore, it produces large quantities of allergenic pollen, which causes severe health problems (Kazinczi et al., 2008b) and diseases more than other allergic plant species. Ragweed pollen cause severe damage to the national economy in many areas. For example, in Hungary the total damage can reach 1% of the annual GDP (Makra et al., 2014). Outpatient and hospital treatment costs for patients suffering from allergic respiratory diseases caused by ragweed pollen (e.g., hay fever, bronchitis, asthma). In addition, *A. artemisiifolia* causes significant yield losses of arable crops, especially in maize crop determined up to 54%, 62%, and 70% at weed densities of 9, 18, and 26 plant/ m^2 (Varga, 2002).

The Hungarian Aerobiological Network (HAN) was established 30 years ago by the predecessor institute of the National Public Health Center, which is coordinating the network. The analysis of the 30 years of data revealed some peculiarities of the ragweed pollen characteristics such as the increasing trend of yearly pollen load in each of the microclimatic regions in Hungary. It was expected the yearly mean pollen load will increase by 30% between 2021 and 2050 and almost double by the end of the century. In this context, and due to the current global climatic changes, *Ambrosia artemisiifolia* pollen concentrations are increasing, and the pollen season is prolonging, the number of allergic diseases is thus increasing globally, and the global public health risk is increasing. As a result, the recent conference "Tackling ragweed: a multidisciplinary and international approach: the world conference of the International Ragweed Society, 8th-9th September, 2022, Budapest, Hungary" (Thibaudon et al., 2022) was aimed to raise the attention to the growing importance of this issue, as well as to provide insight and solutions to the environmental and social challenges caused by the ragweed and its pollen. Common ragweed is also known for causing many socio-economic impacts (Bonini et al., 2016; Plank et al., 2016), thus research and practical efforts has been made to its management (Gerber et al., 2011; Plank et al., 2016). For example; cultural and mechanical management of *A. artemisiifolia* is challenging, as ragweed is fast-propagating can quickly re-grow after cutting. One important weakness of *A. artemisiifolia* species life cycle is that it multiplies only through seeds. Therefore, a simple control strategy can be formulated by prevent the formation of fertile seeds.

All control measures must be designed to prevent of fertile seed production. Any non-lethal control measure must be supplemented with other measures e.g., Integrated Weed Management (IWM) to insure the complete disruption of the ragweed life cycle. In 2013, Europe was confronted with the introduction of the North American native ragweed leaf beetle *Ophraella communa* which offer a potential biocontrol against *A. artemisiifolia* (Buttenschøn et al., 2010; Lommen et al., 2017). The beetle found mainly feeds on *A. artemisiifolia*, causing heavy defoliation and decreasing the amount of produced pollen (Lommen et al., 2017). International research work with the use of *Ophraella* as a successful biological control agent of *A. artemisiifolia* in Asia (Sun et al., 2017) and the rapid spread in Europe greatly boosted studies on the *Ophraella-Ambrosia* interaction.

2.3.2 Cleavers (*Galium aparine* L.)

Description

G. aparine (**Figure 2**) is an annual noxious species belong to the family Rubiaceae, native to Eurasia and Northwest Africa (Ilina, et al., 2019). It is known as a troublesome broadleaved weed in winter wheat fields and other winter-sown crops (Mennan and Uygur, 1994; Defelice, 2002) as a cold germinating plant preferred in autumn and early spring. In Hungary, it has been identified as one of the most important arable weed species mainly in winter cereals and oilseed rape (Novák et al., 2009). It is easy to identify before crop harvest, where the stem climbed over the crop plants pulling down the crop in case of heavy infestation, making the crop harvest very difficult. Seed type is schizocarp, the shape is varied from cylindrical or globose based on the species type with a prominent hole on the ventral side (**Figure 2**).

Figure 2. *Galium aparine* plant (Photo: https://depositphotos.com/stock-photos/galium-aparine.html) and seeds (Photo: https://www.weberseeds.de/).

Effect and importance

G. aparine is described as a serious or principal weed reported as a weed of 19 crops in 31 countries Worldwide (Holm et al., 1977 and 1991). Although the weed commonly occurs in vegetable crops, it is most troublesome in cereals, where it may cause large yield reductions causing potential yield reductions of 30-60% in cereals (Rola,1969), interfere with harvesting, cause lodging, and in some instances smother the entire crop. *G. aparine* is also found to be resistant to phenoxy acetic acid herbicides (Malik and van den Born, 1988). The extended root system results in effectively utilizes the water, and taking up the nutrients (N and K) from the crop to far distances inside the soil that lead to severely competing with the crop (Novák et al., 2009). Moreover, the species can be poisonous for animal grazing. While the leaves and stems of the plant can be cooked as a leaf vegetable if gathered before the fruits appear. They make a useful addition to vegetable soups.

2.3.3 Loose silky bent (*Apera spica-venti* L.)

Description

A. spica-venti **(Figure 3)** is an annual plant native to northern Eurasia. It is most abundant winter annual weeds in Central and Eastern Europe (Soukup et al., 2006). In Hungary, known as foxtail, silk grass, and mostly frequent on the acid soils in West, North, and North-Eastern parts. It has similar life cycle as cereals since they ripen at the same time of crop harvest period, thus become hard to identify till appearance of the spikes. Seed type is grain, enclosed by palea and lemma. The seed size with a slender awn 4-9 mm long; seed without its awn is about 1.6 mm long, the width is 0.5 mm. Seed shape is elongated oval in out line, and elliptical in cross-section **(Figure 3)**. The colour is medium brown (https://gobotany.nativeplanttrust.org/species/apera/spica-venti). Seed production is very high 600-700 seed/plant, survive 1-4 years in the soil and seed weight is (1.2 mg/seed) which has tiny size and loose panicles (Béres et al., 2005).

Effect and importance

This weed species is an important monocot weed of winter wheat fields. The harm effect appears as it germinates at the same time with the crop, developing dense plants and thus competing with crop for water and nutrients (Novák et al., 2009). It is often occurring in winter cereals, e.g., winter wheat and winter barley, but it can also cause damage to winter rape, forage crops, and early sown spring cereals. The reduction of plant numbers caused by frost or usage of short-stalk cereal varieties weakens the competition ability and may lead to higher yield losses caused by this weed species (Melander, 1995), resulting in greater need for effective control measures.

Figure 3. *Apera spica-venti* plant (Photo: https://www.inaturalist.org/taxa/75487-Apera-spica-venti/browse_photos) and seeds (Photo: https://www.weberseeds.de/apera-spica-venti.html).

2.3.4 White goosefoot (*Chenopodium album*)

Description

This leafy vegetable **(Figure 4)** is grown globally as a food and fodder crop in Asian and African nations. It is originated from Europe and introduced to Australia, North America, and Africa. In Hungary, *C. album* is common annual weed widespread everywhere in the country (Németh, 2000) not only in the open fields. It is very common in all kinds of soil in arable land and on plains, along roads, in gardens, in vineyards. Frequent in all types of crops, but mainly in cereals (Vojnich et al., 2018). The fruits are not visible and enclosed in the floral envelope. Plants have high rates of seed production**:** over 150,000 seeds/ plant (Colquhoun et al., 2001). The seed size diameter is approximately 1.5 - 2 mm, the shape is disc-shaped, round or lenticular, or lens-shaped with marginal notch **(Figure 4)**. The seed colour is black or dark, with smooth shiny surface. Seeds are tiny and plants produce two types: smaller seeds are black, while the larger are brown.

Effect and importance

C. album damage is very high, as it grows fast and does not only absorb the food and the water from the sown plant, but it completely overshadows and destroys it (Vojnich et al., 2018). *C. album* is most widely spread weed species in the world and is one of the most successful colonizers of new areas. This weed had a high nutritional composition edible leaf and seeds extensively cultivated and consumed as a food crop in Northern India, the seed is used as poultry and livestock feed (Choudhary and Sharma, 2014). It is a natural source of iron, fiber, vitamins, and protein, which helps the body gain immunity and strength. A tea made from the leaves of this plant can be used to treat lose motions and stomach pains. The plant is used in diet not only to provide minerals, fiber, vitamins, and essential fatty acids but also enhance sensory and functional value of the food.

Figure 4. *Chenopodium album* plant (Photo: http://creativecommons.org/licenses/by-sa/3.0/>) and seeds (Photo: https://www.onlyfoods.net/chenopodium-album.html)

2.3.5 Jimson weed (*Datura stramonium* L.)

Description

D. stramonium L. (**Figure 5**) is an annual plant, belongs to the Solanaceae family. The origin place is unclear, but it has been believed to be originated in South America. Seed has rounded D-shaped, compressed, and flat, or kidney-shaped with an irregularly pitted surface. Seed size as follow: length: 2.5 - 4.0 mm and width: 2.5 - 3.0 mm. The seed weight is 0.009 g/seed. Seed colour is black to dark brown for recently produced; while immature seeds are lighter. Plants may produce 1,300-30,000 seeds, which may survive up to 39 years in the soil (CABI, 2016).

Effect and importance

D. stramonium is an aggressive colonizer of agricultural fields in almost any summer crop. Crop yields losses due to competition from This weed species depend on crop type and climatic conditions. For example, its competition resulted in 56% yield loss in cotton in USA, while only 16% losses in a more competitive soyabean crop (Oliver et al., 1991). The plant is poisonous to human and animals (Royer and Dickinson, 1999). *D. stramonium* also an alternative host for several pests and pathogens of solanaceous crops. But it has valuable contents of atropine and scopolamine used in pharmaceutical industries (Das et al., 2012).

Figure 5. *Datura stramonium* plant (Photo: https://commons.wikimedia.org/w/index.php?curid=430822) and seeds (Photo: https://commons.wikimedia.org/wiki/File:Datura_stramonium-12. JPG)

2.3.6 Barnyard grass (*Echinochloa crus-galli* L.)

Description

E. crus-galli L. **(Figure 6)** is an annual C_4 T grass, native to India, it is widely distributed in tropical and temperate regions of the world (USDA-ARS, 2014). The single plant can produce from 2000 to 40000 seed/plant. Some seeds germinate immediately after harvest, while others remain dormant for 4 to 48 months. Photoperiod duration and intensity both affect seed dormancy. The seeds shape is ovoid or oblong caryopses, the size is around 1.3-2.2 mm long, and 1-1.8 mm wide, the colour is brown (Seinet, 2017). Seed weight of *E. crus-galli* averages 1.13 mg, and approximately 50% of seeds remained afloat after 4-5 d in water (Barrett and Wilson, 1983) indicating that dispersal of seeds by water is likely, particularly in flooded habitats.

Effect and importance

E. crus-galli is a grass considered one of the world's worst weeds. It reduces crop yields and cause forage crops to fail by removing 80% of the available soil nitrogen. It is a serious weed of lowland rice due the rapid growth, competitive ability, and capacity to multiply rapidly. It also acts as a host for several mosaic virus diseases. The risk of introduction of *E. crus-galli* is very high. This weed species is a cosmopolitan weed which has been introduced repeatedly in tropical and subtropical regions to be used as fodder and forage (FAO, 2014).

Figure 6. *Echinochloa crus-galli* plant (Photo: https://ukrbin.com/show_image.php?imageid=46291) and seeds (Photo: Howard F. Schwartz, Colorado State University, Bugwood.org).

2.3.7 Poppy (*Papaver rhoeas* L)

Description

Papaver rhoeas (**Figure 7**) is an annual erect plant, commonly known as corn poppy, belongs to the family (Papaveraceae). It is cosmopolitan weed plant, naturally presented in arable corn fields world wide. The origin is unknown, yet, some authors thought it is native to Europe, North Africa, and West Asia. One plant has 50,000 seeds per one day flowers in a warm season (Reader's Digest, 1981). The seeds are very tiny size and dark colour, thousands of seeds are produced once on the soil surface, and remain dormant for many years (Mabberley, 2008).

Effect and importance

The black seeds are edible either on their own or as an ingredient in bread. Oil made from the seed is highly regarded in France**.** Recently *P. rhoeas* has been included in a drug atlas (Rahfeld, 2017). This weed species infests summer grain crops; occurs in waste lands, gardens, orchards, along roads. Control measures include early ploughing, chemical weeding, removing stubble after harvest. The young leaves and flowers are a popular traditional ingredient in foods and beverages, particularly in Turkey and other Mediterranean countries. Phytochemical studies showed that the leaves of *Papaver rhoeas* contain several minerals, like potassium, sodium, and calcium. Besides, the flowers of poppy are used for medicinal purposes both internally and externally (Mabberley, 2008; Mitich, 2000).

Figure 7. *Papaver rhoeas* plant (Photo: http://luirig.altervista.org/cpm/albums/02b/001620-papaver-rhoeas.jpg) and seeds (Photo: https://www.sciencephoto.com/media/1168088/view/corn-poppy-papaver-rhoeas-seeds).

2.4. Weed management in arable fields: a general view

Weeds as one group of pests compete with field crops and thus reduce their yields and quality (Zimdahl, 2004). They are a major threat to crop production (Adeux et al., 2019), and impose an abiotic constraint on most cropping systems (Oerke, 2006) and act as competitors and intermediate hosts for plants (Barzman et al., 2015). Researchers and farmers have been focused their efforts on reducing the adverse economic effects of arable weeds for a long time. Nevertheless, arable weeds may also provide beneficial services (Fagúndez, 2014), and they also contribute to several important ecosystem services, for example, pest control and soil fertility improvement (Blaix et al., 2018; Smith, et al., 2020; Yvoz, et al., 2021; Merfield, 2022). Furthermore, weeds are often the base of agricultural food webs providing food resources to many organisms, including insect and bird species, thus they are beneficial from a conservation and an agricultural point of view (Bretagnolle and Gaba, 2015; Rollin et al., 2016; Morrison et al., 2021).

The losses in crop yields due to weeds has been amounted up to $95 US billions in agricultural sector, higher than those of plant pathogens ($85 billion), and insects $46 US billion, worldwide (FAO, 2012). In organic production systems, weed control is a big challenge especially without the use of chemical herbicides (Liebman and Davis, 2009). Weed management has been based on wide range measurements including mechanical, physical, agronomic, and biological, and more dominantly on chemical methods. Herbicides, however, effectively reduced weed populations, the overreliance on them resulted in environmental and human health problems, harmful impacts on biodiversity (Riemens et al., 2008; Storkey et al., 2012; Strandberg et al., 2017; Green, 2018) and

development of resistant weed populations (Kudsk and Streibig, 2003; Powles and Yu, 2010; Délye, et al., 2013; Annett et al., 2014). Further, herbicide resistance has reached the level where more research is needed and development of effective management strategies to preserve herbicides as essential tools of agricultural technology (Holt and Lebaron,1990). For instance, in the European Union (EU) herbicides account for more than 40% of the overall consumption of an average of 179,798 tonnes/year of pesticides (FAO, 2021), which has pushed many governments to apply strict restrictions on herbicides utilization (Reuters, 2021). Therefore, there is an urgent need to identify environmentally friendly and sustainable measures for weed management (Bohan et al., 2011). For example, weed regulation through their natural enemies e.g., seed predators has been reported as an important ecosystem service (Altieri, 1999; Losey and Vaughan, 2006).

Seed predators play a key role in weed management by consuming recently produced weed seeds on the soil surface, thus limiting weed soil seedbanks (Gallandt, 2006). Moreover, it was reported that 70-99% of newly produced weed seeds do not emerge as seedlings in the next season which reduce weed infestation (Cardina and Norquay, 1997; Gerowitt and Bodendörfer, 1998). Weed seed predation may thus be an option for chemical weed control (Bohan et al., 2011), as it reduces weed population densities in arable fields as an effective method for weed management (Petit et al., 2018; Sarabi, 2019). Therefore, herbicide reduction and associated land management changed the composition of weed communities in arable fields and thus the functions of weeds related to biodiversity and production (Petit et al., 2015). Many studies have shown that weed management can be supported through limiting weed seed densities created by seed predators.

In this context, seed predation is thought to significantly contribute to weed management. Overall, although, the substantial variability in weed seed consumption rates between studies has been an obstacle to the adoption of weed seed predation in weed management programs (Menalled et al., 2007; Saska et al., 2008; Davis and Raghu, 2010). Weed seed predation could minimize dependence on chemical herbicides and reduce environmental costs, particularly when integrated with other non chemical control methods (Liebman and Davis, 2000; Westerman et al., 2005).

2.5 Integrated Pest Management (IPM)

The integrated pest management aim to achieve economic protection from pest damage while minimizing pesticides impacts on field crops, human health, and on environment (Kogan 1998; Bajwa and Kogan 2002). For example, in the EU level, eliminating the risk of synthetic pesticides to human health and environment was phrased under the Integrated Pest Management (IPM) approach in the Framework Directive on the Sustainable Use of Pesticides (Directive 2009/128/EC, Rotteveel, 2012) that defines IPM as follows: "Integrated pest management involves the careful consideration of all available plant protection methods and then implementing appropriate measures to prevent the development of harmful organism populations, limit the use of plant protection products and other types of treatments to levels that are economically and ecologically acceptable, and lowering the risks to human health and the environment. It also supports healthy crop development with less interruption to the agro-ecosystems and facilitates natural pest control methods".

The strategies of the IPM programs takes the advantage of existing ecosystem dynamics, and involves the participation of new competing organisms or species to manage the levels and population of agricultural pests. As such, biological weed control has come into focus and raised by many researchers and scientists. According to the IOBC (International Organization for Biological and Integrated Control), biological control is widely used in modern pest management since it is the most ecologically friendly, cost-effective, long-term, and safest method of pest control (Vacante, 2012). For instance, Peter, et al. (2001) highlighted that Integrated farming (as phrased by IOBC guidelines) and integrated pest control relies mainly on natural enemies, of which seed predators e.g., Carabid beetles constitute a major group. Therefore, protection of predators via creating and maintaining appropriate habitats plays a key role in integrated pest management (IPM). Besides, it enhances pest management by increasing natural enemy resources through habitat modification (Barbosa,1998) and decreasing pesticide-induced mortality in natural enemy populations (Castle, 2009). IPM approach provide a wide range of pest management options to the farmers and other stockholders including: cultural, biological, chemical, physical, mechanical (Barzman et al., 2015), however they must have enough knowledge and detailed understanding on many relevant key elements as: crops history (variety, sowing dates, tillage system); pest control methods and time; past, present, and future plans for cropping; pesticide use; and yield results, pest identity, growth conditions, damage symptoms, and natural enemies. Beside field scouting, such as systematic sampling and surveys of pest populations.

2.6. Integrated Weed Management (IWM)

In the framework of the IPM approach, the Integrated Weed Management (IWM) practices are necessary to eliminate the negative consequences of herbicides and to reduce the increased incidence of herbicide resistance cases (Norsworthy et al., 2012; Mortensen et al., 2012). IWM is defined as a holistic strategy for weed management that involves different weed control methods to provide the crops with the competitiveness against weeds (Harker and O'Donovan, 2013). Weed seed production by early plants and late emerging plants creates future weed problems. Thus, Integrated weed management programs should include strategies to deplete the decrease of weed seeds present in the seedbank (Menalled, 2008). The main goal of the integrated weed management strategies is to maintain the abundance of weed seedlings under specific thresholds. For example, the threshold level for the weed species *Echinochloa crus-galli* has been fixed to six seedlings (https://www.lfl.bayern.de/ips/unkraut/u_steckbriefe/053955/index.php) in arable maize fields, in Germany. It was found that seed predation has influenced the seedlings number, but did not maintain the weeds populations below this threshold. Practically, farmers should keep extending the exposure period of weed seeds to the predators on the soil surface e.g., delay the tillage and harvest time, to increase and enhance seed predation. Also, cover crops provide dense canopy which prevents seed predators from being predated by higher predators and seeds from entering the soil bank. It is worth also considering that only targeting the seed stage through seed predation is not enough to manage weeds, because weeds plants can compensate for the losses. In order to limit weed population growth, many studies proposed reduction in transitions between all weed life stages must be between 80 to 100% (Westerman et al., 2005; Buckley et al., 2001; Ramula and Buckley, 2010). For example, model analysis investigated the impact of combined weed management measures to minimize herbicides application in *Abutilon theophrasti* Medik. Populations. Results showed that the rate of 40% seed predation, in combination with crop rotation, reduced use of herbicide use, whereas no-till system, reduced weed population (Westerman et al., 2005). Accordingly, the effective weed control programs should be designed to target multiple life stages, rather than focus only on the seed stage.

Application of IWM techniques results in decreasing weeds densities in the field, and weed's ability for competition which help to maintain crops yield, and lower the size of soil seed bank. That can lead to control of the emerged weed seedlings, and therefore, reduce the need for herbicides application (Deytieux et al., 2012). For example, IWM for conservation tillage systems is important so farmers can reduce the environmental impacts of crop production while maintaining effective weed control (Buhler et al., 1995). Moreover, IWM creates a balance of

weed control (reducing their impacts on crops yield and the potential for weed resistance), while preserving the botanical diversity of weed species and weed seed predators. Liebman and Davis (2000) reported that crop diversity and organic amendments can also contribute to weed management by increasing weed seed mortality; delaying weed seedling emergence relative to crop emergence; reducing weed seed and vegetative production; and diminishing variation in weed growth between years. In addition, weed harrowing and interrow hoeing provide are effective as part of a strategy that also involves cultural methods such as fertilizer placement, seed rate and competitive varieties (Melander et al., 2005). It is also important to consider and promote ecosystem services, e.g., weed seed predation, and agro-ecological alternatives (Petit et al., 2015) in order to reduce herbicide usage. Accordingly, IWM may include natural regulating processes such as weed seed predation which can contribute to improve weed management in arable fields.

2.7. The Millennium Ecosystems Assessment (MEA)

The demand for consumption of many ecosystem services has grown over the last 50 years due to the growing world's human population more than the doubled (MEA Synthesis, Hassan et al., 2005), Besides, the substantial improvements in humans was obvious over the last half of the 20th century in many parts of the Globe. Many factors have contributed to these improvements including increased efficiencies of research and technology development and initiation of effective national and international institutions. The nature of ecosystem services utilization has changed because of research and technological advancements, which have facilitated the production and efficient use of essential services such as clean water and food. There are the many causes of the change that affect ecosystems, ecosystem services, and associated human as well (MEA, 2005). The drivers of biodiversity loss are habitat conversion, degradation, and fragmentation on land. While the population growth, economic development, and increasing consumption and production are the indirect drivers of change in ecosystems and ecosystem services. The investigations on ecosystem services are becoming increasingly important recently. The Millennium Ecosystem Assessment (MEA), was announced by the United Nations Secretary-General, Kofi Annan in 2000 and later performed in 2005 with the participation of governments, the private sector, nongovernmental organizations, and scientists. MEA aimed to provide an integrated evaluation of the consequences of ecosystem change on human well-being and to analyse the available alternatives to improve the ecosystems conservation and their contributions to meet human needs. Firbank et al. (2013) mentioned that effective delivery of ecosystem services requires enough information on how ecosystem services are produced.

According to MEA definition: an ecosystem has been defined as a dynamic complex of plant, animal, and microorganism communities and the non-living environment interacting as a one functional unit, whereas humans are considered as an integral component of the ecosystems. While ecosystem services are those benefits whom people receive from ecosystems; contribute to make their life possible and worth living (Daily, 1997; MEA, 2003). The biological structure of the ecosystem services includes many ecosystem functions, such as decomposition of organic matter, pest predation, that determine the supply of vital ecosystem services (e.g., soil generation and fertility, climate stability, crop pollination) (Kremen and Ostfeld, 2005; Oliver et al., 2015). All these functions and services are dependent on biodiversity, any drivers that directly or indirectly reduce biodiversity will also decrease ecosystem service levels and ecosystem functioning (Hector and Bagchi, 2007; Cardinale et al., 2012). Therefore, changes in these services will influence human in many ways. MEA has resulted in important findings including the followings:

- There was rapid and extensive change in ecosystems over the past 50 years of the human history, due to increasing human demands for food, fresh water, and fuel.
- The degradation of ecosystem services might significantly be worsened during the first half of this century which is a barrier to achieving the Millennium Development Goals.
- The restoration of ecosystem degradation is difficult while meeting the growing demands on ecosystem services.

Ecological intensification involves the environmentally friendly replacement of anthropogenic inputs and enhancement of crop productivity, by including regulating and supporting ecosystem services management in agricultural practices (Bommarco et al., 2013). Achievement of successful management of the ecosystem services requires filling the gap on the research between ecosystem services and ecological aspects (Kremen and Ostfeld, 2005). For instance, for the regulation of the ecosystem services of pollination and pest control, many research has used ecological theory (e.g., meta-community dynamics or food web) to explain the mechanisms by which pollinators and natural enemies contributes to these ecosystem services (Memmott, 1999; Hoehn et al., 2008; Lami et al., 2021). However, for the ecosystem services weed seed predation, less research has been conducted to understand the role of seed predators to ecosystem service supply. The potential of weed seed predation for weed control has been raised in the literature during the past two decades (Westerman et al., 2003a, b, 2005; Bohan et al., 2011), yet, weed seed consumption research still seems to be in its infancy compared with other regulating ecosystem services.

2.8. Weed seed predation as an ecosystem service

The successful management of ecosystem services is possible through filling the gap between ecosystem service research and ecological research according to Kremen and Ostfeld (2005). For regulating ecosystem services such as pollination and pest control, an extensive amount of research has used ecological theory, such as meta-community dynamics or food web theory, to understand the mechanisms by which pollinators and natural enemies contributes to these ecosystem services (Memmott, 1999; Hoehn et al., 2008; Lami et al., 2021). However, for other ecosystem services such as weed seed predation, less research has been conducted to understand the role of seed predators to ecosystem service supply. The potential of weed seed predation for weed control has been raised in the literature during the past two decades (Westerman et al., 2003a, b, 2005; Bohan et al., 2011). However, compared with other regulating ecosystem services, weed seed predation research still seems to be less investigated. Ecosystem services such as pest control, weeds and disease management has been associated with farmland biodiversity in agroecosystems (Landis et al., 2000; Luck et al., 2003).

The agricultural intensification during recent decades has led to substantial decline in biodiversity of agricultural areas in the Great Britain and north-west Europe (Robinson and Sutherland, 2002; Kleijn et al., 2006), which has negatively influenced the transfer and reduced efficiency of ecosystem services (Hooper et al., 2005; Tscharntke et al., 2005). Further, species diversity at lower trophic levels improves ecosystem functioning of seed predation (Snyder et al., 2006). However, the impact of greater predator diversity on herbivore regulation is uncertain because predator species both compete for the food source. The biodiversity declining was thought to be because of conversion of adjacent natural and semi-natural habitats into croplands (Benton et al., 2003; Firbank et al., 2008) and higher application of fertilizers and pesticides (Bengtsson et al., 2005; Hole et al., 2005; Geiger et al., 2010). In arable fields, weeds despite causing substantial yield losses due to crop-weed plant competition, they paly beneficial role by enhance functioning of ecosystem services. Conventional weed management widely relies on herbicides to minimize weed species in field crops (Green, 2018). Although, weed control is necessary to increase crops yield, studies on alternative sustainable weed management are essentially required. Since weed seeds constitute a food for several potential seed predators such as arthropods, birds, and mammals (Petit et al., 2014). That would directly and/or indirectly support several ecosystem services such as pests' control e.g., weed seed predation.

Weed regulation by the natural seed predators is an important ecosystem service in agricultural fields strongly impact weed population growth (Crawley, 1990, 2000; Kolb et al., 2007; Begg et al., 2017) thus can help to reduce use of herbicides (Shields, 2019). It also results in decreasing the frequency of weed species in agroecosystems and the spread of invasive species in SNHs as well (Garren and Strauss, 2009). The phrase "seed predation" was first used by Zhang et al. (1997) to describe the cases of animals consuming and/or removing the viable seeds from the soil seed bank, resulting in considerable seed loss both post and prior to seed shedding. Seed predators such as carabids have been found to consume weed seeds under laboratory conditions (Honěk et al., 2007; Petit et al., 2014, Saska et al., 2019) and in arable fields (Honěk et al., 2003, Kulkarni et al., 2015, Petit et al., 2017). Yet to be defined is how cropping systems can be manipulated to enhance the activity of seed predators and maximize their benefit, and thus allowing reductions in other more devastative control methods. In those arable fields dominated by annual weed species; annual plants grow from the seeds in the upper soil profile, and in most cases the annual emergence amounted (1 to 30%) of the weed seeds survive and produce new seeds into the soil seedbank (https://crops.extension.iastate.edu/encyclopedia/fate-weed-seeds-soil).

Thus, most seeds found in the soil seed bank fail to produce seedlings and their fate become unknown and is poorly understood. Historically, the bulk research of the annual weed life cycle has focused on seed dormancy and emergence, study the effect of control measures on weed survival, and weed seed production, but largely ignoring the fate of weed seeds between the period of ripening on the plant parents and entering the seedbank. Seed predation categorised into pre-dispersal seed predation which refers to attacks on seeds before they shed by the parent plant; and post dispersal seed predation occurs on or in the soil surface (Ward et al., 2011) and considered as a determinant of seed survival, species distribution, and community composition (Ashton, 1979), or occurs on other substrate and thus become available as a food source (Janzen, 1971). Generally, there are two common methods to expose weed seeds to seed predators in the field and under laboratory conditions: seed cards (Westerman et al., 2003) and plasticine trays (Honek et al., 2003). Studies showed that seed removal has been observed in the field and in the laboratory depend on the method of seed exposure, the substratum used to expose seeds, and weed seed species. However, both exposure methods, have benefits and disadvantages for use in seed predation studies and may be more useful situations. Some studies showed that the seed cards method is more suitable for short period studies in the field, while tin trays are useful for multi-choice experiments in the laboratory if the targeted predators can remove the seeds.

Weed seeds constitute an important diet of many animals including various invertebrates, small mammals, and birds (Kollmann and Bassin, 2001), and reduced availability of this food resource may cause biodiversity losses in farmed landscapes. For instance, seed predation by insects, is a major factor affect plant species composition and distribution, and plays an important role in regulating weed populations and dynamics (Hulme,1998; Larios et al., 2017). Seed predators reported to decrease seed density by average of 50% (Davis et al., 2011), which could reduce population growth of weed species. For example, 40% of cumulative seed losses due to predation reduced the population density of weed species *Abutilon theophrasti* (Westerman et al., 2005). Seed predation and dispersal both can have severe effects on plant reproduction and community composition (Hulme, 1998), where seed predation removing new plant individuals and seed dispersal influencing the location of new individuals (Pufal and Klein, 2015). In addition, high abundance of carabid seed predators has been negatively associated with weed seedbank change over the cropping season, indicating the potential of seed predators to regulate weed seedbank (Bohan et al., 2011). Therefore, promoting weed seed predation may thus be beneficial to farmland biodiversity (Menalled et al., 2000; Mauchline et al., 2005; Westerman et al., 2005) and contribute to preventive weed management, reducing the need for curative weed control.

2.9. Weed seed predators: an overview worldwide

Seeds are a major source of food for many animals, including vertebrates and invertebrates, such feeding on seeds may affects plant health (Hulme and Benkman, 2002; Kolb et al., 2007). A wide range of animals including birds, rodents, and arthropods, were found to act as seed predators (O'Rourke et al., 2006). Weed seed predators consume weed seeds before seed shedding (pre-dispersal seed predators) or after seed shedding (post-dispersal seed predators) (Sarabi, 2019). The pre-dispersal group includes specialist insects that prey on the seeds of a specific plant species or family and vertebrate species such as birds that remove seeds from plants (Hulme and Benkman, 2002; Crawley, 2013). It induces seed mortality in up to 80% of newly produced seeds in grassland and forest habitats (Janzen, 1971a). The post-dispersal seed predators can be divided into those prey on seeds scattered on the soil surface and those eat seeds buried in the soil seedbank. Post-dispersal seed predators are categorized into two groups, vertebrates (small rodents and birds) and invertebrates (e.g., carabid beetles, crickets, and ants) in the temperate ecosystems (Janzen, 1971; Fischer et al., 2011; Honek et al., 2013). These groups of seed predators vary in size, dispersal ability and activity patterns that lead to seasonal variations in weed seed consumption (Westerman et al., 2003a; Holmes and Froud-Williams, 2005; Westerman et al., 2011; Berl et al., 2017). For example, in temperate agroecosystems, invertebrate species feed on seeds from early spring to late

autumn, as they are active, while the vertebrate's prey on seeds during the whole year. Seed predators are particularly important in organic agriculture systems where use of chemical herbicides is reduced or prohibited (Westerman et al., 2003b; Nanvtoft et al., 2009). Research results showed that seed predators consume up to 53-95% of the annual seed production among studied weed species (Harrison et al., 2003, Honek et al., 2005, Westerman et al., 2011, Davis et al., 2013). Others have reported that predation of 25 to 50% with respect to annually produced seeds might decrease the growth of weed populations of specific weed species (Firbank and Watkinson, 1986; Westerman et al., 2005). Both vertebrates (e.g., birds and rodents) and invertebrates (e.g., ground beetles, ants, crickets) feed on weed seeds (Brust and House, 1988; Cardina et al., 1996; Zhang et al., 1997; Cromar et al., 1999; Crawley, 2019), though differences between seed predator groups shift the magnitude of seed predation each contributes with respect to different plant species (Dudenhoffer et al., 2016). Vertebrates usually play a prominent role as post-dispersal seed predators, whereas invertebrates are more important in pre-dispersal phase, where seeds are attached to the plant (Hulme and Benkman, 2002; Kolb et al., 2007). In the field, the intensity of invertebrate seed predation varies during the years due to the fluctuations in the temperature (Saska and Honek, 2010).

Besides, invertebrates and vertebrates are different in morphology, physiology as well as in their activity. Therefore, understanding global patterns in the importance will help to understand global patterns in the factors shaping plant species' reproductive strategies, including seed mass, defences against seed predators, and temporal variability in seed production (Peco et al., 2014). Besides, larger weed seeds are more likely to be predated by vertebrates compared with invertebrate predators, which seems they prefer smaller seeds (Abramsky, 1983; Brust and House, 1988; Harrison et al., 2003; Booman et al., 2009). Seed predators belong to wide range of taxa including animal's species such as insects, beetles, flies and birds, squirrels, and mice. For instance, insect seed predators caused large reductions in seed viability via decrease number of seeds per fruit (Andersen, 1988). Among insects, both larval and adult insects can function as seed predators: both larvae and adults of the carabid beetle, *Harpalus rufipes*, are seed predators in agroecosystems (Zhang et al., 1997). Ground beetles have been identified as the most abundant invertebrate seed predators in most agroecosystems (Honek et al., 2003). While the ecosystem services provided by insects are generally under-investigated, the general role of ground beetles as seed predators in crop fields is relatively well studied (Petit et al., 2014). Several carabid species have been found to consume a wide range of weed seeds under both laboratory and field conditions (Holland et al., 1999; Harrison et al., 2003; Hough-Goldstein et al., 2004; Lundgren, 2005; Honek et al., 2009; Petit et al., 2017; Saska et al., 2019).

Seed consumption due to invertebrates was determined to be around 80 to 90% in other studies (Westerman et al., 2003a). Besides, invertebrate seed predators, such as carabid beetles (Carabidae), known as a main cause of weed seed predation (Westerman et al., 2003; Kulkarni et al., 2015). Also, Gallandt et al. (2005) stated that many studies confirmed the superior importance of invertebrate seed predators in seed predation compared to vertebrates. Seed consumption by carabids can reduce seed stock of a weed species in the range of 65 to 90% (Honek et al., 2005): in agricultural habitats, up to 74% weed seed predation rates were reported across three weed species, *Viola arvensis*, *Stellaria media* (L.), and *Capsella bursa-pastoris* (L.), for example (Jonason et al., 2013). Assessments of seed consumption by carabid species vary between agroecosystems based on crop type, activity density, seasonality, the presence of non-crop habitats, and the extent of disturbance (Gaines and Gratton, 2010). Seed predation by carabids at soil surface level after seed shedding is the main mechanism by which they limit the entry of seeds into the soil seed bank, consequently affecting the community structure of weed populations (Bohan et al., 2011), and thus studying the factors that affect weed seed predation by ground beetles is necessary to enhance natural suppression of weed populations.

2.10. Effect of seed preference and seed predator behaviour's on weed populations

Seed preference for one species over another is often due to the ease of seed handling rather than textural or chemical structure differences (Lund and Turpin,1977). Selective seed feeding refers to the behaviours by which seed predators can affect the composition of plant populations. If seed predators show no preference to specific weed seeds and consume seed species equally, this will result in a reduced effect on plant species composition within the system. However, many research has shown that seed predators selectively consume specific seed species, though the results of this selectivity are not always guaranteed. Thus, any selective predation will decrease the reproductive ability of the selected species (Janzen, 1971; Harper, 1977), though predation levels are thought to vary with seed species and sizes.

The feeding preferences of seed predators has been associated with various traits such as seed size: for example, ants prefer consuming small-seed species while rodents like larger-seed species (Ferreira et al., 2011). Seed size preference has also been related to body mass, as small seed predators struggle to handle heavy or excessively large seeds. Moreover, seed preference thought to be linked with differences in nutritional value, seed coat strength, and secondary metabolite profile. Seed size may influence the number of consumed seeds, e.g., large seeds (Moegenburg, 1996) provide more food per seed, thus requiring fewer seeds to satisfy the predator.

The shape of a seed might also affect the ease which the predator can open and consume the seed, and thus affect the consumption rates. The levels of seed consumption among predators with respect to germinating and non-germinating seeds has also not been found to be significantly different: Luff (1980) reported that the mean numbers of germinating and non-germinating seeds eaten by ten larvae were 2.8 ± 0.4 and 2.8 ± 0.3, respectively. However, soil disturbances in natural ecosystems can affect the number of soil seed predators resulting in overall reduction in seed predation (Mittelbach and Gross, 1984). There are two types of responses initiated by exploited plant population as a result of seed predation, either by selection for morphological, chemical, temporal, and spatial mechanisms for predator avoidance, or via modification of numerical and spatial occurrence by elimination and redistribution of better-adapted individuals in the population through differential patterns of attack (Louda, 1978).

2.11. Effects of biodiversity on weed seed predation intensity and stability

The relationship between biodiversity and weed seed predation has been indicated by studies on weed seed predation that have revealed seed predation patterns increase as seed predator diversity increases (Jonason et al., 2013). It has been reported by Gray et al. (2021) that higher numbers of trophic relations between predators and prey support higher levels of weed regulation. With respect to the relationship between diversity and weed seed predation stability, Lami et al. (2020) observed that diverse communities of small seed predators reduced weed seed predation variability more than similar numbers of large seed predators. Feeding trials have also suggested that individual seed predators prefer weed seed taxa, though there is considerable overlap between species (Saska et al., 2008; Petit et al., 2014). It is difficult to quantify the contribution of various predator species to field predation across different weed seed taxa. However, recent advances in Molecular Gut Content Analysis (MGCA) allows to measure overlap in resource use between seed predators to a greater extent (Feit et al., 2019), making it feasible to use DNA-based approaches to investigate whether functional redundancy promotes more reliable weed seed predation.

Agricultural intensification may also be expected to have an influence on predator and prey diversity, resource utilisation and availability (Geiger et al., 2010; Sanguankeo and León, 2011; Batáry et al., 2012). Most of the research performed on the effects of agricultural intensity on weed seed predation has focused on the direct impacts of agricultural intensity on predation levels, yet it is critical to understand how agricultural intensity impacts ecosystem service delivery in order to develop predictions of how to improve the stability of weed seed predation and other biodiversity-driven ecosystem services. Lami et al. (2020) reported that there is a relationship between the diversity of predator community and seed predation intensity and stability **(Table 2)**.

The diversity of predator communities was thought to be beneficial to predation intensity and stability. However, a knowledge gap regarding the links between predation intensity and stability and predators' community such as species abundance, species richness, and functional diversity remains (Saska et al., 2008; Trichard et al., 2014), as most research has only focused on analysing seed predation overall (Kulkarni et al., 2015). The high predation intensity and stability was associated with carabid communities showing that higher activity density and species richness being occupied by more large-bodied species (Lami et al., 2020). They have observed obvious interactions between seed predator activity density, species richness, and community body size on predation intensity and stability **(Table 2)**.

Table 2. Effects of predator community structure and seed species on predation intensity and community features (Lami et al., 2020)

Explanatory variables	DFs	F test	p
Predator activity density	1, 1209	103.99	<0.0001
Predator richness	1, 1209	8.32	0.0040
Body size	1, 1209	8.11	0.0045
Seed species	7, 1209	74.63	<0.0001
Predator activity density x Predator richness	1, 1209	1.94	0.1638
Predator activity density x Body size	1, 1209	3.92	0.0481
Predator richness x Body size	1, 1209	26.62	<0.0001
Predator activity density x Predator richness x Body size	1, 1209	3.76	0.0528

2.12. Factors influencing weed seed predation in crop fields

Weed seed predators may not be able to remove seeds of every weed species on the soil surface. Yet many studies have indicated their potential to reduce weed seedbanks via seed consumption. Seed predation on weed species that produce lower seed amounts results in significant reductions in their seedbanks and population dynamics in consecutive seasons. In line with this, pre-dispersal seed predators have greater impact due to seed consumption prior to seed shedding on the soil surface. Therefore, combination of pre-dispersal and post dispersal seed predation is essential for significant weed suppression and the minimising of weed seedbanks. The factors that influence weed seed predation are reviewed in the following sections:

2.12.1. Agricultural practices

The adoption of agricultural practices that preserve and increase weed seed predators' populations must be considered as part of any integrated weed management programme in an agricultural ecosystem: no single method provides sufficient weed seed suppression, while involving many strategies including providing the requirements for predators according to their species and ecosystem structure may deplete weed seedbanks significantly. Seed predation intensity differs across agricultural ecosystems according to the management practices used (Mittelbach and Gross, 1984). For example, seed burial (Hulme, 1994; Hulme and Borelli, 1999) and tillage systems (Cromar et al., 1999) were found to affect the intensity of seed predation, while other studies have reported that factors such as soil cultivation patterns (Carcamo et al., 1995), cropping systems (Carcamo et al., 1995; Carmona and Landis, 1999; Blubaugh et al., 2011), and pesticide use (Lee et al., 2001; Markó and Kádár, 2005) can influence the activity of seed predators.

The literature highlighted that crop management practices such as tillage reduces seed predator populations and affect their foraging behaviours. Incorporating strip-tillage practices may encourage ecosystem services in subsequent generations of weed seed predators (Blubaugh and Kaplan, 2015). In addition, delay soil tillage and cultivation until late fall or spring would increase the exposure period of weed seeds to seed predators (Blubaugh and Kaplan, 2015), which lead to improve weed seed predation services (Menalled 2008; Ward et al., 2008). Tillage practices, mainly stubble cultivation and ploughing, remove weed seeds from the soil surface and bury them at various depths in the soil (Cousens and Moss, 1990). Hulme (1994) reported that weed seeds become unavailable for predation due to post tillage, whereas, in no-till fields, weed seeds are exposed to high levels of predation. Menalled et al. (2007) reported that seed predators carabid species such as *Anisodactylus rusticus* and *Amara aenea* are associated with no-till systems where more seeds were removed in comparison with conventional or organic systems **(Figure 8)**. The equipment used for tillage operations determines the extent of soil disturbance and thus the ultimate effect on seed predators, e.g., carabid populations (Kulkarni et al., 2015). Also, habitat structure determines predators' feeding behavior by providing shelter and protection from being predated (Landis et al., 2000; Kratina et al., 2012). Thus, it is advantageous to leave crop residues in their fields after crop harvest as microhabitats for predators when weed seeds shed.
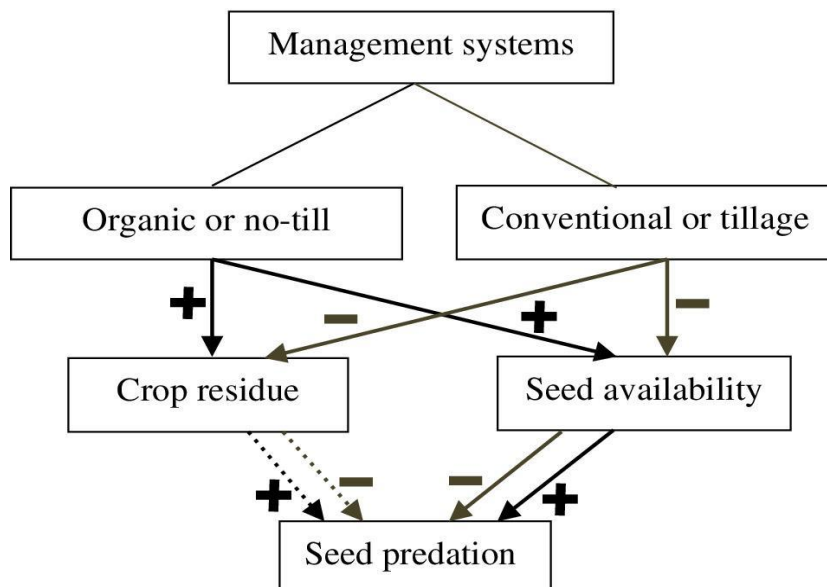
Figure 8. Interactions among management systems and seed predation (Menalled et al., 2007).

2.12.2. Habitat complexity and structure

The complexity levels of the habitat play an important role in increasing seed predator populations especially invertebrates. Birthisel et al. (2014) reported that habitat complexity is an important cause of second-order predation rates. This explains the presence of high densities of seed predators in the field boundaries in comparison within arable fields. Thus, field boundaries must be preserved in order to attract seed predators to return to the fields when the growing season starts. Therefore, it is not recommended to apply chemical pesticides at field boundaries to control pests and weeds. Incorporation of methods to preserve or increase weed seed predator populations to be as integrated weed management programs in agricultural ecosystems is important. Habitat management is a major factor in preserving of predator populations, and it plays similar role in maximizing weed seed predation in agricultural systems (Landis et al., 2005). Habitat structure on the landscape (Menalled et al., 2000), field (Swanton et al., 1999), and local scales (Povey et al., 1993) significantly influences seed predation levels, and the spatial complexity of the landscape, field, and local scale, along with predator preference, also influences seed predation patterns **(Figure 9)** (Booman et al., 2009). Habitat structure may affect seed predator retention and feeding behaviours by providing shelter from predation by other animals (Landis et al., 2000; Kratina et al., 2012). Vegetative cover provides a favourable microclimate (Magura et al., 2001) and habitat for carabid prey (Hawthorne et al, 1998).
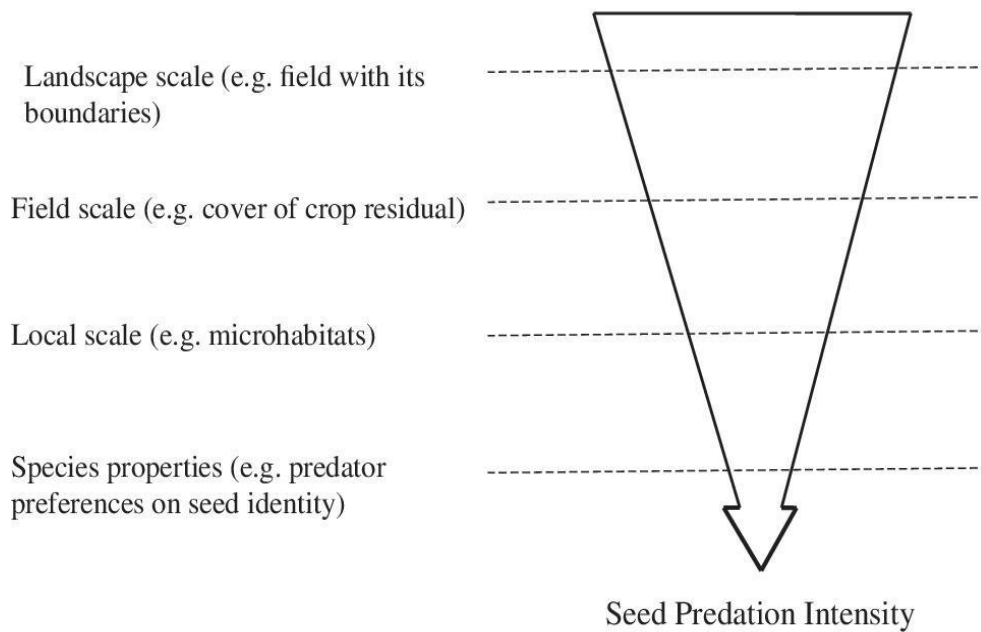
Figure 9. Influences of spatial scale and functional properties on seed predators and predation rates. Arrow width shows relative weight of factors affect predation patterns (Booman et al., 2009).

2.12.3. Crop type and sequence

Seed predation levels are differed due to crop type (O'Rourke et al., 2006), because differences in crop structure and management practices can affect seed predators' activity, density, and predation process accordingly. Also, seed predators' abundance and species composition were found to be affected by the farming system in conventional and organic farming systems in the Netherlands. However, in most cases the type of crop appeared to be of greater importance (Booij and Noorlander, 1992). Thus, the presence and efficacy of the predators' populations is determined by crop structure and crop-related factors. Besides, crop type has significant effect on weed seed removal and seed predator activity-density (Fox et al., 2013; Labruyere et al., 2016a). Invertebrates account for around 80 to 90% of seed predation in maize, soybean, and wheat fields, whereas only 10 to 22% of total seed predation were attributed to vertebrates (Cromar et al., 1999). Similar results were confirmed by Cardina et al. (1996), with vertebrates caused up 13 to 15% predation of weed species (*Abutilon theophrasti*) in maize fields. It has been also reported that weed seed removal rates and activity-density of invertebrate seed predators decreased in maize fields as compared to soybean and hay fields (Heggenstaller et al., 2006; Meiss et al., 2010). While, in terms of crop sequence, cumulative predation rates for the maize-soybean sequence were lower than in the soybean-wheat and wheat/red clover maize sequences (Davis et al., 2013). Model studies estimating seed predation performed by Westerman et al. (2005) revealed that 86% weed control efficacy was achieved in soybeans, without increases in velvetleaf density, in a four-year rotation

(maize-soybean-triticale + alfalfa-alfalfa) pattern that received 82% less herbicides; however, greater than 97% control was required without predation to prevent increases in velvetleaf density in a two-year rotation system (maize-soybean) managed with conventional rates of herbicides.

2.12.4. Application of chemical pesticides

Pesticides are commonly used to control pests and weeds in agricultural ecosystems for many years; however, they have negative effects on beneficial organisms (Straw et al., 2021) such as herbivores, arthropod pollinators, and weed seed predators. Application of chemical pesticides to control pests or weeds should thus be considered only in a reduced doses to avoid mortality of positive organisms, e.g., seed predators. High levels of chemicals have resulted in reductions in wildlife density e.g among birds (Moreby and Southway, 1999; Marshall et al., 2003), a decline mainly due to the reductions in their food supplies, as weeds can serve as both a direct food source (seeds) and as an indirect food source (supported arthropod fauna) for many birds (Squire et al., 2003). Herbicides also influences carabid populations by reduced food availability and habitat suitability (Brust, 1990; Holland and Luff, 2000). Some insecticides, such as synthetic pyrethroids have been reported to cause sublethal toxicity among carabid species (Tooming et al., 2014). Reduced dosage of insecticides can maintain carabid activity (Navntoft et al., 2006). Thus, studies on the impact of pesticides on seed predation and seed predator populations provide valuable information for the development of effective weed management programmes.

2.12.5. Field structure and cropping practices

Leaving a portion of crop residues and cover-cropping practices with low or no-tillage process may affect post dispersal weed seed predator populations (Gallandt et al., 2005); e.g., leaving crop residues after harvest, increased weed seed predation in maize fields (Heggenstaller et al., 2006). Studies showed that vegetation quantity (vegetation cover) is more important than vegetation quality (crop species) for most seed predators (Meiss et al., 2010). Moreover, the vegetation height and vegetation cover were associated with seed predation rates (Pufal and Klein, 2013), while Navntoft et al. (2009) stated that cover-cropping practices increase the abundance and the activity of invertebrate weed predators; the abundance of ground beetles was found to be greater on organic land than on conventional fields (Dritschilo and Wanner, 1980). Also, the predation rate of weed species, *Veronica arvensis* and *Capsella bursa-pastoris*, were significantly higher in winter-wheat with direct drilling and cover cropping than in a tillage system before harvest (Trichard et al., 2014). Also crop vegetative cover provides a favourable microclimate for natural seed predators and facilitates seed consumption (Blubaugh et al., 2016). Therefore, the presence of cover and

forage crops may promote ecosystem services by weed seed predators. However, above a certain level of plant cover (54 to 75%, depending on the distance to the field edges), seed removal rates begin to decrease as the vegetation hinders predators' locomotion and makes it difficult to find prey items. Similarly, crop and plant residues may limit the activity of predators in excess (Cromar et al., 1999; Jacob et al., 2006), restricting their ability to find weed seeds (Harrison et al., 2003).

2.13. Weed seed predation and farming context

Weed species and their regulation seems comprising a complex topic. For example, the borderlines between weeds causing yields and quality losses, their density, and species of non-crop plants (the botanical diversity) in and around crop fields are important to be identified. There is a need to place the crop-weed relation into a spatial context e.g., in the crop field and non-crop habitats (field margins and adjacent SNHs) on a farm and landscape level. Seed predation by ground beetles, found to be higher in perennial non-crop habitats than within potato fields, as those habitats have higher levels of plant structure and diversity (Gaines and Gratton, 2010), that allows beetle populations to become established (Bianchi et al., 2006).

Seed predation found higher in boundary strips than in field interior (Ichihara et al., 2011), as boundary strips are important refuges for seed predators to escape waterlogging. Also, the type of non-crop habitat is important in terms of seed consumption by predators. For example, seed removal rates are higher in fields surrounded by temporary pastures than in fields surrounded by permanent pastures and forest (Trichard et al., 2013). That because temporary pastures are more dynamic and has food availability and shelter than permanent habitats (Kulkarni et al., 2015). Overall, the effects of crop habitat complexity are locally specific, and these can range from having a highly significant effect to no effect at all on weed seed predation (Winqvist et al., 2011). For example: Jonason et al. (2013) showed that seed consumption rates by carabid beetles were higher in a simple landscape with a greater proportion of arable crops, while Kulkarni et al. (2015) stated that an understanding of species dynamics on a given landscape scale with respect to landscape characteristics is important. In order to promote weed seed predation in arable fields, the management options could also be implemented at multiple spatial scales (Labruyere et al., 2016a).

2.14. Impacts of the adjacent (SNHs) at fields edge on seed predation

Despite seed predation cause substantial losses of weed seeds in agricultural fields (Baraibar, 2011), but many factors may affect its effectiveness. For example, the existence of semi-natural habitats (SNHs) at field boundaries affects seed predation on soil surface, as most seed predators need non-agricultural habitats at some stages of their life cycles (Pollard and Relton, 1970; Birney et al., 1976) thus, maintaining such areas would enhance weed seed losses (Menalled et al., 2000). Semi-natural habitats adjacent to the crop fields found to be advantageous for crop protection and sustainability (Hatvani et al., 2001) as their presence is crucial for biological weed control, since they host many beneficial organisms (Altieri and Letourneau, 1982; Thomas et al., 1991; and Collins et al., 2003), birds (Green et al., 1994), and many of them are potential weed seed predators (Westermann et al., 2003). Furthermore, SNHs are important habitats for seed predators and thus a population source for field colonisation (Menalled et al., 2000; Saska et al., 2007). Also, they are sustaining insect natural enemies that move from SNHs into adjacent fields after winter or later in the season (Griffiths et al., 2008). Therefore, good landscape management would improve the role of SNHs and thus support biocontrol functions in agricultural landscapes (Holland et al., 2016). In Hungary, SNHs have been shown as a favourable for beneficial invertebrates seeking to overwinter (Kiss et al., 1994; Kromp, 1999) as they provide valuable ecosystem services (Kádár et al., 2004) such as food and shelter for natural seed predators. Kiss et al. (1993), confirmed the presence of arthropods seed predator individuals mainly Carabidae, both inside winter wheat field and in the field margin in Hungary.

2.15. Impact of weed seed predation on soil seed banks

Management of soil seed banks is an important because weed seed dispersal is essential for both weed population dynamics (Davis et al., 2004) and the development of weed communities in agricultural fields (Fox et al., 2013). Limiting the number of seeds entering the soil seedbank is therefore critical to weed management (Bagavathiannan and Norsworthy, 2012; Fox et al., 2013), and the role of natural seed mortality thus be carefully considered (Petit et al., 2014). Weed seeds are continuously subjected to different types of losses, and among these the ecosystem service of seed predation accounts for a significant quantity of such losses (Heggenstaller et al., 2006). The impact of seed removal from soil seed banks occurs in many ways: at high plant densities, seeds are removed and consumed by many seed predators and dispersers, and high populations of these animals will thus cause significant reductions in the soil seed banks (Hulme, 1998). At low species densities, competition levels will be higher among seed predators and dispersers. Seed predators such as rodents consume seed wherever it is available, directly reducing the seed bank, whereas

the occurrence of seed dispersers, such as ants, in significant populations is beneficial to the recruitment of the next plant generation based on the removal of seeds from the soil surface to other locations, where they may accumulate to develop the next generation (Holmes, 1990). In addition, Cabin et al. (2000) reported that seed predation may strongly affect the distribution of the soil seed bank, and fates of seeds may influence the subsequent structure of plant communities, especially in desert ecosystems where seeds are the most abundant or viable plant species.

2.16. Contribution of weed seed predation to weed management

Weed management in farming systems is a developing research area where seed predators play a beneficial role. Seed predation provides great possibilities for biological weed control by encouraging seed predators (Julien, 1982). Biological weed control is attracting more attention as eliminating the use of herbicides is a priority (Begg et al., 2017; Petit et al., 2018, 2015), and invertebrates that feed on weed seeds, e.g., carabid beetles, reported as contributing significantly to the consumption of weed seeds and thus to weed population regulation (Westerman et al., 2003; Bohan et al., 2011; Blubaugh and Kaplan, 2016). Both experiments and modelling studies suggested that seed predation have strong impact on weed population demography, and another promising means of weed management is thus the conservation of existing weed seed predators (Liebman and Janke, 1990) which has advantages on classical biological control. The conservation process requires sufficient information on life cycle so that IPM programmes can be developed to minimise mortality rates and maximize population growth of seed predators (Bird et al., 1990).

Seed predators such as granivores feed on recently produced weed seeds, reducing the amounts of seeds released to the seed bank (Hulme, 1998), thus minimising the future risk of weed infestations (Holmes, 1990). Identification of potential seed predators is one of the factors affecting prediction of seed predation, and species involved such as granivorous mice, birds, ants, crickets, and carabid beetles, which vary widely in activity patterns, dispersal abilities, food preferences, and, accordingly, in their effectiveness on seed predation. Generally, identity of common seed predators is regionally different, and any single seed predator may not be found in a specific habitat due to a reduction of niche size by biotic interference. To develop effective measures to promote seed predators, enough information must thus be obtained about which factors influence the distribution of such predators in each area. Agricultural ecosystems are thought to affect various external services, including balancing food demands. This is relevant for weed management in low-external input and organic production systems, where post-dispersal weed seed predation is a suitable service with the potential to support weed management.

Seed predation thus, is a natural addition to applied weed management methods, and farmers may consider measures to favour this, as seed consumption by naturally occurring seed eaters contributes to long term suppression of annual weeds in arable farming systems (Cromar et al., 1999), reduces weed population densities (Mauchline et al., 2005), and has an obvious impact on weed population demography (Kauffman and Maron, 2006). Up to 99 % of the weed seeds produced in arable fields do not emerge as seedlings or recover from the soil bank the next season after strong predation (Cardina and Norquay, 1997), and as seed predation is thought to be responsible for such losses (Westerman et al., 2003), it can thus contribute significantly to weed management. Many studies analysed the role of seed predation on weed population dynamics to design measures to maximise this service (Westerman et al., 2003, Heggenstaller et al., 2006; Baraibar et al., 2009). Seed predation together with other non-chemical control methods may reduce use of herbicides (Liebman and Davis, 2000; Westerman et al., 2005), and reduce environmental impacts. As a result, weed seed predation could contribute to weed management (Baraibar, 2011; Navntoft et al., 2009; Westerman et al., 2008), helping to reduce the reliance on herbicides (Shields, 2019). However, its efficacy depends on the predator's ability to respond in a direct density dependent way to increasing weed seed densities (Westerman et al., 2008).

2.17. Enhancement of weed seed predation at fields and landscape levels

Weed seed predators can adapt with the development of agroecosystems; however, agricultural intensity might reduce their abundance, and consequently, limiting weed seed predation. Increases of field management practices via additional pesticide applications and intensive soil cultivation may negatively affect both weed seed predators and seed predation levels (Menalled et al., 2007; Trichard et al., 2013). As such, soil tillage reduces seed availability on the soil surface (Cardina et al., 2002) and directly kills seed predators (Thorbek and Bilde, 2004; Blubaugh and Kaplan, 2015) and destroying their breeding habitats (Witmer et al., 2007; Baraibar et al., 2009). Many studies showed that reduced tillage or no-till systems enhance weed seed predation (Menalled et al., 2007; Baraibar et al., 2009; Petit et al., 2017). In addition, increasing crops diversity and reducing agrotechnical practices and herbicide application reported to increase seed predation levels by diversifying habitats and weed populations, supporting seed predators, and improving weed species suppression (Heggenstaller et al., 2006). Weed seed predation also increases with greater and more diverse crop rotation of fields (Westerman et al., 2005). Cover crops are beneficial to seed predators, enhancing weed seed predation by providing vegetation that creates comfortable habitats and food sources (Meiss et al., 2010; Birthisel et al., 2015; Blubaugh et al., 2016).

On the landscape level, weed seed predators utilise both crop and non-crop habitats to avoid disturbances, and seeking overwintering habitats (Thiele, 1977; Holland, 2002). Consequently, the composition and configuration of habitats at the landscape level can influence seed predator abundance and weed seed predation. It has been reported that increasing landscape heterogeneity, along with high amount of semi-natural habitats, enhances seed predator abundance and their services (Tscharntke et al., 2012; Landis, 2017). However, seed predators are well adapted to the different agroecosystems and some species even spend their whole life cycle within arable fields (Aguilera et al., 2020). Weed seed predation has been found to both increase (Trichard et al., 2013) and decrease (Jonason et al., 2013) with landscape diversity, depending on predator species. Habitat composition can influence seed predator abundance and consequently affect weed seed predation. Therefore, the higher landscape heterogeneity with increasing presence of SNHs, increases seed predator abundance and supports their functions (Tscharntke et al., 2012; Landis, 2017). To improve weed seed predation and to achieve sustainable weed management, more detailed information on direct and indirect mechanisms is thus required in order to successfully predict how local and landscape features may influence weed seed predation and whether these will thus ultimately lead to weed regulation.

3. MATERIALS AND METHODS

3.1. Study location

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) **(Figure 10)**. 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. The soil type is rust-brown forest soil (Chromic Luvisol). The top 40 cm of the soil is made up of 53% sand, 26% loam, and 20% clay fractions. The climate is continental, with frequent 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.

Figure 10. Study area Gödöllő, (Szárítópuszta), Hungary (Source: Google Earth)

3.2. Description of the studied fields and their adjacent SNHs

This study was conducted in two arable fields planted with winter wheat **Figure (11)** and maize crops surrounded by semi-natural habitats at the field borders **(Figure 12)**. Due to the economic value of wheat and maize crops in Hungary and to the related weed infestation problems, they were selected to assess weed seed predation. Seed predation levels were thus measured inside field crops as well as in the adjacent SNHs near Gödöllő, in (Szárítópuszta), Hungary 2020.

Figure 11. Winter wheat field and the adjacent SNHs), Gödöllő, (Szárítópuszta Hungary (Photo: Mohammed Osman 2020).

Figure 12. Example of a SNHs (forest habitat) adjacent to the field crop
(https://www.cropscience.bayer.com)

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

Sampling of vegetation cover (weed cover%) was performed once in summer 28-30 June 2019, by assessing the weed flora of typical weed species and their covering percentages inside crop field e.g., winter wheat **(Table 3)**, and in the adjacent SNHs at the field border **(Table 4)**. Our aim of that was to investigate the link and impact of the ground cover diversity (weed species diversity) on weed seed predation service. The assessment was basically planned to be performed for two years same as of weed seed predation case, that in addition to identification of the involved seed predators. But that was practically a bit challenging on field level as that requires to conduct these assessments all together at same time which is could be hard to manage. Therefore, we accepted to have such single measurement of the weed flora which was not enough, however, it was so far useful to have a simplified picture on weed flora diversity. Nevertheless, we placed this part on our priority list of future research for more investigations.

The method of evaluating of weed covering percentage (weed species frequency) was followed which has the advantage of being simple, easy, and fast (Németh and Sárfalvi, 1998, Zalai et al., 2012). Field-margins at the area of 2 m distance from the sides of the field and the inner area were also investigated. Weed cover percentages were recorded inside the sampling quadrates for each weed species. Sampling quadrates of 1x1 meter area **(Figure 13)** were randomly placed at least 2 meters distance from the field edges, along 8 sampling points for each habitat type (in-field and SNHs), then the weed cover % recorded inside the sampling quadrates for each weed species.

Figure 13. Sampling quadrates (size: 1x1 m) used to determine weed flora in winter wheat field and adjacent SNHs (Szárítópuszta), Gödöllő, Hungary (Photo: Dorner Zita, 2019).

The percentages of weed covering was determined by visual estimation for each weed species inside the sampling quadrates. Table (3) shows the weed species which were frequently occurred in all studied quadrates inside winter wheat field. Weed species presented in different cover percentages as follows; weed species *Polygonum aviculare*, had the highest cover (59%), followed by *Cirsium arvense* with (40%) cover percentage, then *Ambrosia artemisiifolia* (33%), *Convolvulus arvensis* (26%), *Stachys annua* (13%), and the lowest cover percentages has been reported by *Chenopodium album* (9%) *Consolida regalis* (8%).

Table 3. Weed species and their cover percentages in winter wheat field, Gödöllő, Hungary 2019

Weed species	Location codes and weed cover %							
	WT1	**WT2**	**WT3**	**WT4**	**WT5**	**WT6**	**WT7**	**WT8**
Polygonum aviculare	3%	5%	10%	3%	5%	8%	20%	7%
Cirsium arvense	0%	10%	0%	0%	0%	0%	30%	0%
Ambrosia artemisiifolia	5%	2%	0%	0%	7%	0%	11%	8%
Convolvulus arvensis	5%	0%	0%	2%	3%	5%	1%	0%
Stachys annua	0%	0%	0%	0%	0%	0%	3%	10%
Chenopodium album	2%	0%	0%	0%	0%	3%	3%	1%
Consolida regalis	0%	0%	1%	1%	2%	1%	3%	0%

Weed species which frequently presented were recorded in all investigated quadrates in the adjacent semi-natural habitats (SNHs) of winter wheat field are provided in **Table (4)**. Weed species *A. fatua* (73 %), *F. convolvulus,* (64%), and *P. aviculare* (59 %), *S. gigantea* (57 %) were the most frequently species in the adjacent SNHs with highest cover % among all weed species, followed by *A. artemisiifolian* (50 %), *C. canadensis* (29 %), *T. inodorum* (24%) and *S. annua* (17 %). While the lowest species frequency was recorded by *Cyperus* sp (13 %), *S. pumila* (10%) and *G. urbanum* (5%). It worth to mention that in SNHs habitat there were few plant species were neglected (not surveyed) as they were seemed not as weed species (mostly trees seedlings).

Table 4. Weed species and their cover % in SNHs adjacent to wheat field, Gödöllő, Hungary 2019

Weed species	Location codes and weed cover %							
	SNH1	SNH2	SNH3	SNH4	SNH5	SNH6	SNH7	SNH8
Avena fatua	20%	10	10%	15%	6%	4%	3%	5%
Fallpia convolvulus	8%	9%	3%	5%	10%	7%	10%	12%
Polygonum aviculare	7%	4%	7%	24%	2%	6%	0%	10%
Solidago gigantea	26%	10%	5%	8%	0%	5%	3%	0%
Ambrosia artemisiifolia	0%	5%	6%	0%	4%	0%	10%	25%
Conyza canadensis	1%	0%	5%	0%	0%	9%	12%	2%
Tripleurospermum inodorum	7%	0%	9%	0%	1%	2%	0%	4%
Stenactis annua	0%	1%	0%	4%	11%	0%	0%	1%
Cyperus sp	3%	0%	0%	7%	0%	0%	3%	0%
Setaria pumila	0%	0%	0%	0%	5%	0%	0%	5%
Geum urbanum	0%	0%	0%	0%	0%	5%	0%	0%

3.4. Experimental design, and seed predation assessment methods

Seed card method was used as the standard for such types of research of seed predation (Westerman et al., 2003b; Daedlow et al., 2014) and to provide estimates of short-term seed predation rates evaluated directly in the field. Besides, it is time and cost-effective seed predation measurement tools because the final soil dusted surface appears to approximate the surface of an agricultural field. According to Shuler et al. (2008), seed cards are superior to other methods despite being artificial, as they take less effort and time to prepare, and do not fail due to soil seed loss. Also, it inhibits those processes related to long-term cumulative weed seed burial, such as seed washing into soil cracks and gradual seed burial by plant residues (Westerman et al., 2009). The research materials used for seed predation assessment are provided in (**Figure 14**). Seed cards were prepared by gluing (glue Spray Mount adhesive 3M (400 ml/282 g), (ordered from Klingspor, POLAND), 20 fresh seeds (ordered from Herbiseed®, Twyford, UK) of 4 relevant weed species in maize fields as follows: *Ambrosia artemisiifolia, Datura stramonium, Chenopodium album* and *Echinochloa crus-galli* (**Figure 15**), and 3 weed species for winter wheat trial; *Galium aparine*, *Papaver rhoeas*, and *Apera spica-venti* (**Figure 16**) were examined. Weeds seeds were attached to sandpaper (Klingspor, POLAND) which fixed on carton cards by metal clips (**Figure 17**).

The glue was applied first and then 20 seeds of each weed species were fixed on the sticky sandpaper surface (25 cm long/10 cm wide, P=60 roughness (kL361 J-Flex Klingspor). The glue was agreed to be used in some EU partner countries under the research project QUESSA (Quantification of Ecological Services for Sustainable Agriculture). We observed the adhesive glue holed the seeds for (4-5) days not being washed by the rainwater. Also, it seemed that seed predators were able to remove the seeds away and feed on their endosperms. 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 cards placement.

Seed cards covered with metal wire meshes

3M glue spray amount adhesive (400ml/282g)

Sand paper 25/10 cm, P=60 (kL361 J-Flex Klingspor)

Figure 14. Research materials used to prepare the seed cards

The assessment also had to consider of the ability of predator insects to displace the seeds while an exclosure treatment featuring wire mesh (hexagonal mesh, holes size 25 mm was indicated as a maximum diameter) was used to allow easy access of small invertebrates while preventing entry to and securing the seed cards against larger vertebrate predators **(Figure 18)**. The exclusion of vertebrates will ensure and facilitate the actual participation and contribution of invertebrate seed predators to cause seed predation so that we can be able to measure the levels of seed predation which is the focal aim of our presented study. The participation and contribution of invertebrates as seed predators in this research was considered based on a previous study 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 where they confirmed the occurrence of arthropods invertebrate seed predator individuals, mainly Carabidae.

Figure 15. Weed seeds used to assess seed predation inside maize field and adjacent SNHs (Photo: Mohammed Osman, Institute of Plant Protection, Hungary, Gödöllő, 2019).

Figure 16. Weed seeds used to assess seed predation in winter wheat field and adjacent SNHs (Photo: Mohammed Osman, Institute of Plant Protection, Hungary, Gödöllő, 2020).
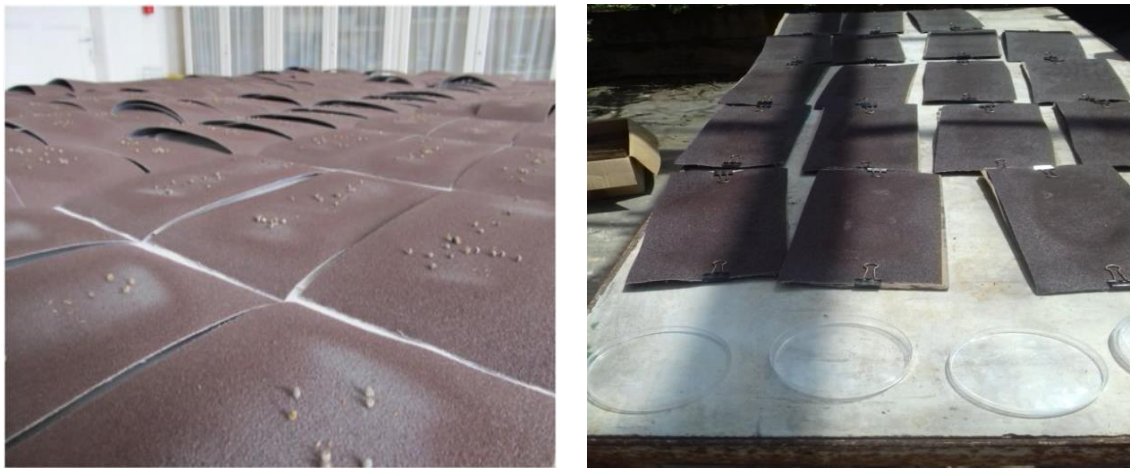

Figure 17. Seeds of weed species *Galium aparine*, glued on sandpaper carton cards (Photo: Mohammed Osman, Institute of Plant Protection, Hungary, Gödöllő, 2019).

Figure 18. Seed cards (covered by wire mesh, hole size 25 mm), placed Horizontally inside wheat fields between crop stands (Photo: Mohammed Osman, Szárítópuszta, Gödöllő, Hungary 2019).

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3.5. Assessment and estimation of weed seed predation

The identification of the optimum exposure period for estimating weed seed predation was challenging aspect. Providing that most of the relevant previous studies assessed seed predation during long term exposure periods, ranging from a couple of weeks to several months (Booman et al., 2009; Deroulers and Bretagnolle, 2019; Ichihara et al., 2021). In maize case, seed consumption estimations 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. 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 case was a bit different, where 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. The investigated fields (maize and winter wheat) were visually checked during the exposure periods to assess the presence of potential weed seed consumers such as (*Microtus arvalis* and other small mammals). Seed cards were checked to detect foot print, excrements of seed feeder birds.

3.5.1 Inside maize field

Using of 4 relevant weed species in maize fields as follows: *Ambrosia artemisiifolia, Datura stramonium, Chenopodium album and Echinochloa crus-galli*, two sampling rounds of seed exposure to invertebrate seed predation were performed in a maize field at the Hungarian University of Agriculture and Life Science (MATE) research farm (Szárítópuszta), in Gödöllő, Hungary, in autumn November 2019 and October 2020, before crop harvest. A total of 100 seed cards were placed horizontally on the soil surface in maize field, at 10 m from the field edge, along 25 transects, with 4 cards/transect (1 card for each weed species), 10 m between transects, 20 seeds/card, and 1 m between cards. locations of the sampling points are provided in **(Figure 19)**. The number of remaining seeds on each card was recorded every 24 hours after the first day of field exposure. Then converted into a proportion representing seed predation relative to the total number of glued-on seeds. 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 (counting of the remains seeds on cards) difficult, that resulted in different exposure lengths.

Figure 19. Locations of sampling points in a maize field and adjacent SNHs at (MATE) research farm (Szárítópuszta), in Gödöllő, Hungary, 2019 and 2020 (Source: Google Erath).

3.5.2. Inside winter wheat field

For winter wheat trial, different 3 weed species were evaluated *Galium aparine*, *Papaver rhoeas and Apera spica-venti*. The sampling rounds were performed twice in a winter wheat field and in the adjacent SNH (**Figure 20 a**), in June of 2019 and 2021, prior to crop harvest and after the seed ripening of the assessed weed species. Locations of the sampling points are showed in (**Figure 20b**). 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 (**Figure 18**), and in the adjacent SNH; 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). Due to the meteorological conditions (continuous rainfall) in the last days of field exposure, 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 was recorded every 24 hours, then converted into a proportion representing seed predation relative to the total number of glued-on seeds. Small size weed seeds e.g., *P. rhoeas* were counted by using a manual magnifier.

Figure 20. (a) winter wheat field and adjacent SNHs (small forest patches) (Photo: Mohammed Osman, Hungary, at Szárítópuszta, Gödöllő, 2020).

Figure 20. (b) Locations of sampling points inside winter wheat field and adjacent SNHs at Szárítópuszta, Gödöllő, Hungary, in 2019 and 2021(Source: Google Erath).

3.5.3. Seed predation of weed species inside fields versus semi-natural habitats

As we used different weed species to measure seed predation in different crops, and according to the literature, 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 semi-natural habitats. For this comparison three seed cards were placed at 20 transects in winter wheat field and the adjacent semi-natural habitat (total of 60 seed cards), each seed card containing of 20 seeds of each of the following weed species: *Apera spica-venti* L., *Galium aparine* L., *Papaver rhoeas* L. The same method was applied in maize field and in the adjacent semi-natural habits, but for different weed species including: *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Datura stramonium* L., *Echinochloa crus-galli* L. Locations of the sampling points are presented in **(Figure 21)**. The data of both trials in maize and winter wheat fields and in the adjacent SNHs were combined and analyzed to investigate if seed predation will differ based on habitat types.

Figure 21. Locations of the sampling points inside maize and winter wheat fields and adjacent SNHs at Szárítópuszta, Gödöllő, Hungary, 2021(Source: Google Earth).

3.5.4. Assessment of seed predation on *Ambrosia artemisiifolia*

Considering the importance of the common ragweed 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 this weed species. During this study, 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) in (Szárítópuszta), Gödöllő, and in the neighbouring semi-natural habitats at the same time, before crops harvest. Using a total of 160 seed cards, 4 sampling rounds of *A. artemisiifolia* seeds exposure to invertebrate seed predators were performed at the Hungarian University of Agriculture and Life Science research farm (Szárítópuszta). 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 the semi-natural habitat (SNH), 10 m from the field border. Twenty fresh seeds of *A. artemisiifolia* were attached with the same repositionable adhesive glue before to sandpaper. Metal wire meshes were used 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 the removal rate of weed seeds starting 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

3.6. Data collection and statistical analysis

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. 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. The same data were also collected in winter wheat fields as well as in the adjacent SNHs, but after 5 and 6 days of exposure in the field. Also, the case of *Ambrosia artemisiifolia* was like the other trials, but with exposure periods of 5, 6 and 7 days after field exposure.

The 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). 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). Tukey test was performed for comparison between groups. Binomial models were fitted and validated at the seed and card levels to compare seed predation between weed species and years (full model: comparing seed consumption across weed species and years). The diagnostic plots were also 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 (in-field vs SNHs), we analysed the data of seed predation 72 h after the 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 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.

4. RESULTS

4.1 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 (**Table 5**). 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 (**Figure 21 and 22**). While the predation levels and their temporal pattern seemed different in the two years, similar pattern was observed for the four weed species, therefore, was selected 3- and 4-days long exposure time, from day 0 to day 3 and day 4, 72 and 96 hours, respectively, for further analysis (**Figure 23**).

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

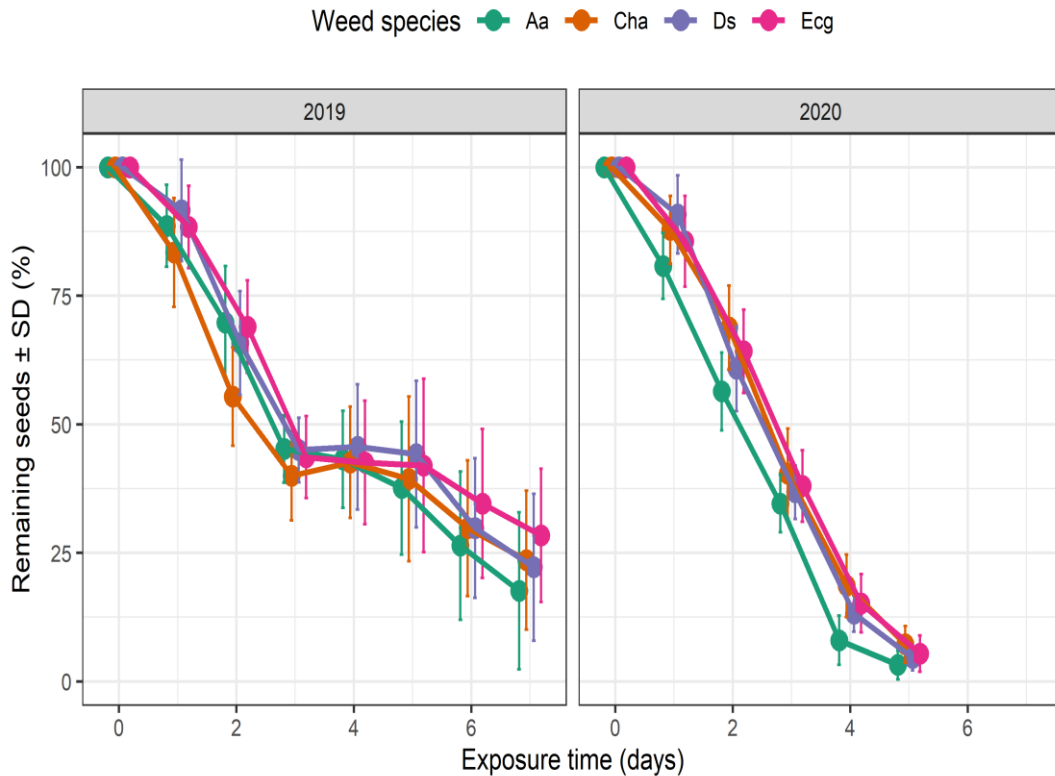| Weed species	Year	Seed predation (%, ± SD)
Ambrosia artemisiifolia	2019	82.40 ± 15.28
Datura stramonium	2019	77.80 ± 14.29
Chenopodium album	2019	76.40 ± 13.50
Echinochloa crus-galli	2019	71.60 ± 12.96
Ambrosia artemisiifolia	2020	96.80 ± 2.84
Datura stramonium	2020	95.40 ± 2.46
Echinochloa crus-galli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

Figure 21. Temporal pattern of seed predation of weed species (Aa: *Ambrosia artemisiifolia,* Cha: *Chenopodium album,* Ds: *Datura stramonium and* Ecg: *Echinochloa crus-galli*) exposed inside maize field in 2019 and 2020, Gödöllő, Hungary.

Figure 22. shows the temporal patterns of seed consumption of weed species (*Ambrosia artemisiifolia, Chenopodium album, Datura stramonium and Echinochloa crus-galli*, exposed inside maize field in 2019 and 2020. The results indicated that 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) between weed species in 2019 and 2020, where all weed seeds were consumed at similar rates, with no large differences in the numbers of predated seeds **(Figure 22)**.
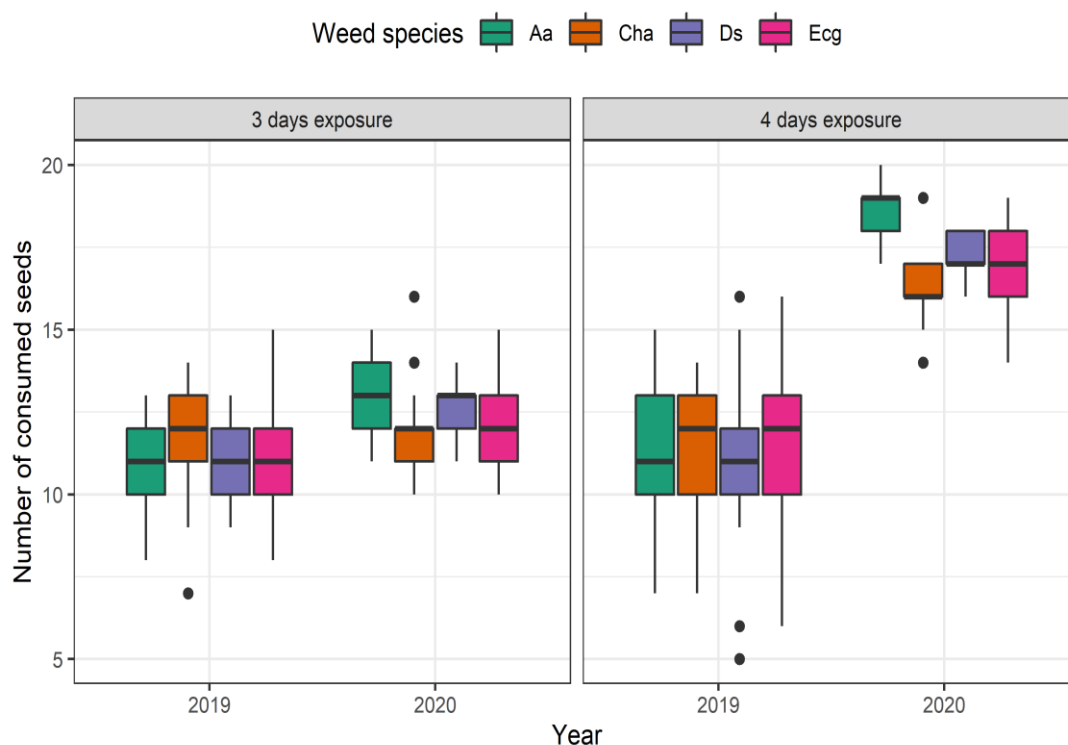
Figure 22. Invertebrate seed predation of weed species (Aa: *Ambrosia artemisiifolia,* Cha: *Chenopodium album,* Ds: *Datura stramonium* and Ecg: *Echinochloa crus-galli*), from day 0 to day 3 and from day 0 to day 4, in 2019 and 2020, in Gödöllő, Hungary.

The statistical analysis was performed based on assessment of seed predation rates on selected day/s during the exposure time in the field. There was a rapid decrease in seed predation over day 3 and day 4, and due to the low numbers of remaining seeds in the last days of field exposure, the data for days 3 and 4 were thus analysed separately **(Figure 23)**. The data analysis of weed seed predation from day 0 to day 3 showed no significant differences (p = 0.962) in seed predation across weed species in both years, whereas significant differences were emerged between the years (p < 0.001). **Figure 23** also shows the differences in seed predation between 2019 and 2020 on day 4. The statistical analysis of the weed seed predation from day 0 till day 4 similarly showed no differences (p = 0.079) in the number of predated seeds across weed species in both years, yet significant differences between years (p < 0.001).
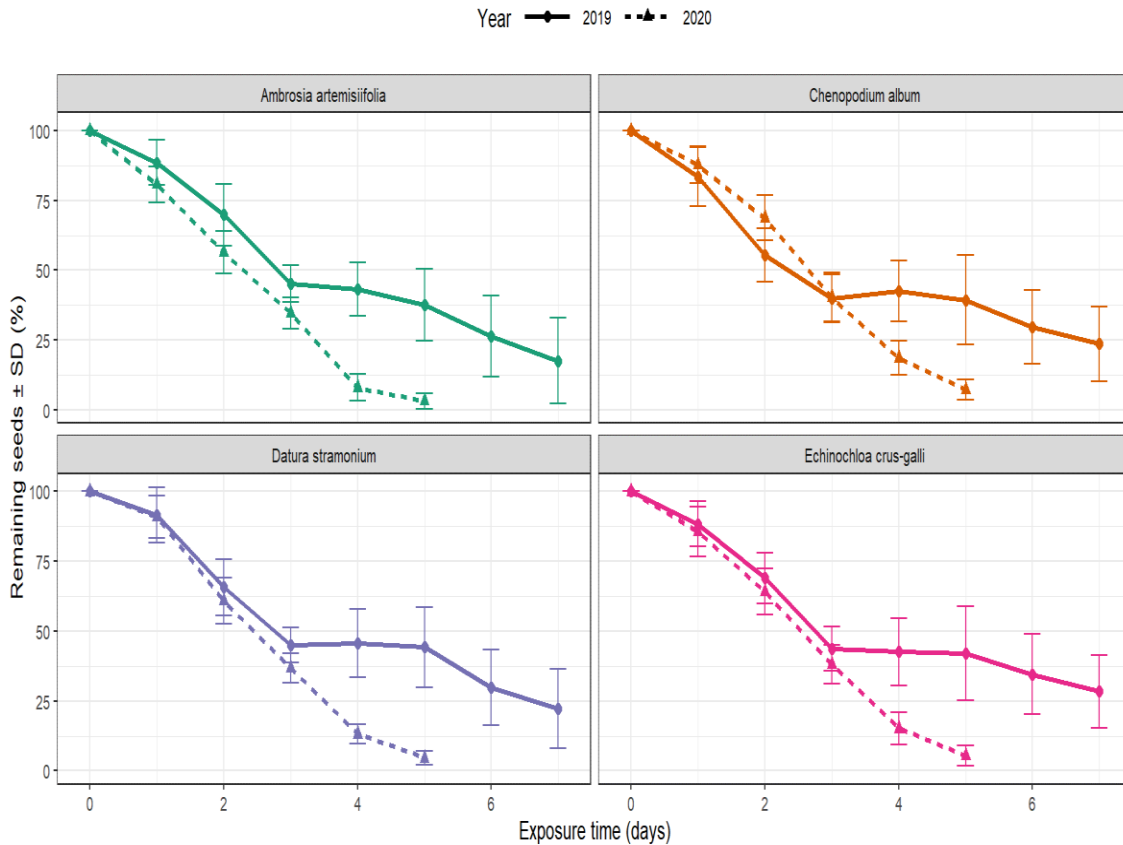
Figure 23. Temporal pattern of invertebrate seed predation of weed species (*Ambrosia artemisiifolia, Chenopodium album, Datura stramonium and Echinochloa crus-galli*) inside maize field in 2019 and 2020, Gödöllő, Hungary.

4.2. Weed seed predation inside wheat field and in the adjacent SNHs in 2019 and 2021

Seed consumption was observed in each seed card (100% of the seed cards) placed inside the winter wheat field and in the adjacent SNH in both study years. Specifically, there was high intensity of seed predation in most cases: there was 100% consumption observed in 75% -100% and 30%-58% of cards during 3 to 6 days of the exposure periods, but higher in 2019 than in 2021, respectively **(Table 6)**. The number of the remaining seeds on the cards (seed consumption) was monotonically decreased from the first day till to the end of exposure periods in both years and habitats. Nearly more than 75% of the weed seeds were consumed on the seed cards after 3 days of exposure e.g., in 2019 (*G. aparine* 17.10 ± 1.66 and 19.62 ± 0.77 for *P. rhoeas*) **(Table 7)**. There were significant differences (p<0.001) in seed predation levels among weed species across years (higher in 2019 than in 2021) where the assessed weed seeds were consumed differently higher in 2019 (100/%) than in 2021 (58%).

Table 6. Percentages of seed cards with 100% consumption after 3 to 6 days of exposure inside wheat fields and adjacent SNHs in 2019 and 2021, Gödöllő, Hungary.

Year	Weed species	after 3 days	after 4 days	after 5 days	after 6 days
2019	*Apera spica-venti*	25%	55%	90%	NA
	Galium aparine	8%	33%	75%	NA
	Papaver rhoeas	73%	95%	100%	NA
2021	*Apera spica-venti*	0%	3%	18%	43%
	Galium aparine	0%	0%	5%	30%
	Papaver rhoeas	0%	0%	15%	58%

NA: Not assessed (no collected data)

Table 7. Average cumulative consumption (20 initial seed/card) and standard deviation (SD N=40 for 1 species / year) after 3-6 days of exposure in winter wheat field and adjacent SNH, in 2019 and 2021 Gödöllő, Hungary

Year	Weed species	Cons. ± SD day3	Cons.± SD day4	Cons.± SD day5	Cons. ± SD day6
2019	*Apera spica-venti*	18.35 ± 1.27 a	19.50 ± 0.6 A	19.9 ± 0.3 α	NA
	Galium aparine	17.10 ± 1.66 a	18.95 ± 1.22 A	19.75 ± 0.44 α	NA
	Papaver rhoeas	19.62 ± 0.77 b	19.95 ± 0.22 A	20 ± 0.0 α	NA
2021	*Apera spica-venti*	15.10 ± 1.37 a	17.23 ± 1.23 A	18.45 ± 1.04 α	19.25 ± 0.74 ́A
	Galium aparine	14.82 ± 1.43 a	16.92 ± 1.12 A	18.2 ± 0.85 α	19.08 ± 0.73 ́A
	Papaver rhoeas	15.18 ± 1.24 a	16.82 ± 1.13 A	18.48 ± 1.04 α	19.45 ± 0.75 ́A

The different alphabet letters mean there are significant differences in seed consumption of weed species by exposure days and years. **NA:** Not assessed (no collected data).

Figures 24, and 25 shows the temporal patterns of seed predation (number of remaining seeds on cards) of weed species Ap: *Apera spica-venti*, Ga: *Galium aparine*, and Pa: *Papaver rhoeas*, exposed to seed predation inside the winter wheat field and the adjacent SNH in 2019 and 2021, Gödöllő, Hungary. The results showed that there were significant differences on seed predation levels among weed species between the two study years ($p<0.001$) of the exposure period in both habitats, but consumption rates were higher in 2019 rather than 2021, that narrowed the options of joint statistical analysis. The number of consumed weed seeds of *Apera spica-venti*, *Galium aparine*, and *Papaver rhoeas*, was higher inside wheat fields and adjacent SNHs in 2019 rather than in 2021 (**Figure 24).** Furthermore, the smoothed trendlines (**Figure 25**) were created by local polynomial regression fitting implemented in R based on the cloess package of Cleveland et al. (1992) to provide an easy-to-grasp descriptive graph of the weed seed consumption difference in among weed species over years.

Figure 24. Temporal pattern of the number of consumed weed seeds Ap: *Apera spica-venti*, Ga: *Galium aparine*, and Pa: *Papaver rhoeas*, inside wheat fields and adjacent SNHs in 2019 and 2021, Gödöllő, Hungary.
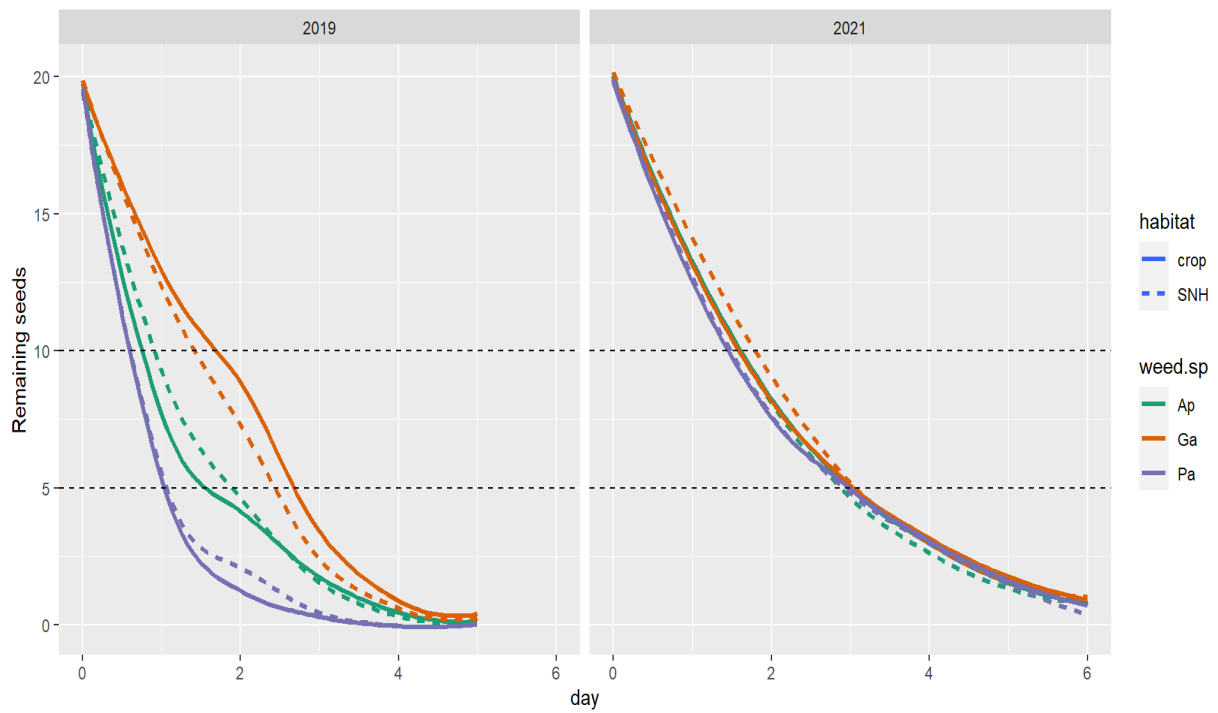
Figure 25. Temporal patterns of seed predation of weed species Ap: *Apera spica-venti*, Ga: *Galium aparine*, and Pa: *Papaver rhoeas*, inside winter wheat field and adjacent SNH in 2019 and 2021, Gödöllő, Hungary.

As there were differences in seed consumption over years, we thus used data from day 2 (48 hours of exposure) for further analysis to quantify and compare seed predation levels among weed species and habitat types with statistical model fitting **(Figure 26).** The binomial model of the weed seed consumption data showed significant differences among weed species, studied years, and their interactions ($p < 0.001$ for all explanatory variables). 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. *P. rhoeas* had the highest consumption levels (p<0.001 compared to the two other species), followed by *A. spica-venti* (p<0.001), and the lowest consumption rate was observed in *G. aparine* in SNH after 48h consumption **(Figure 26)**.
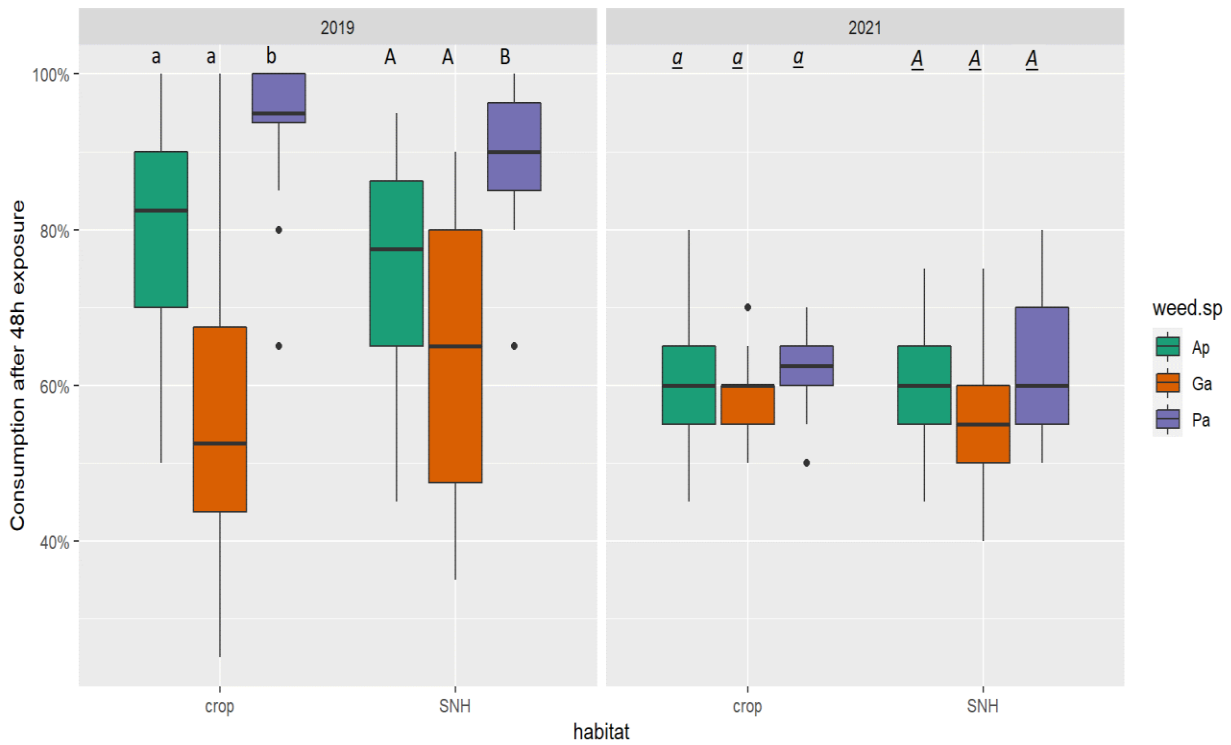
Figure 26. Seed consumption of weed species Ap: *Apera spica-venti*, Ga: *Galium aparine*, and Pa: *Papaver rhoeas* after 48 hours of exposure placed in a winter wheat field and adjacent SNH in 2019 and 2021, Gödöllő, Hungary. Boxplots presenting median, minimum, maximum, 1st and 3rd quartiles with dots as outliers. The different alphabet (and the underlined) letters indicate significant differences in seed consumption of weed species by habitat types and years.

4.3. Weed seed predation inside crop fields versus SNHs

To compare seed predation between the two habitat types (in-field and SNHs), we analysed seed predation after 72 h exposure in both crop fields and in the adjacent SNHs of all sampling rounds. Results showed that the tested weed seeds resulted in variable rates of predation during the 72-h exposure, with an average overall weed 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 adjacent semi-natural habitats. After the statistical analysis of the amount of weed seeds left on the seed cards inside winter wheat as well as in the adjacent semi-natural habitat (SNH), a significant difference ($p < 0.01$) was observed in seed predation levels on each weed species over the years **(Figure 27)**.

This difference in seed consumption was more obvious in 2019 rather than in 2021, clearly showing that the seed consumption of *Papaver rhoeas* (Pa) was higher, followed by that of *Apera spica-venti* (Ap), and then by *Galium aparine* (Ga). While in 2021, there was no statistically significant difference (p = 0.23) were found in seed predation patterns between weed species inside the winter wheat field and in the adjacent semi-natural habitats indicating the rate of seed predation was similar within the field and in the two habitats **(Figure 27)**.
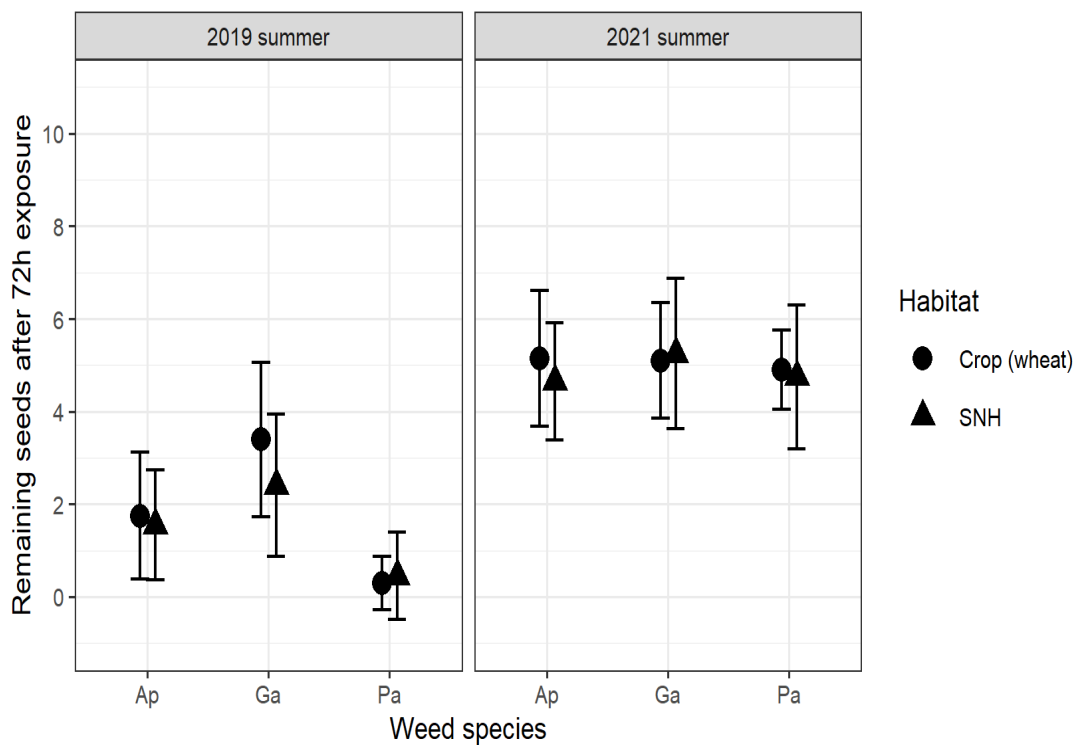

Figure 27. Remaining seeds of weed species *Apera spica-venti* (Ap), *Galium aparine* (Ga), and *Papaver rhoeas* (Pa): 72 hours after exposure in winter wheat field and adjacent SNH, in 2019 and 2021, Gödöllő, Hungary.

While the results of seed losses levels in the maize field (**Figure 28**) were partially different from those obtained in winter wheat field. Seed cards placed in the adjacent semi-natural habitat had nearly the same amount of seed consumption compared to those inside of the maize field during the exposure periods in both years. Specifically, there was no statistically significant difference found (p = 0.22) between weed species in the number of weed seeds remaining on seed cards (seed consumption) placed inside maize field and in the adjacent SNHs in both years. For example: the seeds of weed species *Datura stramonium* (Ds) showed similar levels of seed loss compared to seeds of *Chenopodium album* (Cha), *Echinochloa crus-galli* (Ecg) and *Ambrosia artemisiifolia* (Aa) in both habitats and over the years **(Figure 28)**.
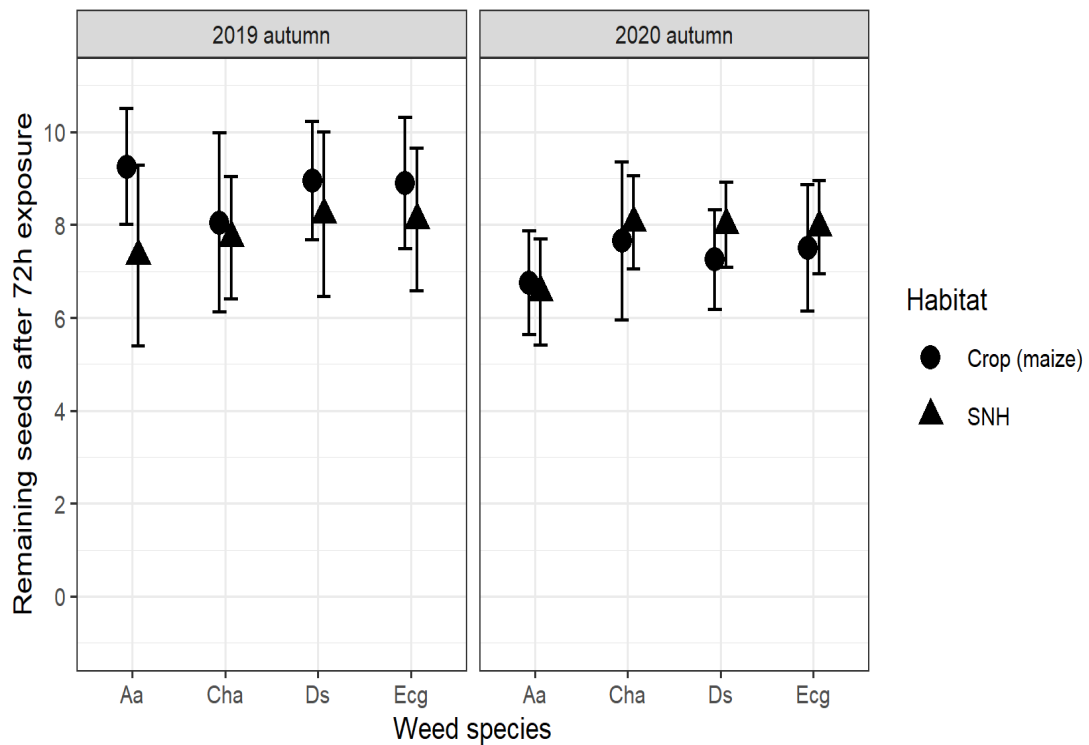
Figure 28. Remaining seeds of weed species *Ambrosia artemisiifolia* (Aa), *Chenopodium album* (Cha), *Datura stramonium* (Ds), and *Echinochloa crus-galli* (Ecg): 72 hours after exposure in maize field and adjacent (SNH) in 2019 and 2021, Gödöllő, Hungary.

When comparing the results obtained in maize field in autumn with the data from winter wheat field in summer, it was observed that the seed predation rate was higher in winter wheat field in the summer period rather than in autumn in maize field. Moreover, when the results of seed consumption in the winter wheat and maize fields and in adjacent semi-natural habitats were combined using a paired t-test, no significant difference ($p = 0.14$) in weed seed predation levels was found between the in-field observations and the adjacent semi-natural habitats. The seed consumption patterns were found to be similar inside both field crop as well as in the adjacent semi-natural habitats.

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

Results revealed that there were high seed predation levels in all common ragweed seed cards placed inside crop fields and in the adjacent SNHs, during the exposure periods of 5, 6 and 7 days, with an overall consumption average of 95.2% ($\pm$ 8.5%). There were 6 cases of negative consumption, which constitute 0.7% of total data. Seed consumption rates were higher inside field crops in summer season e.g., in 2019: $99 \pm 2\%$, than in autumn 2019: $81.7\pm16\%$, with slight difference between SNHs and inside fields in summer **(Table 8)**. Seed cards placed in side the wheat field and in the adjacent SNHs had nearly the same seed predation levels in summer season rather than in maize field in autumn.

Table 8. Averages and $\pm$SD of seed consumptions in the fields and SNHs in 2019, 2020 and 2021.

Year	Season	Habitat	Consumption average	Consumption ±SD
2019	autumn	maize	81.7%	16
2020	autumn	maize	97.2%	2.6
2019	summer	winter wheat	99%	2.0
2021	summer	winter wheat	97%	3.4
2019	autumn	SNH	93.8%	7.7
2020	autumn	SNH	96.3%	3.2
2019	summer	SNH	99.2%	1.8
2021	summer	SNH	96.5%	3.6

Furthermore, the results demonstrated that there was a high increase in the number of consumed seeds in all placed seed cards starting from the first day of exposure periods in winter wheat field in summer (2019) compared to those in other rounds in (2020 and 2021). Seed consumption was higher in summer 2019 in both habitats rather than in autumn 2020 and 2021 for all sampling rounds. Seed consumption rates were decreased over the days 3, 4, and 5 due to the low number of remaining seeds in the cards at the end of field exposure periods **(Figure 29)**. The level of seed consumption was higher but not significantly different in SNHs more than in crop field habitats during all sampling rounds.
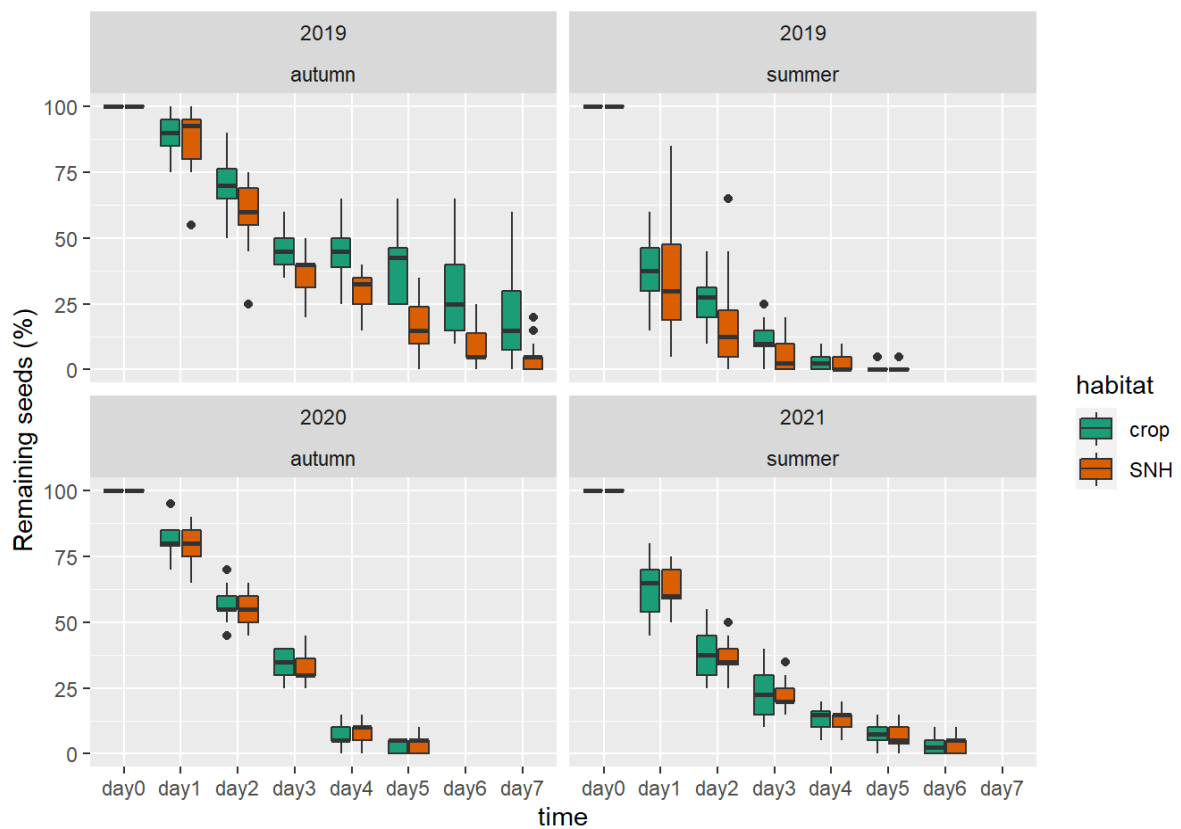
Figure 29. Temporal pattern consumption (remaining seeds %, boxplots) of *A. artemisiifolia* seeds inside crop fields and in the adjacent SNHs, during exposure time of 5, 6 and 7 days in 2019, 2020 and 2021, in Gödöllő, Hungary.

Seed consumption levels on *A. artemisiifolia* seeds were observed inside crop fields as well as in the adjacent semi-natural habitats during the 4 sampling rounds in both seasons, after 72 hours (3 days) after field exposure. The consumption rate of *A. artemisiifolia* seeds was highest (80-100%) recorded in summer of 2019 in winter wheat field in both habitats, higher (60-75%) in summer 2021, while the lowest seed consumption was obtained in maize field as well as in SNHs in autumn of 2019 and 2020 only higher in SNHs rather than inside maize field in 2019 (**Figure 30**). Seed consumption was slightly higher but not significantly different in SNHs more than in crop field habitats. The results further showed that seed consumption was higher in wheat fields in summer rather than maize fields in autumn, and slightly higher in SNH habitats than in crop filed habitats during the 4 rounds, despite the exposure periods were different in each round. The statistical analysis was performed based on the consumption to a selected day, for example in this case we used the data of consumption from (day0 until day3) to assess seed predation levels (72 hours after exposure). Around 50% or even 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 (**Figure 31**).

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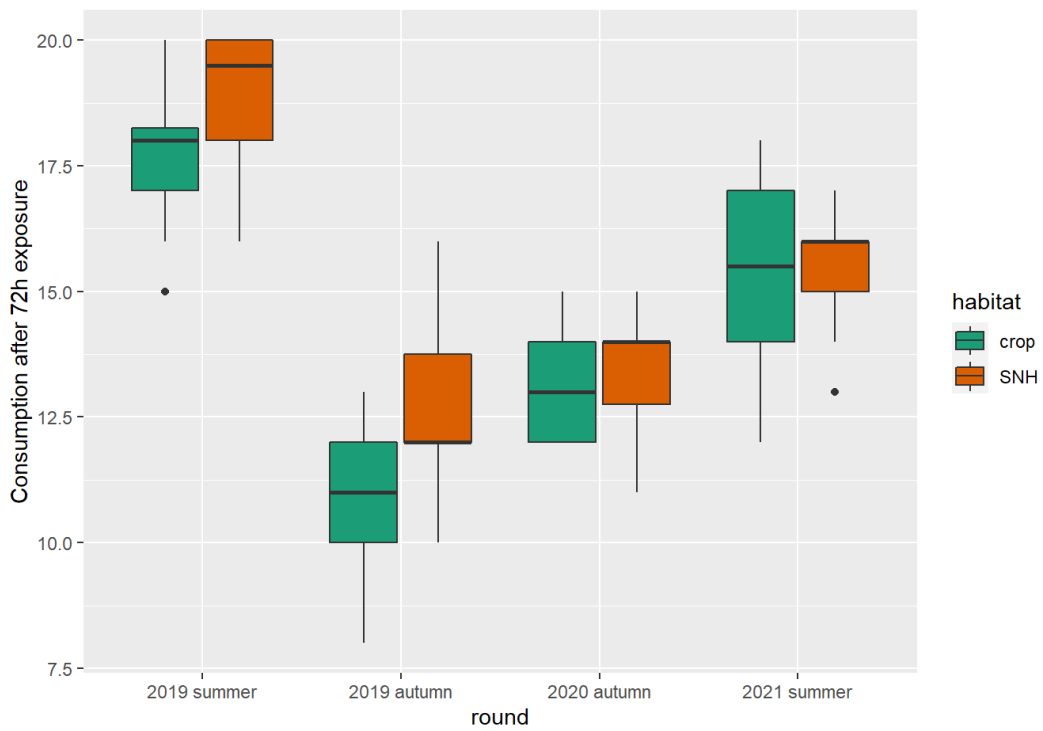
Figure 30. *A. artemisiifolia* seed consumption for the days (day 0 - day3) in summer and autumn in 2019, 2020, and 2021 inside crop fields and adjacent SNHs in Gödöllő, Hungary. Boxplots are presenting median, minimum, maximum, 1st and 3rd quartiles.

Figure 31. *Ambrosia* seeds consumption (boxplots and error bar lines, remaining seeds %) during seasons, and exposure time for 5, 6 and 7 days, in side fields and adjacent SNHs in Gödöllő, Hungary, in 2019, 2020, and 2021.

5. DISCUSSION

Weed species are responsible for causing serious impacts on agricultural production by decreasing crop yields through competition with the crops for the nutrient resources (Barzman et al. 2015; Oerke, 2006), but at the same time they promote a range of ecosystem services necessary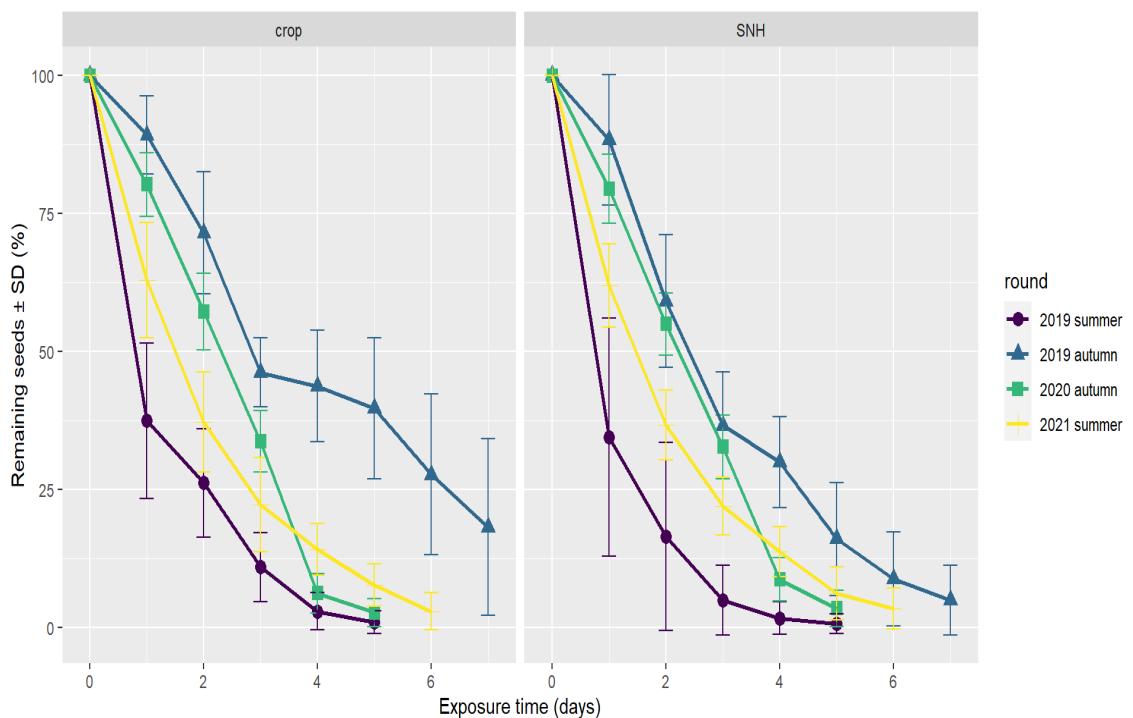 for sustainable crop production. The currently used weed control methods did not provide adequate weed control efficacy and sustainability. This is observed even when chemical, mechanical, cultural, and biological weed control methods are combined in integrated weed management approach (Menegat and Nilsson, 2019). Chemical herbicides however considered as the most common weed control method, but have negative consequences on human health, beneficial organisms, and on the environment (Van Bruggen et al., 2018; Zhang et al., 2019; Straw et al., 2021). Besides the high distribution of herbicide-resistant weed populations, that all alerts for alternative options for weed management based on ecological features (MacLaren et al., 2020). Also, the agricultural intensification introduced during the Green Revolution in the 1950s, was characterised by agronomic advances, but unfraternally also with the intensive use of fertilisers and pesticides in order to increase food production (Godfray et al., 2010). But this process resulted in great un wanted impacts of pesticides use to the environment, biodiversity (Donald et al., 2001; Hörnfeldt, 2004) and human health. Therefore, there is a need for environmentally friendly management methods and to identify ecological and sustainable alternatives for weed control e.g., ecosystem service weed seed predation.

During the period of 1960-1970s there was gradual increase in use of ecological principles in crop production systems (Altieri et al., 2012; Gallardo-López et al., 2018). At the same time, Integrated Pest Management (IPM) was suggested to depends more on natural regulators and mechanisms for weeds regulation and management (Stern et al., 1959). Enhancing the biodiversity and promoting potential and key ecosystem services (e.g., seed predation), can reduce the dependence on anthropogenic inputs in agriculture while maintain crops yield and sustainability. Predation of recently produced weed seeds is a key regulating ecosystem service that has the potential to support weed regulation. It provides a possibility for classical biological control of weed species by potential seed predators (Julien, 1982). Consequently, weed seed predation can be used for weed control, reducing the current reliance on herbicides to minimise weed-related crop losses. Also, the results provided in this study may improves understanding of weed seed predation, by providing evidence that increased levels of weed seed predation can result in weed regulation. However, the unexpected abundance of seed predators and other biological weed control measurements might hinder their adoption, acceptability, and direct use in weed management

programs. It is highly expected to find this phenomenon by measuring seed predation in the field and on the adjacent SNHs. The importance of seed predation as an ecosystem service is rapidly growing among ecosystem ecologists, agroecologists, and plant population ecologists (Westermann et al., 2003b; Kulkarni et al., 2015; Blubaugh et al., 2016; Petit et al., 2017). Yet, its adoption in weed management programs requires clarification on temporal and spatial patterns, information on seed predators, their response to increasing seed densities, feeding preferences and the mechanisms. That besides to the high variability of seed predation rates between studies (Menalled et al., 2007; Saska et al., 2008 and Davis and Raghu, 2010). To understand the ecological mechanisms that explain the variation in weed seed predation estimates, it is important to consider the impact of both alternative prey and seed availability on weed seed predation. The potential of weed seed predation for weed control has been addressed in the last two decades (Westerman et al., 2003a, b, 2005; Bohan et al., 2011). However, weed seed predation research seems insufficient compared with other regulating ecosystem services.

The role of vertebrates and invertebrates to weed seed predation has been a subject of debate, where previous studies showing higher predation rates by invertebrates (Harrison et al., 2003; Mauchline et al., 2005), while others by vertebrates (Westerman et al., 2003b; Tschumi et al., 2018). Therefore, the combined seed predation by both groups of seed predators is essential and will be the best way to suppress weed population growth in the fields. Besides, invertebrate seed predators, such as carabid beetles has been known as dominant organisms (Westerman et al., 2003; Kulkarni et al., 2015), and have been observed consuming weed seeds in the laboratory (Petit et al., 2014; 2017; Saska et al., 2019; and Kulkarni et al., 2015) as well as under field conditions. Also, Gallandt et al. (2005) stated that many studies have confirmed the superior importance of invertebrate seed predators in seed consumption compared to vertebrates; Cromar et al. (1999) and Westerman et al. (2003) reported that invertebrates account for 80 to 90 % of seed predation in maize, soybean, and wheat fields. In Hungary, among the invertebrate seed predators ground beetles are common in agricultural fields, play important role in lowering pest populations (Lövei and Sunderland, 1996). The most common Carabid species in transgenic and isogenic maize plots in Sóskút, Hungary were *Calathus ambiguous* (Paykull), *Dolichus halensis* (Schaller), *Harpalus distinguendus* (Duftschmid), *H. rufipes* (De Geer), *Poecilus sericeus* (Fischer von Waldheim) and *Trechus quadristriatus* (Schrank) (Szekeres et al., 2006).

In our study, we investigated the potential of the ecosystem service of weed seed predation, and how far can be a successful alternative for weed regulation in arable fields, using data from couples of sampling rounds conducted inside crop fields (maize and winter wheat) and in the adjacent SNHs. Field experiments were performed in a maize field in November 2019 and October 2020, to measure the post-dispersal invertebrate seed predation levels of the most relevant weed species in maize fields, by estimate seed removal of artificially exposed weed seeds (Westerman et al., 2003a, b) for short periods similarly as reported by Brust and House (1988) Honek et al. (2003) and Davis et al. (2011). Despite, we did not collect data on seed predators during this study, but we used the exclusion approach (wire meshes) as a confirmation step to prevent vertebrate predators from consuming the seeds, as that was insured by less of damaged or missing seed cards. The exclusion of vertebrates was to ensure and facilitate the actual participation of invertebrate seed predators to cause seed predation so that we can measure the levels of seed predation which is the focal aim of our presented study. The contribution of the invertebrates as seed predators in our study was thus in consideration with a previous findings reported by Kiss et al. (1993) on the activity density, key mixed feeder species, and their phenology in a winter wheat field and in the adjacent SNHs, in Kartal, Northern Hungary, near to the region of our study area. They indicated the occurrence of arthropods seed predator individuals, mainly Carabids such as *Harpalus* and *Amara* species. As a result, we consider the present results on seed predation indicate the potential role of invertebrate seed predators as their activity density was justified in Hungary earlier.

The results obviously showed high patterns of weed seed predation on the soil surface as a result of consumption of the exposed weed seeds. This result could be supported by similar local scale studies performed across Europe which showed actual levels of weed seed predation on the soil surface (Westerman et al., 2005; Trichard et al., 2014; Carbonne et al., 2020). Specifically, we observed high levels of seed predation (100% of seed cards were affected and 86% of weed seeds were consumed), which confirms our initial hypothesis that the examined weed seeds might be consumed when exposed to the relevant seed predators. Also, these results are agreed with the findings of several studies (Brust and House, 1988; Swanton et al., 1999; and Menalled et al., 2000) where seed predation has been 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 the weed species (*Stellaria media* (L.) and other weed species in cereal fields on conventional and organic farms in Sweden. Also, more than 70% of seed predation rates of the weed species *Stellaria media*, *Chenopodium album*, and *Sinapis arvensis* L. were recorded in spring barley field at Reading University's Farm at Sonning, Berkshire, UK, Mauchline et al.

(2005) reported. These high levels of weed seed predation could be due to the positive relations between the ecosystem service seed predation and the invertebrate predators' activity density. As seed predation occurred if seed predators are presented in high densities and active enough, or considered as a direct response to the high availability of weed seeds (Frank et al., 2011). Thus, we assume that seed predation has the potential to reduce weed seeds on the soil surface and could contribute to weed management. In accordance with Petit et al. (2018), Sarabi (2019) and Westerman et al. (2006), who stated that seed predation is a potential biological control process that limits weed population densities and growth, here we expect that the population densities and growth of the assessed weed species might be decreased because of the high seed predation levels estimated by our study. However, Westerman et al. (2008), mentioned that the efficacy of seed predation is partially depends on predator's response in a direct density dependent way to increasing weed seed densities. Also, according to Blubaugh and Kaplan (2016) who showed that seedling emergence of *Chenopodium album* and its biomass were reduced by 38% to 81% due to seed predation, similarly here we expect that seedling emergence and weed biomass of this weed species and the others would be greatly decreased as a result of seed predation.

Furthermore, our results showed that seeds of all tested weed species were similarly consumed inside maize filed. That means weed seeds were equally preferred and consumed by the seed predators as seed consumption rates in maize field were not significantly different from those observed in the adjacent semi-natural habitats (SNHs). However, Dennis and Fry (1992) reported that the adjacent SNHs have positive effects on natural enemies. Moreover, SNHs are beneficial for the abundance of predators and their amount highly depends on the amount of non crop habitats at landscape scale (Langellotto and Denno, 2004; Bianchi et al., 2006; Tscharntke et al., 2008; and Attwood et al., 2008). In addition, Schneider et al (2013) found that predation rates can be higher in the areas closer to the forest at field edge than inside the field, as forests can host more seed predator species (Lövei and Sunderland, 1996). Our finding is also in line with those of Gaba et al. (2019) and Moles et al. (2003), who mentioned that seed predation levels are thought to vary due to weed species considering the morphological and physiological characteristics and the nutrient value of the seeds of each weed species. Ferreira et al. (2011), reported that the feeding preference of seed predators has been associated with various traits such as: seeds size: e.g., ants prefer consuming small-seed species while rodents like larger-seed species. Seed size preference has also been related to body mass, as small seed predators struggle to handle heavy or large seeds, which may influence the number of consumed seeds, as large seeds provide more food per seed, thus requiring fewer seeds to satisfy the predator.

Moreover, seed preference thought to be linked with differences in nutritional value and seed coat strength. The shape of a seed might also affect the ease with which the predator can open and consume the seed, and thus affect consumption rates. This situation is so far applicable to the results we observed as we used different seeds which are morphologically seem differs in size, mass, shape, and even in their nutritional values. On the other side, seed predators are expected to be presented in diverse densities and populations and species at field level, that would make their food preference is different for each weed seed.

The results further showed that there were significant differences in seed consumption rates between the studied years. This result agrees with researchers who stated that weed seed losses caused by seed predators can be substantial (Zhang et al., 1997) but vary between years (Cardina et al., 1996; Tooley et al., 1999a). The variability in seed predation patterns between time e.g., years could be attributed to the different foraging and feeding behaviours of the involved seed predator species/groups; for example, Westerman et al. (2008) reported that some vertebrate species are quick in respond to changes in seed availability than invertebrate seed predators. Besides, seed preference and availability of weed species and alternative preys as food sources could directly affect seed predation rates over time factor. Besides, the involvement of seed predation as a standard measurement in weed management programs remains difficult due to the high variability in time and space (Westerman et al., 2003b, Saska et al., 2008, and Kulkarni et al., 2015). However, promoting weed seed predation is still beneficial to farmland biodiversity and contributes to preventive weed management and decreases the usage of chemical herbicides.

For winter wheat, we measured seed predation levels as in maize case, but on three different weed species including: *G. aparine*, *P. rhoeas*, and *A. spica-venti*, during two sampling rounds inside the field and in the adjacent SNH, in June of 2019 and 2021. Results showed the potential of weed seed predation on the soil surface, in both habitats and studied years, where soil dwelling arthropods as weed seed predators expected to play a significant role by consuming weed seeds. This result is in consistent with those findings reported by Westerman et al. (2005); Trichard et al. (2014); and Carbonne et al. (2020) which showed relevant patterns of weed seed predation on the soil surface. We certainly found high intensity (100%) of seed predation on all seed cards placed inside the field and in the adjacent SNHs. This indicates the potential contribution of invertebrate seed predators on consuming the assessed weed seeds. Our result agreed with those studies reporting that invertebrates were described as the most important seed predators' group during crop growth (Harrison et al., 2003; Mauchline et al., 2005), while others showed that vertebrates were the most significant (Westerman et al., 2003; Tschumi et al., 2018). However, we expected

vertebrates may have participated on the observed seed predation levels observed in our study. Our results further agreed with those of Daouti et al. (2022) who observed high seed predation rates (89% ±2% SE) on weed species *Alopecurus myosuroides*, exceeding the 78% required to prevent population growth in that species. Similarly, Ichihara et al. (2021) reported that the seed loss due to predation over four months was consistently high (>99%) in two years (maximum proportion of seed predation per 2 weeks = 80.0 to 86.7%). Also, Diekötter et al. (2010, 2014) showed that seed predation levels on arable weeds were higher in conventional winter wheat fields compared with organic fields, though Tschumi et al. (2018) found seed predation levels to be higher in some cereal fields when the contribution of vertebrate predators was considered. Westerman et al. (2003) also mentioned that seed predation is an important depletion factor for weed seedbanks, reporting the total seed loss due to seed predation 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 the seed banks of the tested weed species will be reduced due to the high levels of seed predation rates observed in winter wheat field.

As weed seed predation is thought to differ due to weed species and time, our findings confirmed this case by showing significant differences in seed predation levels among weed species across years. This result is in line with those obtained by Gaba et al. (2019) who demonstrated that seed predation levels vary according to weed species. Meiss et al. (2010) also found different seed predation rates between different weed species, with the highest for *Viola arvensis*, then *Alopecurus myosuroides*, and the lowest for *Sinapis arvensis*. Although, we observed different results from those reported in maize field where seeds of different weed species being similarly consumed inside maize fields, but we found seed consumption levels were significantly deferred over years which agree with others (Cardina et al., 1996; Zhang et al.,1997; Tooley et al., 1999a; DeSousa et al., 2003; Davis et al., 2011) who reported that seed losses by seed predators can be substantial and vary between years. The variations in seed predation among weed species over years found by our study are due to the different foraging mechanisms and feeding behaviours of certain seed predators. For example, some vertebrate species in some cases are quicker in respond to changes in seed availability than the invertebrate seed predators (Westerman et al., 2008).

Regarding the influence of habitat type on seed predation intensity: our results revealed that habitat type (SNHs versus in-field conditions) did not influence seed predation levels. Seed cards placed in the adjacent SNHs have received nearly the same levels of seed predation with no significant difference detected compared to those inside of the maize field. When the results of seed consumption in the winter wheat and maize fields and in adjacent SNHs were combined, no difference was found between the field and the adjacent habitats. Thus, the variability in seed predation was not explained by habitat type, but may be influenced by the different levels of seed predators' populations and seed bank in each habitat. This result agreed with that reported by Ichihara et al. (2011) who found that seed predation patterns at field edges were like those in the field interior areas. The occurrence of such similar levels of seed predation in both habitats was contrary to our expectations as we were assuming that seed predation will vary due to habitat types. That could be due to the presence of same species/communities of seed predators in both habitats, or both habitats were comfortable and preferred by the potential seed predators which resulted in similar rates of seed consumption. Whereas, other authors observed different cases, for example, Navntoft et al. (2009) reported that vegetation cover at the field edges did not affect seed losses, as seed consumption decreased when moving from field edge towards the interior of organic fields. Further, Jacob et al. (2006) found that seed predation rates were higher in the field borders near to the adjacent habitats than those in the field centre and within bordering vegetation. However, our results are inconsistent with the findings of González et al. (2020), who observed different levels of seed predation across habitat types. Despite we measured seed predation inside winter wheat field in a specific (narrow) period (48 h after field exposure) in both 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, relevance, and consistency with previous findings.

When comparing the obtained results on maize field with those of winter wheat field, there was a difference in seed predation levels among weed species only inside winter wheat field, while no significant difference was observed inside maize field nor in the adjacent SNHs. That means crop type may influence seed predation levels. These results could be supported by those of O'Rourke et al., 2006; Fox et al., 2013; and Labruyere et al., 2016a, who reported that seed predation levels are differed due to crop type. This is because the differences in crop structure and management practices can directly affect invertebrate seed predators' activity and density, and seed predation process accordingly. For instance, each arable crop has a specific agronomic characteristic, cultural and management practices that influence seed predators' activity and later seed predation. In addition, our study indicate that crop seasonality can play a considerable role in seed predation

estimations; we observed that seed predation rates were higher in the summer season rather than in autumn, when weed seed predators were more active in their movements. This result agrees with those found by González et al. (2020), they mentioned that seed predation levels may increase in summer season. This could be due to the positive relations between ecosystem services and carabid predator's activity-density, since some seed predators e.g., ground beetles are more abundant in summer due to the seasonality nature of their life cycles (Labruyere et al., 2016a), or could be considered as direct response to an increase in food resource availability (Frank et al., 2011). For example, seed losses of the Italian ryegrass found to be 32-70% in summer season in organic wheat fields in the Netherlands due to the predation process (Westerman et al., 2003a).

On the other part of our study, we assessed the importance of the invertebrate seed predation on *Ambrosia artemisiifolia* seeds. The common ragweed is the most rapidly spreading invasive weed species in Eastern European countries with severe negative impacts on agriculture, biodiversity, and on human health (Burbach et al., 2009; Smith et al., 2013; Essl et al., 2015). Chemical and mechanical control methods have been used, but sustainable measures are still required to minimize the abundance of this weed. Invertebrate weed seed predation has been identified as a potential biocontrol option and an ecological weed management tactic, and the main cause of weed seed losses in agricultural fields. The results showed temporal patterns of seed predation on *Ambrosia artemisiifolia* seeds caused by invertebrate seed predators on the soil surface, confirming the same finding reported across Europe on seed predation observed on the soil surface (Trichard et al., 2014; Carbonne et al., 2020). The occurrence of seed predation on all *Ambrosia artemisiifolia* seed cards placed inside wheat and maize fields and in the adjacent SNHs, during exposure periods 5, 6 and 7 days, in both seasons confirm our hypothesis that *A. artemisiifolia* seeds might be consumed by seed predators. We specifically observed high seed predation rates in summer rather than in autumn season, which are in consistence with the findings of González et al. (2020) that seed predation levels may increase in summer but differ between habitat types. Moreover, we found that seed predation levels were a bit different between habitat types, being slightly higher in the adjacent SNHs, but not significantly different from those reported inside crop fields. This is possibly because the field boundaries constitute an important habitat and safe for seed predators to avoid waterlogging and burning. Marino et al. (1997) mentioned that the inability to detect differences in seed removal in edge vs. interior may be due to the low rates of seedling emergence, the presence of crop residue inside the fields, and to the small size of these fields and the diversity of the surrounding landscape. However, Ichihara et al. (2011) reported 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 showed lower levels of seed predation near to the field edges (Saska et al., 2008). That could be attributed to other factors beside the activity-density of carabids can influence seed predation. For example, seed density can be high at field boundaries, that makes seed consumers less hungry and less attracted to the exposed seeds (Du Toit et al., 1999). Moreover, other invertebrates, e.g., crickets, ants, and slugs, can be important and competitive seed predators in arable fields (O'Rourke et al., 2006).

Overall, the results of this study showed that weed seed predation is a promising ecosystem service provided in agricultural fields, and it is possible to achieve sustainable weed regulation at the field level. Our findings could be thus counted as a new reported case of post-dispersal invertebrate seed predation on *Ambrosia artemisiifolia* and other serious weed species in arable crops (maize and winter wheat) and in the adjacent SNHs in Hungary. These findings confirm the potential of weed seed predation contributing to sustainable weed management and thus could be included in the ecological intensification toolbox to reduce the current dependence on herbicides for weed control. Seed losses via seed predation could be improved by allowing weed seeds on the soil surface for long time to increase exposure period to predators, according to Westerman et al. (2006 and 2009). Support weed seed predators and then enhance weed seed predation requires reduce the intensive field management practices and design more diversified cropping systems at both field and land scape levels. While, from the farmers point of view, it is necessary to clearly explain how weed seed predation can reduce weed-crop related losses and protect crops yield.

6. CONCLUSIONS AND RECOMMENDATIONS

- This study presents promising results on weed seed predation in arable fields and adjacent SNHs, which could be a potential strategy to regulate weed species in arable crops. Our findings confirm the potential of weed seed predation to achieve sustainable weed control.

- Although, we did not collect data on seed predators during this study, but we thought the exclusion approach (wire meshes) as a confirmation step to prevent vertebrates from consuming the seeds, which was ensured by no damaged or missing seed cards. This would indicate that the estimations of seed predation obtained through the mesh were indicative of invertebrate seed predation. Thus, its possible to accept invertebrates as a potential weed seed predators and the present work as a participation of invertebrate seed predation

- **In maize field**, results showed significant levels of invertebrate seed predation (100% of the seed cards were affected, where 86% of the weed seeds were predated), on the evaluated weed species *A. artemisiifolia, D. stramonium, C. album* and *E. crus-galli*. This signifies the potentiality of this ecosystem service to be useful in weed management.

- 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 SNH. 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.

- When the data from the maize field were compared to those from 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.

- The variability in weed seed predation levels was not explained by habitat type, since the combined assessment of seed consumption data from both 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.

- 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 field habitats.

- It could be recommended that future research should investigate the impact of weed seed predation on crops yield, which will help to design of cropping systems that enhance weed seed predation for weed control 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 on the combined effects of pre and post dispersal weed seed predation to increase the predictability of the influence of weed seed predation on weeds population dynamics, and identify the relevant seed predator groups.

- More knowledge is necessary to develop cropping systems that count on ecological intensification of beneficial organisms and their regulating and yield supporting services.

7. NEW SCIENTIFIC RESULTS

- **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%.

- The optimum exposure period to measure weed seed predation in maize field was identified as 3 or 4 days after field exposure (as a first record in Hungary).

- 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.

- **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.

- The period 48 h after field exposure was identified to measure weed seed predation levels in winter wheat field (first report in Hungary).

- Significant differences in seed predation rates were observed in winter wheat field among weed species, studied years, and their interactions. While, no differences were found between habitat types, interaction of weed species and habitats.

- The variability in seed predation levels was not explained by the habitat type, as there were no differences found between the fields and the adjacent SNHs.

- **In *Ambrosia* case**: there was high seed predation in all *Ambrosia artemisiifolia* seed cards placed inside the fields and SNHs, during 5, 6 and 7 days, each year, and seasons, where *Ambrosia* seeds were consumed with overall consumption average of 95.2% (±SD 8.5%).

- Seed consumption was largely higher in wheat fields in summer rather than maize fields in autumn, and slightly higher but not significantly different in SNHs more than in crop field.

8. SUMMARY

Weed infestations are significantly reduce crops yield and quality. For example, in Hungary, the ragweed *A. artemisiifolia* has become a noxious species in agricultural areas and landscapes. But, reliance on herbicides affects human health, beneficial organisms, and ecosystem services delivery. Thus, sustainable, and long-term weed management solutions are required to limit weed abundance. Weed seed predation is a potential ecosystem service provided in cultivated areas and in semi-natural habitats (SNHs). It causes substantial weed seed losses, decrease weed seedlings emergence next season which may minimize herbicides use. This study aimed to estimate seed predation levels of 4 weed species *A. artemisiifolia*, *D. stramonium*, *Ch. album*, and *E. crus-galli*, in maize field, and 3 weed species, *G. aparine* L., *P. rhoeas* L., and *A. spica-venti* L, in winter wheat field, and in the adjacent SNHs, in Gödöllő, Hungary. Weed seeds were assumed to be predated, but predation levels may differ by weed species and habitat type. Two sampling rounds were performed, in November 2019 and October 2020, by placing total of 100 seed cards/round on soil surface inside maize field, before harvest, and in the adjacent SNHs, along 25 transects, with 4 cards/transect. 20 seeds/weed species were glued on sandpaper (25×10 cm, P-60), covered by wire mesh to exclude vertebrate predators. Seed removal was measured every 24 h, for 7 days in 2019 and 5 days in 2020, and seed predation was estimated using number of removed seeds on each card. Results showed high seed predation on all seed cards, with overall average $85.9 \pm 13.7\%$. The optimum period for measuring seed predation was observed over day 3 and 4, though the number of predated seeds significantly differed between years ($P < 0.001$), with higher predation in 2020 than in 2019. No differences detected in predation rates among weed species ($P = 0.962, 0.079$). Similarly, in winter wheat, the sampling was performed twice, in 2019 and 2021, but by placing of 240 seed cards (120 card/round), with 60 seed cards placed inside crop field and in adjacent SNHs, along 20 transects, with 3 cards/transect. Seed removal was assessed on each card every 24 hours, for 5 days in 2019 and 6 days in 2021. Results revealed high intensity (100%) of seed predation on weed species. Seed consumption was observed in all seed cards in both habitats during both years, though weed seeds were significantly consumed ($p < 0.001$) in 2019 as opposed to 2021. The differences were not significant ($p=0.802$) between habitat types, interaction of weed species and habitats ($p=0.353$), and between habitats and years ($p=0.842$). A short period of 48 h of exposure, was identified to quantify weed seed predation rates.

In *Ambrosia artemisiifolia* case, seed predation was quantified inside wheat field in summer (June 2019, 2021), and maize field in autumn (November 2019 and October 2020), and in adjacent SNHs. Using a total of 160 seed cards, 4 sampling rounds of seeds exposure to invertebrate seed predators were performed in Gödöllő, Hungary. Number of 40 seed cards/round were placed on

soil surface with 20 cards inside crop field and 20 in SNHs. Twenty seeds of *A. artemisiifolia* were attached to the sandpaper which covered with metal wire meshes. Seed removal was assessed every 24 hours of exposure for 5 days in June 2019 and October 2020, 6 days in June 2021, and 7 days in November 2019. Results showed high consumption rates (overall average 95.2, 8.5% ± sd) on *A. artemisiifolia* seeds in all sampling rounds. Seed consumption rates were higher inside field crops in summer in 2019: 99 ± 2%, than in autumn 2019: 81.7±16%, with slight difference between SNHs (99.5±1.8%) and inside fields (93.9 ±7.8%). These findings indicate the potential of seed predation to contribute on weed management inside crop fields and in adjacent SNHs. Seed predation reduced number of exposed seeds on soil surface and thus decreased weed seed bank. Weed seed predation could be considered in Integrated weed management framework to reduce the heavy use of herbicides.

9. ÖSSZEFOGLALÁS (SUMMARY IN HUNGARIAN)

A gyomosodás általában jelentősen csökkenti a terméshozamot és a minőséget. Magyarországon például a parlagfű *A. artemisiifolia* a mezőgazdasági és ruderális területeken is káros fajjá vált. A gyomirtó szerektől való függés azonban hatással van az emberi egészségre, a hasznos szervezetekre és az ökoszisztéma-szolgáltatásokra. Ezért fenntartható és hosszú távú gyomszabályozási megoldásokra illetve a gyomnövények mennyiségének korlátozására van szükség. A gyommag fogyasztás olyan potenciális ökoszisztéma-szolgáltatásnak tekinthető, amely mind a művelt területeken, mind a természetközeli élőhelyeken jelen van. A gyommagok magpredátorok általi fogyasztása csökkentheti a gyomnövények egyedszámát a következő vegetációs időszakban, és ezáltal a gyomirtó szerek használatát. Ebben a kutatásban négy gyomfaj (*Ambrosia artemisiifolia* L., *Datura stramonium* L., *Chenopodium album* L. és *Echinochloa crus-galli* L.) magjainak predációját vizsgáltuk kukoricában és három gyomfaj (*Galium aparine* L., *Papaver rhoeas* L. és *Apera spica-venti* L.) magjainak predációját őszi búzában valamint a szomszédos természetközeli élőhelyeken (SNH) Gödöllőn, Magyarországon. Feltételeztük, hogy a gyomnövények magpredációjának mértéke fajonként és élőhelytípusonként eltérő lehet. A vizsgálatokat két évben, 2019 novemberében és 2020 októberében végeztük. Mindkét évben 100 magkártyát helyeztünk el a talajfelszínre a kukoricatáblákon belül a betakarítás előtt, és 25-öt a szomszédos SNH-kon. Gyomfajonként 20 magot ragasztottunk homokpapírra (25×10 cm, P=60), amelyet dróthálóval fedtünk le, hogy megakadályozzuk a gerinces ragadozók hozzáférését. A megfigyelés a magkártyák kihelyezése után 24 órával kezdődött, és a magveszteséget 2019-ben 7 napon keresztül, 2020-ban pedig 5 napon keresztül minden nap feljegyeztük. Az eredmények azt mutatták, hogy minden magkártyán magas volt a magveszteség, átlagosan (85.9 ± 13.7%). Mindkét évben a kártyákon maradt magok aránya az első naptól kezdve csökkent. A magveszteség mérésének optimális időpontja a 3. és 4. nap volt, de a magveszteség szignifikánsan különbözött az egyes évek között (P < 0.001), 2020-ban magasabb volt, mint 2019-ben. A gyomfajok között nem volt különbség a predációs arányok között (P = 0.962, 0. 079). Hasonlóképpen, az őszi búzában kétszer, 2019-ben és 2021-ben végeztünk mintavételt, 240 magkártya (120 kártya/kör) talajfelszínre helyezésével. 60 magkártyát a szántóföld belsejében, 60-at a szomszédos SNH-kban helyeztünk el. Minden egyes kártyán 24 óránként értékeltük a magveszteséget, 2019-ben 5 napon keresztül, 2021-ben pedig 6 napon keresztül. Az eredmények a gyommagfogyasztás magas intenzitását (100%) mutatták. A magfogyasztás mindkét évben mindkét élőhelyen, minden magkártyán megfigyelhető volt, bár a gyommagfogyasztás 2019-ben szignifikánsan nagyobb volt (p < 0.001), mint 2021-ben. Az élőhelytípusok, a gyomfajok és az élőhely (p = 0.353), valamint az élőhely és az év (p = 0.842) között nem volt szignifikáns különbség (p = 0.802). A

gyommagfogyasztás számszerűsítésére rövid, 48 órás expozíciós időszakot határoztunk meg. Az *Ambrosia* esetében a magpredációt nyáron (2019 júniusában és 2021-ben) egy búzatáblában, ősszel (2019 novemberében és 2020 októberében) pedig egy kukoricatáblában, valamint a szomszédos SNH-kon belül számszerűsítettük. Összesen 160 magkártya felhasználásával 4 mintavételi fordulóban vizsgáltuk a gyommagok gerinctelen magpredátoroknak való kitettségét Gödöllőn. A talajfelszínre 40 magkártya/kör került kihelyezésre, 20 kártyát a szántóföldön belül, 20-at pedig az SNH-kban helyeztünk el. Húsz *A. artemisiifolia* magot rögzítettünk a homokpapírra, amelyet fémdróthálóval borítottunk. A magok eltávolítását 24 óránként értékeltük a kitettséget követően 2019 júniusában és 2020 októberében 5 napon keresztül, 2021 júniusában 6 napon keresztül, 2019 novemberében pedig 7 napon keresztül. Az eredmények magas fogyasztási arányt mutattak (összességében átlagosan 95.2, 8.5% ± sd) az *Ambrosia* magvakra vonatkozóan minden mintavételi fordulóban. A magfogyasztási arányok magasabbak voltak a szántóföldi kultúrákban 2019 nyarán: 99 ± 2%, mint 2019 őszén: 81.7±16%. Enyhe különbség volt az SNH-k (99.5±1.8%) és a szántóföldi kultúrákon belüli eredmények vonatkozásában (93.9 ±7.8%). Ezek az eredmények azt mutatják, hogy a magpredáció hozzájárulhat a gyomszabályozáshoz a kukorica- és őszi búza táblákon és a szomszédos SNH-kon. A gyommagpredáció csökkentette a talajfelszínen lévő magok számát, így csökkentheti a talaj gyommagkészletét és a herbicidhasználatot.

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2- **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

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