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# Seed Dispersal by Rooks (*Corvus frugilegus*) - with Focus on Non-Native Species

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### Abstract

Seed dispersal is an essential ecological function, and many plants depend on dispersal mediated by animals (zoochory). Zoochory is important for the natural succession and regeneration of plant communities but may also play a role in the dispersal of non-native and invasive species with damaging ecosystem effects. The rook (Corvus *frugilegus*) is an omnivorous species eating a broad variety of seeds and therefore potential to disperse a wide range of seeds in both natural and urban habitats. In this study seed dispersal by rooks in Central and Northern Jutland, Denmark was examined through examination of regurgitation pellets and vegetation studies. The aim of the study was to investigate i) to what extent rook regurgitation pellets found under rookeries contain seeds; ii) which seed species are found, including whether pellets contain seeds of non-native species; iii) if the plant species found in the regurgitated pellets were found in the vegetation under rookeries. Regurgitation pellets were collected from April to June beneath rookeries. In total 124 seeds representing 8 taxa were found in 153 pellets. Most of the seed species were cereals and other dry-fruited species, mainly weeds and ruderal species. The study showed that Danish rooks canact as seed dispersers. Cereals and other dry-fruited species such as weeds, and ruderal species were the dominant seeds found in rook pellets. No non-native species were found. However, this does not exclude the possibility of the rooks being able to disperse them. The lack of exotic fruit found in the pellets of Danish rooks may be due to waste management in Denmark, not having open waste dumps and open compost heaps with spill overs from the kitchen is not recommended. A vegetation survey under rookeries showed that only Stellaria media was found both in regurgitation pellets and in the vegetation beneath rookeries, suggesting that many dispersed seeds are unable to germinate or survive under the tree canopies at the rookeries.

Keywords: vegetation survey; regurgitation pellets; invasive species; zoochory.

#### الملخص

تشتت البذور هو وظيفة بيئية أساسية، وتعتمد العديد من النباتات على التشتت بوساطة الحيوانات .(zoochory) تعتبر Zoochory مهمة للتعاقب الطبيعي وتجديد المجتمعات النباتية، ولكنها قد تلعب أيضًا دورًا في تشتت الأنواع غير الأصلية والغازية مع تأثيرات النظام البيئي الضارة. الرخ (Corvus frugilegus) هو من الأنواع النهمة التي تأكل مجموعة واسعة من البذور وبالتالي فهي قادرة على تفريق مجموعة واسعة من البذور في كل من الموائل الطبيعية و الحضرية. في هذه الدراسة تم فحص نثر البذور بالغراب في وسط وشمال جوتلاند ، الدنمارك من خلال فحص حبيات كل من الموائل الطبيعية و الحضرية. في هذه الدراسة تم فحص نثر البذور بالغراب في وسط وشمال جوتلاند ، الدنمارك من خلال فحص حبيات كل من الموائل الطبيعية و الحضرية. في هذه الدراسة تم فحص نثر البذور بالغراب في وسط وشمال جوتلاند ، الدنمارك من خلال فحص حبيات على بذور ؛ 2) أنواع البذور التي تم العرف من الدراسة هو التحقق من: 1) إلى أي مدى تحتوي كريات ارتجاع الرخ الموجودة تحت مغلفات النباتات على بذور ؛ 2) أنواع البذور التي تم العرفر عليها، بما في ذلك ما إذا كانت الكريات تحتوي على بذور من أبريل إلى بونيو من تحت مغلف النباتي تحت المصانع. تم جمع كريات القاس من أبريل إلى يونيو من تحت مغارف الغربان. النباتية الموجودة في الجنور التي تم العرف من الدراسة في ذلك ما إذا كانت الكريات تحتوي على بذور م ازواع غير محلية ؛ 3) ما إذا كانت الكريات تحتوي على بذور من أنواع غير محلية ؛ 3) ما إذا كانت الأنواع في بذور ؛ 2) أنواع البذور التي تم العربان الموجودة في للعاء النباتية الموجودة في العربي إلى يونيو من تحت مغارف الغربان. في المجموع ، تم العثور على المجموع ، تم العثور على الغربان. في المجموع ، تم العثور على المعنور على إلى أولي الغربان الدماركية يمكن أن تعمل كمشتات البذور على دات ثمار جافة ، وخاصة العربان الغربان الموات الغربان الدمار كية يمكن أن تعمل كمشتور على أنواع الخرى ذات ثمار حاف في أور الغربان الدماركية ومع ذالي فالغربان. في المجموع مالخران الخرين من الغربان الحفري على ألنواع البرين والأواع البرية ، في الغربي أور المحمودة في كريات الرخ . لم يتم العثور على أنواع غرب فال هان هان ها وي الغربان في الغرور على ألفرا والغذري ي الغربي المحمور الخلي مان والغان ألفرا الجنور على في ألغري الغرار الحفي في مالمدين أولالغان مكنور على م

# Introduction

A growing human population, globalization, and urbanization has increased the spread and distribution of non-native and invasive species that threaten global biodiversity (Millennium Ecosystem Assessment 2005; Rai & Singh 2020). Biodiversity loss may over the next 50 years affect environmental quality, ecosystem services, and ultimately human welfare and invasive species expenses (Rai & Singh, 2020; Pyšek et al. 2020; Richardson et al. 2000; Kettunen et al. 2008). Introduction of invasive plant species to novel habitats may occur by various seed dispersal mechanisms, and especially zoochory including

ornitochory (dispersal of seeds by animals and birds, respectively) has gained increased focus recently (Gosper et al. 2005; Benvenuti 2007 Kitowski et al. 2017; Green et al. 2019). Birds are effective seed dispersers being responsible for the dispersal of around 40% of trees and shrubs in temperate regions and around 25% of invasive plant species (Jordano 2000: Gosper et al. 2005; Green et al. 2019). Rooks are important Corvid seed dispersers associated with both urban and agricultural environments (Czarnecka et al. 2013; Kitowski et al. 2017). Rooks are omnivores that eats small invertebrates, fruits and a broad variety of seeds (Fog 1963; Gromadzka 1980; Czarnecka & Kitowski 2013; Kitowski et al. 2017). In Poland, they have been found to disperse seeds of e.g. the alien walnut (Juglans regia), exotic kiwi fruit (Actinidia deliciosa) and pepper (Capsicum annuum), most likely originating from human food waste (Lenda et al. 2012; Czarnecka et al. 2013). Other studies have found large proportions of fleshy-fruited species such as cherry (Prunus avium) and strawberries (Fragaria sp.), and also cereals and weed species such as barley (Hordeum vulgare) and chickweed (Stellaria media) in rook regurgitation pellets (Czarnecka & Kitowski 2010, 2013). In previous studies, seeds of between 19 and 60 plant taxa have been found in rook regurgitation pellets, most of which were also found in the vegetation below the rookeries (Czarnecka & Kitowski 2010, 2013; Czarnecka et al. 2013; Kitowski et al. 2017). Consequently, rooks are potential dispersers of non-native species and invasive species in Denmark as well as other countries, which could have detrimental effects on biodiversity and human health.

The aim of this study was to investigate 1) to what extent rook regurgitation pellets found under rookeries contain seeds, 2) which seed species are found in regurgitated pellets of Danish rooks and whether seeds of non-native species are among; 3) if the rook-mediated seed dispersal is altering the vegetation beneath the rookeries.

# Materials and Methods

# Study areas and collection of regurgitation pellets

Regurgitation pellets from six rookeries containing between 20 and 90 nests in mostly agriculturedominated landscapes in Central and Northern Jutland, Denmark were collected from April to June 2021. Three rookeries were close to anthropogenic organic waste sources; one in the city of Aalborg (L1), one close to the city of Aarhus (L2) and one located next to a motorway rest area (L5) (Table 1). Some of the rookeries were visited more than once and all available non-crushed pellets were collected. Collected pellets were stored in coffee filters in sealed or unsealed plastic bags marked with location and collection date at approx. 20°C until sorting in spring 2022.

**Table 1.** Location of rookeries, the collection date of the regurgitation pellets and the number of collected pellets (n). The total number of pellets was N=153.

Sampling sites	Rookery location and sample date					
	Coordinates (DDM)	Collection date (number of pellets				
L1 <sup>a</sup>	57°2.3352 N, 9°53.9369 E	08-05-2021 (n=28)	03-06-2021 (n=2)			
L2 <sup>a</sup>	56°7.3556 N, 10°13.1531 E	25-04-2021 (n=1)	13-06-2021 (n=27)			
L3	56°24.3799 N, 10°33.4193 E	29-04-2021 (n=30)	14-06-2021 (n=23)			
L4	56°11.2199 N, 10°11.6788 E	01-05-2021 (n=1)				
L5 <sup>a</sup>	56°26.9796 N, 9°59.9474 E	03-08-06-2021 (n=18)	22-06-2021 (n=12)			
L6	55°52.1339 N, 10°0.2440 E	13-06-2021 (n=13)				

a. Access to human wastes from either larger town or motorway rest area

# Analysis of regurgitation pellets

The pellets (one pellet = one sample) were weighed and examined using a stereo microscope under 10x magnification in petri dishes. The pellets were systematically sorted through with tweezers and all seeds and animal items were sorted and stored in 1.5 mL Eppendorf tubes at around  $22^{\circ}$ C for further investigation. Both intact and damaged seeds were counted and identified as a minimum to family level, and some were identified to genus- or species level. The proportion of seeds, animal items, pebbles and anthropogenic items in the pellets were noted to nearest 5%. However, no anthropogenic items were found in the pellets.

Identification of seeds was carried out using own reference collection of 84 seeds from common Danish

berries, grasses, and herbaceous plants - both wild and cultivated species. Also, relevant literature and websites containing photos and illustrations of seeds was used (Lindman & Keuck 1999; Groningen Institute of Archaeology & Deutsches Archäologisches Institut 2006; International Seed Morphology Association 2019; Department of Agroecology at Aarhus University & SEGES Innovation 2022).

### Vegetation survey

To evaluate whether regurgitation pellets lead to vegetation changes beneath rookeries, a vegetation survey was conducted on the ground below the surveyed rookeries at three locations (L1, L3 and L5). The vegetation survey was carried out in late April 2022. The method of the vegetation survey followed Fredshavn et al. (2022) where all species in a test field measuring  $0.5 \times 0.5$  meters were carefully recorded; supplementary species (species not noted in the smaller test field) were additionally registered in a circle measuring 5 meters in radius (78.5 m2); and surrounding vegetation, shrubs and trees, were registered to provide an overview of the area. At each location, four test fields and one 5-metre circle were applied beneath the rookeries and in a control area at least 50 m away from the nearest rook nests.

# Data Analysis

The data for weight of the pellets was tested for normality with Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. A one-way ANOVA was performed to evaluate the differences between months or locations. Chi squared test was applied to evaluate if pellets containing seeds varied between months and locations and if the number of species found in the vegetation survey differed between locations (L1, L3, L5). Sample values <5 in the Chi squared test were excluded, which resulted in the exclusion of location 4 (L4). Kruskal Wallis test was applied to evaluate if number of seeds per pellet varied between month and location. A Mann-Whitney U test (pairwise) with Bonferroni correction was applied to the data for number of seeds per pellet for locations.

### Results

### Seeds in regurgitation pellets

A total number of 153 pellets was analyzed and 29% of the pellets contained seeds. In these pellets there were on average 2.76 seeds pr. pellet. Out of all pellets, there were on average 0.8 seeds per pellet which for locations ranged from 0.3-3 seeds per pellet and for months from 0.4-1.3 seeds per pellet (Table 2). The number of seeds was significantly higher in location L5 than L3 (p=0.02). No significant difference was found when comparing number of seeds per pellet between months (p=0.10). Of all found seeds (N=124), 51% were intact and 49% were damaged in varying degrees. The seeds represented 8 plant families, with the majority being Poaceae sp. (Figure 1, Appendix table A1). Cereals (barley (*Hordeum vulgare*), oat (*Avena sativa*), wheat (*Triticum aestivum*) comprised 64% of the total number of seeds. Barley alone, which was found in 14% of the pellets, included 50% of the total number of seeds. All the seeds identified to species level were dry-fruited species and apart from the cereals, associated with disturbed or urban areas. None of the identified seeds were from invasive species. The number of pellets with or without seeds did not differ between months (p=0.17) nor locations (p=0.06) (Table 2).

**Table 2**. Number of pellets for each location and the month the pellet was sampled. Also, the total number of seeds, number of intact seeds, the mean number of seeds per pellet, frequency of pellets with seeds and the mean mass of the pellet.

			Locati	ons				Months	
-	L1	L2	L3	L4	L5	L6	April	May	June
Number of pellets (N=153)	30	28	53	1	28	13	57	29	67
Total number of seeds (N=124)	14	16	17	3	62	12	22	15	87
Number of intact seeds (N=63)	11	11	11	1	18	11	13	10	40
Mean number of seeds per pellet	0.5	0.6	0.3	3	2.2	0.9	0.4	0.5	1.3
Frequency of pellets with seeds (%)	23	25	21	100	50	39	23	24	37
Mean mass of pellet (g)	1.9	1.8	2.0	1.7	1.6	1.8	1.8	1.9	1.8





The majority of pellets (94%) contained small stones ranging in size from 0.1-10 mm. Stones constituted on average 10% ranging from 0-70% of the pellet material. Animal items were found in 87% of the pellets and 26% of the pellets contained visible mould. The mean weight of the pellets was 1.85 g ranging from 0.12-5.14 g. Pellet size/weight did not vary with month (p=0.91) or location (p=0.47).

#### Vegetation survey

The vegetation survey for the three locations (L1, L3 and L5) resulted in an average of 3.8 species under the rookeries compared to 3 species in the control areas (Figure 1). In the 5-meter circle additional 10 species were recorded under rookeries whereas 14 species were found in the control area (Table 3). There was no significant difference in the vegetation below rookeries compared to the vegetation in the control areas when comparing the pooled number of species in the three locations (p=0.86). Chickweed was the only species found in both the pellets and beneath the rookeries. None of the species observed in the survey were non-native Table 3.

No. of species	Area and total/average	L1	L3	L5	Total
Test field	Rookery (average)	3.25	3	5.25	3.83
	Control (average)	3	2	4	3
5-metre circle	Rookery (total)	3	5	2	10
	Control (total)	5	2	7	14
Additional	Surroundings (total)	4	4	6	14

**Table 3.** Number of species found in the test field (0,5x0.5 meter), the 5- meter circle and control areas. The numbers for the test fields are averages based on four test fields in each location.

#### Discussion

#### Seeds in regurgitation pellets

A study by Green et al. (2019) found that regurgitation pellets from two corvids species contained a higher number of intact seeds than droppings. Therefore, seeds from pellets is expected to have more effects on the vegetation beneath rookeries than droppings, suggesting that regurgitation pellets is the best method for analyzing seed dispersal by rooks.

The average number of seeds per pellet found in this study (0.8) was lower than the average 3.9 seeds per pellet discovered in spring pellets in Poland (Czarnecka & Kitowski 2010). However, another study by Czarnecka & Kitowski (2013) found on average 1.84 seeds per pellet ranging from 0.27 to 4.60 seeds per pellet across months with the number of seeds being lowest in April and highest in June. Although no significant differences were found between the number of seeds per pellet between the month in this

study the tendency of seeds being fewer per pellet in April (0.4 seeds) than in June (1.3 seeds) with May having an intermediate value (0.5) was found was the same as in the previous study (Table 2). The increasing number of seeds per pellet from April to June is probably due to more plant species with ripe seeds as the spring temperatures are getting warmer. A study by Kitowski et al. (2017) in the spring in Poland and Romania found on average 2.16 seeds per pellet ranging from 1.90 to 2.95 seeds per pellet across locations, which is high compared to this study. A possible explanation for this difference in the number of seeds per pellet may be attributed to the differences in cultivation level in the countries. Denmark is the most intensively cultivated country in Europe where more than 60% of the total area constitute to agricultural areas compared to 46% in Poland (Holmstrup et al. 2018). This could lead to an increased foraging for invertebrates instead of seeds causing the rooks in Denmark to have a higher consumption of animal items compared to Poland and Romania. The overall proportion of pellets containing seeds (29%) is similar to the proportion (34%) found by Czarnecka et al. (2013).

The pellets mainly comprised plant material and chitin fragments from invertebrates (in 87% of the pellets). Rooks eat both plant and invertebrates, with invertebrates being most important during spring (Fog 1963; Gromadzka 1980; Kitowski et al. 2017). Czarnecka et al. (2013) also found human remnants such as paper, plastic bags, glass, and aluminium foil, which they found in 65.7% of the pellets. This was not the case in this study which could be ascribed to the time of year. Czarnecka et al. (2013) collected the pellets in December and January and human remnants are more common in pellets from winter roosts due to the rooks foraging on garbage dumps (Czarnecka & Kitowski 2010).

The significant higher number of seeds per pellet at location L5 compared to location L3 may be due to differences between the two locations. L3 is sited in a beech forest with dense canopies surrounded by agricultural areas while location L5 is sited in a large tree on a lawn by a pull-up along the motorway surrounded by primarily meadows and a large river bench. This difference between the locations may have influenced the number of cereals (barley and wheat) especially found in the pellets found at L5, indicating that the rooks at L5 feed on cultivated land to a greater extent than the rooks in L3. The lack of significant differences in number of seeds per pellet between months maybe due to low sample size.

In the current study, a total of 124 seeds representing 8 different taxa (families) was found (Figure 1, Appendix table A1). The number of taxa is lower than in other studies, that found 19-60 taxa with the number of seeds ranging from 571-2,257 (Czarnecka & Kitowski 2010, 2013; Czarnecka et al. 2013; Kitowski et al. 2017). The much larger seed pool in the studies from Poland and Romania most probably reflects a larger sample size in the studies from Poland and Romania (188-739 pellets). However, the larger proportion of agricultural areas in Denmark than in Poland may also lead to a lower seed diversity in the rook's diet (Holmstrup et al. 2018).

In comparison with other studies (Czarnecka & Kitowski 2010, 2013; Czarnecka et al. 2013; Kitowski et al. 2017), this study also found barley, chickweed, dandelion (Taraxacum officinale), oat, proso millet (Panicum miliaceum), wheat, wild buckwheat (Fallopia convolvulus), yellow foxtail (Setaria pumila), and the following genera: Echinochloa sp., Polygonum sp., Rubus sp., and Rumex sp (Appendix table A1). The majority were the dry-fruited species to be from either cultivated meadows or ruderal habitats (Appendix table A1). Holland et al. (2006) investigated the food of farmland birds in Europe, found the most important plant genera (not including cereals) to be Stellaria sp., especially chickweed, Polygonum sp., especially common knotgrass and several genera belonging to the Poaceae. In addition, cereals are an important food source throughout the year. Poaceae sp. comprised 38% of the rooks' diet (Holland et al. 2006). Dry-fruited species comprise a stable component of the rook's diet, which has also been found for other seed-eating birds (Holland et al. 2006; Orłowski & Czarnecka 2009). The reason for the low occurence of fleshy-fruited species in this study is most probably due to time of year for collecting samples, as most fruits and berries are present from mid-summer and autumn. Species from the genera Rubus as well as the families Solanaceae and Rosaceae have fleshy-fruited seeds, and these comprised around 10% of the total seed pool in this study. Fleshy fruits have been found to be an important and stable element of the diet of breeding rooks in Poland (Czarnecka & Kitowski 2013; Czarnecka et al. 2013. Kitowski et al. (2017) observed a higher frequency of fleshy-fruited species in urban areas (54-66%) compared to agricultural areas (12-38%). Fleshy-fruited species may occur year around in human leftovers, however Denmark does not have open waste dumps, whereas these can still be found in Poland and constitute a food source for omnivores such as the rook (Czarnecka & Kitowski 2010; Czarnecka et al. 2013). Consequently, the access to exotic fleshy-fruited seeds from human garbage may be higher in Poland compared to Denmark.

Several species of birds such as the American robin (*Turdus migratorius*), three-wattled bellbird (*Procnias tricarunculata*), magpie (*Pica pica*), hooded crow (*Corvus cornix*), common raven (*Corvus corax*) and rook have been found to disperse seeds to disturbed habitats such as edges and gaps of forests (Gosper et al. 2005; Bartuszevige & Gorchov 2006) rather than to understory sites (Bartuszevige & Gorchov 2006).

In the vegetation study only chickweed occurred in both pellets and under rookeries. In Poland (Czarnecka & Kitowski 2010, 2013; Czarnecka et al. 2013) chickweed was found in the vegetation under the rookeries - together with several other species. In Czarnecka & Kitowski (2013) chickweed was present under more than a half of the investigated rookeries. Chickweed is a common and dominant weed species in cereal fields in Scandinavia and in Denmark it has a frequency of more than 10% in all fields (Bitarafan & Andreasen 2019). Therefore, the presence of chickweed under the rookeries can probably be attributed to this as well as its persistence.

Most rookeries in this study were located in forests, where the forest floor was covered by leaf litter with scattered occurrence of herbs, bushes and small trees, providing poor germination conditions for most species (Valladares et al. 2016). For instance, locations L1 an L3 were beech forests, where the mature trees had formed a dense canopy out shading many other plant species. In the study by Nogales et al. (1999) where ravens nests were in the edges of forests or woodlots, 75% of ingested seeds were found in the vegetation.

In this study a mean number of 3.8 species was recorded under the rookeries. In Poland vegetation studies beneath rookeries found a mean number ranging from 21 to 31 species /Czarnecka & Kitowski 2013; Czarnecka et al. 2013. The significant difference between vegetation beneath rookeries in Denmark and Poland may be due to different methods for vegetation study and season, factor potentially affecting the number of species found in the vegetation survey. Czarnecka & Kitowski (2013) and Czarnecka et al. (2013) conducted their vegetation surveys in July, while the vegetation analyses in this study was in April. However, the difference in mean number of species found in Denmark is much probably due to a general higher biodiversity in Polish woods than in Danish beech forests. It has been observed that the presence of rookeries causes a rise in the soil acidity, nutrient level, and the humidity, which can affect plant species differently depending on the type of habitat (Weir 1969; Borkowska et al. 2015). The lack of significant differences between the vegetation under the rookeries in Denmark compared to the control areas, may be due to much smaller rookeries in Denmark than in Poland. In Poland, rookeries of almost 600 nests have been observed whereas the rookeries in this study ranged from 20-90 nests. Additionally, rookeries in Denmark are known to move about and spread to smaller size due to high culling of rooks during the breeding season, preventing long time effects of from regurgitation pellets and droppings (Fog 1963).

# Conclusion

This study showed that Danish rooks act as seed dispersers. Cereals and other dry-fruited species such as weeds, and ruderal species were the dominant seeds found in rook pellets. No non-native species were found; however, this does not exclude the possibility of the rooks being able to disperse them. The lack of exotic fruit found in the pellets of Danish rooks may be due to waste management in Denmark, not having open waste dumps and open compost heaps with spill overs from the kitchen is not recommended by the Danish authorities. Chickweed was the only species present both in regurgitation pellets and in the vegetation below the investigated Danish rookeries.

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# **Author's Contributions**

The three authors contributed to data collection, preparation, development and publication of this manuscript.

No animal was harmed, and Danish legislation has been followed during the performance of this study.

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# Table A.1. Latin, family name, fruit type for the species found in rook regurgitation pellets this study.

			Habitat type	Fruit
Latin name	Family	Native/Non-native		type
Asteraceae sp.	Asteraceae	—	_	_
Avena sativa P	Poaceae	Non-native	Cultivated	Dry
Bupleurum sp.	Apiaceae	—	_	_
Carthamus tinctorius	Asteraceae	Non-native	Cultivated	Dry
Echinochloa sp.	Poaceae	—	_	Dry
Fallopia convolvulus	Polygonaceae	Native	Meadow/ruderal	Dry
Helianthus annuus	Asteraceae	Non-native	Cultivated	Dry
Hordeum vulgare P	Poaceae	Non-native	Cultivated	Dry
Oxybasis sp.	Amaranthaceae	_	_	
Panicum miliaceum	Poaceae	Non-native	Cultivated	Dry
Persicaria sp.	Polygonaceae	_	_	
Poaceae sp.	Poacea	_	_	Dry
Polygonum aviculare	Polygonaceae	Native	Meadow/ruderal	Dry
Polygonum sp.	Polygonaceae	_	-	Dry
Rosaceae sp.	Rosaceae	_	-	Dry
Rubus sp.	Rosaceae	_	-	Fleshy
Rumex sp.	Polygonaceae	_	-	_
Setaria pumila	Poaceae	Native	Meadow/ruderal	Dry
Solanaceae sp.	Solanaceae	_	-	_
Stellaria media	Caryophyllaceae	Narive	Meadow/ruderal	Dry
Taraxacum officinale	Asteraceae	Native	Meadow/ruderal	Dry
Triticum aestivum	Poaceae	Non-native	Cultivated	Dry