

Ambrosia artemisiifolia L. seeds in bird feed in Southern Belgium, 10 years after the EU regulation

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Description of the subject. Common Ragweed (*Ambrosia artemisiifolia* L.) is a problematic invasive plant which population has recently spread/increased throughout Belgium. Previous studies in European countries have pointed out wild bird and poultry feed trade as a vector of introduction. In 2011, a maximum limit of contamination was imposed by the European commission.

Objectives. The goal is to quantify the level of bird seed contamination sold in Southern Belgium.

Method. In 2021, we analyzed 42 bags of bird feed, purchased in various retailers in Southern Belgium. We distinguished wild bird seed mixes, poultry seed mixes and sunflower seeds.

Results. Regardless of the moisture of the samples (not measured), the results of the samples were not near the legal limit. However, 13 out of 42 samples contained common ragweed seeds and 0.75 ± 0.265 seeds (mean \pm standard error) per kilo of bird feed were found on average, of which 82.4% were viable.

Conclusions. Although the legal limit seems to be respected, the bird feed trade still represents a substantial introduction pathway for ragweed.

Keywords. Asteraceae, weeds, invasive species, regulations, feed contamination, introduced species.

Graines d'*Ambrosia artemisiifolia* dans la nourriture pour oiseaux en Wallonie, 10 ans après la réglementation européenne

Description du sujet. L'ambrosie annuelle (*Ambrosia artemisiifolia* L.) est une plante exotique envahissante problématique, en nette expansion en Belgique. Des études antérieures en Europe ont démontré que le commerce de graines pour oiseaux était un vecteur d'introduction et en 2011, une valeur maximale de contamination a été imposée par la Commission européenne.

Objectifs. L'objectif est de quantifier le niveau de contamination des graines pour oiseaux vendues en Wallonie.

Méthode. En 2021, nous avons analysé 42 sacs de graines pour oiseaux, achetés auprès de différents vendeurs en Wallonie. Nous avons distingué les mélanges de graines pour oiseaux sauvages, les mélanges de graines pour volailles et les graines de tournesol.

Résultats. Nos résultats, même s'ils ne tenaient pas compte de l'humidité des échantillons, n'ont pas indiqué que les échantillons s'approchaient de la limite légale. Néanmoins, 13 des 42 échantillons contenaient des graines d'ambrosie et $0,75 \pm 0,265$ graines (moyenne \pm erreur standard) étaient retrouvées par kilo, dont 82,4 % viables.

Conclusions. Malgré le respect des limites légales, la nourriture pour oiseaux semble rester une voie d'introduction conséquente pour l'ambrosie.

Mots-clés. Asteraceae, mauvaise herbe, espèce envahissante, réglementation, contamination des aliments pour animaux, espèce introduite.

1. INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.) is a very problematic invasive plant in Europe. Its pollen is highly allergenic, and the plant is known as a weed in different crops, particularly sunflower (Chauvel et al., 2004). Originating from North America, its accidental introduction is thought to be related to worldwide movements of contaminated seed lots, fodder, and soil (Chauvel et al., 2006). The analyses of invasion patterns, coupled with genetic structure analyses, strongly suggest that there have been multiple introduction events in European countries (Chauvel et al., 2006; van Boheemen et al., 2017).

Bird seed mixes/feed, particularly those including sunflower seeds, were repeatedly pointed out as a vector of spread. Between 2008 and 2012, several studies assessed the presence of ragweed in bird feed available on the market. Vitalos & Karrer (2008) found ragweed seeds in 7 out of 19 samples and concluded that bird seed should play a minor role in the primary introduction in non-invaded countries. However, in 2011, Frick et al. (2011) documented the presence of ragweed seeds in 21% to 75% of the bird seed mixes/feed on the market in Switzerland, Germany, Slovenia and Denmark. The same year, an EU commission regulation amended the annex 1 of the directive 2002/32/EC to better address the risk of ragweed spread due to bird feed transportation. It imposed a maximum limit of 50 mg·kg⁻¹ of *Ambrosia* spp. seeds in unground grains and seeds with a moisture content of 12%. Krajšek & Novak (2013) found ragweed in 8 out of 24 samples bought in Slovenia in 2011, of which three were exceeding the legal limit. Between 2007 and 2012, Ries et al. (2013) found ragweed seeds in 7 out of 33 samples bought in Luxemburg, of which 2 were exceeding the legal limit.

Ten years ago, common ragweed was hardly considered as a problem in the Walloon region (Southern Belgium). However, due to an increasing number of records of the species, it has progressively been considered as a significant threat to human health. In 2019, the Walloon Ragweed Observatory (WRO) was launched to notably monitor the progress of the species and reduce the spread as much as possible. As field observations were repeatedly made in sites where birds were fed (poultry farm, bird feeders in gardens, etc.), the WRO analyzed bird feed samples bought in 2021 in Wallonia to determine:

- what is the proportion of contaminated sample;
- how much ragweed seeds are found per kilo of bird feed;
- is there a difference in the contamination level between poultry feed, wild bird seed mixes and pure sunflower seeds?

2. MATERIALS AND METHODS

In 2021, bags of 21 different products were bought in eight retail chains (ranging from specialized feed retailer to supermarkets). Among the products, eight were sold as poultry seed mixes; seven as wild bird seed mixes and six were exclusively sunflower seeds. After ca. one month, bags of the same 21 products were bought again, either in the same or in a different shop, resulting in 42 samples (**Table 1**). In each bag, a composite sample of 2 kg (four random subsamples of 500 g) was collected and sieved through a 3 mm mesh. This mesh size is intermediate between the sizes used in Krajšek & Novak (2013) (2.3 mm) and Jakovac et al. (2013) (4 mm), and Frick et al. (2011) found that seeds larger than 3.5 mm were rare. Ortmans et al. (2016) found no seeds (out of 900) with a width above 3 mm. The sieved fraction (< 3 mm) was inspected with a stereomicroscope and all *A. artemisiifolia* achenes (referred to as seeds) were collected and weighted to the nearest 10⁻³ g. It must be noted that samples were analyzed at their actual moisture level, not at a homogenized 12% moisture level. The viability of the seeds was assessed with a crush test (following Karrer et al., 2016). The crush test is a simple and widely accepted means of testing seed viability, though overestimation is possible (Hall et al., 2021). An ANOVA was performed on the number of seeds per kilo and the mass of seed per kilo after the responses were rank-transformed. The feed type was fixed, and product was random, nested in feed type.

3. RESULTS

Ambrosia seeds were found in 13 out of 42 samples, with maximal values of 7.5 seeds·kg⁻¹ and 34 mg·kg⁻¹. **Table 1** summarizes the results. The ANOVA revealed no significant effect of the feed type (poultry seed mixes, wild bird seed mixes and sunflower seeds) on the number of ragweed seeds per kilo and the mass of seed per kilo (respectively Df = 2; F = 0.27; P = 0.770; and Df = 2; F = 0.32; P = 0.731), but a significant effect of product (respectively Df = 17; F = 3.27; P = 0.005; and Df = 17; F = 3.59; P = 0.003). As a reminder, a product is a particular bird feed as it is sold on the market, *i.e.* two bags of the same product look identical (except for the lot number).

4. DISCUSSION

The proportion of contaminated products in our study (13/42, 31%) was far from negligible and comparable to previous studies (Vitalos et al., 2008; Frick et al., 2011; Krajšek et al., 2013; Ries et al., 2013). Though,

Table 1. Results of the bird feed samples analysis. The two replicates are different bags of the same product, bought with approximately one month of delay and either in the same or a different shop of the retailer chain — *Résultats de l'analyse des échantillons de nourriture pour oiseaux. Les deux réplicats sont deux sacs d'un même produit, achetés avec approximativement un mois d'écart et soit dans le même magasin, soit dans un autre magasin de la même chaîne de magasin.*

Product	Feed type	Retail chain	Replicate	Ragweed seeds·kg ⁻¹ of bird feed	Mass of ragweed seeds·kg ⁻¹ of bird feed (mg·kg ⁻¹)	Proportion of viable seeds (%)
Product 1	Poultry seed mix	A	1	1.5	6	33.3
			2	3	15	100
Product 2	Poultry seed mix	B	1	1.5	5.5	100
			2	0.5	2.5	100
Product 3	Poultry seed mix	C	1	0.5	2.5	100
			2	1.5	4	66.7
Product 4	Poultry seed mix	B	1	0	0	-
			2	0.5	3.5	100
Product 5	Poultry seed mix	D	1	0	0	-
			2	0	0	-
Product 6	Poultry seed mix	C	1	0	0	-
			2	0	0	-
Product 7	Poultry seed mix	E	1	0	0	-
			2	0	0	-
Product 8	Poultry seed mix	E	1	0	0	-
			2	0	0	-
Product 9	Wild birds seed mix	C	1	3	20	66.7
			2	6.5	34	84.6
Product 10	Wild birds seed mix	C	1	0.5	2.5	100
			2	0	0	-
Product 11	Wild birds seed mix	C	1	0	0	-
			2	0.5	2	100
Product 12	Wild birds seed mix	D	1	0	0	-
			2	0	0	-
Product 13	Wild birds seed mix	F	1	0	0	-
			2	0	0	-
Product 14	Wild birds seed mix	G	1	0	0	-
			2	0	0	-
Product 15	Wild birds seed mix	C	1	0	0	-
			2	0	0	-
Product 16	Sunflower	E	1	7.5	26	86.7
			2	0	0	-
Product 17	Sunflower	C	1	4.5	13.5	33.3
			2	0	0	-
Product 18	Sunflower	E	1	0	0	-
			2	0	0	-
Product 19	Sunflower	B	1	0	0	-
			2	0	0	-
Product 20	Sunflower	H	1	0	0	-
			2	0	0	-
Product 21	Sunflower	F	1	0	0	-
			2	0	0	-

the number and mass of seed per kilo was generally low and no sample exceeded the legal limit of 50 mg·kg⁻¹. This contrasts with previous studies and suggests that across years, seed producers have taken up measures to reduce the contamination by agricultural practices and cleaning techniques. It must be noted, however, that our results may be underestimated, as Frick et al. (2011) found a small fraction of seeds larger than our sieve mesh, so analyzing both sieved fractions may have increased the number or recorded seeds. In addition, moisture level of our samples was not measured and may differ from the 12% reference. Based on our study, 0.75 ± 0.265 seeds (mean ± SE) are on average found per kilo of bird feed, 82.4% of which being viable. In other words, one viable seed is on average introduced on the territory with every 1.6 kg of bird feed that is imported. The proportion of imported bird feed in our samples was not documented, but is expected to be very high as the production of sunflower and grain corn are low in Wallonia: respectively < 0.1% and < 1% of arable fields (Direction générale Statistique, Service public fédéral Économie [Statbel], 2022). With an estimated number of chickens of 8.7 million (Direction générale Statistique, Service public fédéral Économie [Statbel], 2022) in Wallonia (a region where, besides, feeding wild birds in private gardens is popular), the quantity of bird feed that is annually imported is important. As a population can establish from only one germinated seed, the risk of introducing a ragweed population over time at a given feeding location is real. This is likely to represent a substantial introduction pathway in Wallonia, where still relatively few ragweed populations are established to date. No clear difference was found between feed types, which is surprising given the well-established link between sunflower and ragweed contamination. The different products analyzed show variable levels of contamination. For four of them, the two replicates were contaminated. This is notably the case for the two products from organic farming, with one standing out as the most contaminated product when considering both samples. It is however difficult, based on the present study, to guide or advise the customers when purchasing bird seeds. Instead, communication campaigns should target those persons to make them aware of the risk and to engage them in active surveillance of the ragweed invasion. In the future, it would be interesting to quantify the level of contamination by other invasive plants, such as *Datura stramonium*.

5. CONCLUSIONS

Although the legal limit of 50 mg·kg⁻¹ seems to be well respected in the bird feed available on the market, this trade still represents a substantial introduction pathway

for ragweed in Southern Belgium due to the high proportion of contaminated bags.

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