

Ragweed (*Ambrosia* sp.) seeds in bird feed

Geneviève Frick⁽¹⁾, Heinrich Boschung⁽¹⁾, Gudrun Schulz-Schroeder⁽²⁾, Gabriele Russ⁽²⁾, Igor Ujčič-Vrhovnik⁽³⁾, Breda Jakovac-Strajn⁽³⁾, Daniela Angetter⁽⁴⁾, Ingrid John⁽⁴⁾, Jan Sten Jørgensen⁽⁵⁾

⁽¹⁾ Agroscope Liebefeld-Posieux Research Station (ALP). Tioleyre, 4. P.O. Box 64. CH-1725 Posieux (Switzerland).

E-mail: genevieve.frick@alp.admin.ch

⁽²⁾ Chemisches- und Veterinäruntersuchungsamt Rhein-Ruhr-Wupper (CVUA-RRW). Deutscher Ring, 100. D-47798 Krefeld (Germany).

⁽³⁾ University of Ljubljana. Veterinary Faculty. National Veterinary Institute (NVI). Unit for Pathology of Animal Nutrition and Environmental Hygiene. Gerbiceva, 60. SLO-1115 Ljubljana (Slovenia).

⁽⁴⁾ Landeslabor Berlin-Brandenburg (LLBB). Templinerstraße, 21. D-14473 Potsdam (Germany).

⁽⁵⁾ The Danish Plant Directorate (DPD). Skovbrynet, 20. DK-2800 Lyngby (Denmark).

Five years of monitoring and control for ragweed seeds in bird feed done by members of the IAG working group (International Association for Feedingstuff Analysis, Section Feedingstuff Microscopy).

In 2005, the Swiss official feed inspection of Agroscope Liebefeld-Posieux Research Station (ALP) was mandated to check bird feed and raw materials for the presence of *Ambrosia* sp. seeds. Indeed, such seeds were found in varying amounts when analyzed in the feed microscopy laboratory. The producers were informed, and a limit of intervention ($50 \text{ mg}\cdot\text{kg}^{-1} \approx 9$ to $10 \text{ seeds}\cdot\text{kg}^{-1}$) was finally set for this undesirable component. The results of five years of controls show, at first, around 50% of contaminated samples. With appropriate measures, the level of contamination could be lowered in the following years. In parallel, the size of *Ambrosia* sp. seeds and the sieves to be used for routine analyses were checked. *Ambrosia* sp. seeds found in feed checked in Switzerland were seldom larger (“wider”) than 3.5 mm and never smaller than 1.5 mm. Several EU Member States started their own control and monitoring programs. The results of studies from Germany, Slovenia and Denmark, presented by their feed microscopists at the IAG meetings, indicate the presence of *Ambrosia* sp. seeds in 21 to 75% of the products put on the market.

Keywords. *Ambrosia artemisiifolia* L., ragweed, bird feed, contaminant, seeds, microscopy, pollen, allergy.

1. INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.) is an invading plant with highly allergenic pollen and important seed production. Originating from North America, its spread throughout different European countries has been thought to be linked in the past to the import of cereals (Kiss, 2007), and more recently with contaminated bird feed and the germination of seedlings at the foot of the trough. Other ways of propagation are movements of soil and vehicles together with contaminated seed lots. In 2005, Delabays et al. (2005) and Tamarcaz et al. (2005) described the situation in Switzerland and Brandes et al. (2006) similarly observed the distribution and dispersal of ragweed in Germany. The latter authors suggested: “At the present climatic conditions a continued occurrence of *A. artemisiifolia* in Central Europe is highly possible, even more so under global change conditions of elevated temperature and/or carbon dioxide”. As a consequence, and although ecological or economical damage (on the

crop yield) was considered low, control measures were suggested because of the allergenic properties of ragweed pollen. Several measures to be applied on growing plants of known populations were elaborated, together with preventive measures concerning the dispersion of seeds (seed stock in soil or in vehicles, seed import as seed lot contaminant).

At this juncture, presence of *A. artemisiifolia* in bird feed found on the market had been confirmed, especially in those mixtures containing sunflower seeds (Delabays et al., 2005; Brandes et al., 2006).

In Switzerland, mandate was given to the official feed inspection unit of ALP (Agroscope Liebefeld-Posieux, the Federal Research Station) to monitor and control bird feed and raw materials with respect to the presence of *A. artemisiifolia*. Therefore, samples were collected on the market or in feed mills and the producers were instructed to respect the new intervention limit: no sample with more than $50 \text{ mg}\cdot\text{kg}^{-1}$ of whole *A. artemisiifolia* seeds would be tolerated. Shortly after this, the IAG

feed microscopy working group, as a network for exchanging information and reference material, together with developing and standardizing methods, started to write a protocol for the detection and quantification of *A. artemisiifolia* in bird feed, and several control authorities in Europe started their own screening.

Although the screening focuses on *A. artemisiifolia* (the most abundant species of the genus *Ambrosia* in Europe) we cannot exclude that some of the selected seeds belong to other *Ambrosia* species, *A. trifida* for example. This could happen as the seeds have similarities. Consequently, whenever presenting results of the feed control, we will mention *Ambrosia* sp. In any case, *A. trifida* is also known to produce allergenic pollen (D'amato et al., 2007).

This paper gives an overview of the results for the presence of *Ambrosia* sp. in bird mixed feed or raw materials analyzed in Switzerland (years 2005 to 2009) and in four other laboratories in Europe (two laboratories in Germany, one in Slovenia and one in Denmark; years 2006 to 2009). These laboratories presented their work at IAG meetings as posters or lectures, and their results are summarized here.

This paper also describes the records done on the size of the *Ambrosia* sp. seeds found in Swiss samples.

2. MATERIALS AND METHODS

2.1. ALP analyses

When the bird feed analysis started at ALP in summer 2005, no protocol was available for this screening.

First, reference material was studied. *A. artemisiifolia* seeds were observed under the stereomicroscope at magnification six times or more. The seeds are enclosed individually in the flower bracts which form a 3-4 mm long and 2 mm wide non-fleshy fruit presenting one apical thorn and several other spines placed as a crown (**Figure 1**). This characteristic rough skin is usually still present on the seeds isolated from the feed samples; but in some cases, the more or less naked and glossy seeds are found (**Figure 2**).

Second, bird feed samples were taken on the market and an amount of at least 500 g was sieved in several fractions. In the years 2005 to 2007, the three following fractions were exhaustively analyzed by searching under a stereomicroscope: ≤ 2.5 mm; > 2.5 to ≤ 3.5 mm; > 3.5 to ≤ 4.5 mm. The fraction larger than 4.5 mm was not analyzed.

2.2. Method improvement

To consolidate the protocol, the size of the *Ambrosia* sp. seeds collected in the checked samples from the official control and private clients in the first three years was



Figure 1. Typical *Ambrosia artemisiifolia* fruit containing one seed. The hairy fruit displays several characteristic thorns.



Figure 2. Several *Ambrosia* sp. seeds selected from feed samples and showing diversity in size, color and level of destruction of the fruit envelop.

analyzed in more detail: seeds were fractionated through three sieves with a decreasing mesh-size by steps of 0.5 mm (2.5 mm to 1.5 mm) to look for the lower limit.

In the years 2008 and 2009, following the IAG method, only the fraction between 1.5 and 4 mm was analyzed.

2.3. Monitoring in other European laboratories

The four other laboratories presenting results on *Ambrosia* sp. seeds in bird feed followed the IAG method (IAG, 2009) using sieves and a sub-sample of

500 g, except for the group LLBB (Germany) where the sub-sample size was only 25 g in 2008 and the first part of 2009. All results were collected as number of *Ambrosia* sp. seeds·kg⁻¹.

3. RESULTS

3.1. ALP results

In **table 1**, the results of all analyses done at ALP in the frame of the official control in Switzerland on the detection of *Ambrosia* sp. seeds in bird mixed feed or in their components (raw materials) are summarized. In general, the proportion of contaminated mixed feed samples varied from 57% to 22% with a slight decreasing tendency over the years. The percentage of the raw material samples which were contaminated varied between 0 and 100%.

In 2005, from the 32 samples collected (9 mixed feed and 23 raw materials), some contained large number of *Ambrosia* sp. seeds. From the 9 mixed feed samples, 4 (44%) were contaminated above the intervention limit (set at 50 mg·kg⁻¹, corresponding to approximately 9 seeds per kg) with a maximum of 367 per kg. One sample contained less than 9 *Ambrosia* sp. seeds per kg and 4 were *Ambrosia* sp.-free. From 23 raw materials, 4 (17%) were contaminated above the intervention limit, with a maximum of 133 seeds per kg. One sample was contaminated under the intervention limit and 18 were not contaminated. The large number of *Ambrosia* sp.-free raw materials can be explained by the fact that, at first, all possible components were checked whereas in following years only the most at-risk components (sunflower, sorghum, millet, hemp, etc.) were selected to be screened. In 2006, an improvement of the situation was observed in the level of contamination of the mixed feed samples: from 14 mixed feeds, 57% were contaminated, but only 2 (14%) showed a contamination slightly above the intervention limit (11 and 19 *Ambrosia* sp. seeds

per kg). Six mixed feeds contained a very low number of *Ambrosia* sp. seeds and 6 were *Ambrosia* sp.-free. Only 4 raw materials were checked in 2006, but 2 were highly contaminated (up to 303 seeds per kg) and 2 were *Ambrosia* sp.-free. In the following three years, the proportion of mixed feeds contaminated above the intervention limit staid relatively low: 14% in 2007, 22% in 2008 and 11% in 2009. However, some mixed feeds and raw materials showed high numbers of *Ambrosia* sp. seeds (up to 220 seeds per kg). Clearly, some raw materials are highly contaminated, but it seems to be possible to reduce as well the percentage of contaminated samples as the concentration of *Ambrosia* sp. seeds in the majority of the samples.

3.2. Method improvement

One goal of the different groups working on the contamination of bird feed with *Ambrosia* sp. being the ability to check numerous samples in a low time-consuming manner, some work was invested in the method of screening. To reduce the size of the sample to be checked and to facilitate the work, the compulsory sieve-fractions were determined. At ALP, the total number of seeds found in the different sieve-fractions in 2005 to 2007 was recorded. **Figure 3** shows that the proportion of the seeds smaller than 2.5 mm was strongly reduced with time (434 from 498 seeds = 87% in 2005; 84 from 179 seeds = 47% in 2006; 55 from 163 seeds = 34% in 2007). *Ambrosia* sp. seeds larger than 3.5 mm were very seldom found (only two seeds from a total of 840).

To ascertain the lower size limit of the *Ambrosia* sp. seeds, the seeds collected during 3 years were put through a series of sieves. The repartition of the seeds in the sieves is presented in **figure 4**. No seeds smaller than 1.5 mm were found. Ten per cent (88 seeds) of the *Ambrosia* sp. seeds were found in the sieve between 1.5 and 2.0 mm. Most of the seeds were found between 2.0 and 2.5 mm (506 seeds = 59%) which reflects the mean width of the seed. Another 31% (= 263 seeds) of the

Table 1. Results of the bird mixed feed and raw materials analysis done at ALP (Switzerland) in the years 2005 to 2009.

		<i>Ambrosia</i> sp. contamination				
		2005	2006	2007	2008	2009
Contaminated samples*	Mixed feed	56% (9)	57% (14)	39% (28)	50% (18)	22% (18)
	Raw materials	22% (23)	50% (4)	100% (3)	50% (2)	0% (1)
> 9 seeds·kg ⁻¹ (% of all samples analyzed)	Mixed feed	44%	14%	14%	22%	11%
	Raw materials	17%	50%	33%	0%	0%
Highest contamination level (seeds·kg ⁻¹)	Mixed feed	367	19	109	220	100
	Raw materials	133	303	220	5	0

* The contaminated samples are given in percentage of all analyzed samples, the number of analyzed samples are in brackets.

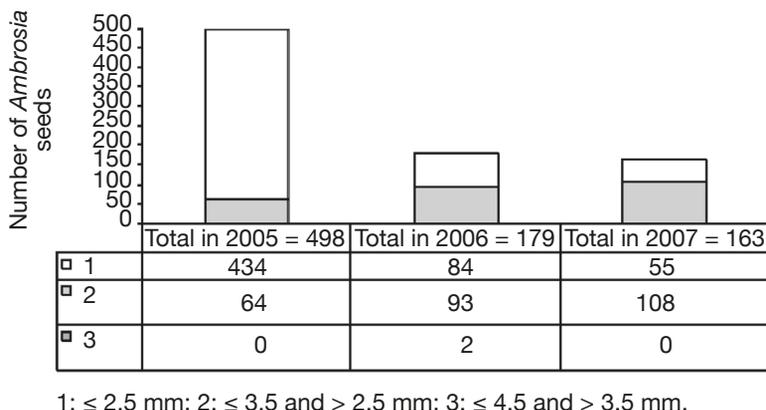


Figure 3. Pooled results of the bird feed and raw materials analysis done at ALP (Switzerland) in the years 2005 to 2007 considering the size-fraction where the seeds were found. Results are expressed in number of *Ambrosia* sp. seeds.

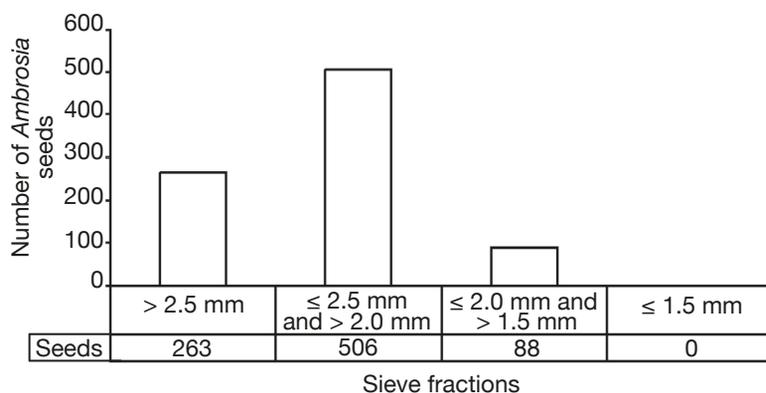


Figure 4. Size repartition of *Ambrosia* sp. seeds selected between 2005 and 2007 from bird feed and raw materials at ALP (Switzerland). Four sieve-fractions were analyzed and the number of seeds recorded.

seeds were larger than 2.5 mm. These results, together with other experiences in the practicing laboratories, led us to set the size-limit of the compulsory fraction to analyze by 1.5 and 4 mm in the IAG method. This way, most of the sunflower seeds, for example, are discarded with little chance of missing *Ambrosia* sp. seeds.

3.3. European laboratories screening results

In **table 2**, the results of four European control laboratories who performed *Ambrosia* sp. screening are presented. In general, the situation resembles the one in Switzerland with a relatively high percentage of contaminated samples and high concentration of *Ambrosia* sp. seeds at first and a tendency towards lower levels.

CVUA-RRW (Chemisches- und Veterinäruntersuchungsamt Rhein-Ruhr-Wupper, Krefeld, Germany). The percentage of contaminated mixed feed samples

varied from 92 to 11% over the years. In 2006, 2007 and 2008, this German group stated respectively 42, 57 and 0% of the mixed feeds with contamination level above the Swiss intervention limit of 9 seeds per kg, and no really high contamination was noted (up to 31 *Ambrosia* sp. seeds per kg). Concerning the raw materials, one contained 61 *Ambrosia* sp. seeds per kg in 2006, but in the following years, the level of contamination was low (maximum 20 seeds per kg). In 2009 only four samples were analyzed, from which three were contaminated at low levels (maximum of 12 *Ambrosia* sp. seeds per kg).

NVI (Veterinary Faculty, National Veterinary Institute, Ljubljana, Slovenia). An analysis of the situation in Slovenia in the winter season 2007/2008 showed a high percentage of contaminated mixed feeds (70%) and raw materials (60%). The highly (above the Swiss intervention limit) contaminated mixed feeds (40% of the analyzed mixed feeds) contained up to 292 seeds per kg, whereas by the raw materials, the five highly contaminated samples (50% of the analyzed raw materials) contained up to 470 *Ambrosia* sp. seeds per kg. In the winter season 2008/2009, the percentage of contaminated samples and the level of contamination was lower: 50% of the mixed feeds and 30% of the raw materials were contaminated with *Ambrosia* sp.; 30% and 10%, respectively, of the mixed feeds and the raw materials contained more than 9 seeds per kg. The highest level of contamination was also lower than the year before: 42 seeds per kg for the mixed feeds and 56 for the raw materials.

DPD (The Danish Plant Directorate, Lyngby, Denmark). The evolution in Denmark in the first two years of control seemed to tend in the direction opposite to the general trend. In 2008 more mixed feeds (64%) and raw materials (44%) were highly contaminated compared to 2007 (50% and 13% for mixed feeds and raw materials, respectively). The highest level of contamination in mixed feeds and in raw materials was high over the two years 2007 and 2008: 185 and 252 seeds per kg, respectively, in 2007, and 112 and 847 in 2008 (Jørgensen, 2008). In the year 2009, the percentage of contaminated samples for both mixed feeds and the raw materials came back to the levels of 2007 (70% of contaminated mixed feeds and 29% of contaminated raw materials), but the average contamination level of seeds in contaminated

Table 2. Results of the bird feed and raw materials analysis done in laboratoris in three European countries in the years 2006 to 2009.

		<i>Ambrosia</i> sp. contamination					
		CVUA-RRW				NVI	
		2006	2007	2008	2009	2007/08	2008/09
Contaminated samples	Mixed feed	92% (12)	71% (7)	11% (9)	67% (3)	70% (10)	50% (10)
	Raw materials	25% (4)	100% (2)	100% (1)	100% (1)	60% (10)	30% (10)
> 9 seeds·kg ⁻¹ (% of all samples analyzed)	Mixed feed	42%	57%	0%	33%	40%	30%
	Raw materials	25%	0%	100%	0%	50%	10%
Highest contamination level (seeds·kg ⁻¹)	Mixed feed	24	31	2	12	292	42
	Raw materials	61	3	20	2	470	56
		DPD			LLBB		
		2007	2008	2009	2008*	2009/1*	2009/2
Contaminated samples	Mixed feed	75% (8)	73% (11)	70% (10)	33% (3)	20% (10)	11% (18)
	Raw materials	25% (8)	67% (9)	29% (7)	29% (7)	25% (4)	100% (1)
> 9 seeds·kg ⁻¹ (% of all samples analysed)	Mixed feed	50%	64%	30%	33%	20%	11%
	Raw materials	13%	44%	14%	29%	25%	0%
Highest contamination level (seeds·kg ⁻¹)	Mixed feed	185	112	101	40	160	144
	Raw materials	252	847	32	1040	80	6

CVUA-RRW (Chemisches- und Veterinäruntersuchungsamt Rhein-Ruhr-Wupper, Krefeld) and LLBB (Landeslabor Berlin-Brandenburg, Potsdam) are German analysts, NVI stands for Veterinary Faculty, National Veterinary Institute, Ljubljana, Slovenia and DPD for The Danish Plant Directorate. For the mixed feeds and raw materials, the contaminated samples in percentage of all analyzed samples, the number of analyzed samples (in brackets), the percentage of all samples with a contamination above nine *Ambrosia* sp. seeds·kg⁻¹, as well as the highest contamination found in the samples (in *Ambrosia* sp. seeds·kg⁻¹) are given; *Analysis done on 25 g.

samples was very much lower than in previous surveys (Jørgensen, 2009): only 30% of the mixed feeds were contaminated above the intervention limit.

LLBB (Landeslabor Berlin-Brandenburg, Potsdam, Germany). In this other German laboratory, analyses were started in 2008 with 10 samples which were not assigned directly by official authorities to the monitoring of *Ambrosia* sp. contamination but had been collected for other analytical parameters. Therefore, the available sample weight (25 g) was lower than the weight recommended in the IAG-method for *Ambrosia* sp. determination. In 2008, 33% of the samples analyzed were contaminated with *Ambrosia* sp., the highest contamination was found in a raw material (1,040 *Ambrosia* sp. seeds per kg). In the first part of 2009 (**Table 2:** 2009/1) (again on a basis of 25 g sample amount) the percentage of contaminated samples was low (20% and 25% respectively for mixed feeds and raw materials), but the level of contamination was high (160 and 80 seeds per kg respectively). In the second part of 2009, after change-over to an amount of 500 g examination sample (**Table 2:** 2009/2), 11% of the mixed feeds were again highly contaminated (maximum: 144 seeds per kg), and the only raw material

was found to be contaminated with *Ambrosia* sp. at low level (6 seeds per kg).

4. DISCUSSION

The analysis of bird feed and raw materials (mostly sunflower seeds) in Switzerland and three other European countries in the years 2005 to 2009 confirmed that a large proportion of these mixtures were contaminated with *Ambrosia* sp. In the southern and central parts of Europe, the seeds of *Ambrosia artemisiifolia* which reach the ground and stay in the soil can germinate and the plant originating from these seedlings may develop up to flowering and produce a large quantity of allergenic pollen. It seems logical to try to reduce this way of dispersal, even more when considering the fact that these plants often grow in private or public gardens. In Switzerland and Germany (BVL, 2009), pressure set on the producers of bird feed and measures taken had a noticeable effect on the level of contamination in both raw materials seed lots intended for bird feed and in the bird feeds themselves, although a low level of contamination seems unavoidable. In Slovenia the decreased number of contaminating seeds in the bird

feed put on the market was also noticed. On the other hand, in Denmark the situation did not improve in 2008 compared to 2007, but improved in 2009 when considering the contamination level of the contaminated samples. In this country, the climatic situation might still prevent the ragweed plants to reproduce by seeds, but pollen production is possible and the allergenic consequences for the population will still be present. This situation prevails in Finland too (Pohto, personal communication). Also, *Ambrosia artemisiifolia* seeds can survive in soil for more than 40 years (Baskin et al., 1977) and be capable of germinating.

To help the analysts with the task of analyzing bird feed, an official standardization of the method is welcome. One parameter to check was the choice of the fraction to analyze. Fractionating the sample by sieving through a column of sieves is little work and allows the removal of all particles of irrelevant size. This is particularly interesting when analyzing samples of sunflower seeds, for example, because of the different seed size of *Ambrosia* sp. and sunflower. Checking particles of mostly the same size under the stereomicroscope or a magnifying lamp is also less tiring and more efficient than analyzing a heterogeneous sample. As well, removing the dust and small particles makes the shifting of the seeds in a dish with a spatula more convenient.

Recording the size of the seeds found in bird feed was done at ALP during the first three screening campaigns (2005 to 2007). Together with the determination of the limits of seed size (minimum 1.5 mm; maximum 4 mm), these records brought unexpected information: the distribution of the individual seed size varied with time toward larger seeds. Although it might be due to pre-harvest growing conditions or the presence of different species of the genus *Ambrosia* sp., this could also be a consequence of a post-harvesting cleaning treatment of the raw materials which eliminated the smaller but not the larger seeds. In this regard, it would be important to check what is done with the residue after cleaning, to make sure that it does not end up being discarded in the open nature.

The presence of larger seeds in a sample with low contamination level was also noted in a ring test organized to check the IAG method (data not yet published).

A general remark is the fact that the method is relatively easy to set up thanks to the characteristic features of the *Ambrosia* sp. seed (botanically speaking a fruit, **figure 1**). Also, only whole seeds and not the fragments are of interest because the germination potential solely is relevant (no direct toxicity). Still, the analysis of large samples can be tedious if the mixture contains mostly seeds of a size similar to *Ambrosia* sp. (for example: hemp, sorghum or millet seeds). Also, as described above and documented in **figure 2**, the possible

diversity in the size, color and level of destruction of the fruit around the seed can represent a challenge to the microscopists.

Finally, as a side effect to this work, all analysts mentioned a valuable increase of knowledge on the possible contaminants of seed mixes: *Datura stramonium* seeds were recorded, sclerotia from fungi, other weed seeds and stones were observed in some samples.

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