SCIENTIFIC OPINION

Scientific Opinion on the effect on public or animal health or on the environment on the presence of seeds of *Ambrosia* spp. in animal feed[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) and EFSA Panel on Plant Health (PLH)[2, 3]

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ABSTRACT

The European Commission requested EFSA to provide a scientific opinion on the effect on public or animal health or on the environment on the further distribution of *Ambrosia* spp. in the European Union and on the importance of feed materials, in particular bird feed, in the dispersion of *Ambrosia* spp. The genus *Ambrosia* (Asteraceae family) is distributed worldwide. *Ambrosia artemisiifolia* (common ragweed) has heavily colonised several areas of South-East Europe. *Ambrosia* spp., both in their native range and in invaded areas, are of public health concern due to the allergenic properties of their pollen. The NDA Panel concluded that inhalation of the plant pollen causes rhino-conjunctivitis and asthma, with skin allergies and food allergy playing minor roles. *Ambrosia* may cross-sensitize patients to other allergens, including food allergens. There is some evidence for allergenicity of *Ambrosia* pollen in animals. With regard to the effects on the environment of the further distribution of *Ambrosia* spp. in the European Union, the PLH Panel concluded that there is no direct evidence that *Ambrosia* spp. cause extinction of plant species. However, there are some indications that *A. artemisiifolia* could become highly invasive in certain environmentally-valuable habitats and might be linked to an impoverishment of species richness, therefore further ecological studies are needed. The CONTAM Panel focused on the relative importance of animal feed, bird feed in particular, on the dispersion of *Ambrosia*. *Ambrosia* seeds may contaminate feed. However, animal feed materials compounded for use in livestock are extensively processed. This processing destroys *Ambrosia* seeds and hence the contribution of compounded feed to the dispersion of *Ambrosia* is considered to be negligible. Bird feed often contains significant quantities of

1 On request from the European Commission, Question No EFSA-Q-2009-00655 adopted on 19 March 2010 by the CONTAM Panel, Question No EFSA-Q-2010-00890 adopted on 4 June 2010 by the NDA Panel and Question No EFSA-Q-2010-00820 adopted on 31 May 2010 by the PLH Panel.

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Ambrosia seeds and remains unprocessed. Therefore, bird feed seems to play an important role in introducing *Ambrosia* to new, previously not infested areas.

KEY WORDS

Allergenicity, *Ambrosia*, animal toxicity, bird feed, dispersal, distribution, ragweed

SUMMARY

The genus *Ambrosia* (family Asteraceae) is distributed worldwide. In Europe, *Ambrosia artemisiifolia* (common ragweed) is the most common *Ambrosia* species and has heavily colonised several areas including the French Rhône valley, Northern Italy and South-East Europe (Bulgaria, Hungary, Southern Russia and Ukraine).

A. artemisiifolia is a weed of waste ground and agricultural land that flourishes wherever soil is newly disturbed. Dispersion of *Ambrosia* occurs naturally through seed drop, movement by animals and surface water, and often follows human activities entering the different regions by transport with agricultural machines and excavated material.

With regard to the effects on the environment of the further distribution of *Ambrosia* spp. in the European Union, the Panel on Plant Health (PLH Panel) concluded that there is no direct evidence that *Ambrosia* spp. cause extinction of plant species. However, there are some indications that *A. artemisiifolia* could become highly invasive in certain environmentally-valuable habitats and that under certain conditions, generally in habitats disturbed by human activities, *A. artemisiifolia* might be linked to an impoverishment of species richness, therefore further ecological studies are needed.

The Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) concluded that the major adverse health effects of *Ambrosia* are related to the allergenicity of inhaled plant pollen causing rhino-conjunctivitis and asthma, with skin allergies and food allergy playing minor roles. *Ambrosia* may cross-sensitize patients to other allergens, including food allergens.

The Panel on the Contaminants in the Food Chain (CONTAM Panel) noted that there is some evidence for allergenicity in animals, particularly in relation to the obstructive airway diseases in horses. There is no evidence that *Ambrosia* species form secondary plant metabolites that are of clinical significance for livestock.

Animal feeds, including maize, wheat, sunflowers, millet, peanuts, soybean, peas and beans may contain seeds of *Ambrosia*. Commercial feed for livestock is processed prior to use and the procedures of grinding, pelleting and/or heating almost completely destroy the *Ambrosia* seeds. In contrast, bird feed used for the feeding of wild and ornamental birds, which is often contaminated with seeds of *A. artemisiifolia*, is generally not processed and hence may contribute to the dissemination of viable ragweed seeds.

The CONTAM Panel noted that the contribution of processed commercial feed materials to the further dispersion of *Ambrosia* seems to be negligible as seeds are destroyed during the processing of compound feeds. In contrast, the CONTAM Panel concluded that bird feed may be an important route of ragweed dispersal especially in not infested areas. Therefore, the prevention of the use of contaminated bird feed is likely to contribute to an attenuation of the further dispersion of *Ambrosia* in Europe.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The genus *Ambrosia* (family *Asteraceae*) is distributed worldwide. The common English name of the genus is ragweed. These plants are native to North America and some species in the genus are considered weeds, both in their native area and other parts of the world, including Europe. The most abundant species in the European Union is *Ambrosia artemisiifolia* L. (common ragweed) but also *Ambrosia trifida* L. (giant ragweed) and *Ambrosia psilostachya* DC (perennial ragweed) have been observed.

Ambrosia spp., both in their native range and in invaded areas, are known to cause impact on agriculture and are of particular concern due to the allergenic properties of their pollen. Pollen allergenic persons are often allergic to several types of pollen. The pollen season of *Ambrosia* spp. is late, August to October. Therefore, *Ambrosia* pollen will affect allergic individuals at a time when many would normally be experiencing relief from their symptoms. *Ambrosia* spp. thus extend the "problem season" for the pollen allergic population. If *Ambrosia* spp. become common in Europe, *Ambrosia* pollen allergy-related rhinitis, asthma and food allergy manifestation are likely to become a significant public health problem.

It seems important for the protection of public health to limit the further distribution of *Ambrosia* in the European Union as much as possible. Bird seed and sunflower seeds used for the feeding of birds have been reported to contain significant quantities of *A. artemisiifolia* seeds.

The Scientific Panel on Plant Health of the European Food Safety Authority (EFSA) adopted on 7 April 2007 an opinion on the pest risk assessment made by Poland on *Ambrosia* spp.[4] and an opinion on the pest risk assessment made by Lithuania on *Ambrosia* spp.[5] The Panel on Plant Health concluded in both cases that no sufficient evidence was provided to assess on a scientifically sound basis whether *Ambrosia* spp. qualify as quarantine pests.

The Scientific Panel on Plant Health did not perform an in depth evaluation of impacts of *Ambrosia* spp. on human health as such an evaluation is outside of the Panel's activity. A specialist on human allergies has been consulted and asked to summarize the existing knowledge on the *Ambrosia* pollen induced allergy. It is mentioned in the opinion that there is scientific evidence that *Ambrosia* spp. can cause detrimental impacts on human health due to their allergenic properties. A pathway analysis within the pest risk assessment is performed in both opinions confirming the importance of bird feed and other seeds for animal feeding for the distribution of *Ambrosia* spp. in the environment.

Therefore risk management measures are considered to reduce the presence of seeds of *Ambrosia* spp. in (bird) feed. One of these measures is the possible inclusion of seeds of *Ambrosia* spp. in the annex to Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. The Directive provides that any provision that may have an effect upon public or animal health or on the environment can be adopted only after consultation with EFSA.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority to provide a scientific opinion

- on the effect on public or animal health or on the environment on the further distribution of *Ambrosia* spp. in the European Union;

[4] Opinion of the Scientific Panel on Plant Health on a request from the Commission on the pest risk assessment made by Poland on *Ambrosia* spp. The EFSA Journal (2007) 528, 1-32. Available from http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/plh_op_ej528_ambrosia-pl_en.pdf?ssbinary=true.

[5] Opinion of the Scientific Panel on Plant Health on a request from the Commission on the pest risk assessment made by Lithuania on *Ambrosia* spp. The EFSA Journal (2007) 527, 1-33. Available from http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/plh_op_ej527_ambrosia-lt_en.pdf?ssbinary=true.

- on the importance of the pathway of bird feed and more in general of feed materials (including seeds) for animal feeding in the distribution of *Ambrosia* spp. in the environment.

APPROACH TAKEN TO ANSWER THE TERMS OF REFERENCE

After having received this request from the European Commission, EFSA allocated the mandate to the Panel on Contaminants in the Food Chain (CONTAM Panel), the Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) and the Panel on Plant Health (PLH Panel). The part of the scientific opinion falling into the remit of the CONTAM Panel was adopted on 19 March 2010. The chapter on Human health risks (Chapter 7) and its specific conclusions were adopted by the NDA Panel on 4 June 2010 and the chapter on Effects of *Ambrosia artemisiifolia* on the environment (Chapter 8) and its specific conclusions were adopted by the PLH Panel on 31 May 2010.

ASSESSMENT

1. Introduction

Ambrosia is a large genus within the Asteraceae family. Its more than 40 species occur mainly in America, with only one species (*Ambrosia maritima*) native to South Europe, West Asia and North Africa. Several of the American *Ambrosia* species have been introduced to Europe, in particular *Ambrosia artemisiifolia*, *Ambrosia trifida* and *Ambrosia psilostachya* DC.

A. artemisiifolia (common ragweed) is the most widespread *Ambrosia* species in Europe. Heavily colonised areas in Europe include the French Rhône valley, Northern Italy, Hungary, some countries of the Balkan peninsula (Bulgaria, Croatia, Serbia, etc.) as well as Ukraine and Southern Russia.

The major adverse health effects of *Ambrosia* are related to the allergenicity of inhaled plant pollen causing rhino-conjunctivitis and asthma, with skin allergies and food allergy playing minor roles (Dechamps, 1995).

A. artemisiifolia is present as a weed in many crops but is more important in spring-sown crops, especially sunflower, than in winter crops (EFSA, 2007a, b).

Seeds of common ragweeed may contaminate feed materials such as maize, wheat, sunflowers, millet, peanuts, soybean, peas and beans and may be imported with grains from infested regions. There is evidence that commercially available bird feed is often contaminated with seeds of *A. artemisiifolia* (Alberternst et al., 2006; Bohren, 2006) and that this can contribute to the dispersion of ragweed into non invaded areas.

An update of the literature published until December 2009 and related to the occurrence of *Ambrosia* in feed materials, the effects in farm animals, the human health risks and the effects of *Ambrosia artemisiifolia* on the environment was performed using selected databases (Pubmed, Medline, Web of Science).

2. Previous evaluations and risk assessments on *Ambrosia*

2.1. Pest risk assessments from Lithuania and Poland and evaluation by the Panel on Plant Health

Poland and Lithuania presented risk assessments related to three species of the genus *Ambrosia*: *A. artemisiifolia* L. (common ragweed), *A. trifida* L. (giant ragweed) and *A. psilostachya* DC (perennial ragweed) and proposed that *Ambrosia* spp. should be included as harmful organisms in the Council Directive 2000/29/EC[6] (Annex I, Section II, point C). Both the Polish and the Lithuanian pest risk assessments concluded that *Ambrosia* spp. are quarantine pests justifying the use of phytosanitary measures to exclude *Ambrosia* spp. from the pest risk analysis area (Poland and Lithuania respectively). Following the requests of the European Commission, EFSA's Panel on Plant Health (PLH Panel) was asked to issue a scientific opinion on the pest risk assessments as presented by Poland and Lithuania (EFSA, 2007a, b).

In the introduction chapters to these opinions (EFSA, 2007a, b), the PLH Panel presented an overview of literature regarding the impact of *A. artemisiifolia*, *A. trifida* and *A. psilostachya* on agricultural production, biodiversity and human health.

The PLH Panel examined in detail the documents provided by Lithuania and Poland. The review was based on the principles and terminology of the International Standard for Phytosanitary Measures ISPM N°11: Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms (2004) by the International Plant Protection Convention (FAO-IPPC,

[6] OJ L169, 10.07.2000, p. 1-159.

2006b). According to the International Standard for Phytosanitary Measures ISPM N°5: Glossary of phytosanitary terms (FAO-IPPC. 2006a), a pest is "*any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products*". Quarantine pest is defined as "*a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled*" (FAO-IPPC, 2006a).

The PLH Panel concluded that the Lithuanian and the Polish documents did not provide sufficient evidence to assess whether *Ambrosia* spp. qualify as quarantine pests for both countries. However, there was sufficient scientific evidence, both in the provided documents and the existing literature, that *Ambrosia* spp. can cause detrimental impacts on human health due to their allergenic properties. It was also stated that the control of *Ambrosia* spp. is difficult in certain crops and in non-cultivated areas. The PLH Panel recommended that the pest risk assessments should be revised and updated, including consideration of alternative pathways, as bird seed and spreading by soil, machinery and tyres.

2.2. Evaluation by the European and Mediterranean Plant Protection Organization (EPPO)

EPPO is an intergovernmental organization responsible for European cooperation in plant health. Founded in 1951 by 15 European countries, EPPO now has 50 members, covering almost all countries of the European and Mediterranean regions. Its objectives are to protect plants, to develop international strategies against the introduction and spread of dangerous pests and to promote safe and effective control methods. As a regional plant protection organization, EPPO also participates in global discussions on plant health organised by Food and Agriculture Organization (FAO) and the IPPC Secretariat. *A. artemisiifolia* figures on the list of invasive alien plants (EPPO-OEPP, 2007). In addition, EPPO advises its member states to monitor, eradicate or contain the species (EPPO-OEPP, 2008).

2.3. Evaluation by the United States Department of Agriculture - Animal and Plant Health Inspection Service (APHIS)

APHIS lists and regulates federal noxious weeds under the authority of the Plant Protection Act. Listed species may not be imported into the United States, nor moved between states, without a special permit. At present, *A. artemisiifolia* is not a federal noxious weed however it is officially identified as a noxious weed in Illinois, Michigan and Oregon (APHIS, online).

2.4. Evaluation by the Delivering Alien Invasive Species Inventories for Europe (DAISIE) project

The DAISIE project was funded by the sixth framework programme of the European Commission.[7] The project has identified approximately 10,800 alien species in Europe. Only about one in ten is considered invasive in the sense of having a negative effect on its environment. Due to its health and economic impacts, *A. artemisiifolia* is identified by the DAISIE project as being one of the hundred worst invasive aliens in Europe (DAISIE, online).

2.5. Evaluation by the Global Compendium of Weeds (GCW)

In this compendium, *A. artemisiifolia* is defined as being a noxious weed, meaning a species subject to legal restrictions (i.e. control, eradication, containment, etc.) and for some countries this term also encompasses quarantine species (i.e. US Federal Noxious Weed Compendium). It is also defined as a native weed, meaning a species that is native to the country and which invades the native ecosystems (GCW, online).

2.6. Evaluation by the Global Invasive Species Programme (GISP)

A. artemisiifolia is not on the list of the 100 worst invasive species. However, its impacts on human health as well as on biodiversity are noted (GISP, online).

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[7] Contract number SSPI-CT-2003-511202.

2.7. North European and Baltic Network on Invasive Alien Species (NOBANIS)

NOBANIS is a gateway to information on alien and invasive species in North and Central Europe. *A. artemisiifolia* is listed as invasive in four and as potentially invasive in a further three of the eleven countries listed (NOBANIS, online).

3. Current legislation and national control programs

At present, *Ambrosia* spp. are not covered by European Union (EU) legislation. However, as mentioned above, the EFSA PLH Panel has recommended in its scientific opinions (EFSA, 2007a, b) that the pest risk assessments on *Ambrosia* spp. should be revised and updated.

3.1. Current control programs in EU member states

Several countries in Europe are currently actively working on establishing prevention and control measures against *Ambrosia.* Several international conferences have reported these ongoing activities. Most recently, an international workshop on "Invasive Alien Species in EU countries" was held in Budapest from 6th to 8th of October, 2009.[8] Experts from 13 EU Member States (Austria, Bulgaria, Czech Republic, Germany, France, Hungary, Ireland, Luxembourg, Malta, The Netherlands, Poland, Romania, Slovenia) collated experiences on plant health aspects of distribution, monitoring, control and legal regulation of common ragweed (*A. artemisiifolia*).

The experts reported on national control programs against *Ambrosia* and agreed that the following factors should be considered: (1) The spread of *Ambrosia* into areas not yet infested may be by means of bird seed, some seeds intended for cultivation, wastes from processing facilities, transport of infested commodities (leakage), soil or manure. (2) The ongoing threat to EU countries from the spread of *A. artemisiifolia* requires continuing attentions. Subsequently, the International Ragweed Society was founded in autumn of 2009.

In France, ragweed invasion in the Rhône-Alpes region has increased over the past 30 years despite several eradication campaigns. Documentation and practical recommendations on how to fight ragweed and to reduce pollen counts for various areas within the Rhône-Alpes region are available online.[9]

In Italy, ragweed is widespread, and little progress has been made to contain its expansion. The consequences for public health are significant. For example, in the very heavily infested area of Busto Arsizio, 12 % of the local population are allergic to ragweed pollen. Here the pollen concentration often exceeds 200 pollen grains/m^3 during the flowering season and may reach 700 pollen grains/m^3 for several days (Zanon et al., 2002).

In Germany, the "Action Programme *Ambrosia*" was initiated by an interdisciplinary working group led by the Julius Kühn Institute (Starfinger, 2008, 2009). The program is not legally binding, but encourages authorities and the public to report pioneer plants of *Ambrosia*, to prevent further dispersal and to eradicate or control stands of the plant. The success of the program is reviewed annually in meetings of the working group.[10]

3.2. Swiss control program

Switzerland declared *A. artemisiifolia* an undesirable plant in 2006,[11] meaning that there is now an obligation to notify and to eradicate *A. artemisiifolia*. As a result, the federal government can

[8] Report: Invasive Alien Species in EU countries. International Workshop, Budapest, 6-8 October 2009 (Circulated to the Swedish Presidency, EU Council Secretariat, EU Commission and to the EU Member States). Available from http://www.fvm.hu/main.php?folderID=1683&articleID=15282&ctag=articlelist&iid=1.

[9] Available from www.ambroisie.info.

[10] Reports available from www.jki.bund.de/ambrosia.html.

[11] Ordinance on Plant Protection, available from http://www.blw.admin.ch/themen/00012/index.html?lang=en

reimburse proprietors who suffer losses of crops as a result of ragweed and - under certain circumstances - also provide compensation for extra expenditure on measures to eradicate ragweed. In addition, Switzerland supports research in the development of new criteria and methods to facilitate enforcement in the areas of early detection, monitoring, control and outcome evaluation. The Swiss Federal Authorities are responsible for raising awareness, providing information and promoting cantonal enforcement with regard to invasive alien species, including *A. artemisiifolia*.

A nationwide campaign was initiated in 2005: flyers about ragweed, containing information on how to recognise and eradicate the plant were sent to the 2800 municipalities of the country, aimed at making ragweed known to the population, eradicating it in gardens and so reducing seed production (Bohren, 2006).

Moreover, Swiss official feed inspection was mandated to check bird feed and raw materials for the presence of *Ambrosia* seeds. Since March 2005, commercially available bird seed has been required to be virtually free of ragweed seeds.[12] The current limit of intervention is 50 mg/kg (= 9 seeds /kg).

3.3. Canadian control program

In Canada, the historically successful eradication campaign in Gaspesia, launched in 1938, has been mainly based on systematic hand weeding of the ragweed plant with the help of school-children. There are several geographic reasons that may explain this success that still prevails today. Gaspesia is located on the shore of the Atlantic Ocean, which limits the spreading of the weed and is situated at the northern limit of the ragweed distribution. However, a similar campaign in 1950, in other parts of Canada and in particular in Montreal, was unsuccessful.

The campaign against the spread of ragweed in Quebec was re-evaluated in 1990 and has demonstrated that repeated yearly campaigns implementing deep mowing or hand weeding before July are effective. Seed production has been reduced and, as a result, the pollen production has diminished by 88 %, thereby insuring some relative relief for people allergic to ragweed pollen (Vincent et al., 1992; Dechamps, 1995).

4. Ambrosia artemisiifolia

4.1. Taxonomy

The genus *Ambrosia* belongs to the tribe *Heliantheae* in the Asteraceae family (= Compositae, daisy family). It consists of about 40 species, 22 of which occur naturally in North America. *A. artemisiifolia* is native to most of the United States and parts of Canada. In the pre-settlement vegetation it grew in disturbed sites in the prairies. Today it is common in agricultural ecosystems, in urban-industrial ruderal sites and along roadsides (Lavoie et al., 2007; Otto et al., 2008).

Several *Ambrosia* species have been introduced to other parts of the world, most notably *A. artemisiifolia* is now found in Europe, Japan and Australia. Several varieties of *A. artemisiifolia* are described in the native range. The only variety found in Europe is *A. artemisiifolia* var. *elatior*. *A. elatior* L. and *Ambrosia glandulosa* Scheele are considered synonyms of *A. artemisiifolia*.

Amongst other species that occur as non-natives in parts of Europe are the perennials *A. psilostachya* (syn. *Ambrosia coronopifolia*) in most of West and Central Europe, and *Ambrosia confertiflora* in West Asia and Israel. The annual *A. trifida* occurs in many European countries but seems to be less common (DAISIE, online).

[12] Feedstuff Book Ordinance, available from http://www.kpmg.ch/Topics/13965.htm

4.2. Life history and ecology

A. artemisiifolia is a tall erect summer annual. *Ambrosia* is among the few genera in the Asteraceae family that are wind pollinated and are lacking showy flowers. Male flower heads are found in terminal racemes: female flowers are found in leaf and branch axils on the same plants. Flowering is triggered by decreasing day length. The flowering period begins in July or early August and peaks in August and September. The male flowers produce large quantities of pollen and a single plant can produce >100 million pollen grains (Puc, 2004; Fumanal et al., 2007a).

The seeds are around 3-4 mm long and 2 mm wide. They are enclosed in the flower bracts which have characteristic blunt spines. The diaspore is thus an achene (Bassett and Crompton, 1975). The number of seeds produced by one plant varies widely. Hungarian studies found that plants germinating in April produce 3000-4000 seeds, whereas those germinating in August produced only 14-16 seeds (Szigetvári and Benkö, 2008). The highest number reported is 62,000 (Bassett and Crompton, 1975). They require cold temperatures for breaking dormancy and can remain viable in the soil for several decades (Bassett and Crompton, 1975).

A. artemisiifolia occurs in a wide range of soils and habitat types. It needs open soil for germination and becomes less common in the course of undisturbed vegetation succession.

4.3. Occurrence of *Ambrosia artemisiifolia* in Europe

A. artemisiifolia was introduced into several European countries around the mid 19th century (Chauvel et al. 2006). Available distribution maps (e.g. Bretagnolle and Chauvel, 2006) show the presence of ragweed in many European countries with an increase in the distribution pattern (see Appendix for a selection of maps). Although these maps give some indications of where ragweed has been found, they do not provide details on the relative abundance of *Ambrosia* in these regions.

A more reliable estimate of the actual frequency and abundance in Europe can be derived from air pollen counts (Figure 1). These data show that high densities of ragweed occur in Hungary and neighbouring countries, in the South-East of France and the Po region of Northern Italy. Towards the North the species becomes rarer: Szigetvári and Benkö (2008) give 55 °N as the northern limit. Dahl et al. (1999), however, report incidental findings of *A. artemisiifolia* in Sweden.

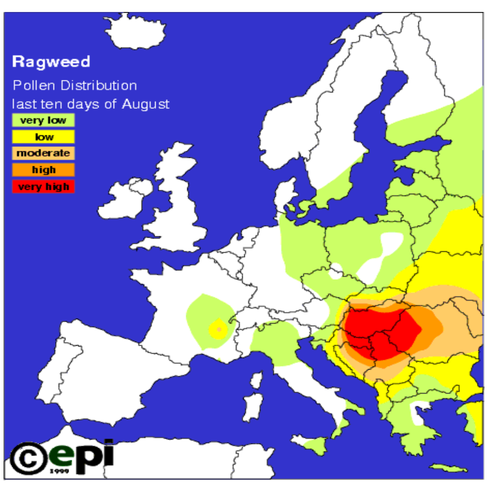

Figure 1: Pollen distribution in Europe[13]

13 Available from www.polleninfo.org.

4.4. Dispersal of ragweed seed

The fruits of *Ambrosia* are relatively heavy and lack adaptation to specific modes of dispersal. Natural dispersal is by gravity (barochory) or by water (hydrochory), since the seeds may float on water for extended periods of time (Fumanal et al., 2007b). Animal dispersal (zoochory) may occur but no detail is available on its importance.

Because ragweed flourishes in newly disturbed soils, it tends to follow human activity. In addition to bird feed the following have been suggested as important mechanisms in its dissemination:

- import of clover and cereal seed from America and other countries where ragweed is a common agricultural weed;
- around docks where imported grain is transferred;
- around oil seed mills (Brandes and Nitzsche, 2006);
- along railways as ballast weed (Essl et al., 2009);
- along roadsides, moved by traffic (Lavoie et al., 2007; Essl et al., 2009);
- movement of US troops and horses in the First World War (Chauvel et al., 2006);
- moved with mowing machinery (Vitalos and Karrer, 2009);
- moved with agricultural equipment (Chauvel et al., 2006);
- moved with soil.

Ragweed increased in abundance and range in Canada long before people commonly put out seeds for wild birds, initially along river corridors, later through the use of contaminated crops and more recently along roadways (Lavoie et al., 2007).

5. Occurrence of *Ambrosia* in feed materials

Ambrosia is determined by the visual identification of the fruits and seeds. Details are specified by the International Association of Feedingstuff Analysis.[14]

5.1. Occurrence in bird feed

A variety of sources from different countries have implicated bird feed as a possible vector in the dissemination of ragweed (Bohren et al., 2005; Alberternst et al., 2008; Vitalos and Karrer, 2008; Essl et al., 2009). In this context, the term bird feed refers to feed materials used, in an unprocessed form, for feeding wild and caged birds. Processed compound feeds used in commercial large scale poultry production are not included here, as professional processing reduces the number of viable *Ambrosia* seeds significantly (see section 5.3).

If bird feed is an important vector in the spread of ragweed, then one would expect to find that:

- bird feed can be contaminated with ragweed seeds;
- ragweed seeds found in bird feed are viable;
- there is a possible route between bird feed and ragweed establishment;
- the route occurs frequently enough to make ragweed establishment a probable event;
- ragweed grows in gardens where feed is put out for birds;
- the spatial distribution of recorded occurrences of ragweed is consistent with bird feed as an origin;
- the genetic diversity of ragweed found in different places is consistent with multiple introductions;
- bird feed is an important route of ragweed spread compared with other dispersal vectors;
- a reduction of ragweed seed in bird feed leads to a reduction of ragweed pioneers in gardens.

[14] The International Association of Feedingstuff Analysis, available from http://www.iag-micro.org/

The earliest reference to bird feed as a source of alien weeds was in Germany (Muller, 1950). The first reference to bird feed as a source of *A. artemisiifolia* is Hanson and Mason (1985), who succeeded in growing and identifying plants from seed found in commercial bird-food mixtures. Since then, bird seed mixtures have been investigated in more detail (Table 1).

On average, more than half the samples of bird feed investigated contained ragweed seed. A kilogram of typical bird feed contained 86 ragweed seeds. The studies generally found that sunflower seed was the most likely source of ragweed contamination (because sunflower and ragweed belong to the same plant family, i.e. Asteraceae, and this makes it difficult to control ragweed infestation on sunflower fields).

In five of the studies listed in Table 1, it was found that between 2 and 25 % of the ragweed seeds germinated. However, even low germination rates do not exclude the possibility that seeds are viable and have the potential to germinate at a later date.

Ragweed seeds may be spread directly or indirectly through bird feed. Direct routes include people spreading bird seed on the ground to feed domestic poultry or game birds, discarding bird cage sweepings on rubbish tips. Because bird seed is cheaper than specialist nursery seed, sunflower seeds intended for use as bird feed are sometimes sown to create cut-your-own flower gardens (Alberternst et al., 2008).

Indirect distribution of ragweed seeds may occur as a result of birds and arboreal mammals selecting and discarding seeds presented in feeders, birds and mammals carrying seeds away to feed young, or to hide them away, and seeds passing unharmed through the guts of seed eaters. Fallen seeds may be consumed or hidden by ground feeders such as game birds, poultry, mice, voles and rats.

The study of Vitalos and Karrer (2008) in Table 1 estimates that only 10 % of bird feed samples contained significant numbers of ragweed seed, and that only 2 % of them were viable. They concluded that the role of bird seed was probably overestimated as a cause of ragweed spread. However even if we accept that the probability of ragweed contamination of, and germination from, bird feed is small, it needs to be recognised that the practice of feeding wild birds is very common in Europe. In the UK, for example, it has been estimated by the Royal Society for the Protection of Birds that half of all adults regularly feed wild birds. This amounts to several million households putting out seed in their gardens, so that even if the probability of ragweed establishment is small, this may be outweighed by the large number of opportunities presented.

There are many references, albeit anecdotal, to ragweed found in gardens. Dahl et al. (1999) note that, in the course of preparing a new flora of Southern Swedish province of Skane, about 70 occurrences of ragweed have been documented and that “most occurrences reported to the authors have been in connection with bird tables”. Bohren (2006) states that, in Switzerland, “beside a few known foci in arable fields, ragweed grows mainly in private garden sites all over the country”. Rich (1994) observes that ragweed in the UK has been found in chicken runs. A survey conducted in a local Bayreuth newspaper (Lauerer et al., 2008) found 18 instances of ragweed in gardens, all of which could be traced back to the presence of bird seed. A more formally conducted German monitoring study found that 55 % of 283 ragweed reports were found in the close vicinity of bird feeders (Starfinger, 2008). These reports indicate that ragweed can move from bird feeds into the surrounding environment. It is less clear how often this occurs.

Ragweed is now well established in many European countries and it is not easy to deduce from presented maps alone what were the initial mechanisms by which they became naturalised. In the UK however, although ragweed has been found in many places, its appearances are casual and there is little evidence that it persists (Appendix, Figure 5). The majority of ragweed occurrences in Germany are similarly dominated by groups of less than 20 plants (Appendix, Figure 4).

Table 1: Summary of 12 recent studies of seeds intended for bird feed and their degree of contamination with ragweed seed. % positive is percentage of samples in which at least 1 ragweed seed was found. The mean 'n' of seeds per sample has been calculated in 2 ways: 1) for all samples including samples with zero seeds, 2) for only those samples with at least 1 ragweed seed was found.

			All samples	Samples containing ragweed					
Food type	**number of samples**	**% positive**	**mean number of ragweed seeds/kg**	**mean number of ragweed seeds/kg**	**Min[(a)]**	**Max[(b)]**	**Sd[(c)]**	**% germination**	**Reference**
Bird seed	16	50	36.9	73.9	7.6	195	77.18	Not tested	Jørgensen, 2008
Bird seed	17	71	5.4	7.6	1	23	7.5	Not tested	Bohren et al., 2005
Sunflower seeds	7	71	440.9	617.2	23	2781	1211.21	11.8	Chauvel et al., 2004
Bird seed	(13.8 kg)		23.8			34		Not tested	Brandes & Nitzsche, 2006
Bird seed						170		Not tested	Alberternst et al., 2006
Single variety seeds	286	20			6	418		Not tested	German Govt. Fact sheet, 2008
Mixed bird feed	22	41			1	629		Not tested	German Govt. Fact sheet, 2008
Other animal feed	11	45			1	26		Not tested	German Govt. Fact sheet, 2008
Bird seed	19	37	35.6	96.7	1	531.2	195.37	2	Vitalos and Karrer, 2008
Bird seed	33	70	21	30.1	1	374	76.93	19.5	Alberternst et al., 2006
Bird seed	25	68	12.6	18.6	1	54	18.64	25.1	Lauerer et al., 2008
Sunflower seeds	11	64	194.5	305.7	1	1143		Not tested	Thommes, 2008
Mixed bird feed	11	91	56.1	61.8	2.5	243		Not tested	Thommes, 2008
Fatball	9	44	30.1	67.8	55.8	133.3		Not tested	Thommes, 2008
								11.4	Thommes, 2008
Birdfeed	18	84				285		Not tested	Klein, 2007
Birdfeed						301		Not tested	Albertenst and Nawrath, 2008
mean		**58**	**85.7**	**142.1**				**13.9**	

(a) Minimum number of ragweed seeds/kg;
(b) Maximum number of ragweed seeds/kg;
(c) Standard deviation.

Ragweed, although relatively rare in the UK, has often been recorded in the Atlas of the Botanical Society of British Isles (BSBI, 2002). The data suggest that there has been an increase in ragweed occurrences from 94 10 km squares (3.4 % of all UK 10 km squares) recorded in the years 1987-1999, to 124 10 km squares (4.5 %) in the years 2000-2009. It is also noticeable that only 25 % of the squares recorded in 1987-1999 were still found positive for ragweed in 2000-2009. This indicated that ragweed failed to persist in at least 75 % of its recorded sites, suggesting that ragweed in the UK has been introduced on many independent occasions but has usually failed to establish itself as a source for further dispersal. Groom et al. (in press) have conducted a variogram analysis of ragweed distribution and found that it is typical of plants that are randomly distributed and casual, rather than clumped as one would expect if they were dispersing naturally.

Multiple fresh occurrences of the weed across a wide geographical area are at least consistent with bird feed as a source for new introductions. However, without further analysis of the BSBI data it is not possible to rule out other mechanisms. Rich (1994) suggests that the failure of ragweed to spread in the UK is because the climate has cooler and damper springs and autumns, and the small seasonal temperature range may discourage seed germination.

Plants from different origins differ more in their genetic structure than those from the same origin. If ragweed is typically introduced into a new territory in only a few places and subsequently spreads out from these foci, then we might expect the genetic patterns of these ragweed plants to be more similar to each other than those of ragweed plants introduced from many different original sources (such as bird feeding stations). In a comparison of genotypes between ragweed collected in France and samples from the USA, Genton et al. (2005) conclude that "our results are consistent with a scenario of multiple sources for the introduction of common ragweed in the Rhône-Alpes region". A high genetic diversity does not necessarily implicate bird feed as the origin (and the spread of ragweed in France probably pre-dates the recent habit of feeding wild birds), but it does imply that ragweed has been introduced on numerous independent occasions rather than spreading out from a single successful pioneer. And it suggests that, if ragweed is an unwelcome alien, then effort should be focused on preventing new introductions and not simply on containment and eradication of established populations.

The invasion of Austria by *A. artemisiifolia* and the changing habitat of ragweed were analysed in detail by Essl et al. (2009), based on a survey of available records. In total, 697 records were obtained from more than 30 sources. For 247 records, habitat data were not available. The study suggests that ruderals linked to bird feeding places were particularly important in the period between 1950 and 1979, but are less important now. Currently, more than 50 % of ragweed records in Austria are found along roadsides (Table 2). A species distribution model indicated that temperature, topography, roads and human settlements were statistically important predictors of the presence of naturalised ragweed populations.

Table 2: Habitats colonised by *Ambrosia artemisiifolia* during the different periods of its invasion. Numbers of records for each habitat are shown followed by its percentage contribution to the total number of records in the given period (n = 450); for 247 records, habitat data were not available. The category "other ruderal habitats" includes all ruderal habitats not associated with traffic infrastructure, waste sites or bird feeding places (Essl et al., 2009).

Period	Road-sides	%	Rail-ways	%	Other ruderal habitats	%	Fields	%	Waste sites	%	Bird feeding places	%	Gardens	%	Total
1883–1949	0	0.0	8	80.0	2	20.0	0	0.0	0	0	0	0	0	0.0	10
1950–1979	2	3.1	15	23.4	27	42.2	3	4.7	4	6.3	8	12.5	5	7.8	64
1980–1994	10	10.8	12	12.9	46	49.5	9	9.7	9	9.7	4	4.3	3	3.2	93
1995–2005	142	50.2	42	14.8	69	24.4	17	6.0	2	0.7	3	1.1	8	2.8	283
Total	**154**	**34.2**	**77**	**17.1**	**144**	**32.0**	**29**	**6.4**	**15**	**3.3**	**15**	**3.3**	**16**	**3.6**	**450**

According to Vitalos and Karrer (2008, 2009), the role of bird seed has been overestimated as an important ragweed vector in Austria. Their work suggests that the most dramatic increases in ragweed numbers have been along the main arterial roads in lowland Austria. They believe that the main distribution vectors are mowing machines rather than road traffic. Seed traps placed along the roadside indicated that ragweed seeds did not often move much beyond 25 m, whereas they found an average of 28 ragweed seeds per 100 g of material clinging to mowing machines.

The authors acknowledge that bird seed could play a role in "primary introduction in countries where ragweed is not yet established". Even in countries where ragweed populations have established themselves it is possible that bird seed could open up new centres from which ragweed could naturalize and spread.

As mentioned above, in Switzerland the use of bird feed contaminated with *Ambrosia* seed has been prohibited since 2006. At that time, according to Popow (2008), there were 594 reports on occurrences of ragweed and two thirds of them (397 reports) described occurrences of ragweed in domestic gardens. In 2007, the number of reports in gardens fell to 65 and, in 2008, there were only 19 reports. The obvious explanation for this decline is that ragweed is now less common in bird seed. Other possible explanations for the decline are milder winters in which less bird seed were sold, and a decline in reporting rate rather than a decline in new ragweed pioneers.

There is strong evidence that ragweed can and does establish itself in a new terrain as a contaminant of bird seed. Once established it is likely that other dispersal mechanisms become more important. If ragweed could be eliminated from bird seed (by physical removal or by sterilisation), then the regions most likely to show immediate benefits would be those where ragweed has, as yet, few naturalised populations. The benefits of such a measure might be less obvious in regions where persistent ragweed populations exist. However, even in these regions, control of ragweed in bird seed may prevent pioneer plants from establishing new bases from which to spread.

5.2. Occurrence in feed materials other than bird feed

Feed materials used in the composition of farm animal feeds include maize, wheat, sunflowers, millet, peanuts, soybean, peas and beans, all of which have been reported to be potentially contaminated with seeds of *Ambrosia.* In a recent study in Germany (BELV-BLV, 2008), *Ambrosia* seeds were identified in sunflower and sorghum imported into the EU in 2006 and 2007 (Table 3).

Table 3: Number of *Ambrosia* seeds in different feed materials collected in Germany in 2006 and 2007.

	Number of samples tested	Number of positive samples	*Ambrosia* seeds per kg of feed	
			minimum	maximum
Sunflower	49	15	6	108
Millet	179	39	7	418
Sorghum	57	2	27	27

This study shows that *Ambrosia* seeds were found in both small-grained and large-grained feed materials. Millet is not widely used in rations for farm livestock, although sorghum is being used increasingly. Sunflower seeds are fed, but usually only as sunflower meal following oil extraction. Although physical separation is possible as a means of removing *Ambrosia* seed from other grains, in the case of sorghum this is technically difficult because the weight and size of the seeds are similar to that of sorghum.

In addition to cereal grains and oilseeds/oilseed meals, ruminant livestock consume large quantities of forage crops such as grass, maize and alfalfa, either in the fresh state or conserved by drying or ensiling. Since *Ambrosia* may be present as a weed in these crops, they represent a potential source of exposure to *Ambrosia*. There are no published data indicating the likely scale of this contamination.

However, since infestation with *Ambrosia* can significantly reduce yields or the feed value of these crops, most farmers will attempt to control the weed with appropriate herbicides. As discussed below, it appears that *Ambrosia* seeds do not survive the ensiling process and so the largest contribution of weed seed in animal diets is therefore likely to arise from contaminated hay and grain. A portion of weed seed present in feed can remain viable after passing through an animal's digestive tract. Weed seeds that are present in bedding or in spilt-feed may bypass the animal and directly enter the manure stream. Both of these weed seed sources may result in manure containing viable weed seeds.

The presence of *A. artemisiifolia* has also been shown in feed, other than bird feed namely in mixed feed containing straw. In a study undertaken in The Netherlands (van Denderen, 2008), samples were analysed for the presence of *A. artemisiifolia*. Seventeen samples were purchased in local shops and analysed. *A. artemisiifolia* was present in 65 % of the samples, but mostly at levels of <10 seeds per 0.5 litre (values ranging from 0 to 8 seeds per 0.5 litre). Only 3 samples showed high or very levels of seeds (respectively 61, 66 and 194 seeds per 0.5 litre).

5.3. Effects of feed processing and storage

Grains fed to livestock generally undergo some form of processing, including grinding and, in the case of compound feeds, exposure to heat and/or high pressure. Cash et al. (1998) have estimated that for compound feeds less than 1 % of weed seed will survive grinding and pelleting. However, studies that have directly measured the viability of *Ambrosia* seeds during processing are not available.

In contrast, grain seed fed to birds generally undergo minimal processing. Bird feed is a mixture of seeds, nuts, fruits, vegetables and fat provided to birds for sustenance. Making bird seed is a relatively simple manufacturing process. Following procurement of the seeds, the grains and the fruit that make up the various mixes from processors, the grains are cleaned, typically using a multi-step air cleaning system that sorts the quality foodstuff from the waste (also known as Chilton). The air sorter separates the lighter debris such as sunflower hulls and stems from the raw materials used in the bird seed mixes. Many manufacturers use the Chilton process to make other animal feed such as pellets for companion birds. Additional waste products may also be sold to local farmers for use in their animal feed. The second phase involves blending and packaging of these materials, and then shipping them to retailers. Guidelines for the manufacture of bird feed have been produced by the FAO and other organisations.[15]

5.4. Estimation of the intake by farm livestock

The probability that *A. artemisiifolia* occurs on natural grassland is very low. In a French study of infested agro-ecotypes the species was not found on grasslands (Fumanal et al., 2008). The vegetative parts of *A. artemisiifolia* are unpalatable to grazing livestock and, where adequate supplies of alternative feeds are available, the intake of *Ambrosia* is likely to be low.

Since it can infest practically all field crops, it may be present in forage crops harvested for preservation. While it is likely that seeds in dried forages (e.g. grass or alfalfa) will remain viable, Woodward (1940) reported that seeds of *A. artemisiifolia* failed to germinate after ensiling in corn (maize) silage.

The greatest risk of exposure to *Ambrosia* seed is from the contamination of grains, and in particular sunflower. Due to the botanical similarity of *Ambrosia* to sunflower, herbicide use is limited for this crop. This is particularly problematic in areas where sunflower is a major crop plant. However, whole sunflower seeds are seldom fed to farm livestock, but are de-husked and conditioned by heating to 80-90 ^{o}C prior to oil extraction. The residual meal is exposed to more heating, followed by grinding and pelleting to produce sunflower meal. These processes reduce the viability of *Ambrosia* seeds (Cash et al., 1998).

[15] Available from http://www.fao.org/docrep/008/y5831e/y5831e01.htm.

Recent data indicate that approximately 4.5 million tonnes of sunflower meal are used as livestock feed in the EU, but only about one third is from EU-grown sunflowers (FEFAC, 2007). In the EU, France, Hungary, Spain, Romania and Bulgaria produce 4.2 million tonnes of sunflower seeds, which account for 88 % of EU-27 production (EUROSTAT, 2008). To put this into context, this sunflower meal contributes only about 5 % of the protein used in livestock feeds. The remainder comes predominantly from whole seeds imported into, and subsequently processed in, the EU. These imports therefore represent a risk of introducing viable *Ambrosia* seeds into the EU, but only as far as the processing plant. Once the oil has been extracted the risk that the remaining meal contains viable seeds is minimal.

The presence of *Ambrosia* seeds in consignments of sorghum and millet has been attributed to the fact that it is almost impossible to physically separate the seeds due to their similar size and weight. Data on the EU grain sorghum market show that EU production of sorghum has remained relatively constant and is low, with about half a million tonnes annually. Between 1988 and 2006, total consumption averaged about one million tonnes, with most of the imports coming from the USA and Mexico. In 2007, imports and consumption increased to 5.7 and 6.1 million tonnes respectively,[16] largely due to reduced production of cereal grains in the EU in that year. Almost all of the sorghum is used as feed for livestock and birds.

5.5. Summary on the role of bird feeds in the main dissemination scenarios

The available data indicate that:

- between 20 % and 91 % (see Table 1) of commercially available bird feeds can be contaminated with ragweed seeds. Seed counts vary between 0 and 2781 seeds per kilogram bird feed;

- between 0 % and 25 % of the ragweed seeds found in these commercially available samples (see Table 1) were germinated, but even low germination rates do not exclude the possibility that seeds are viable retaining the potential to germinate at a later date;

- the possible correlation between bird feed and ragweed establishment can be demonstrated by a German monitoring showing that up to 55 % of the reported ragweed were found in private gardens in the close vicinity of bird feeders. This indicates that ragweed can move from bird feeds into the surrounding environment and that bird feed has to be considered as a route of dissemination in previously non infested areas;

- in follow-up studies on the spatial distribution of *Ambrosia* only 25 % of the recorded sites were found positive after several years. However, as one *Ambrosia* plant can produce between 3000 and 4000 seeds (maximum recorded seeds is 62,000), this relatively low incidence of established and continuing habitats may be outweighed by the number of opportunities for further spreading by the newly established (pioneer) plants;

- ragweed seeds may be spread directly or indirectly through bird feed: direct routes include individuals spreading bird seed on the ground to feed birds, or discarding bird cage sweepings. Indirect distribution of ragweed seeds may occur as a result of birds and arboreal mammals selecting and discarding seeds. Fallen seeds are consumed or hidden by ground feeders such as game birds, poultry, mice, voles and rats. Seeds passing unharmed through the guts of seed eaters may also contribute to the dispersal.

[16] Available from http://www.depts.ttu.edu/aged/Sorghum/text/Bulletin2%20-finished.pdf.

6. Effects in farm animals

6.1. Hypersensitivity

Cattle and horses are believed to be allergic to pollen antigens, but detailed information is lacking. In horses, the so-called heaves are associated with the inhalation of fungal spores (*Aspergillus fumigatus*) and it is likely that a similar reaction occurs following inhalation of *Ambrosia* pollen. The pathogenesis of allergic airway obstruction differs, however, between horses and humans (Laan et al., 2007). In horses, an immunoglobulin E (IgE)-mediated allergic reaction has been related to dermal sensitivity and it is likely that they develop dermatitis as well following direct skin contact with pollen allergens, but clinical reports are lacking.

As a model of allergic nasal congestion, beagle dogs were experimentally sensitized to *Ambrosia* seeds by means of intranasal applications (Skorohod and Yeates, 2005). The introduction of ragweed caused a dose-related decrease in nasal cavity volume without adverse systemic effects.

6.2. Other adverse reactions

There is no evidence that *A. artemisiifolia* produces secondary metabolites that cause clinical intoxications in livestock. Studies on the performance of grazing sheep and goats on pastures contaminated with common ragweed indicated small differences in grazing time as compared to non-contaminated areas, but no adverse reactions were observed (Animut et al., 2005). Other Asteraceae, such as *Ambrosia tenuifolia* and *A. maritima,* contain biologically active sesquiterpenes and lactones. For example, a molluscicidal activity has to be attributed to *A. maritima* (Geerts et al., 1991, 1994) and trypanocidal and leishmanicidal effects to *A. tenuifolia* (Sulsen et al., 2008a, b). Hepatoprotective and antioxidant effects of *A. maritima* have been reported by Ahmed and Khater (2001). In contrast, when *A. maritima* shoots were fed to chicken at 2 or 10 % of the basic diet for 6 weeks, the average body weights and efficiency of feed utilization were depressed in chicken fed a diet containing 10 % of *A. maritima* (Bakhiet et al., 1996).

6.3. Carry over and residues

No studies could be identified addressing distribution and residue formation of secondary plant metabolites present in *Ambrosia* spp. Historical reports (Spencer, 1957) describe that cattle, consuming large amounts of ragweed due to feed shortage, produce milk with an unacceptable taint.

7. Human health risks

Ambrosia spp. produces pollen grains in large quantities which easily become airborne and may reach the upper respiratory tract (Stepalska et al., 2002). *Ambrosia* pollen is known to be a particularly strong allergen (Smith, 1984; Lewis et al., 2000). Rhino-conjunctivitis and asthma due to inhalation of airborne allergens are the most common clinical manifestations of allergic reactions to *Ambrosia* spp. (Dechamps, 1995), and represent the major health risk caused by *Ambrosia*.

Skin exposure to *Ambrosia* allergens may be airborne (Epstein, 1960; Hjorth et al., 1976; Schumacher and Silvis, 2003) or by direct contact with the plant e.g. during manual weeding and handling of *Ambrosia*-contaminated crops (Guin and Skidmore, 1987; Gordon, 1999). The most common clinical manifestations of skin exposure to *Ambrosia* allergens are atopic dermatitis (IgE-mediated, Type I) and contact dermatitis (cell-mediated, Type IV). Ragweed contact urticaria (due to IgE-mediated allergy) is rare (Dechamps, 1995).

The present statement focuses on allergic reactions triggered by the oral exposure to *Ambrosia* allergens in food and on allergic reactions to *Ambrosia* allergen-free foods because of preexistent sensitization to *Ambrosia***.**

7.1. Contamination of food with *Ambrosia*

Direct oral exposure to ragweed pollen, apart from pollen in inhaled air, is recognised as a potential problem only in relation to the consumption of beehive products, which contain a large number of allergens that come from the bodies of the bees and the plant materials they collect (nectar and plant exudates). Beehive products (honey, royal jelly and propolis) contain pollen grains.

Pollen allergies and in particular to Compositae *(Artemisia* spp., *Ambrosia* spp., *Matricaria* spp., *Tripleurospermum* spp., *Anthemis* ssp., *Taraxacum officinalis*) are a risk factor for honey and royal jelly allergies (Bousquet et al., 1984; Helbling et al., 1992). While uncommon, allergies to honey can involve reactions varying from mild discomfort to anaphylaxis (Kiistala et al., 1995). The incidence of honey allergy has been reported to be 2.3 % in a group of 173 patients with food allergies (Bauer et al., 1996). Similarly, propolis and royal jelly may trigger allergic reactions. Avoiding beehive products and food products thereof is the only effective prophylaxis for the allergic individuals. No specific data regarding *Ambrosia* pollen as a cause of allergic reactions to beehive products and food products thereof are currently available.

Exposure to *Ambrosia* allergens via cow's milk has not been reported, possibly because cattle apparently avoid eating *Ambrosia*. No reports on allergic reactions due to *Ambrosia* pollen contamination of seed, grain or other food or feed products could be found.

7.2. Allergic reactions to foods because of preexistent sensitization to *Ambrosia*

The same IgE antibody molecule may bind to different antigen (allergen) molecules if they share similar epitopes (Løvik, 2009). This phenomenon (i.e., the capacity of one antibody molecule to bind similar epitopes from different allergens) is called cross-reactivity and may lead to clinical allergic reactions, although these are usually weaker than allergic reactions triggered by the primary sensitizing allergen.

The oral allergy syndrome (OAS) is caused by cross-reactivity between proteins present in fresh fruits and/or vegetables and pollen. OAS due to ragweed pollen sensitization has been described in relation to the consumption of Cucurbitaceae such as melon, watermelon, cucumber, and zucchini, as well as Musaceae such as banana (Anderson et al., 1970; Enberg et al., 1987; Felber et al., 2003; Egger et al., 2006). Most people with OAS have symptoms such as itching, burning, tingling and occasionally swelling of the lips, mouth, tongue and throat which have been in contact with the fresh fruit or vegetable. Symptoms usually last between few seconds and few minutes, and only rarely progress into any serious discomfort. OAS is diagnosed in subjects with the symptoms above and history of seasonal allergic rhinitis to ragweed or other pollens as a trigger. Positive skin testing to the suspected food can support the diagnosis of OAS. However, skin testing with food extracts obtained commercially could lead to false negatives since the proteins triggering OAS are often broken down during processing. Therefore, it may be necessary to use fresh fruit or vegetables in skin tests ("prick to prick" procedure). Because of the small but real chance of a more severe allergic reaction, avoidance of the incriminated fresh fruits and/or vegetables is advised. Many subjects already avoid the suspect foods spontaneously since OAS symptoms are unpleasant. Thus, sufferers of the OAS have a reduced number of choices with regard to plant-derived food products. However, no data are available on the frequency of OAS reactions triggered by Cucurbitaceae and Musaceae (Anderson et al., 1970; Enberg et al., 1987; Felber et al., 2003; Egger et al., 2006).

8. Effects of *Ambrosia artemisiifolia* on the environment

A. artemisiifolia is classified as an epoecophyte. i.e. a xenophyte species established only in ruderal or segetal (arable) vegetation (Protopopova et al., 2006). In its current range, *A. artemisiifolia* occurs predominantly in disturbed and open habitats and its constant presence can be expected in locations where disturbance is repeated regularly, i.e. in arable fields, roadsides and generally in places where human activities cause disturbance (Bazzaz, 1974; Mihály and Botta-Dukát, 2004; Szigetvári and Benkö, 2008). Normally it decreases in the course of succession (Szigetvári and Benkö, 2008). According to Bonnot (1967) *A. artemisiifolia* grows wherever competition is low and also in ecosystems regularly disturbed by humans. Fumanal et al. (2008) found *A. artemisiifolia* with other non-native species and with species from early successional stages. They conclude therefore that *A. artemisiifolia* does not present a threat to the plant biodiversity of the different invaded areas, but is an alien generalist species occupying a free ecological niche. They refer with regard to *A. artemisiifolia* to a "winner species" (as defined by McKinney and Lockwood, 1999) rather than a "transformer species" (according to Richardson et al., 2000).

Some studies show that *A. artemisiifolia* has allelopathic abilities, which might have negative impacts on the germination of other species (Siniscalco et al., 1992; Siniscalco and Barni, 1994; Brückner et al., 2003). Allelopathic effects were also found by Hodişan et al. (2009) for *A. artemisiifolia* on wheat, rye, barley and rape (aqueous extracts from roots and leaves of mature plants had a significant inhibiting influence on the germination of these plants; stem extracts only inhibited germination of wheat and rye). Lucerne was not inhibited at all. These allelopathic effects may also inhibit germination of other plant species.

General or indirect statements are made in literature regarding the environmental impact of *A. artemisiifolia*, particularly with regard to threat to biodiversity by competition with other species (Bohren et al., 2008; Chauvel et al., 2006; Delabays et al., 2008; van Vliet et al., 2009) but published evidence on this aspect is sparse.

Brandes and Nitzsche (2006) did not find any evidence of a replacement of native species by *Ambrosia* spp. in Germany. They found *A. artemisiifolia* only scarcely at river banks in Germany and explain this with the assumption that the summer fluctuations of the water level may be the main reason for hampering the establishment in these habitats, because the species seems not to be tolerant to inundation. For torrential rivers with an extended period of summer drought this is different, as *A. artemisiifolia* often grows in dried up river beds. In one case cited in Alberternst et al. (2006), *A. artemisiifolia* had an environmental impact on a sand dune. In the vicinity of a nature conservation area near Daßfeld (lower Bavaria), 10 cm of soil contaminated with *A. artemisiifolia* seeds had been deposited illegally. Since 1993, around 20 mature specimens were found. In 2000 and the following years, the number of specimens suddenly increased to around 10000, growing on 200-300 m^2 of an adjacent, so far undisturbed sand dune under nature conservation, threatening rare species like *Teesdalia nudicaulis*, *Veronica verna*, *Veronica dillenii* and others. Due to nature conservation measures (mainly hand pulling), the population could be reduced by 75 % in 2005 and seems to be eliminated by now.[17] According to Brandes and Nitzsche (2007), the increase of the population was caused by inappropriate management measures. They assume that *A. artemisiifolia* did not invade the sand dune actively but was introduced by substrates for soil amelioration.

A. artemisiifolia is reported as invasive on river banks of South France (Doutriaux, 1997; Faton and Montchalin, 2007), particularly in new artificial embankments or in occasion of floods when the secondary river beds (usually dry) are flooded and then dried again (Doutriaux, 1997). Seeds of *A. artemisiifolia* can be easily dispersed by water but also human activities as movement of soil and gravel are very active pathways of dispersal. Use of herbicides being not allowed in river banks, in natural reserves *A. artemisiifolia* is controlled by sheep grazing with the objective to reduce pollen production (Faton and Montchalin, 2007). However, no impacts of *A. artemisiifolia* on biodiversity have been documented in France.

In Austria, the majority of *A. artemisiifolia* populations is expected to grow on fields and associated habitats in the near future (Essl et al., 2009), no evidence is given for environmental impacts. In Hungary, *A. artemisiifolia* is also reported in forest, where the common ragweed prefers the disturbed and light rich habitats as cut road margins, ditches, fields for game and feed troughs (Hirka and Csoka, 2009).

[17] Available from http://www.flora-niederbayern.de/fundorte.html#trag_du.

With respect to a similar study conducted in 1969-1970 (Ujvarosi, 1975, cited in Pál, 2004), Pál (2004) showed in southern Hungary a pauperization of the segetal (arable) weed flora in terms of number of species. For the 2001-2002 surveys, he reported a 90 % coverage by *A. artemisiifolia* of stubble fields and summer annual crops. However, from the data presented in this paper, it is not possible to distinguish whether the pauperization is due to a change in agriculture practice, to a transforming effect of the invasive weeds or to both. More details with regard to environmental impacts of *A. artemisiifolia* in Hungary are given by Pinke et al. (2008), who studied the weed vegetation on extensively managed fields in western Hungary. The surveyed vegetation contained 41 International Union for Conservation of Nature (IUCN) red list weed species (among which, two “critically endangered” (CR), four “endangered” (EN), seven “vulnerable” (VU), 22 “near threatened” (NT), and 6 “data deficient” species)[18], showing that extensively managed fields represent refugia for threatened weed species. Furthermore, these species support the agro-ecosystem food chain. This indicates a high value for the conservation of biodiversity of these habitats, which are considered by the author threatened by the increasing spread of *A. artemisiifolia* in two ways: by invading these habitats, *A. artemisiifolia* could replace these species, and a total weed control as well as an early ploughing of stubbles to remove *A. artemisiifolia* would also remove these valuable red list species.

Maryushkina (1991) has conducted studies to analyse the species strategy of *A. artemisiifolia* in the steppe in Ukraine. A reduction in the number of species was observed when ragweed seedlings were not removed in an experiment on a freshly ploughed plot of an abandoned field. Also in the steppe zone in Ukraine, *A. artemisiifolia* demonstrated high invasiveness and wide ecological amplitude (it occurs in eight synanthropic and two natural floristic complexes) (Protopopova et al., 2006). *A. artemisiifolia* penetrated even into dense stands of *Festuca sulcata*, especially if the communities were overgrazed and trodden by cattle and other livestock (Solomakha et al., 1992 cited in Protopopova et al., 2006). On pastures, large stands of *A. artemisiifolia*, as well as of other invasive weeds, prevented the process of restoration of steppe communities, which were replaced by various synanthropic communities, especially under proceeding overgrazing pressure (Protopopova et al., 2002 and 2003, cited in Protopopova et al., 2006). During the processes of restoration of completely or partly transformed vegetation, alien species as *A. artemisiifolia* raised the level of competition for ecotopes and are stronger competitors than native plant species acting in the newly formed ruderal communities as dominants (Protopopova et al., 2006).

Regarding other ragweeds, *A. trifida* is categorised in Japan as a Rank A species, i.e. one of the 16 most invasive species, having demonstrated strong adverse effects on biodiversity and ecosystems. Most of these 16 species dominate large areas of riparian habitats. Uraguchi et al. (2003) found allelopathic effects of *A. trifida* against an endemic floodplain plant in Japan. Kong et al. (2007) state that *A. trifida* could release allelochemicals into the soil to act as inhibiting the growth of wheat both in rhizosphere and non-rhizosphere soils in which *A. trifida* had been grown to different growth stages. In the *A. trifida* non-rhizosphere soil, however, the growth of wheat was considerably more reduced compared to that in the rhizosphere soil, implying that soil phytotoxicity did not result primarily from *A. trifida* root exudates, but from residues. As a result, there appear to be phytotoxins in the *A. trifida* infested or amended soils. A possible beneficial effect of *A. trifida* is mentioned in a study by Liang et al. (2007) in China, where, investigating the temporal dynamics of soil nematode community structure under invasive *A. trifida* and native *Chenopodium serotinum*, the number of nematode genera was higher in soil under *A. trifida* than under *C. serotinum*.

In Great Britain, *A. psilostachya* naturalised on sand dunes on the coast, but no environmental impacts were described (Rich, 1994). Yaacoby (2008) states that another ragweed *A. confertifolia* (generally

[18] The IUCN Red List is the world's most comprehensive inventory of the global conservation status of plant and animal species. It uses a set of criteria to evaluate the extinction risk of thousands of species and subspecies. These criteria are relevant to all species and all regions of the world. With its strong scientific base, the IUCN Red List is recognized as the most authoritative guide to the status of biological diversity (http://www.iucn.org/about/work/programmes/species/red_list/about_the_red_list/).

considered as a synonym of *A. psilostachya* according to Szigetvári and Benkö, 2008) severely infests nature reserves and reduces biodiversity in Israel, but no details were provided.

The Panel concludes that there is no direct evidence that *Ambrosia* spp. cause extinction of plant species. However, there are some indications that *A. artemisiifolia* could become highly invasive in certain environmentally-valuable habitats and that under certain conditions, generally in habitats disturbed by human activities, *A. artemisiifolia* might be linked to an impoverishment of species richness, therefore further ecological studies are needed.

9. Uncertainties

The high variability of data on the occurrence of *Ambrosia* in Europe, the lack of structured listings on habitat and status, and the uncertainties in the interpretation of the few structured surveys on migration patterns of *Ambrosia* (addressed more in detail in the previous opinions of the Plant Health Panel), hinder a quantitative attribution of sources of distribution of *Ambrosia* in Europe. In turn, the absolute contribution of *Ambrosia* seeds in bird feeds in comparison to other routes of dispersal cannot be quantified.

Due to lack of ecological studies, there are uncertainties whether *A. artemisiifolia* causes an impoverishment of species richness in certain habitats and conditions.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- The genus *Ambrosia* (family Asteraceae) is distributed worldwide. These plants are native to North America and are expanding their range in both their native areas and other parts of the world, including Europe. The most abundant species in Europe is *Ambrosia artemisiifolia* L. (common ragweed). *Ambrosia* may exert adverse health effects in humans due to the strong allergenicity of the pollen shed by the plants and distributed into the air.

- There is some evidence for allergenicity in animals, particularly in relation to the obstructive airway diseases in horses. There is no evidence that *Ambrosia* species form secondary plant metabolites that are of clinical significance for livestock.

- The Panel on Dietetic products, Nutrition and Allergies (NDA Panel) concluded that the major adverse health effects of *Ambrosia* are related to the allergenicity of inhaled plant pollen causing rhino-conjunctivitis and asthma, with skin allergies and food allergy playing minor roles. *Ambrosia* may cross-sensitize patients to other allergens, including food allergens.

- The Panel on Plant Health (PLH Panel) concluded that there is no direct evidence that *Ambrosia* spp. cause extinction of plant species. However, there are some indications that *A. artemisiifolia* could become highly invasive in certain environmentally-valuable habitats and that under certain conditions, generally in habitats disturbed by human activities, *A. artemisiifolia* might be linked to an impoverishment of species richness, therefore further ecological studies are needed.

- Ragweed follows human activity. Important mechanisms in its dissemination are import of grain and cereals, movement of mowing machinery and agricultural equipment. Movements of soils and traffic along the roadsides also contribute to the dispersion of the plant.

- The Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that bird feed (seeds) used for wild and ornamental birds may be an important route of ragweed dispersal especially in non infested areas. The relative contribution of bird feed (seeds) on the dispersion of *Ambrosia* compared with other routes of dissemination cannot be determined from the available information.

- The CONTAM Panel noted that *Ambrosia* seeds may contaminate feed materials containing maize, wheat, sunflowers, millet, peanuts, soybean, peas and beans and may be imported with raw feed materials from infested regions. However, animal feed materials compounded for use in livestock are extensively processed. This processing almost completely destroys *Ambrosia* seeds and hence the contribution of compounded feed to the dispersion of *Ambrosia* is considered to be negligible.

RECOMMENDATIONS

- There is a need for additional information on the actual status of distribution and sources of the dispersion of *Ambrosia* spp. in Europe.

- Further ecological studies are needed on the effects of *A. artemisiifolia* on the environment, particularly to investigate whether *A. artemisiifolia* causes an impoverishment of species richness in certain habitats and conditions.

- *Ambrosia* can establish itself in new territories as a contaminant of bird feed*.* Mechanical procedures can reduce the rate of contamination of sunflower seed. Cleaning techniques for all seeds used in bird feed need to be developed.

- Efforts to eliminate ragweed seeds from bird feed should be focused most on those areas where there are, as yet, few naturalised populations of *Ambrosia*.

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APPENDIX - DISTRIBUTION MAPS OF *AMBROSIA ARTEMISIIFOLIA* IN EUROPE

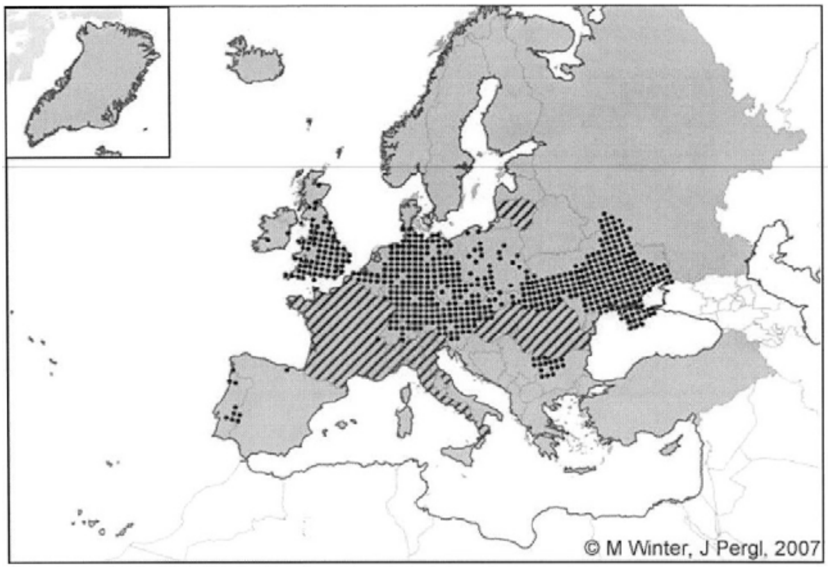

Figure 1: Distribution map in Europe from DAISIE project

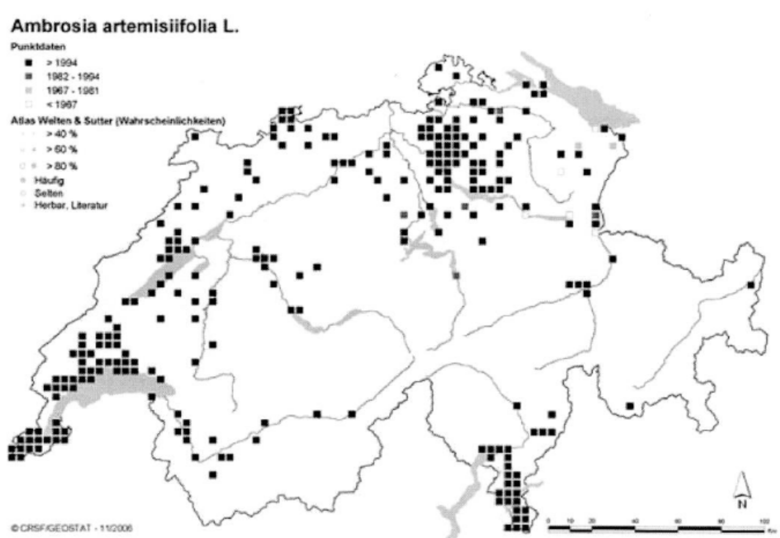

Figure 2: *Ambrosia artemisiifolia* distribution in Switzerland updated in December 2003. Elaborated by the CPS/SKEW (Commission suisse pour la conservation des plantes sauvages) and the CRSF (Centre du réseau suisse de floristique) on a mandate of the SAEFL (Swiss Agency for Environment, Forest and Landscape).

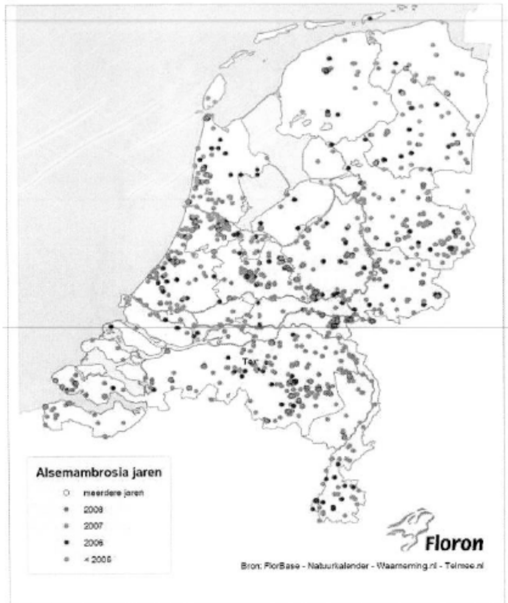

Figure 3: van Vliet et al. (2009) showing ragweed distribution and the increase in number of sightings in the Netherlands in the recent years.

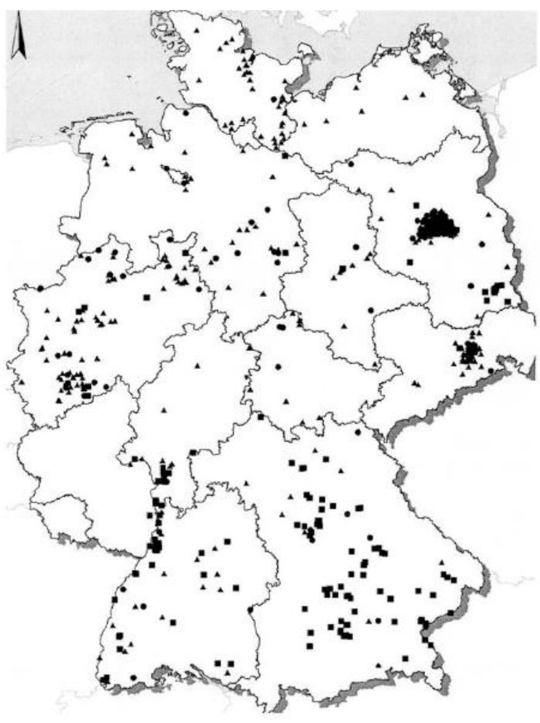

Figure 4: Occurrence of *A. artemisiifolia* in Germany according to the monitoring in the action programme *Ambrosia*. Triangles – small stands of simple plants; round dots – stands of 20 - 100 plants; squares – stands of <100 plants (from Starfinger, 2009). The map shows that many small stands (<20 plants) occur in regions that are otherwise free from the species. A concentration of small stands is found in cities. Since small stands are generally believed to be casual occurrences, it can be assumed that these result from release on the spot such as by feeding birds.

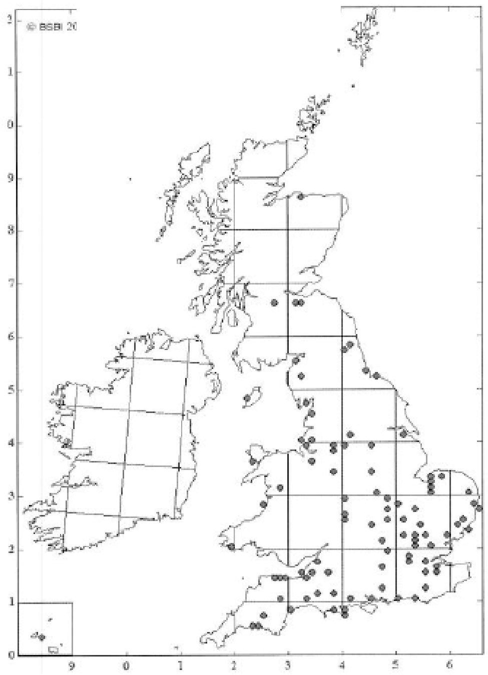

Figure 5a

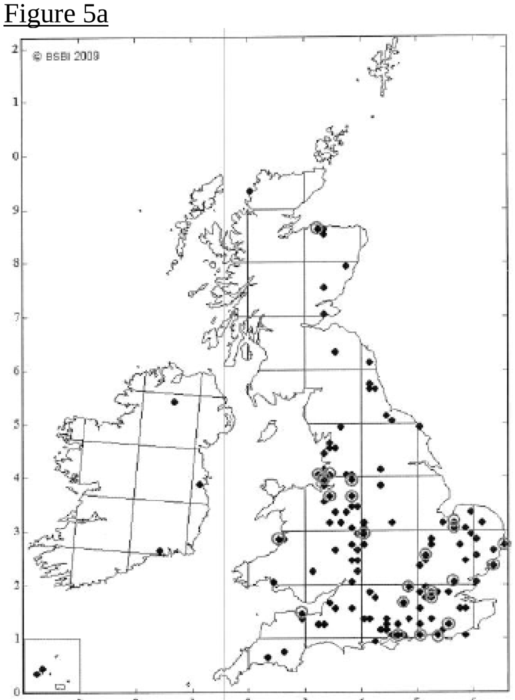

Figure 5b

Figures 5a and 5b: Data from BSBI Atlas showing the occurrence of ragweed in the United Kingdom. Pink dots represent 10 Km squares in which ragweed was identified in the 13 years from 1987 to 1999. Blue dots are for the (nearly) 10 years up to 2009. Blue dots with orange rings around them are those 10 Km squares where ragweed was found in both the earlier and later surveys.

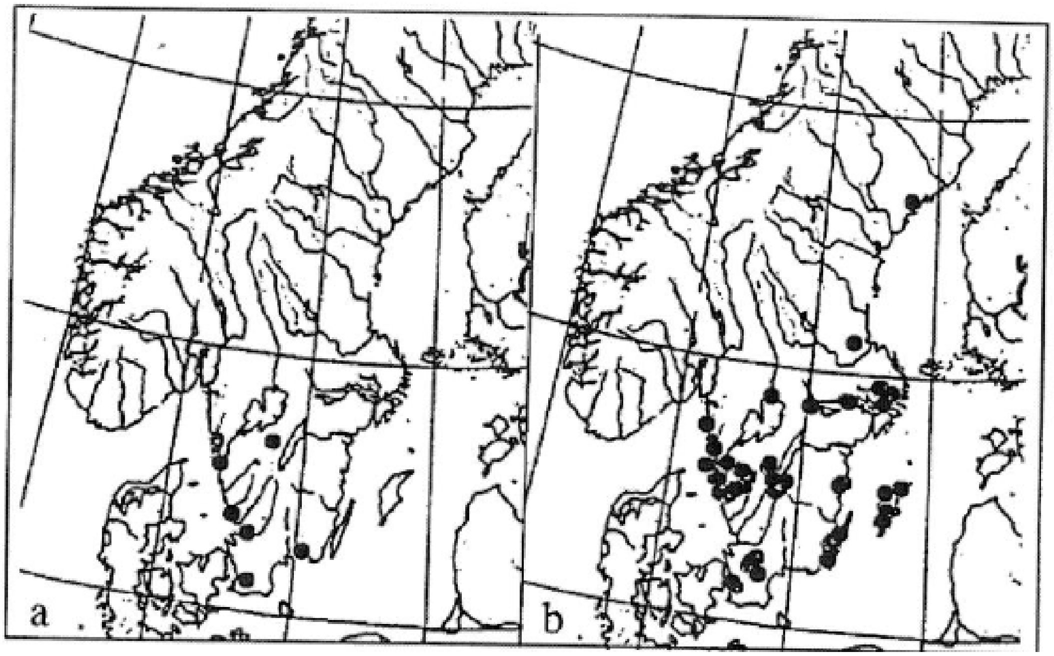

Figure 6: *Ambrosia* distribution in Sweden (Dahl et al., 1999). (a) Locations of Burkard 7-day volumetric spore traps in South Sweden where *Ambrosia* pollen has been registered during 1995-1998; (b) *Ambrosia artemisiifolia* - occurrences in Sweden during 1992-1998, as reported to the authors. One dot may represent a number of observations.

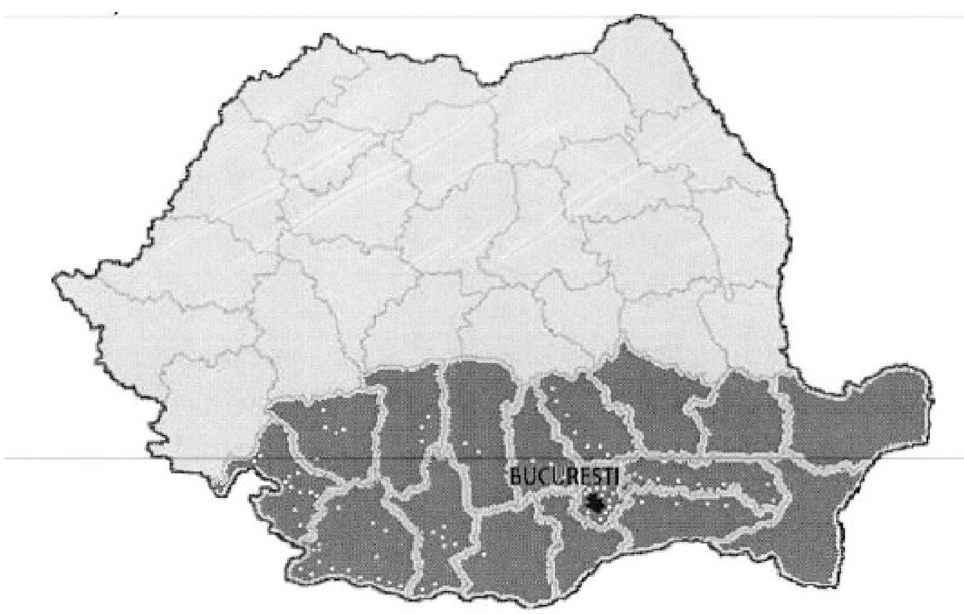

Figure 7: The spreading of *Ambrosia artemisiifolia* L. species (ragweed) in the south and south-eastern Romania, 2007 (Hodişan and Morar, 2008).

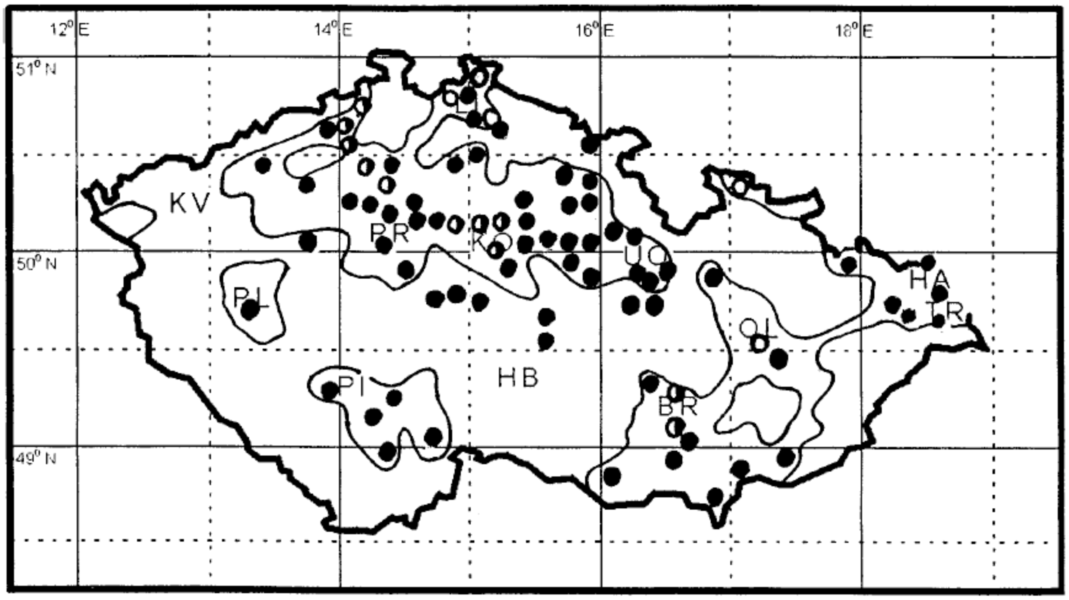

Figure 8: Present occurrence and potential future permanent area of distribution of ragweed species in Czech Republic (Rybnicek et al., 2000).

Black circles: recent findings of *Ambrosia artemisiifolia*
White circles: recent findings of *Ambrosia trifida*
Half black and white circles: both species

ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
BSBI	Botanical Society of British Isles
CONTAM Panel	Panel on Contaminants in the Food Chain
CPS/SKEW	Commission suisse pour la conservation des plantes sauvages
CR	Critically endangered
CRSF	Centre du réseau suisse de floristique
DAISIE	Delivering Alien Invasive Species Inventories for Europe
EFSA	European Food Safety Authority
EN	Endangered
EPPO, OEPP	European and Mediterranean Plant Protection Organization
EU	European Union
FAO	Food and Agriculture Organization
GCW	Global Compendium of Weeds
GISP	Global Invasive Species Programme
IgE	Immunoglobulin E
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
IUCN	International Union for Conservation of Nature
NDA Panel	Panel on Dietetic Products, Nutrition and Allergies
NOBANIS	North European and Baltic Network on Invasive Alien Species
NT	Near threatened
OAS	Oral allergy syndrome
PLH Panel	Panel on Plant Health
SAEFL	Swiss Agency for Environment, Forest and Landscape
sd	Standard deviation
VU	Vulnerable