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Allergenic invasive plant *Ambrosia artemisiifolia* L. in Poland: threat and selected aspects of biology

Barbara Tokarska-Guzik*, Katarzyna Bzdęga, Katarzyna Koszela, Izabela Żabińska, Barbara Krzuś, Małgorzata Sajan & Agnieszka Sendek

Department of Plant Systematics, Faculty of Biology and Environmental Protection, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland, e-mail: *barbara.tokarska-guzik@us.edu.pl

Abstract: The study presents the current state of research on *Ambrosia artemisiifolia* in Poland within its wider scientific background and gives the results of some field and laboratory investigations. This annual plant is characterised by a high level of production of seeds, easily dispersed by different vectors. Ragweed pollen is a strong allergen considered to be one of the most dangerous pollen allergens in the world. Being a serious hazard to human health, it is also considered as an "environmental weed" causing economic threat. The main aim of the study is to verify the status of the species in the flora of Poland, to assess the threat and discuss possibilities to prevent its future spread.

Keywords: alien invasive species, distribution, soil seed bank, seed germination, human health, control

1. Introduction

Invasion by plants, animals or fungi is one of the most pressing issues of nature considered on a global scale (Tokarska-Guzik 2005). Some authors even deem it to be the single most important problem in protecting biodiversity in the 21st century (Vitousek *et al.* 1996, 1997; Mooney & Hobbs 2000; Hulme *et al.* 2009). The International Convention on Biological Diversity contains a special provision calling upon country-signatories to eradicate or at least control invasive alien species which could be of danger to native habitats, communities or species. These circumstances have contributed to an evident increase in the interest in these issues among the theoreticians and practitioners in nature conservation.

Due to the extent of anthropogenic changes in plant cover, the monitoring of species of alien origin has acquired an ever-increasing importance in recent times. It is crucial to control quantitative changes in the population size of these species not only from the point of view of natural sciences, but also from economic as well as medical perspectives (Mack *et al.* 2000; Pimental 2002; Gadermaier *et al.* 2004; Tokarska-Guzik *et al.* 2009). However, it will not be possible to prevent their spread without knowledge of their biology and habitat requirements as well as their geographical range of distribution.

Among numerous invasive alien plants, of particular interest in many regions of the world, is Ambrosia artemisiifolia L. - Common ragweed - one of 40 species of the Asteraceae genus which has representatives originating both from the Americas and Africa (Willis 1973). Ragweed pollen is a strong allergen considered to be one of the most dangerous pollen allergens in the world (Comtois 1998; Jäger 2000; Bousquet et al. 2001). In spite of the species being recorded in some regions of Europe since the second half of 19th century, pollen of Ambrosia had hitherto not been considered a threat in Europe. Since the 1960s, this pollen has been recorded in the atmosphere of several European countries (Chłopek & Tokarska-Guzik 2006, and literature cited therein). Recently, A. artemisiifolia has been recognized as one of the most dangerous invasive alien species in many regions of the world, including southern and central Europe (e.g. Laaidi et al. 2003; Makra et al. 2004; Peternel et al. 2005, 2008; Testi et al. 2009), causing environmental, economic and medical problems. Common ragweed is also a weed in arable crops both in its natural and introduced range. Its seeds have been transported to Europe with imported seeds of cultivated plants and with ship ballast. Currently, most frequently, it is a contaminant of crop (cereals) seeds, grain, maize, sunflower and soybean seeds as well as clover, alfalfa and fodder materials.

All these are reasons for a growing interest in research in the fields of chemistry, allelopathy, cytogenetic, genetics, biology, ecology, palinology and different control methods. Only between 2001 to 2010, approx. 100 papers were published (author's own publications review).

Three species from the genus have hitherto been recorded from Poland: Ambrosia artemisiifolia L., Ambrosia psilostachya DC. = A. coronopifolia Torr. et Grey and Ambrosia trifida L. The first two taxa have the status of naturalized species in the country, while A. trifida L. is regarded as a sporadically introduced species (Zając et al. 1998; Mirek et al. 2002; Tokarska-Guzik 2001, 2005; Chłopek & Tokarska-Guzik 2006; Chłopek et al. 2008; Weryszko-Chmielewska & Piotrowska 2008, and literature cited therein). Although Ambrosia psilostachya and A. trifida are rare species on the territory of Poland, the monitoring of all species is required. Nevertheless the two latter species have more limited distribution compared with A. artemisiifolia, which has uniquely raised awareness as an invasive plant in Europe (DAISIE "100 of the Worst", http://www.europe-aliens.org/ speciesTheWorst.do; European and Mediterranean Plant Protection Organization (EPPO), http://www.eppo.org), occurring most frequently both in Europe and in Poland.

Although almost the entire area of Poland appears to be suitable for its establishment and spread (Karnowski 2001), common ragweed is not currently spreading on a large scale. New, isolated places of occurrence are sometimes found, mainly along communication routes and around warehouses and places of re-loading. One should assume, however, that this species could become very invasive in the future, as it already has in some other regions in Europe (Chłopek & Tokarska-Guzik 2006; Chłopek *et al.* 2008).

In view of the above assessment, the main ideas behind the present study were: (*i*) to investigate changes in distribution of *Ambrosia artemisiifolia* on a regional scale, (*ii*) to provide new evidence concerning selected aspects of its biology, important from the point of view of assessing the current threat caused by this plant species, (*iii*) to identify the status of the species on the basis of germination strength values, (*iv*) to estimate participation of *A. artemisiifolia* seeds in a soil seed bank and (*v*) to predict future trends of spread in Poland and to identify management options.

2. Material and methods

2.1. Distribution

Botanical recording was carried out on a regional scale in the Silesian Uplands and surrounding areas

during the growing seasons of years 2007-2009. The data were stored in the regional database ATPOL Silesia, which is fully compatible with the database of the Atlas of distribution of vascular plants in Poland (ATPOL). In ATPOL, the basic cartogram unit is a square 10 x 10 km; these are combined in "large" 100 x 100 km squares (Zając 1978). The area of the Silesian Uplands is limited to two ATPOL large squares designated CF and DF. Each ATPOL square of 10 x 10 km was subdivided into 25 smaller squares of a 2 x 2 km side, which are the basic units in ATPOL Silesia cartograms. Input sources for the database include original data, published papers and unpublished manuscripts as well as herbarium data. A map of the distribution of A. artemisiifolia in Poland was published in "Distribution Atlas of Vascular Plants in Poland" (Tokarska-Guzik 2001). The map shown here contains further additions.

2.2. Biology

A germination experiment was performed on a sample of 600 seeds gathered in September 2009 from a roadside population of *A. artemisiifolia*, located in Żory (R). 300 seeds were germinated without any treatment, while the other 300 were first stratified (4°C, 90 days) (Fig. 1). The collected seeds were placed on moist germination paper in Petri dishes. Germination was conducted for three weeks in conditions of 19°C and 14 hours light and recorded daily for 21 days. After finishing the experiment, the percentage of germinated seeds and the dynamics of germination (i.e. average time period needed for germination of one seed) were examined.

The dynamics of germination was calculated using Pieper's formula (Pieper 1952):

$$W = \frac{\Sigma(d \times pd)}{k}$$

W-germination rate (Pieper's coefficient), d-day of seed germination, pd – number of germinated seeds in each counting day, k – total number of germinated seeds

The seedlings obtained were grown further in a common environment in a glasshouse at the University of Silesia. Those plants were later used for the regeneration capability experiment (Fig. 1). Additionally, observations of seed germination were undertaken in the field (in two different habitat sites) during the Spring of 2010 (Fig. 1).

2.3. Ecology

Horizontal and spatial distribution of *Ambrosia* seeds – syconia (*A. artemisiifolia* produces so-called 'syconia' which consist of an achene and its associated woody coat) – was evaluated in two populations, present in different habitat types: a roadside population (R - Zory)

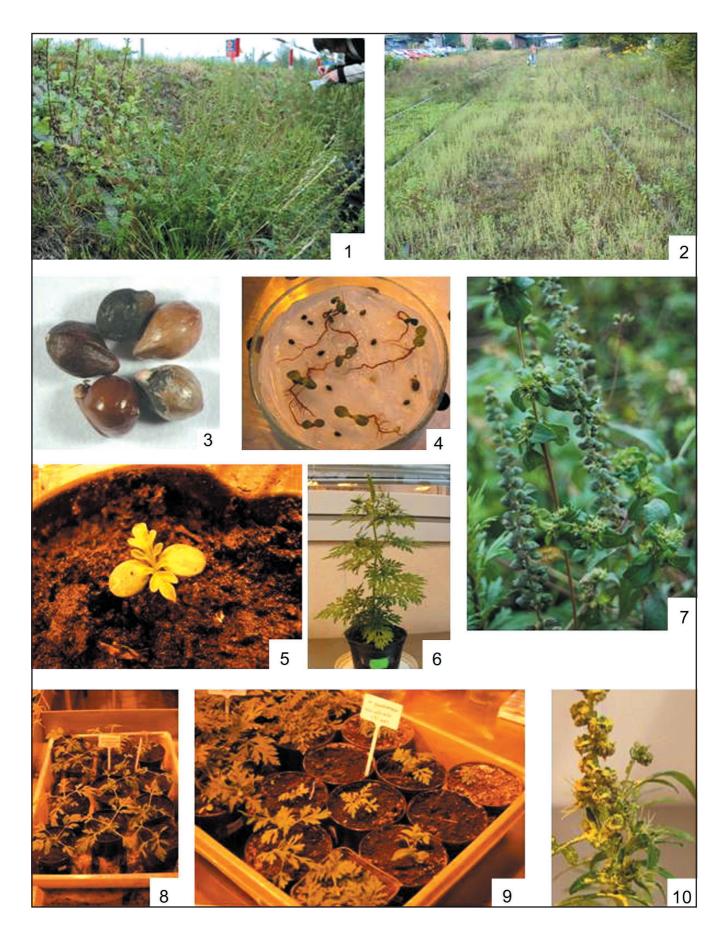


Fig. 1. *Ambrosia artemisiifolia* in the Silesian Upalnds: selected aspects of biology Explanations: 1 – the roadside populations (R) in Żory, 2 – the railway sidetrack population (S) in Tarnowskie Góry, 3 – seeds, 4 – germinating seeds, 5 – seedling, 6 – plant grown from a seed in laboratory conditions, 7 – inflorescences, 8 – plant cut in regeneration capability experiment, 9 – plants after two weeks, 10 – numerous pollen grains

and a railway sidetrack population (S - Tarnowskie Góry). The sampling method combined a subjective choice of Ambrosia stands with a haphazard placement of 27 sampling quadrats (1 m x 1 m) within one stand (in total 54). From each quadrat 20 soil cores (sized 10 x 10 x 5 cm) were sampled at two different depths. The upper soil layer (0-5 cm) corresponds to the active layer of germination (Munier-Jolain et al. 2002), while the lower one (5-10 cm) corresponds to the dormant seed bank which may be activated by soil disturbances (Radosevich et al. 1997; Fenner & Thompson 2005). During the experiment, 1 080 soil samples were collected and examined. Fieldwork on both sites was done during early spring (March 2010), before seed germination but after the natural stratification period. Seeds of Ambrosia were separated from the soil substrate using two sieves: the upper one with 2 mm mesh, employed to eliminate coarse particles and the lower one with 1 mm mesh, used to retain Ambrosia seeds, which were afterwards counted and utilized in the germination experiment.

2.4. Regeneration capability

An assessment of regeneration capability was applied to selected seedlings, obtained from the germi-

nation experiment. Chosen plants were uniform in size, with between six and eight leaves. Altogether 60 plants were used for the experiment: (i) - 30 obtained from seeds without any treatment and (ii) - 30 from stratified seeds. Plants were cut at different height: 10 beneath the first leaves, 10 above the first leaves and 10 above the second leaves in both series respectively. The appearance of new branches was recorded each week.

3. Results

The species is scattered all over lowland Poland (Fig. 2). At present, it occurs most often in south-western part of the country. Some of the isolated localities still retain their ephemeral character. On regional scale, *Ambrosia artemisiifolia* was recorded at several new localities, especially along roads (Figs. 3-4). The size of local populations at particular stations varied from a few to thousands of individuals. Only one locality known since the 1970s was confirmed during the field survey. A few of the earlier recorded stations were not confirm recently. This fact should be attributed to insufficient oldest data or their ephemeral character in the past.

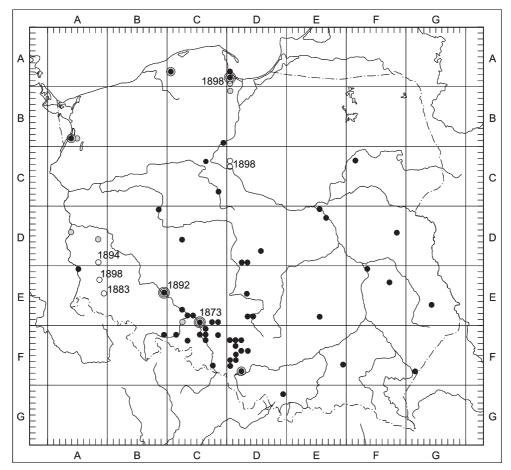


Fig. 2. Recorded history of the spread of *Ambrosia artemisiifolia* in Poland over the consecutive time periods Explanations: \bigcirc – between 1851 and 1900, \bigcirc – between 1901 and 1950, \bullet – between 1951 and 2009

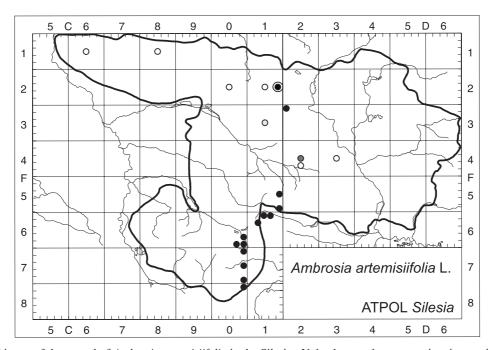


Fig. 3. Recorded history of the spread of *Ambrosia artemisiifolia* in the Silesian Uplands over the consecutive time periods Explanations: \circ – between 1970 and 1980, \circ – between 1981 and 1990, \bullet – between 1991 and 2000, \bullet – recently recorded localities – 2007-2010, \circ – confirmed localities

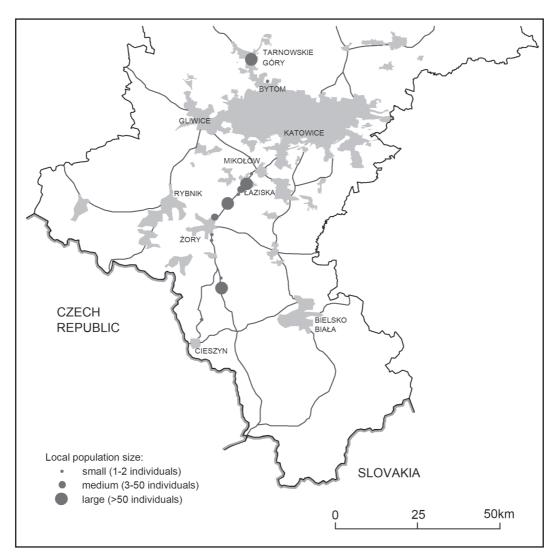


Fig. 4. Confirmed and recently recorded localities of Ambrosia artemisiifolia at a regional scale with a background of the road map and the distribution of built-up areas

Table 1. Germination results for a roadside population (R) of Ambrosia artemisiifolia from Żory

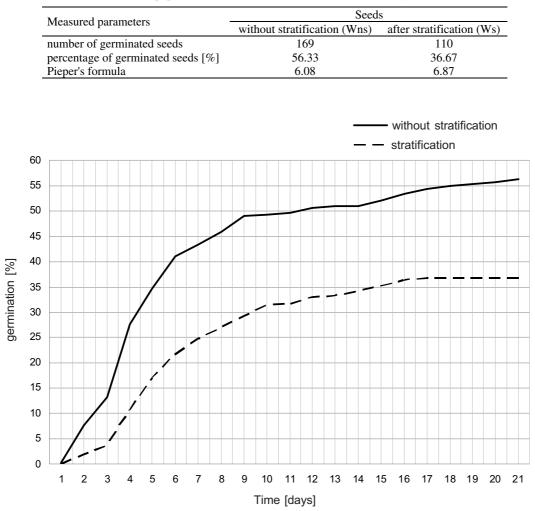


Fig. 5. Germination percentage of Ambrosia artemisiifolia seeds

Seed germination percentage exceeded 55%. Among seeds exposed to thermal stratification, this value was much lower (36%) (Table 1; Fig. 5). Average time for germination, assessed on the basis of number of seedlings germinating each day, was longer after stratification (Ws = 6.87), than without it (Wns = 6.08) (Table 1).

Seeds of *Ambrosia* started to germinate in the field in different time periods: in the railway sidetrack population (S – Tarnowskie Góry) at the end of March and in the roadside population (R – Żory) – in mid April. Seeds of *A. artemisiifolia* were present mainly in the topsoil (0-5 cm). The number of seeds present in the soil of the two populations was higher at Tarnowskie Góry (S). The total topsoil sample at Tarnowskie Góry stored 208 seeds (average number of seeds per sampling quadrat, i.e. per square metre was 7.7) and 16 seeds at Żory (R) (average number of seeds per sampling quadrat was 0.6), while the lower depth (5-10 cm) accumulated 3 seeds in the S population. No seeds were found at this depth in the R population (Table 2).

Table 2. The size of soil seed bank of Ambrosia artemisiifolia populations

Depth of sample [cm]	Number of seeds	Percentage [%]
0-5	208	33.65
5-10	3	33.33
0-5	16	25.00
5-10	0	0.00
	0-5 5-10 0-5	0-5 208 5-10 3 0-5 16

Explanations: S - railway sidetracks population (Tarnowskie Góry), R - roadside population (Żory)

	Cut beneath first leaves (10 plants)		Cut above first leaves (10 plants)		Cut above second leaves (10 plants)	
Treatment	No of regenerative plants	No of plants with flowering branches	No of regenerative plants	No of plants with flowering branches	No of regenerative plants	No of plants with flowering branches
without stratification	4	0	10	8	10	9
after stratification	8	0	10	6	10	8

 Table 3. Regeneration ability experiment

Regeneration capability was higher in plants cut above the first or second leaves (Table 3). After two weeks of growth, most new branches started to flower. There was not much difference between plants obtained from stratified seeds or seeds without stratification. The lowest regeneration capability was observed in plants obtained from seeds germinated without any treatment and cut beneath the first leaves. Only vegetative branches appeared during two weeks of observation.

4. Discussion

The results achieved during the present study provide some new, important evidence for the potential spread of *Ambrosia artemisiifolia* in Poland.

Since the first record of Ambrosia artemisiifolia in Poland, the rate of its spread, both at a regional and national scale, was rather low. In spite of this, the species is considered as one of the "oldest" arrivals among the neophytes (i.e. alien species arrived since the end of 1500) in Poland (Tokarska-Guzik 2005) and was mentioned as present in Poland in one of the oldest sources, the work by Syreński (Sirenius) from 1613 (Sir. Vol.III/Chapter 50) (Syreński 1613). The number of localities in Poland increased in the most recent 50 years (Tokarska-Guzik 2005) (Fig. 2). Detailed recording at a regional scale indicated that A. artemisiifolia had a great potential to spread from accidental introductions into built-up areas or on railway routes and along roads. Recently, a new station in the Silesian Uplands was recorded in 2006 in Zory town, along part of reconstructed road. After the first record of a new station in 2006, in the following years several new stations were found, mainly along the same main road, linking Katowice with Cieszyn, which is located on the country border with the Czech Republic. These new stations could have been a result of seed transport via vehicles, most probably from southern regions of Europe (the Czech Republic or Hungary), where A. artemisiifolia is considered as a common weed (Rybniček et al. 2000; Kazinczi et al. 2008a, 2008b, 2008c). Many authors concluded that linear corridors such as the network of major roads and railways facilitate the invasion of weedy alien species (e.g. Christen & Matlack 2006). In Austria, the invasion by A. artemisiifolia was strongly associated with railways and, since the 1970s, with roadside habitats, i.e. generally associated with human transportation activities (Christen & Matlack 2006; von der Lippe & Kowarik 2007). Records from roadsides increased strongly there and now predominate (Essl *et al.* 2009). This fact should result in constant monitoring of main roads which can play a role as transmitter corridors for the spread of *A. artemisiifolia* seeds.

Descriptions of the plant morphology and some aspects of its biology were published in numerous papers as early as in 1960s and 1970s (e.g. Payne 1963; Tacik 1971; Basset & Crompton 1975). There are no detailed data on the biology of *A. artemisiifolia* in Poland.

Research on the biology of flowering of *A. artemisiifolia* was undertaken by Weryszko-Chmielewska and Piotrowska (2008) under conditions of cultivation and some years earlier in the botanical garden of the Medical Academy in Poznań (Karnowski 2001, and literature cited therein). Plants of this species grew, flowered and fruited without any complication or special treatment. In the natural range, plants flower in August-September while in Central Europe inflorescences occur usually from August to October and mature seeds are produced in October (Poscher 1997). There is no vegetative propagation, so that all reproduction occurs through seed (Basset & Crompton 1975).

In monitored populations, the flowering period depends on local weather and habitat conditions. In the railway sidetrack population (S) monitored in the present study, during two consecutive growing seasons plants started to flower and terminated flowering earlier (10-14 days) than in the roadside population (R). Similarly, seeds of Ambrosia started to germinate in the field in different time periods: in the railway sidetrack population at the end of March and in the roadside population in mid April. Special conditions in the railway sidetrack habitat, such as a high insolation and dry soil, appeared to facilitate relatively rapid growth of plants. Because there was no agreement between botanists in Poland regarding the status of the species in the country, the question which needed to be addressed was connected with the ability of seeds to germinate in a newly established populations. Our results confirmed that Ambrosia artemisiifolia in local populations in the Silesian Uplands was well-established and produced viable seeds, with a strong capability for germination.

From recent research, it is known that a small ragweed plant can produce 3 000 seeds per year, whereas large plants produce up to 62 000 seeds, which can remain viable in the soil for more than 30 years (Gadermaier *et al.* 2004).

The seed of *A. artemisiifolia* is not adapted for dispersal by wind or animals (Basset & Crompton 1975); longdistance dispersal is mostly dependent on humans. Several pathways (contaminated crops and bird seed, agricultural machines, transport of soil) have been found to contribute to high levels of propagule pressure (Chauvel *et al.* 2006; Brandes & Nitzsche 2007; Vitalous & Karrer 2008) and successful invasion (Essl *et al.* 2009). The appearance of new stations along roads in the study area could also be connected with human activity, especially transport. After that, local spread was most probably attributable to other vectors, such as water (during heavy rains, floods). Mowing of roadsides also enhances spread, since significant numbers of seeds stick to mowing machines (Vitalous & Karrer 2008).

In the natural range and also in the introduced one the plant produces large numbers of dormant seeds, which are able to survive for up to 40 years in the soil. A portion of the seeds germinate in spring, while the rest of the seeds enter secondary dormancy (Fumanal et al. 2006). The same study reported that the total soil seed bank (recorded to a depth of 20 cm) ranged from 250 to 5 000 seeds/m². The top 5 cm of soil held more seeds (200 to 2 800 seeds/m²) than deeper layers. Large differences in seed density between sites were mostly explained by the type of habitat (differences in soil perturbation, competition, etc). The results of the present research confirmed the concentration of seeds in the topsoil, while the total soil seed bank as well as number of seeds per square meter were much lower than in other studies. This fact can be explained by the type of habitat examined. High concentrations of seeds were recorded mainly in arable habitats, where soil layers were disturbed by ploughing at depth of 20 cm (Fumanal et al. 2006). The present investigation also suggests that comparatively recent establishment of a population may also account for a relatively small soil seed bank. The topsoil (270 000 cc) at Tarnowskie Góry - a station known since 1970s – stored 208 seeds, while the topsoil at Zory – a recent station, recorded for the first time in 2007 - stored only 16 seeds. The Ambrosia artemisiifolia soil seed bank could be also influenced by local habitat conditions and by the type of management on a particular site. In the railway sidetrack habitat, the observed plants grew in dense patches but were shorter, probably because of cutting injuries from trains and herbicide treatment. The roadside population was located on a scarp with an angle of depression of 45°,

which affected seed dispersal, causing many to fall down to its base. This result was clearly seen where the most dense concentration of seedlings appeared during seed germination.

Common ragweed is able to adapt to mowing, trampling and grazing. The present experiment showed that after a week or two 52 of 60 plants cut above the first or second seedling node were growing quite normally, and 31 were starting to flower. Similar results were obtained in Canada (Karnowski 2001). The regeneration capability depends on the height of the cutting. Plants cut below the first node regenerate slowly. According to Vincent and Ahmim (1985) and Meiss et al. (2008, 2009), cutting of the A. artemisiifolia is only effective if plants are either cut very close to the ground surface or when flowering has already started. These facts have an implication for control options. Several cuttings, therefore, are likely to be required in early August to prevent flowering and seed development or (in small populations) the uprooting of the whole plants is recommended.

5. Conclusions

As global trade and travel increase every year, Ambrosia artemisiifolia, which is listed on the EPPO List of invasive alien plants for Europe, has more opportunities to hitchhike as a crop and birdseed contaminant, in ballast soil or in any other cargo. These circumstances, together with climate changes, may cause future spread of this species in Poland. The results reported in this paper suggest that detailed and comprehensive collections of floristic data are of considerable practical importance. They provide scientific background for the planning of nature protection-related activities as well as phytosanitary alerts on regional scale. The present investigation also suggests that a wide knowledge of the biology and ecology of a species and its adaptability to particular environmental conditions can also provide a background for developing methods for its effective control.

The strong influence of *Ambrosia* pollen on human health is among the most important reasons for systematic monitoring of the existing and newly appearing stations. Both traditional methods of monitoring its spread as well as new approaches, such as the use of genetics, modeling further spread and helping to identify a possible range edge, will play an increasingly important role in solving problems associated with invasions and in finding improved and novel ways to deal with them (Genton *et al.* 2005; Friedman & Barret 2008).

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