Bur-Chervil Anthriscus caucalis M. Bieb. (Apiaceae) - Potentially Invasive Species in **Forests**

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Puchalka, R., Rutkowski, L., Popa, M.-O., Pliszko, A. and Piwczyński, M. 2018. Bur-Chervil Anthriscus caucalis M. Bieb. (Apiaceae) - Potentially Invasive Species in Forests. Baltic Forestry 24(2): 189-200.

Abstract

In Poland, Anthriscus caucalis reaches the north-eastern limit of its secondary geographical range in Central Europe and is a declining archaeophyte in ruderal habitats. However, in recent years, new localities of A. caucalis were found in the north-western and western Poland in forest communities. A high number of individuals of A. caucalis in these localities suggests that it finds optimal growth conditions in these strongly eutrophic forests, exhibiting an invasive potential in this habitat. To compare the floristic composition and habitat conditions between ruderal and forest plant communities occupied by A. caucalis in Poland, a total of 23 relevés were collected. For each vegetation plot, median Ellenberg's indicator values (EIV) were estimated in order to evaluate the environmental conditions. The differences in EIV and floristic composition between the localities were analyzed using the Non-Metric Multi Dimensional Scaling (NMDS). In addition, we examined the ITS1-5.8S-ITS2 sequence variation for five individuals from four populations to analyze the association of particular genotype with ruderal or forest habitat. The results showed that the specific configuration of environmental factors, including moderate shade, mild and stable thermal and moisture conditions as well as high concentration of nutrients in the soil, is more important to A. caucalis occurrence than the floristic composition of the plant communities. Therefore, we hypothesize that the vanishing of A. caucalis in the ruderal habitats is a response to the currently observed increase of the extreme weather events. Likely, it finds more suitable and stable microclimate conditions in the forest areas. The results of the molecular analyses revealed two ribotypes which occurred in both types of habitats. The lack of correlation between genotype and habitat supports our conclusion from EIV analysis that the colonization of forests should be treated rather as a niche tracking than as an adaptive shift in habitat preference.

Keywords: alien species, Central Europe, Ellenberg's indicator values (EIV), ITS rDNA

Introduction

The invasion of alien plant species is a current problem concerning the floras around the globe (Richardson and Pyšek 2012, van Kleunen et al. 2015). Invasive plant species can spread rapidly, forming numerous and abundant populations that pose a threat to the native biodiversity in various habitats (Richardson et al. 2000, Richardson and Pyšek 2012). Forest areas in Europe are prone to alien plant invasions, especially when exposed to disturbance, fragmentation, alien propagule pressure and high soil nutrient levels (Wagner et al. 2017). A particular attention is drawn

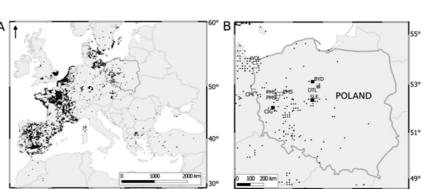
to neophytes, the species introduced after AD 1500, which are commonly distributed in various forest communities, e.g. Echinocystis lobata Torr. & A.Gray, Erechtites hieraciifolius Raf. ex DC. and Impatiens parviflora DC. (Tokarska-Guzik 2005a, b, Wagner et al. 2017). Interestingly, due to the fact that the predicted climate changes could accelerate plant invasions, some archaeophytes rare in Central Europe (species introduced between the Neolithic and the late Medieval Ages) should also be considered as potentially invasive (Jarošik et al. 2011).

Bur-chervil, Anthriscus caucalis M. Bieb. (Apiaceae), is an annual or biennial plant distributed in Western, South-

ern and Central Europe, South-Western Asia, and Northern Africa (Tutin et al. 1968, Reduron and Spalik 1995, Spalik 1997). It was introduced as a contaminant, herbal or ornamental species to Northern, Central and Eastern Europe, Eastern Asia, North America, South America, and Australia, becoming naturalized or invasive in many countries (Wallace and Prather 2016, Randall 2017 and literature therein). In Europe, within and outside of its native range, it is associated with nitrophilous synanthropic herb-rich communities of shaded woodlands and riparian fringes, Robinia-dominated shrubbery, and ruderal vegetation (Brandes 2007, Mucina 1997, Spalik 1997). Moreover, it occurs in arable fields as a weed of canola and cereals (Randall 2017 and literature therein).

In Poland, A. caucalis is an established alien species that belongs to archaeophytes (Mirek et al. 2002, Tokarska-Guzik et al. 2012). It is distributed mainly in the western part of the country (Koczwara 1960, Zając and Zając 2001) and occurs mostly in anthropogenic habitats, including ruderal grounds and arable fields. Many authors claim that A. caucalis is a vanishing and vulnerable species in Poland (Markowski and Buliński 2004, Zając et al. 2010, Jarzembowski et al. 2011, Tokarska-Guzik et al. 2012). However, some populations in the north-western Poland were observed in eutrophic forests (Piotrowska 1985, Kujawa-Pawlaczyk and Pawlaczyk 1999, Piwczyński et al. 2016). Also, recently discovered populations from the north-central part of the country occupied forests and thickets (Figure 1). The abundance, healthy appearance of the plants, and a high number of generative individuals indicate optimal growth conditions in these habitats.

Figure 1. Distribution of Anthriscus caucalis in Europe and adjacent territories (GBIF 2017, Tekin et al. 2017) (A) and in Poland (Zając and Zając 2001) (B): grey and open squares - localities of phytosociological releves; grey and black squares - populations sampled for molecular studies; black spots - general distribution of Bur-chervil; BYD - Bydgoszcz, CPK - Cedynia Landscape Park, CIG - Cigacice, OTL - Otłoczyn, PMS Międzychodzko-Sierakowskie Lakeland, SLE – Ślesin, WOL – Wolin



Anthriscus caucalis in Poland reaches the optimum in rather limited number of habitats. However, recent expansion into forests may indicate a significant niche shift which will potentially lead to the invasion. Therefore, in this study, we aim to quantify this shift by estimating differences between niches of A. caucalis in populations occurring in forests and those in open areas in Poland. We also examine if recent expansion is associated with the invasion of a new

genotype by analyzing the relationship between the genotypes of selected individuals sampled from different populations and habitats based on polymorphism of rDNA ITS (ITS1-5.8S-ITS2; hereinafter ITS) sequences.

Materials and Methods

Phytosociological data

A total of 23 phytosociological relevés (Table S1, see Appendix), in accordance with the Braun-Blanquet method, were used to compare the floristic composition and habitat conditions among the open and forest habitats, in which A. caucalis occurs in Poland (Table 1). Vegetation plots were divided into four groups based on their locations in four different regions of Poland and based on habitat type (Figure 1, Table 1). The first group included five relevés made in managed Robinia pseudoacacia-Pinus sylvestris forest near Otłoczyn (OTL) village. The second group included nine vegetation plots collected from the southern part of Cedynia Landscape Park (CPK) in the vicinity of Gozdowice and Bleszyn village. All relevés from CPK were made in forests and with R. pseudoacacia (Kujawa-Pawlaczyk and Pawlaczyk 1999). The third group comprised five vegetation plots made in alluvial elm forest on Wolin Island (WOL) in NW Poland (Piotrowska 1985). The first three groups were considered collectively as occupying forest habitats. The last, fourth group included four relevés made in weed vegetation in the vicinity of Głażewo, Zatom Stary and Chrzypsko Wielkie village in Międzychodzko-Sierakowskie Lakeland (PMS) (Wojterska 2003). The relevés from PMS and WOL were acquired from the Polish Vegetation Data-

base (Kącki and Śliwiński 2012). The limitation of study area to the north-western and north-central Poland resulted from the relevé availability.

For each species registered in the vegetation plots, Ellenberg's Indicator Values (EIV) (Ellenberg et al. 1992) were retrieved from the JUICE 7.0 software (Tichý 2002) and then the median and range values were calculated for each plot. The estimated variation in EIV within the vegeta-

Table 1. Characteristics of vegetation plots with *Anthriscus caucalis* used in the study

Name of locality (abbreviation)	Number of taken relevés	Relevé size [m²]	Number of species in relevés	Vegetation type	Geographical coordinates in decimal degrees [dd]	Collection date	Reference
Otłoczyn (OTL)	5	15	17-22	Robinia pseudoacacia- Pinus sylvestris forest	18.69 E, 52.91 N	June 2015	Own, unpubl. data
Cedynia Landscape Park (CPK)	9	100- 400	11-32	forests with Robinia pseudoacacia	14.32 E, 52.76 N	May-June 1999	Kujawa- Pawlaczyk, Pawlaczyk (1999)
Wolin (WOL)	5	100- 150	31-38	alluvial elm forest	14.43 E, 53.86 N	1-2 June 1977	Piotrowska (1985)
Miedzychodzko- Sierakowskie Lakeland (PMS)	4	15-50	14-22	non-woody weed vegetation	16.23 E, 52.63 N; 15.97 E, 52.63 N; 16.23 E, 52.63 N; 15.92 E, 52.533 N	16 June 1984; 16 July 1991; 16 June 1984; 20 June 1984	Wojterska (2003)

tion plots was compared to the original values assigned to *A. caucalis* (Ellenberg et al. 1992). The differences in EIV and floristic compositions between the localities were analyzed using *Non-Metric Multi Dimensional Scaling (NMDS)*. In the case of EIV, a Euclidean distance was used, whereas, the floristic composition was based on a Jaccard coefficient. The differences between four localities (groups of relevés) in medians of EIV were analyzed using *tie corrected Kruskal-Wallis* test and post-hoc *Mann-Withney U test*. Statistical analyses were performed using the R 3.4.2 (R Core Team 2015) and PAST (Hammer et al. 2001) software.

Molecular analyses

Five specimens of *A. caucalis* from four populations occurring in north-western and western Poland were used to examine their ITS sequence variation (Table 2). Fresh leaves of three specimens were sampled in the field and immediately preserved in silica gel. In the case of the two remaining specimens, herbarium material and seedlings derived from cultivation were used for DNA extraction (Table 2). N-nc18S10 and C26A primers were used to am-

Table 2. Five accessions of *Anthriscus caucalis* used for molecular analyses, with corresponding voucher information, the origin of material used for DNA extraction and GenBank reference numbers

Locality abbreviation	Voucher information	Plant material used for DNA extraction	Accession No.
CIG	Poland, Lubuskie Province, Cigacice village near Sulechów, weed vegetation, Lamińska s.n. 6 July 1973 (TRN)	Dried leaf from Herbarium specimen	MH102001
BYD	Poland, Bydgoszcz, thickets with <i>R.</i> pseudoacacia, Rutkowski s.n. 25 April 2016	Leaves preserved in silica gel	MH102000
OTL	Poland, Otłoczyn near Toruń, P. sylvestris-R. pseudoacacia forest, Piwczyński & Puchałka s.n. 21 April 2016	Leaves preserved in silica gel	MH101998
OTL	Poland, Otłoczyn near Toruń, P. sylvestris-R.pseudoacacia, Piwczyński & Puchałka s.n. 21 April 2016	Leaves preserved in silica gel	MH101999
SLE	Poland, Ślesin near Konin, euthrophic urban pine forest <i>Piwczyński & Ostrowski s.n.</i> 17 June 2017	Fresh seedling derived from cultivation	MH102002

plify the ITS region (Wen and Zimmer 1996). Details of the DNA extraction, PCR amplification and sequencing are provided elsewhere (Piwczyński et al. 2015). BLAST searches were performed against the GenBank database at NCBI for each new DNA sequence to determine its similarity with deposited sequences. Since there was no full ITS sequence available in GenBank at the time of writing of this manuscript, we compared the ITS1 and ITS2 separately. Additionally, we retrieved from GenBank eight sequences (ITS1+ITS2) belonging to seven species from the genus Anthriscus and two species from other closely related genera, Myrrhis odorata (L.) Scop. (ITS) and Geocaryum macrocarpum (Boiss. & Spruner) Engstrand (ITS1+ITS2). We aligned all sequences using MUSCLE (Edgar 2004), through the graphical interface in Seaview 4.4.0 (Gouy et al. 2010). The phylogenetic analysis was performed using maximum likelihood (ML) implemented in Phyml version 20120412 (Guindon and Gascuel 2003). The substitution model was selected using jModelTest 2.1.6 software and the Akaike information criterion (Darriba et al. 2012). Node support values for the best ML tree were assessed using bootstrap analysis with 1000 replicates. The plant material used for the molecular studies was deposited in Herbarium of Department of Ecology and Biogeography in Nicolaus Copernicus University.

Results

Analysis of EIV for vegetation plots

In the majority of relevés (87%), the median value of the light indicator (L) ranged from seven to eight (Figure 2, Table 3) suggesting semi-light conditions, whereas median value of the moisture indicator (M) ranged from five to six in 82.6% of the relevés indicating fresh conditions. Similar range of values, from seven to eight in 69.6% of relevés, was also found for the soil reaction (R) indicating moderately acidic to neutral conditions. The widest range of the median value (from five to nine) was revealed for the nutrient indicator (N) suggesting very fertile and fertile conditions in 39.1% and 30.4% of relevés, respectively (Figure 2,

Table 3). The temperature (T) indicator, by contrast, equalled six for all relevés.

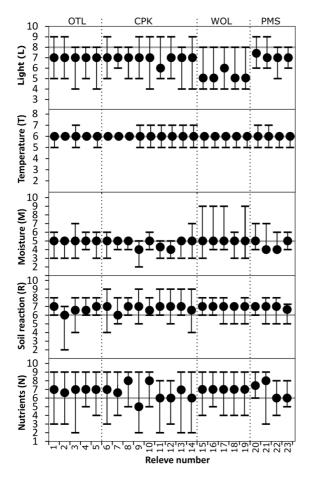


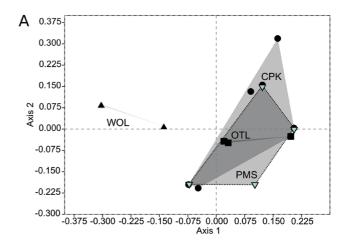
Figure 2. Analysis of Ellenberg's indicator values (EIV) for vegetation plots: black spots – median value, whiskers – minimal and maximal values, grey horizontal lines – original values (Ellenberg et al. 1992), OTL – Otłoczyn, CPK – Cedynia Landscape Park, WOL – Wolin, PMS - Międzychodzko-Sierakowskie Lakeland

Table 3. The percentage of relevés in median classes of Ellenberg's Indicator Values (EIV). L: 6-7 – partial shade to semi-light; 7-8 – half-light, 8-9 – half-light to full light; T: 6-7 – temperate; M: 4-5 – dry to fresh; 5-6 – fresh; R: 5-6 – moderately acidic; 6-7 – moderately to weak acidic; 7-8 – moderately acidic to neutral; N: 5-6 – moderate; 6-7 – average; 7-8 – fertile; 8-9 – very fertile

Median value of	Percentage of vegetation plots in median classes of EIV													
EIV	4-5	5-6	6-7	7-8	8-9									
Light (L)	-	-	8.7	87.0	4.3									
Temperature (T)	-	-	100	-	-									
Moisture (M)	17.4	82.6	-	-	-									
Soil reaction (R)	-	4.3	26.1	69.6	-									
Nutrients (N)	-	8.7	21.7	30.4	39.1									

Differences in EIV and floristic composition between the vegetation plots

Non-metric Multidimensional Scaling of vegetation plots showed good segregation along the first two axes for floristic composition while poor for the EIV median values (Figure 3). The range of variation of the median values of EIV in the case of vegetation plots made in OTL was almost fully included within the range of vegetation plots made in PMS and partially within the range of plots made in CPK (Figure 3A). These three sets of vegetation plots were strongly overlapping, whereas the vegetation plots made in WOL represented a separate group (Figure 3A). The tie corrected Kruskal-Wallis test (Table 4) showed statistically significant difference in the median values of EIV among sites only in L (χ =19.59, p=0.0002). According to post-hoc Mann-Withney U test (Table 5), this difference was due to



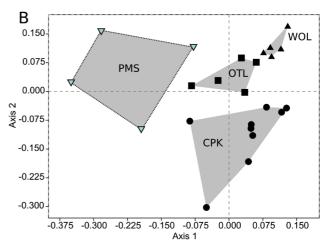


Figure 3. Non–Metric Multidimensional Scaling of mean EIV values (A) and floristic composition (B) of the vegetation plots: inverted triangles connected with spotted line – non-woody ruderal vegetation; black triangles, circles and squares – forest communities, OTL – Otłoczyn, CPK – Cedynia Landscape Park, WOL – Wolin, PMS – Międzychodzko–Sierakowskie Lakeland. The lines indicate convex hulls

WOL, which was characterized by lower median values of L. The analysis of floristic composition revealed that the range of variation in floristic composition of the vegetation plots between the studied sites did not overlap, showing four separate clusters (Figure 3B).

Table 4. The tie corrected Kruskal-Wallis tests for medians of Ellenberg's Indicator Values (EIV) for vegetation plots between four study localities. NAN - not analyzed (all samples have the same value); statistically significant values are

χ²	p-value
19.59	0.0002
NAN	NAN
3.62	0.3053
4.22	0.2385
1.24	0.7434
	19.59 NAN 3.62 4.22

Table 5. Post-hoc Mann-Withney U test for Light (L) median values for study localities (groups of relevés). Statistically significant values are bolded

p-value\test U	OTL	CPK	WOL	PMS
OTL		0.0	0.0	7.5
CPK	1.000		0.0	13.5
WOL	0.006	0.001		0.0
PMS	0.371	0.182	0.013	

Molecular analysis

There were two ITS ribotypes among the five individuals sequenced in this study, which differed by three substitutions in the ITS1 region. The first ribotype was found in two individuals inhabiting thickets and open forest habitats (ribotype 1, AH006067, MH102000, MH102002), while the second one was identified in three individuals also derived from two types of habitats (ribotype 2, MH102001, MH101998, MH101999). There were only three sequences of A. caucalis in GenBank which contained various parts of

ITS. Two of them contained the 5.8S-ITS2 part of ITS (KX167857, KX166739), whereas one accession contained ITS1 and ITS2 (AH006067). The ITS1 regions of ribotype 1 and ribotype 2 were 100% and 99%, respectively, identical to accession AH006067 of A. caucalis. In the case of ITS2 region, both ribotypes were 100% identical to three accessions of A. caucalis deposited in GenBank, namely KX167857, KX166739, and AH006067. According to BLAST, the most similar sequences after A. caucalis accessions belonged to A. sylvestris subsp. sylvestris (AH008912) and were 94% and 91% identical to the ITS1 and ITS2 regions of the obtained sequences. jModelTest with the Akaike information criterion selected the SYM + G model. The ML analysis showed that all accessions of A. caucalis were placed in one clade with maximal bootstrap support in which two clusters were present corresponding to ribotype 1 (haplotype I) and 2 (haplotype II) (Figure 4).

Discussion

Ecological niches of Anthriscus caucalis differ only marginally among ruderal and forest habitats

Although A. caucalis is a species associated typically with the moderately shaded forests and their fringes on light sandy soils (Mucina 1997, Pimenov and Ostroumova 2012), it has been recorded mainly from the ruderal and segetal habitats in Poland (Jarzembowski et al. 2011). The sites of this suboceanic species (Elleberg et al. 1992) in Poland are located at the eastern verge of its geographical range (Zajac and Zajac 2001). Hence, its further spread to the east is likely to be limited by the strong influence of the continental climate in this part of Europe (Woś 1999). On the other hand, the observed disappearance of this species in ruderal areas of Poland (Zając et al. 2010, Jarzembowski et al. 2011, Tokarska-Guzik et al. 2012) may be associated

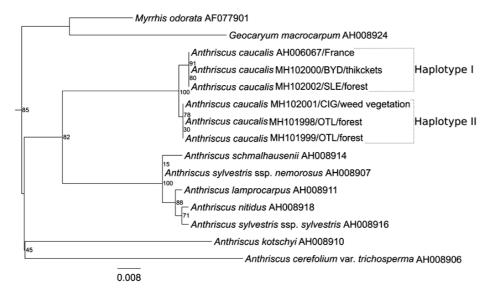


Figure 4. The best maximum likelihood tree inferred from the ITS sequences of genus Anthriscus including the selected populations of Anthriscus caucalis from Poland and outgroups. Branch lengths were estimated using the SYM + G substitution model

with the progressing urbanization causing their transformation and loss of habitats. In addition, extreme thermal conditions and summer droughts in urban areas, which have intensified in recent years as a result of the climate change, may also contribute to the decline of A. caucalis in the urban areas (Skelhorn et al. 2014, Harris et al. 2014). However, the ruderal sites and railways close to which there are populations found in the Polish forests may be places of introduction and start of invasion in the adjacent areas. This can be confirmed by the records of A. caucalis in the communities with R. pseudoacacia at railway stations in eastern Germany (Brandes and Braunschweig 2004, Brandes 2007). Interestingly, all analyzed vegetation plots from Poland, except PMS and WOL, were made in forest communities with a noticeable share of R. pseudoacacia (Table 1 and S1), while spreading of the Bur-chervil under the Black locust tree stands were observed also in other areas of central Europe (Vítková and Kolbek 2010, Krumm and Vítková 2016, Vitková et al. 2016). According to our own field observations, A. caucalis most likely avoids light deficiency by early phenology. Its life cycle ends immediately after the development of leaves by R. pseudoacacia. Considering the original EIV values for A. caucalis (Ellenberg et al. 1992), the studied sites in Poland showed lower values of L indicator (Figure 2). The results of EIV analysis show that this species can also occupy more shady riparian forests as exemplified by WOL populations (Figure 2) and that its distribution in Poland might be strongly limited by thermal, climate, precipitation, and moisture factors. For example, the median value for the temperature indicator (T) was the same for all vegetation plots and equaled to the value proposed by Ellenberg et al. (1992). Similarly, the majority of vegetation plots showed the same value of the moisture indicator (M) as proposed by Ellenberg et al. (1992). It should be pointed out that A. caucalis occurs on the light sandy soils, which are characterized by high values of the soil reaction (R) and nutrient (N) indicators (Figure 2). Such specific configuration of the edaphic factors is found in places dominated by the intensively spreading in Europe R. pseudoacacia, which by atmospheric nitrogen fixation, contributes to soil fertility and changes soil pH (Vítková and Kolbek 2010, Piwczyński et al. 2016). The analysis of EIV suggests that Bur-chervil has a narrow ecological niche (Figure 2). The results of NMDS indicate that for A. caucalis, the habitat parameters (which are similar in presented sites, except WOL) are more important than the floristic composition which is different in all four groups of sites (Figure 3). The results of Kruskal-Wallis test (Table 4) and Mann-Whitney U test (Table 5) indicate that any statistically significant differences in EIV were only noted for the L indicator. In this case, only WOL differed from all other forest and non-forest sites (OTL, CPK and PMS). Therefore, we conclude that the observed spread of the species in the forest areas with R. pseudoacacia is a niche tracking rather than

the niche shift as the differences in the environmental conditions among them and the open regions are insignificant. On the other hand, the documented occurrence of this species in the more shaded alluvial elm forests in WOL indicates that the species is also able to inhabit habitats with significantly lower L indicator values than those indicated by the original L indicator value than it would emerge from the original value of the indicator (Ellenberg et al. 1992). This suggests that in the future this species may become a permanent component of the herbaceous layer of various types of Polish forests.

The lack of association between genotype and habitat in Anthriscus caucalis

Many recent studies indicate that numerous invasions are associated with multiple introductions and subsequent mixing (Bock et al. 2015). Bur-chervil was introduced to Poland, likely from Western Europe, between the Neolithic and the late Medieval Ages. However, the origin and exact time of its arrival remain unknown (Jarzembowski et al. 2011, Tokarska-Guzik et al. 2012, Zając and Zając 2014). The fact that Bur-chervil was recorded mainly from the ruderal habitats in Poland suggests that the spread of this species was caused by human activities, most likely by unintentional, independent and numerous introductions. This is corroborated by our results. We found two ITS sequences which is a high number as this marker is usually conserved at intraspecific level and at such small geographic scale. For example, the genetic analysis of wild parsnip (Pastinaca sativa L.), an invasive species in Nearctic and Australasian regions, showed that 79% of the analyzed individuals sampled from North America, New Zealand and Europe shared one ribotype (Jogesh et al. 2015). All other observed ITS variants were unique to no more than 5% of individuals. In this study, on the other hand, the unique ribotypes were almost equally divided between the sampled individuals. This result may indicate a high genetic variation of A. caucalis in Poland generated by multiple immigration events and subsequent intraspecific genetic admixture. This variation may trigger future adaptation and geographic spread by preventing genetic bottlenecks and generating genetic novelties through recombination (Lavergne and Molofsky 2007). However, as results from the ecological analyses suggested, the shift from open to forest habitats has not required significant adaptive change. The lack of association of particular genotype with ruderal or forest habitats also confirmed this scenario.

Although the sampling and marker used for the genetic analysis do not allow for detailed study of migration paths of *A. caucalis* in Poland, some interesting patterns are noticeable. For example, the newly discovered population in Otłoczyn shared an identical ribotype with the geographically distant population from Cigacice (W Poland) (Figure 4), while it had a distinct ribotype from the indi-

vidual sampled from the closely located population in Bydgoszcz. It is possible that the populations from Bydgoszcz and Otłoczyn originated due to the introduction by railway transport (both populations are located near railway tracks). The importance of railway tracks in the long-distance dispersal of plant species was documented in many studies in central Europe (e.g. Pyšek et al. 1998, Tikka et al. 2001, Jehlík et al. 2013, Puchałka et al. 2015). The role of the railway tracks for spreading and establishing plant communities with *A. caucalis* was confirmed in Germany (Brandes and Braunschweig 2004, Brandes 2005, 2007).

Acknowledgments

We would like to thank Zygmunt Kącki and Grzegorz Swacha for giving us access to the phytosociological relevés with Anthriscus caucalis deposited in the Polish Vegetation Database. The study was supported by institutional research funding of the Chair of Ecology and Biogeography of the Nicolaus Copernicus University.

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Appendix

Table S1. Relevés with Anthriscus caucalis used for calculation of Ellenberg's Indicator Values (EIV) and differences in species composition between four study sites (Figure 2 and 3)

Locality			OTL				СРК								WOL							PMS			
Source		OV	vn, unpi	ıbl.			Pawlaczyk and Kujawa-Pawlaczyk (1999)								Piotrowska (1985)							Wojterska (2003)			
Vegetation type				oacacia- is forest			Forest with Robinia pseudacacia									Allu	vial elm f	orest		Non-	woody w	eed veg	etation		
Relevé number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
No. table in publ.															1	1	1	1	1	63	63	68	68		
No. relevé in table						161	162	163	175	176	177	178	188	204	4	5	6	12	17	2	13	6	11		
Date (d/m/y)	0.6	0.6	0.6	0.6	0.6	08.05.	08.05.	08.05.	03.06.	03.06.	03.06.	03.06.	05.06.	09.07.	01.06.	01.06.	01.06.	02.06.	01.06.	16.06.	16.06.	16.06.	20.06.		
	2015 15	2015 12	2015 15	2015 15	2015 15	1999 100	1999 100	1999 100	1999 200	1999 100	1999 400	1999 400	1999 400	1999 400	1977 100	1977 150	1977 150	1977 120	1977 150	1984 15	1984 20	1984 20	1984 50		
Relevé area (m²)	0	0	0	0	0	SW	SW	SW	S	S	E	E	E	E	SWW	SSW	SWW	SSE	SW	0	0	0	0		
Aspect	0	0	0	0	0	30	30	30	30	20	10	10	10	0	50	50	50	35	40	0	0	0	0		
Slope (degrees)	52.91	52.91	52.91	52.91	52.91	52.76	52.76	52.76	52.76	52.76	52.76	52.76	52.76	52.76	53.87	53.87	53.87	53.87	53.87	52.63	52.63	53.63	52.53		
Latitude	18.69	18.69	18.69	18.69	18.69	14.32	14.32	14.32	14.32	14.32	14.33	14.33	14.33	14.33	14.33	14.33	14.33	14.33	14.33	16.23	15.97	16.23	15.92		
Longitude	40	70	80	60	80	40	70	70	40	70	70	80	70	60	0	50	50	75	50	0	0	0	0		
Cover tree layer (%)	1	5	1	5	1	50	30	60	30	30	30	20	5	70	80	60	50	20	40	0	0	0	0		
Cover shrub layer (%) Cover herb layer (%)	85	60	65	60	60	90	80	80	90	90	30	80	80	70	90	95	80	95	95	100	70	60	100		
Trees and shrubs																									
Acer platanoides s1				1										٠.											
Acer platanoides hl	+	+		+		+		*																	
Corylus avellana hl					,													+							
Crataegus monogyna t2																3	1								
Crataegus monogyna s1														. '	3		3	1	2	٠.					
Crataegus monogyna s2															1	1	2								
Crataegus monogyna hl																+	+								
Crataegus sp. hl												+			.,										
Euonymus verrucosa s1								2							+	1									
Euonymus verrucosa s2									<u>,</u> .							+	+	+	+						
Euonymus verrucosa hl															+		+		+						
Fraxinus excelsior t1								1																	
Fraxinus excelsior s1								1																	
Hedera helix t1		. *						*								1	+								
Hedera helix s1		•													1	1		+	1						
Hedera helix s2															+	+	+								
Hedera helix hl							•			•					1	+	+		+						
Ligustrum vulgare s2															+	+	+								
Ligustrum vulgare hl Lycium barbarum s1						3	1											100							
Lycium barbarumhl						+	1						+												
Malus domestica hl	+																					4			
Padus serotina s1		1	+		+																				
Pinus sylvestris t1	3	4	4	3	3				1																
Prunus spinosa s1														1											
Prunus spinosa s2												1				+	+	+							
Prunus spinosa hl															+		+	+	+						
Pyrus pyraster t1																	3								
Pyrus pyraster s2															+		2			***					
Pyrus pyraster hl										•	•	+			+	+	+					7			
Quercus rubra hl												+	*			*									
Rhamnus cathartica s1								*							2	2	1	2				1			
Rhamnus cathartica s2															+	1	2	+	+						
Rhamnus cathartica hl	*														+			+							
Ribes rubrum hl								*								-						1			
Ribes spicatum s2	,														+		+	:	+						
Ribes spicatum hl															+		+			•					
Robinia pseudoacacia t1						3	4	3	3	1	4	5	4	4						•					
Robinia pseudoacacia t2	3	3	4	3	5		3				3	2	4	3											
Robinia pseudoacacia s1	+	*			+	1	3		,					+											
Robinia pseudoacacia hl		+			Τ,										1										
Rosa canina s1															+	+	+								
Rosa canina s2															+	+	+								
Rosa canina hI				•											1										
Rosa dumalis hl									3																
Rosa glauca s1						1		1		2				2		2	+		+						
Sambucus nigra s1										-				-		2	+	+	2						
Sambucus nigra s2 Sambucus nigra hl																+	+	+							
Syringa vulgaris hl								1																	
Ulmus glabra t1																1	1	4							
Ulmus glabra s1															1										
Ulmus glabra s2							٠.								+	+		1							

BALTIC FORESTRY

BUR-CHERVIL ANTHRISCUS CAUCALIS /.../ - POTENTIALLY INVASIVE SPECIES IN FORESTS R. PUCHAŁKA ET AL.

Table S1. (Continued)

Ligustrum vulgare s2								-							+								
Ligustrum vulgare hl								-							+	+	+						
Lycium barbarum s1						3	1																
	-					+	1						+										
Lycium barbarumhl										-													
Malus domestica hl	+																	•	•				
Padus serotina s1		1	+		+			*											•				
Pinus sylvestris t1	3	4	4	3	3				1														4
Prunus spinosa s1														1									
																+	+	+					
Prunus spinosa s2															+		+	+	+				
Prunus spinosa hl																	2						
Pyrus pyraster t1																	3						
Pyrus pyraster s2															+		2						
Pyrus pyraster hl												+			+	+	+						
												+											
Quercus rubra hl															2	2	1	2					
Rhamnus cathartica s1															+	1	2	+	1				
Rhamnus cathartica s2															-		2	7	-				
Rhamnus cathartica hl															+			+					
Ribes rubrum hl																						1	
Ribes spicatum s2															+		+		+				
															+		+						
Ribes spicatum hl				,			4	3	3	1	4	5	4	4									
Robinia pseudoacacia t1						3	4	3	3		*	5	**	7									
Robinia pseudoacacia t2	3	3	4	3	5							*				•							,
Robinia pseudoacacia s1	+	+			+	1	3		1	1	3	2	1	3		2							
Robinia pseudoacacia hl		+			+					+	+	+		+									
															1								
Rosa canina s1							-								+	+	+						
Rosa canina s2															_	_		*				-	-
Rosa canina hl									•						+	+	+						
Rosa dumalis hl	+																						
Rosa glauca s1									3	4			*										
						1		1		2				2		2	+		+				
Sambucus nigra s1																2	+	+	2				
Sambucus nigra s2													•			+	+	_					
Sambucus nigra hl				*																			
Syringa vulgaris hl				-		*		1															
Ulmus glabra t1																1	1	4					
Ulmus glabra s1															1								
															+	+		1					
Ulmus glabra s2																							
Ulmus glabra hl										4													
Ulmus laevis t1										4													
Ulmus laevis s1								1											- 1				
Ulmus minor t1															-	1	1		4				
Ulmus minor s1						1		3											2				*
Ulmus minor s2															*	+	+		1				
						+	+	+	+										+				
Ulmus minor hl																							
Herbs																							
Achillea millefolium														-							-		
Adoxa moschatellina																		+					
Agrostis capillaris			+											+									
Alliaria petiolata	1	1	1	1	1									+	2	2	2	1	2				
															+			+	1	1			
Allium oleraceum													-									+	
Anagallis arvensis	•																					2	
Anchusa arvensis									-													-	
Anchusa officinalis									+														
Anthriscus caucalis	3	3	4	4	4	2	3	2	+	1	+	+	1	+	1	+	+	+	+	3	r	+	1
Anthriscus sylvestris									+							+		2	+				
•																				. "	2	1	1
Apera spica-venti									•											1			
Arctium lappa																·				1			
Arrhenatherum elatius			+									+	+										
Artemisia absinthium																					+		
Artemisia campestris									+														
Artemisia vulgaris	+					+								+						+		1	
											+												
Asparagus officinalis							4					•	-	1	+	1	1	1	1		3		
Ballota nigra						•	1		*				7	1	-	1	1	'			J		
Berteroa incana	+	+												+					•				
Brassica napus																							5
Bromus hordaceus																					+		
				2					3			1				+	1		1				
Bromus inermis	T			2						4		4	5	1				1					+
Bromus sterilis			: 1			4	4	3	3			1	J	'				'					-
Bromus tectorum			+		1													•					
Bryonia alba										•		2		+		+							
Bryonia dioica						1	+																
Calamagrostis epigejos		20.							1		4			2							*		
Campanula rapunculoides															2		+						
varriuariula rabunculoides																							

BALTIC FORESTRY

BUR-CHERVIL ANTHRISCUS CAUCALIS /.../ - POTENTIALLY INVASIVE SPECIES IN FORESTS R. PUCHAŁKA ET AL.

Table S1. (Continued)

Capsella bursa-pastoris	+	+																				1	1
Centaurea cyanus						*					i											1	+
Centaurea stoebe									+									1.0					
Cerastium holosteoides						+																	
Chaenorhinum minus																						+	
Chaerophyllum temulum						2		+		3			1		2	1	+	2	2				
Chamomilla suaveolens																					1		
Chelidonium majus	+		+	2	+	1	+	1		1				3		2	+	2	+				
Chenopodium album															100							+	
Cirsium arvense																				1			
Convallaria majalis		1															i.						
Convolvulus arvensis									+					+						1			
Conyza canadensis									+					+								+	
Cynoglossum officinale									+							+							
Dactylis glomerata	1	1	·	Ċ	1		Ċ			·				•	i	i	1	2		•			
							•											2				2	
Daucus carota Deschampsia flexuosa		+																				2	
		·							•			,											
Descurainia sophia				•								,									т.		
Echium vulgare	2								7														
Elymus repens	2		-		*							т								2		1	+
Erigeron acris	,						•	•		•	Τ.									-			
Erodium cicutarium											•		•								ı.		
Euphorbia cyparissias				•					+			+		*									
Euphorbia esula	+						•				•									•		,	
Falcaria vulgaris				•				*			*		+	1									
Fallopia convolvulus													• ,	**								1	
Fallopia dumetorum	*																+						
Festuca heterophylla												+			. 7			•					
Festuca rubra	2		1	+								1			•								
Festuca trachyphylla			+											1									
Ficaria verna															4	4	3	2	5				
Fragaria vesca			+		2																		
Fumaria officinalis	. "																					2	
Gagea lutea		1																1	1				
Galanthus nivalis	4.																	+		.,			
Galeopsis speciosa								. 1						+									
Galeopsis tetrahit															r								
Galinsoga parviflora														+							+		4
Galium aparine		1	1	2	2	1	1	1		1			1		2	1	+	1.	+	1			+
Geranium lucidum											1	4											
Geranium molle							1							+									
Geranium pusillum			+						+								. 1				1		+
Geranium robertianum									3					+	+	+			+				
Geum urbanum					+					+			+		+	+	+	+	+		4		
Glechoma hederacea										1													
Heracleum sibiricum	+																		1	+			
Hieracium murorum		4	+																				
Impatiens parviflora														1		4							
Lactuca serriola									+					+									
Lamium album						+		1	+					1									
Lamium purpureum					+										1	1	1	1	1				+
Lapsana communis															+	1	+	2	1				
Leonurus cardiaca	+					+	+	1		+			1								2		
Lithospermum arvense									+													+	
Malva neglecta																					+		
Marrubium vulgare																					2		
Matricaria maritima ssp. inodora																					+		1
Melandrium album						+	+											r		+			
Moehringia trinervia															+	+		+					
Oenothera biennis									+														
										+										+			
Onopordum acanthium Omithogalum umbellatum																		1					
						+																	
Papaver argemone							+	·							7								
Papaver dubium									+	•													+
Papaver rhoeas									2														
Phleum phleoides									2								•			•	3		
Poa annua												4								•	J		•
Poa compressa		٠				1			-		+	1							•	•			
Poa nemoralis				i					•			•						2		•			
Poa palustris								•							+	+	2		+				
Poa pratensis				٠				•	+	•	1	+	+							,			•
Poa sp.						*												+					

BALTIC FORESTRY

BUR-CHERVIL ANTHRISCUS CAUCALIS /.../ - POTENTIALLY INVASIVE SPECIES IN FORESTS R. PUCHAŁKA ET AL.

Table S1. (Continued)

Poa trivialis																					1		
Polygonum aviculare										;												2	
Ranunculus bulbosus	+						·	•						4				0.8					
Ranunculus repens		4							h.		4					•				r			
Rubus caesius			+																	+			
Rubus saxatilis					+											,							
Rubus sp.		+																					
Rumex acetosa											+			+									
Saponaria officinalis							+		2														
Secale cereale															+		+						
Sedum maximum										•				1			$i \propto$						
Senecio vernalis						+			+								r	r					
Setaria viridis																						+	4
Sinapis arvensis							•					+											
Solidago gigantea				1																			
Stachys recta								8	2	1													
Stachys sylvatica																+			+				
Stellaria media		3	2		1			1			+		+		3	4	4	2	4	2			2
Taraxacum officinale	+	+	+	+	1	+	+	+					+		+	+	+	+					
Thlaspi arvense	٠.																	٠.					+
Trifolium pratense										1						r						*	
Triticum aestivum																-							1
Urtica dioica	+													+		+		1		4	1		
Urtica urens								*						*		. "				7	+		
Valerianella locusta								1	1														
Verbascum densiflorum									1	•													
Veronica hederifolia	1	1		+		1		+							2	2	2	2	2			+	
Veronica polita		4					4			1					.7		2			1		+	
Veronica triphyllos									+														
Vicia tenuifolia													+										
Vicia tetrasperma																	r		r				
Viola arvensis			+		1																	1	1
Viola odorata										1			*		1	+	+	2	1				
Viola tricolor												1					,						

Explanations: OTL – Otłoczyn; CPK – Cedynia Landscape Park; WOL – Wolin, PMS - Międzychodzko-Sierakowskie Lakeland; hl – herb layer; s1 – higher shrub layer; s2 – lower; t1 – higher tree layer; t2 – lower tree layer; 'r, +,1 , 2, 3, 4, 5' – Braun-Blanquet cover scale.