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The twinflower in the Augustów Forest: occurrence, condition and threats

Dorota Zawadzka[1]*, Grzegorz Zawadzki[2], Jerzy Bednarek[3], Joanna B. Bednarek[3], Dorota Piechowska[4], Artur Mikitiuk[5]

[1]University of Łódź, Branch in Tomaszów Mazowiecki, Institute of Forest Sciences, ul. Konstytucji 3 Maja 65/67, 97–200 Tomaszów Mazowiecki, Poland; [2]Okółek 14, 16–506 Giby, Poland; [3]Wigry National Park, Krzywe 82, 16–402 Suwałki, Poland; [4]Płaska Forest District, Sucha Rzeczka 60, 16–326 Płaska, Poland; [5]The Cardinal Wyszyński University in Warsaw, Faculty of Mathematics and Natural Sciences, ul.Wóycickiego1/3, 01–938 Warszawa, Poland

Tel. +48 44 7252905, fax. +48 44 7249720, e-mail: dorota_zaw@wp.pl

Abstract. The twinflower *Linnaea borealis* L. is a rare, boreal plant species that grows in coniferous forests and is partly protected in Poland. We studied its distribution and the condition in the Augustów Forest (NE Poland) describing 22 twinflower patches in total. The following features were assessed: (1) patch area, (2) distance between individual twinflowers, (3) patch density, (4) intensity of flowering, (5) fruiting, (6) main accompanying plant species, (7) plant community structure, (8) species composition and age of the forest stands, (9) canopy cover of the forest stands, (10) existing and potential threats. In the northern part of the forest where suitable pine sites dominated, twinflowers were distributed irregularly and unevenly. Twinflowers in the Augustów Forest occur in relatively big areas with individual patches covering from 100 m^2 to 3000 m^2, but most commonly 500–1000 m^2. The mean distance to the nearest neighbouring twinflower was 1600 m (range from 250 m to 6035 m). This pattern of distribution and separating distance does allow for sexual reproduction within the studied forest stand and blooming was confirmed in 21 out of 22 patches. In four locations, intensive blooming with >50 flowers shoot/m^2 was observed. We did not observe a dependency of the flowering intensity on patch size.

Despite the intensive and frequent flowering, as well as the presence of fruits, which were found in 15 patches, the twinflower reproduces mainly vegetatively. Twinflowers grew in 34–166 years-old pine-spruce stands with moderate canopy cover, but there was no relationship between the age of forest stands and the size of a flower patch. Plant species, which commonly occurred with twinflowers were: *Vaccinium myrtillus*, *Calamagrostis arundinacea*, *Goodyera repens*, *Rubus saxatilis, Fragaria vesca, Pleurosium schreberi.* Twinflower communities are threatened by invasive species in the forest understory in two locations only, while in the other locations no active protection is needed and the plants should rather be protected and monitored through multifunctional forest management. We emphasise the importance of the Augustów Forest for the twinflower in Poland, because about 10% of the Polish population grows in this forest complex.

Keywords: abundance, Augustów Forest, condition, *Linnaea borealis*, protection

1. Introduction

The twinflower *Linnaea borealis* L. is classified in the honeysuckle family *Caprifoliaceae*. It occurs in northern Europe, in the mountains of Central Europe as well as in northern Asia and parts of Mongolia and China (Piękoś-Mirkowa, Mirek 2003, Witkowska-Żuk 2013). The species is placed in the Holarctic element of the Circum-Boreal sub-element. The southern limit of the species range runs through Poland. The twinflower is most abundant in the north of the country; however, scattered locations of this species have been found in other Poland's regions. More than 200 locations are known from Poland (Zając, Zając 2001). In the southern regions, *L. borealis* is considered extinct or critically endangered, whereas in Central Poland and in the north of the country (Western Pomerania), it is threatened with extinction (Jakubowska-Gabara, Kurowski 2012). The Red List of Threatened Species in Poland classifies *L. borealis*

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occurring in the eastern part of Poland (Podlasie) as vulnerable (VU) (Sokołowski, Wołkowycki, in print).

L. borealis is a small, evergreen shrub categorised as modular, stoloniferous species. It forms large but spatially isolated patches that occupy the areas of several to several hundred square metres (Kurowski 2004). This species propagates primarily by vegetative reproduction, and its sexual reproduction is rarely observed (Wróblewska 2013). The twinflower is a polyploid plant – self-incompatible, which prevents self-fertilisation (Scobie, Wilcock 2009, Wróblewska 2013). The flowers are pollinated mostly by Diptera and Hymenoptera insects. Seed production in twinflower is low (Scobie, Wilcock 2009). The species is characterised by low genetic diversity, because of the rarity of sexual reproduction as well as post-glacial, fast-paced colonisation of large areas of Eurasia (Wróblewska 2013).

This plant requires moderate light exposure. It declines in heavily shaded areas as well as in those areas that are too strongly exposed to the sunlight. Twinflower populations often endure in well-exposed areas, on division lines or dune embankments (Puchałka et al. 2015). The twinflower takes over or recedes new locations relatively easily (Zaręba 1986, Piękoś-Mirkowa, Mirek 2003). It occurs in the groundcover in various coniferous forest communities (Witkowska-Żuk 2013). It is a rare forest species, characteristic for the class *Vaccinio-Piceeta*, the sub-continental pine forests *Peucedano-Pinetum* association as well as the dry coastal pine forest *Empetro-nigri Pinetum* (Matuszkiewicz 2002, Matuszkiewicz et al. 2012).

The aim of the present study was the inventory of *L. borealis* in the Augustów Forest along with the determination of the condition and threats to locations of this species. The condition of twinflower individual patches (width, density, flowering intensity and fruit presence), plant associations, stand characteristics, threats and distance between patch locations were evaluated. Considering the geographic position of the study area, high numbers of twinflower patches with plants in good health condition were expected. It was anticipated that in the vast forest complex, spread in the sub-boreal climate zone, comprising forest communities with high proportion of boreal spruce (Sokołowski 2010), the twinflower with the circum-boreal origin would occur relatively abundantly on vital locations. Actions were proposed to preserve existing twinflower locations.

2. Study area

In Poland, the Augustów Forest (23°15′E, 53°54′N) spreads on the area of about 114,000 ha. The whole forest complex lies on a sandy, flat sandstone plain – formed in the last glacial period (Weichselian glaciation) (Kondracki 1994). In terms of natural-forest regionalisation, it is the Mazursko-Podlaska Region, the District Augustów Primeval Forest (Zielony, Kliczkowska 2012). According to the geobotanical criteria, the Augustów Forest is located in the Masurian-Belarusian Northern Division, the Augustowsko-Suwalski Region (Matuszkiewicz 1993). Amongst the types of forest habitats, the largest area is covered by fresh coniferous forest (40%), fresh mixed coniferous forest (31%), then by fresh mixed forest (6%), alder forest (5%) and wet mixed coniferous forest (4%). The Scots pine *Pinus sylvestris*, which occupies 78% of the forest area, is the dominant species in stands. The black alder *Alnus glutinosa* constitutes 9%; Norway spruce *Picea abies,* 8%; *Betula* spp., 5%; and pendulate oak *Quercus robur,* 1%. The average age of managed tree stands in the forest districts is 60–65 years and within the Wigry National Park is 79 years. The distinctive climatic conditions of this area are characteristic of strong continental influence and show features specific to the sub-boreal zone. The average annual air temperature is 6.6°C, the average annual temperature in January is 4.7°C and the annual average temperature in July is from 17.5 to 18.0°C. The length of the growing season is 190–195 days, and the snow cover lasts for 90–100 days (Sokołowski 2010). In the north-western part of the forest, there is designated the Wigry National Park. The remaining forest part is managed by the State Forests (forest districts: Augustów, Głęboki Bród, Płaska, Pomorze, Suwałki and Szczebra). There are 14 nature reserves within the area managed by the State Forests, and 13 of these protect parts of valuable forest associations. The total area of the reserves is 4,083.62 ha.

3. Material and Methods

This paper presents the description and comparison of 22 twinflower locations – evaluated during the nature inventory in the Augustów Forest, carried out in the years 2005–2015, all through the whole forest complex. The following parameters were evaluated: (1) twinflower patch area (m^2), (2) the distance (m) between adjacent patches in order to determine patch spatial pattern, (3) patch density according to the Braun-Blanquet abundance scale (strong > 75%, moderate 50–75%, weak 25–50%), (4) flowering (strong > 50 flower shoots/m^2, moderate 10–50 flower shoots/m^2, weak > 10 flower shoots/m^2, not any) and fruiting (presence/absence), (5) main herbaceous species within the patches, (6) plant association – based on the assessment of species composition and vegetation coverage, (7) stand species composition and undergrowth share (assessed under field conditions), (8) tree age (based on the Forest Digital Map), (9) the canopy density in accordance with the Forest Management Manual (Instrukcja Urządzania Lasu 2012) and (10) existing and

Table 1. The characteristic of twinflower patches in the Augustów Forest

Locality (forest subcompartment), number means location on the fig. 1	Area (m^2)	Patch density 1 strong, 2 moderate, 3 loose	Flowering 1 strong, 2 moderate, 3 loose, 4 lack	Fruiting 1 present, 2 lack	Threats 1 shading by soil cover, 2 shading by shrub layer, 3 shading by trees, 4 clear cutting
o. 242bWPN (1)	100	1	3	2	
o. 253d WPN (2)	100	3	3	2	1
o. 277b WPN (3)	1500	2	3	1	1
o. 310a WPN (4)	700	1	3	1	1
o. 311d WPN (5)	400	1	3	2	1, 3
o. 295c WPN (6)	700	2	3	1	1
o. 330b WPN (7)	500	1	2	1	
o. 359a WPN (8)	300	1	2	1	
o. 253a GB (9)	150	2	3	2	1
o. 281d GB (10)	900	1	2	1	
o. 263d GB (11)	300	2	3	2	1
o. 306c GB (12)	1000	1	1	1	4
o. 395f GB (13)	500	1	1	1	
o. 431c GB (14)	3000	2	3	1	4
o. 695k PO (15)	100	3	4	2	3
o. 790c PO (16)	950	1	1	2	
o. 923g PO (17)	2000	2	3	1	2
o. 922h PO (18)	500	3	3	1	2
o. 967l PO (19)	600	2	3	1	1, 4
o. 56d,f PŁ (20)	2500	2	3	1	
o. 59a PŁ (21)	500	2	1	1	
o. 124i PŁ (22)	2000	2	2	1	1

Explanations: PO – Pomorze Forest District, GB – Głęboki Bród Forest District, PŁ – Płaska Forest District, WPN – Wigry National Park

potential threats, including groundcover shading, canopy shading and tree felling operations. Measurements and assessments were conducted in the growing seasons 2014 and 2015. The classification of forest habitats by Matuszkiewicz et al. (2012) was used. The values of Pearson's correlation coefficients and Student's t-test were calculated within the R environment.

4. Results

In the years 2005–2015, 22 twinflower locations were found in the Augustów Forest. The species was identified in the area of the Wigry National Park as well as in the forest districts: Głęboki Bród (GB), Płaska (Pł) and Pomorze (Po) (Fig. 1, Table 1). Eight locations were observed within borders of the Wigry National Park, one in the 'Łempis' reserve and 13 within the area of forests managed by the State Forests. The area of twinflower patches ranged from 100 to 3,000 m^2, on an average 877 m^2. The majority of locations (50%) were covered by twinflower patches of size 500–1,000 m^2, whereas those with the area smaller than 500 m^2 constituted 32% of the total patch area (Fig. 2). The only patch exceeded 2,500 m^2 (it was 3,000 m^2).

In the study area, irregular twinflower distribution was observed. Plants of this species were found in the northern and central parts of the forest complex, whereas not found at all in its western and southern parts. In general, several closely spaced patches as well as those single and isolated were observed (Fig. 1). Local aggregations of 2–5 patches were recorded in the eastern part of the Wigry National Park, in the Głęboki Bród Forest District and in the eastern

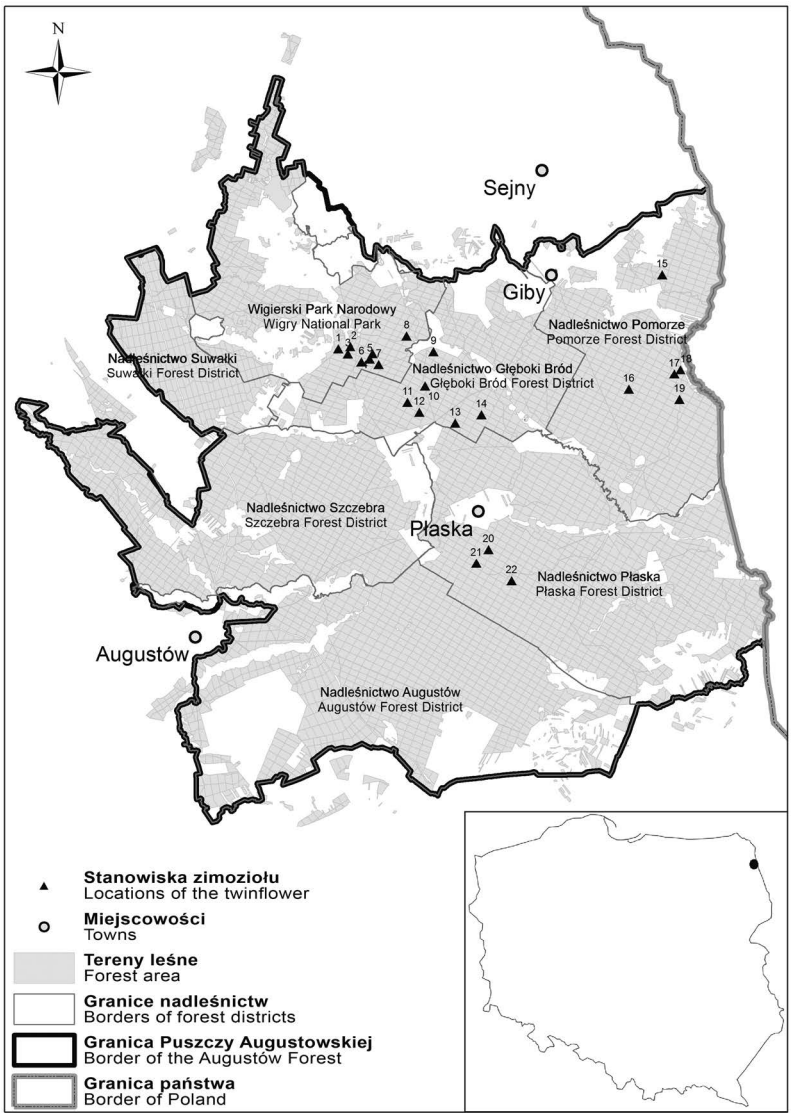

Figure 1. Distribution of twinflower localities in the Augustów Forest

Numbers on the map mean localities described in Table 1.

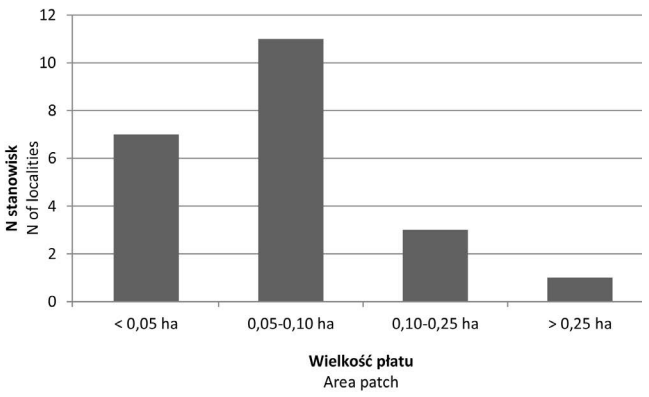

Figure 2. The distribution of the twinflower patch size in the Augustów Forest.

part of the Pomorze Forest District. The distance between individual patch positions was from 215 to 6,035 m (on an average 1,550 m). No correlation was shown ($r = -0.07$) between the patch size and the distance between adjacent ones. This parameter was not statistically significant ($t = -0.311$, $p = 0.759$, df = 20). Nearly all (77%; N = 17) patches were found in forest gaps or stand with the sparse canopy layer, in exposed areas, but usually surrounded by compact, shaded stands. The presence of flowers was observed in 21 out of 22 twinflower locations. In the Augustów Forest, in the 'Łempis' reserve, the twinflower in one patch only (with the relatively smallest area 100 m^2) showed no flowering. In all the remaining twinflower patches examined, flowers were observed, and in four of these, flowering was assessed as

Figure 3. Flowering twinflower in the compartment 395 f Głęboki Bród Forest District. Fot. Grzegorz Zawadzki

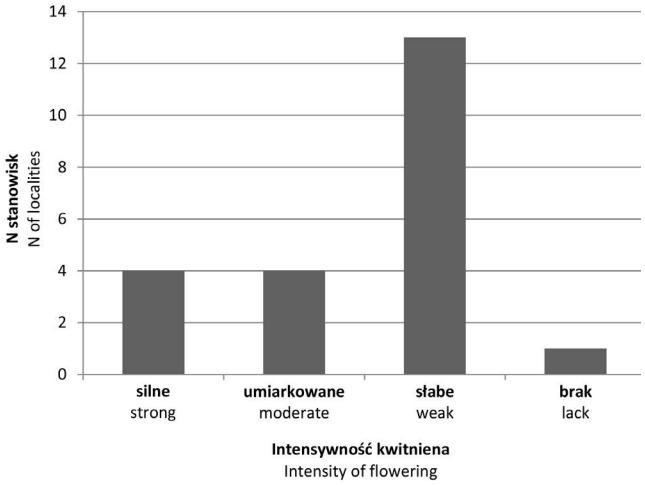

Figure 4. The distribution of the intensity of flowering of twinflower in the Augustów Forest.

Figure 5. Fruits of the twinflower. Fot. Grzegorz Zawadzki

abundant (Fig. 3). In the utmost number of patches (59%, N = 13), the density of flowers did not exceed 10 shoots/m^2 (Fig. 4, Table 1). No relationship was found between the intensity of flowering and the size of twinflower patch (r = −0.017). The result was not statistically significant (t = -0.07, p = 0.93, df = 20). The value of Pearson's correlation coefficient showed a weak negative correlation between the flowering intensity and stand age (r = −0.39), and the Student's t test showed a statistical trend (t = −1.8955, p = 0.073, df = 20). This result indicates a higher number of flower shoots in younger stands. In nearly all locations (81%, N = 17), flowering was observed twice in the season: first, more intense, occurred in the first half of June, and second, with single flowers, in August. Flower abundance did not translate into the efficiency of sexual reproduction. The presence of fruit – just a few per patch – was recorded in 15 locations (71% flowering, Fig. 5, Table 1). In all the locations examined on the forest outskirts, numerous single shoots outside the main area of the plant occurrence were observed, indicating the processes of vegetative reproduction.

In the Augustów Forest, the twinflower was observed in Scots pine and Norway spruce stands aged from 34 to166 years (Table 2). The species did not show any significant preference with respect to the age of stands but did not appear in stands younger than 30 years. The largest twinflower patch (3,000 m^2) was found in one of the oldest stands, but no relationship was observed between the area occupied by the plant and the stand age (r = −0.02). The result was not statistically significant (t = −0.060, p = 0.952, df = 20). The canopy density in the stands with twinflower patches ranged from full (18%, N = 4) to lose (9%, N = 2) and discontinuous (5%, N = 1). The largest patch number was observed in tree stands with moderate canopy density (68%, N = 15, Table 2).

In the Augustów Forest, the twinflower occurred in two plant communities. Nine locations were recorded in *Peucedano-Pinetum* (continental fresh pine forest) and 13 in *Serratulo-Pinetum* (sub-boreal mixed coniferous forest) (Table 2). In the twinflower locations examined (or in their close vicinity), the accompanying plant species such as bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), common wood sorrel (*Oxalis acetosella*), chickweed-wintergreen (*Trientalis europaea*), lily of the valley (*Convallaria majalis*), reed grass (*Calamagrostis arundinacea*), stone bramble (*Rubus saxatilis*) and hairy wood-rush (*Luzula pilosa*) and also mosses such as knights plume moss (*Ptilium crista-castrensis*), red-stemmed feathermoss (*Pleurozium schreberi*), broom forkmoss (*Dicranum scoparium*) and glittering woodmoss (*Hylocomium splendens*) were most frequently recorded. The common heather (*Calluna vulgaris*) was observed only in 3 twinflower locations, and the common cow-wheat (*Melampyrum pratense*) was observed in 2 loca-

Table 2. The characteristic of twinflower habitat in the Augustów Forest

Locality	Species composition of stands so – pine, św – spruce, stand age (years),	Density of tree layer 1 complete, 2 moderate, 3 loose	Species composition of shrub layer, covering (%)	Species of soil cover within patch	Plant community
o.242b WPN (1)	9 so 1św (68)	2		*Vaccinum myrtillus, Trientalis europaea, Luzula pilosa, Pleurosium schreberi*	*Peucedano -Pinetum*
o.253d WPN (2)	8 so, 2 św (63)	2	*Picea abies,* 10%	*Calamagrostis arundinacea, Convallaria majalis, Peucedanum oreoselinum, Trientalis europaea, Vaccinum myrtillus, Rubus saxatilis, Sorbus aucuparium, Oxalis acetosella, Pleurosium schreberi*	*Serratulo -pinetum*
o. 277b WPN (3)	10 so (56)	2		*Calamagrostis arundinacea, Rubus idaeus, Fragaria vesca, Convallaria majalis, Peucedanum oreoselinum, Trientalis europaea, Vaccinum myrtillus, Sorbus aucuparium, Oxalis acetosella, Veronica officinalis, Urtica dioica, Mycelis muralis, Moehringia trinervia, Dryopteris filix-mas, Pleurosium schreberi, Ptilium crista-castrensis, Climacium dendroides*	*Serratulo -pinetum*
o.310a WPN (4)	10so (81)	2		*Calamagrostis arundinacea, Vaccinium myrtillus, Rubus saxatilis, Fragaria vesca, Convallaria majalis, Pleurosium schreberi, Climacium dendroides*	*Serratulo -pinetum*
o.311d WPN (5)	5so 5św (166)	1		*Calamagrostis arundinacea, Oxalis acetosella, Dryopteris filix-mas, Vaccinium myrtillus, Rubus saxatilis, Fragaria vesca, Pleurosium schreberi, Climacium dendroides*	*Serratulo -pinetum*
o.295c WPN (6)	4so 6św (166)	2		*Vaccinium myrtillus, Calamagrostis arundinacea, Rubus idaeus, Trientalis europaea, Goodyera repens, Lycopodium annotinum, Pleurosium schreberi, Climacium dendroides, Ptilium crista-castrensis*	*Serratulo -pinetum*
o.330b WPN (7)	10so (39)	2		*Vaccinium myrtillus, Calamagrostis arundinacea, Rubus saxatilis, Pleurosium schreberi, Ptilium crista-castrensis*	*Serratulo -pinetum*
o. 359a WPN (8)	8so 2św (61)	2		*Vaccinium myrtillus, Calamagrostis arundinacea, Rubus idaeus, Mycelis muralis, Goodyera repens, Pleurosium schreberi*	*Serratulo -pinetum*

Locality	Species composition of stands so – pine, św – spruce, stand age (years),	Density of tree layer 1 complete, 2 moderate, 3 loose	Species composition of shrub layer, covering (%)	Species of soil cover within patch	Plant community
o.253a GB (9)	10so (104)	2		*Vaccinium myrtillus, Calamagrostis arundinacea, Trientalis europaeus, Lusula pilosa, Ptilium crista-castrensis, Pleurosium schreberi*	*Serratulo -pinetum*
o.281d GB (10)	10so (37)	2		*Vaccinium myrtillus, Calamagrostis arundinacea, Trientalis europaeus, Goodyera repens*	*Serratulo -pinetum*
o.263d GB (11)	8so 2św (121)	2	*Picea abies,* 10%	*Vaccinium myrtillus, Vaccinium vitis -idaea, Calamagrostis arundinacea, Mycelis muralis, Viola silvestris, Pteridium aquilinum, Ptilium crista-castrensis, Pleurosium schreberi, Climacium dendroides*	*Serratulo -pinetum*
o. 306c GB (12)	10so (126)	3		*Vaccinium myrtillus, Vaccinium vitis-idaea, Calluna vulgaris, Melampyrum pretense, Calamagrostis arundinacea, Fragaria vesca, Viola silvestris, Goodyera repens, Ptilium crista-castrensis, Pleurosium schreberi*	*Peucedano -pinetum*
o.395f GB (13)	9 so 1św (37)	1		*Vaccinium myrtillus, Vaccinium vitis-idaea, Calamagrostis arundinacea, Calluna vulgaris, Oxalis acetosella, Ptilium crista-castrensis, Pleurosium schreberi, Climacium dendroides*	*Peucedano -pinetum*
o. 431c GB (14)	9 so 1św (121)	2	*Picea abies,* 20%	*Vaccinium myrtillus, Vaccinium vitis-idaea, Oxalis acetosella, Goodyera repens, Ptilium crista-castrensis, Pleurosium schreberi, Hylocomium splendens*	*Peucedano -pinetum*
o. 695k PO (15)	6 św 4 so (79)	1	*Picea abies,* 30%	*Vaccinium myrtillus, Calamagrostis arundinacea, Oxalis acetosella, Goodyera repens, Ptilium crista-castrensis, Pleurosium schreberi, Climacium dendroides*	*Peucedano -pinetum*
o. 790c PO (16)	10 so (59)	2		*Vaccinium myrtillus, Vaccinium vitis-idaea, Calluna vulgaris, Calamagrostis arundinacea, Oxalis acetosella, Veronica officinalis, Melampyrum pratense, Lusula pilosa, Convallaria majalis, Chimaphila umbellata, Goodyera repens, Ptilium crista-castrensis, Pleurosium schreberi, Climacium dendroides*	*Peucedano -pinetum*

Locality	Species composition of stands so – pine, św – spruce, stand age (years),	Density of tree layer 1 complete, 2 moderate, 3 loose	Species composition of shrub layer, covering (%)	Species of soil cover within patch	Plant community
o.923g PO (17)	7 so 3 św (89)	2	*Picea abies, Frangula alnus, Corylus avellana,* 30%	*Vaccinium myrtillus, Vaccinium vitis-idaea, Rubus idaeus, Calamagrostis arundinacea, Lusula pilosa, Goodyera repens, Ptilium crista-castrensis, Pleurosium schreberi, Climacium dendroides, Hylocomium splendens,*	*Serratulo-pinetum*
o. 922h PO (18)	6so 4św (109)	2	*Frangula alnus, Corylus avellana,* 40%	*Vaccinium myrtillus, Rubus idaeus, Rubus plicatus, Fragaria vesca, Mycelis muralis, Lusula pilosa, Goodyera repens, Dryopteris filix-mas, Ptilium crista-castrensis, Pleurosium schreberi, Climacium dendroides, Hylocomium splendens*	*Serratulo-pinetum*
o. 967l PO (19)	10 so (129)	3	*Juniperus communis, Picea abies,* 20%	*Vaccinium myrtillus, Vaccinium vitis-idaea, Fragaria vesca, Arnica montana, Lusula pilosa, Calamagrostis arundinacea, Convallaria majalis, Pteridium aquilinum, Ptilium crista-castrensis, Pleurosium schreberi, Climacium dendroides, Hylocomium splendens*	*Peucedano-pinetum*
o. 56d,f PŁ (20)	10 so (42)	2		*Vaccinium myrtillus, Vaccinium vitis-idaea, Goodyera repens, Lusula pilosa, Calamagrostis arundinacea, Ptilium crista-castrensis, Pleurosium schreberi, Hylocomium splendens*	*Peucedano-pinetum*
o. 59a PŁ (21)	10 so (34)	1		*Vaccinium myrtillus, Vaccinium vitis-idaea, Lusula pilosa, Calamagrostis arundinacea, Ptilium crista-castrensis, Pleurosium schreberi,*	*Peucedano-pinetum*
o.124i PŁ (22)	9 so 1 św (61)	2	*Picea abies, Juniperus communis,* 30%	*Vaccinium myrtillus, Vaccinium vitis-idaea, Trientalis europaea, Goodyera repens, Lusula pilosa, Calamagrostis arundinacea, Pteridium aquilinum, Ptilium crista-castrensis, Pleurosium schreberi, Hylocomium splendens*	*Serratulo-pinetum*

tions. The twinflower patches were frequently accompanied by other plants of protected species. In 11 locations, individuals of creeping lady's-tresses (*Goodyera repens*) were observed, whereas in individual patches, umbellate wintergreen (*Chimaphila umbellata*), mountain arnica (*Arnica montana*) and stiff clubmoss (*Lycopodium annotinum*) were recorded. No alien plant species were observed.

The threats were reported in 13 locations of twinflower (Table 1), but in most cases, these were not serious. Two locations were considered as critically threatened: one situ-

ated in the forest management unit 263d GB (No. 11) – because of the expansion of reed grass– and the second – in the 'Łempis' reserve (No.15) – because of the Norway spruce shade. One location is potentially at risk as a result of turf developing on the groundcover because of increased light access attributable to tree cutting performed in the vicinity. Shading due to some expansive plant species, such as common bracken (*Pteridium aquilinum*), raspberry (*Rubus idaeus*) and undergrowth of alder buckthorn (*Frangula alnus*) and common hazel (*Corylus avellana*), was considered as unfavourable for twinflower development. However, just 1 location was seriously threatened due to these factors. No threat associated with alien species was documented. Nevertheless, harvest cutting poses a potential threat to the twinflower in the Augustów Forest (Table 1).

5. Discussion

In Poland, the twinflower is prevalent in small areas, with strong spatial and genetic isolation (Piękoś-Mirkowa, Mirek 2003). As expected by the study authors, the Augustów Forest turned out to be an area of numerous incidence of this low-growing boreal shrub, attributable to the geographical position of the forest, continental climate and boreal character of its plant communities. Other boreal species of flora, such as creeping lady's-tresses, Greek valerian (*Polemonium caeruleum*) and downy willow (*Salix lapponum*) are present here (Sokołowski 2010). The representatives of boreal fauna, such as mountain hare (*Lepus timidus*) (Zbyryt et al. 2013), capercaillie (*Tetrao urogallus*), hazel grouse (*Tetrastes bonasia*), nutcracker (*Nucifraga caryocatactes*) and redwing (*Turdus iliacus*) also occur (Zawadzka et al. 2011). Sokołowski (2010) reported 7 twinflower sites in the Augustów Forest but did not specify any locations. Most probably, this author observed other locations than those described in the present paper. One twinflower location was found a few years ago in the Szczebra Forest District, in the south of the study area (M. Orzechowski, personal communication). It can be expected that the actual number of twinflower locations in the Augustów Forest is higher than that shown in this paper. The uneven and cluster distribution of known locations on the scale of the whole forest somewhat confirms such a conclusion. The obtained data on the location of the twinflower in the Augustów Forest could be influenced by a slightly stronger penetration rate in the northern part of the forest and the presence of smaller areas suitable for the twinflower in the southern part, with large areas covered by deciduous, alder and swamp forests, as well as milder climatic conditions. The twinflower is difficult to detect because of its small size and form of growth; thus it is poorly recognised by the Forest Service staff. Taking into account the published national data, the Augustów Forest has the largest number of twinflower locations. There occurs almost 10% of the national population of this species, known from more than 200 posts (Zając, Zając 2001, Piękoś-Mirkowa, Mirek 2003).

The twinflower populations observed in the Augustów Forest showed good health and vitality. Most of them occupy large areas, with strong or moderate canopy closure. There dominate 500–1,000 m^2 twinflower patches, whilst in Poland, the size of the areas occupied by this species usually do not exceed 100 m^2 (Wójcik 2010, Jakubowska-Gabara, Kurowski 2012, Wróblewska 2013). Single larger patches – from 100 to 3,000 m^2 – are known from the Tucholskie Forests (Grzywacz, Pietrzak 2012), the Napiwodzko-Ramuckie Forests (Środa, Dąbrowski 1999) and the Vistula Spit (Endler et al. 2008), as well as from Central Poland (Jakubowska-Gabara, Kurowski 2012). In the Augustów Forest, flower shoots were observed in almost all the twinflower locations examined. In most of them, small, single fruits were also found – and this is rather uncommon in this part of Europe (Ciosek et al. 2015). There are no published data on the distances between neighbouring twinflower locations within the forest complex. In the Abernethy Forest, Scotland, the species patches were separated by 0.25–3 km, whilst in the Balmoral Estate, the distance ranged from 30 m to 1 km (Scobie, Wilcock 2009). The distances observed in the Augustów Forest are comparable to those in the Abernethy Forest. Such positioning with respect to each other allows pollinating of plants by insects and sexual reproduction, which promotes the preservation of high genetic diversity of the species. It would be advisable to verify the presence of sexual reproduction in genetic research.

In Poland, habitat changes (increase in fertility of forest habitats and change in composition of vegetation) as well as succession (Jakubowska-Gabara, Kurowski 2012, Wolańska-Kamińska et al. 2014, Ciosek et al. 2015) are considered most serious threats. One of the most important factors is the availability of light. According to Zaręba (1986), the twinflower is a light-loving plant species. Most researchers believe that moderate sunshine is good for this species, but too strong, full irradiation as well as strong shading affect it negatively (Lorens 1993; Wolańska-Kamińska et al. 2014; Ciosek et al. 2015). In shady locations, the twinflower responses positively to the increase in light availability on a small scale (Niva et al. 2006). Potential threat is the utilisation of forest, leading to twinflower habitat destruction as a result of increased sunlight and mechanical damage due to felling operations and tree skimming (Piękoś-Mirkowa, Mirek 2003, Puchałka et al. 2015). In the central parts of Poland, the twinflower population was lost because of changes in flora composition and phytocenose structure, in consequence of regeneration pro-

cesses of previously disturbed communities, as well as expansion of invasive species (Wolańska-Kamińska et al. 2014). The species retrograded because of excessively limited light access on account of lush development of undergrowth (especially buckthorns and hazel) as well as groundcover plants (Kurowski 2004, Jakubowska-Gabara, Kurowski 2012). As a plant requiring moderate shade, after tree stand thinning, it can be displaced by blackberries (*Rubus* spp.), forest reeds and other expansive groundcover species. In the Augustów Forest, in the locations examined, the twinflower was threatened to a small extent. In individual cases, unfavourable factors were too strong shade and expansion of forest reed or shrubs and too much light because of tree cutting performed in the vicinity of twinflower locations. Potential threat is tree harvesting performed within managed forests, and this concerned more than half of the locations examined.

The twinflower is listed in the regional red lists of threatened with extinction and endangered plants in the provinces: Kujawsko-Pomorskie, Lubelskie, Łódzkie, Małopolskie, Opolskie, Podlaskie, Pomorskie, Śląskie and Wielkopolskie (Jakubowska-Gabara, Kurowski 2012, Wolańska-Kamińska et al. 2014; Puchałka et al. 2015; Sokołowski, Wołkowycki in print). A small number of twinflower locations are protected in national parks and reserves. In Central Poland, several reserves were created specifically to protect this species. Seven twinflower locations have been protected as nature monuments (Grzywacz, Pietrzak 2012). Active protection measures have been undertaken to increase the exposure to sunlight of twinflower patches in forest stands through reducing canopy closure as well as 'metaplantations' of this species have been established in the 'Jawora' and 'Jaksonek' reserves (Kurowski 2004, 2015, Witosławski 2004, Wolańska-Kamińska et al. 2014). In the Augustów Forest, there is no need to create reserves for the protection of this species, but the status of twinflower populations should be monitored, especially in managed forests. The protection of twinflower locations should be taken into account in the planned and implemented economic activities (Wójcik 2010). Given category VU (vulnerable), the twinflower is included in the Polish red list of ferns and flowering plants (Kaźmierczakowa 2016). Until 2014, it was subject to the strict protection in Poland, but since October 2014, it has been only partially protected (Regulation 2014). The authors of this paper and also other authors conducting research on the twinflower (e.g. Ciosek et al. 2015) believe that it should be strictly protected.

6. Conclusions

1. In the Augustów Forest, there occurs the most abundant and vital twinflower population on Poland's scale, comprising at least about 10% of known locations in the country. Most patches do not require special protective measures, only long-term monitoring.

2. It would be valuable to perform genetic studies on the twinflower in the Augustów Forest, in order to assess the degree of genetic diversity of the population and sexual reproduction.

3. Within the area managed by the State Forests, twinflower locations should be protected from accidental destruction during economic activities by taking into account the natural sites of this species in conservation programmes included in forest management plans. In some forest districts, this postulate has been already implemented.

4. Light conditions in too much shaded twinflower locations should be carefully improved by moderate, gradual thinning of shady vegetation in the lower and upper layers of forest stands. Excessive light access caused by canopy thinning should be avoided. It would be valuable to conduct an inventory of the twinflower in the Augustów Forest as a whole by employees of the State Forests – after a relevant training to raise their knowledge on the identification of this species.

Conflict of interest

Authors declare no potential conflicts.

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Authors' contribution

D. Z., G. Z. – concept and article preparation, literature review, fieldwork, graphic materials; J. B., D. P., J. B. B., A.M. – field work.