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How wrong are we in estimating the abundance of mistletoe occurring on Scots pine? – a case study from Central Europe

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Abstract

This paper presents data on the occurrence of the semi-parasitic mistletoe plant (*Viscum album* L.) in a pine stand in central Poland. The number of mistletoe shrubs, the percentage of mistletoe in the crown volume, and the location of its occurrence were determined from the ground level on 193 pine trees growing in 212c in the Głuchów Forestry, Rogów Forest Experimental Station. In addition, 15 trees were analysed in detail after felling to verify the previously obtained results. The age, diameter, and location (top, middle, and bottom of the tree crown) of the shrubs were measured. The study showed that the pine stand analysed was strongly infested by mistletoe. The number of mistletoe shrubs recorded on standing trees from the ground level was significantly lower than that found on trees after felling. An in-depth visibility analysis showed that in some cases, an operator assessing mistletoe shrub abundance from the ground level may miss a very large number of juvenile individuals, as well as mistletoe shrubs with large diameters. This is probably related to the clumping of perennial individuals growing in proximity to a single shrub that is visible from the ground. *V. album* mainly colonizes the apical zone (observations from the ground) and middle zone of the host crown (detailed analysis of mistletoe abundance on the felled trees). Older shrubs were most abundant at the apex of the crown, while younger shrubs were most likely to colonize the middle and bottom of the host crown. During the survey of felled trees, 289 mistletoe individuals were found on the most infested tree. Also, it was found that the most numerous shrubs of *V. album* were young, aged between three and six years. The increase in mistletoe infestations in forests observed in recent years may pose a serious threat to pine stands weakened by abiotic factors.

Keywords: spread of mistletoe; monitoring methods; *Viscum album*; forest condition monitoring; forest; Poland

Introduction

Scots pine is one of the most important forest-forming species in Poland from economic and environmental perspectives. In recent years, mass emergence of mistletoe has been reported, and the economic losses in forests caused by it are increasingly severe (Iszkuło et al. 2020). Although forests are adapted to a certain level of disturbance, they are exposed to new factors such as climate change, air pollution, and new invasive organisms (Anderegg et al. 2015, Trumbore et al. 2015). The increasing negative impact of mistletoe on forest management is beginning to be considered as a significant threat to stand stability.

In 2018, Polish forests experienced severe drought, which contributed to lower groundwater levels. This has resulted in a significant weakening of many tree species, including our main forest-forming species, Scots pine (Kędziora et al. 2020). The stress caused by climate change increases the vulnerability of trees to various biotic agents (Griebel et al. 2017), such as fungi and insects, as well as parasitic and semi-parasitic plants, including mistletoe. Pine mistletoe infection leads to crown degradation (Rigling et al. 2010). Until recently, mistletoe was not considered a real threat to stands and its presence was rarely noted in stand descriptions (Kędziora et al. 2020). Recent research considering climate change are showing possible expansion of mistletoe in Europe (Walas et al. 2022).

According to current knowledge, the genus mistletoe (*Viscum* L.) is classified in the mistletoe family (Visca-

ceae) in the order Santalales and includes approximately 100 species. The common mistletoe (*Viscum album* L.) is a species found in Europe, of which three subspecies are found in Poland: *Viscum album* L. subsp. *album*, *Viscum album* subsp. *austriacum* (Wiesb.) Voll and *Viscum album* subsp. *abietis* (Wiesb.) Janch. In Europe, another subspecies of mistletoe, *Viscum album* subsp. *creticum* Böhling et al., is found only in Crete, where its host is Calabrian pine (*Pinus halepensis* ssp. *brutia*) (Böhling et al. 2002).

The subspecies occurring in Poland are morphologically very similar, which makes it difficult to distinguish between them. However, molecular studies have confirmed the distinctiveness of individual mistletoe subspecies (Zuber and Widmer 2009). An important feature that makes it possible to distinguish subspecies without detailed laboratory tests is their close association with specific host species (Stypiński 1997).

Among the mistletoe subspecies found in Poland, the typical one, *Viscum album* subsp. *album*, showed the greatest diversity in terms of host plants. It grows on trees and shrubs that belong to 31 genera and 118 deciduous species. *Viscum album* subsp. *abietis* is mainly located on the silver fir (*Abies alba* Mill.). Exceptionally, it grows on Japanese larch (*Larix kaempferi* (Lambert) Carriere) and silver maple (*Acer saccharinum* L.). In contrast, *Viscum album* subsp. *austriacum* shows definite attachment to Scots pine (*Pinus sylvestris* L.). Although extremely rare, it was also found in Norway spruce (*Picea abies* (L.) Karsten) and occasionally on goat willow (*Salix caprea* L.), rowan (*Sorbus aucuparia* L.), and *Genista cinerea* (Vill.) DC. (Stypiński 1997).

Mistletoe is an ornithochoristic species, meaning that its seeds are mainly spread by birds. Mistletoe fruit is an extremely attractive food for birds because it remains on the bush for a very long time (even several months), is abundant, and contains up to several tens of percent carbohydrates in the dry matter (Watson 2001). Watson (2001) listed as many as 57 bird families worldwide, whose representatives feed on the fruit of plants of the order Santalales. Of the European species, these are mainly Bohemian waxwings (*Bombycilla garrulus*) and mistle thrushes (*Turdus viscivorus*), whose Latin name indicates that it is a consumer (and at the same time a disseminator) of *Viscum* fruit (Iszkuło et al. 2020).

Observations of mistletoe made in Poland are most often associated with *Viscum album* subsp. *album* (Stypiński 1997). The common mistletoe growing on pine trees (*Viscum album* subsp. *austriacum*) has been the subject of many research. One of the first analyses of the mass occurrence of this variety of mistletoe was conducted in 2013. At that time, an infestation of 46% of the over 300 analysed trees was found in the Turek Forest District in eastern Wielkopolska Voivodeship (Kołodziejek and Kołodziejek 2013). Inventories conducted in the State Forests show a successive increase in the area of infested conifer stands (Szmidla et al. 2019). However, it is expected that the results given by the State Forests are significantly underestimated. This may be indicated by an analysis of surveys conducted in the Katowice Regional Directorate of State Forests. An inventory of forest areas infested by mistletoe was conducted thrice. In the summer of 2018, 7.5 thousand ha of infested forests were observed. In the winter of the same year (when the undergrowth made up of deciduous species was leafless and crown observation was much easier), 9.1 thousand ha were observed, and in the summer of 2019, 12.5 thousand ha were observed. It follows that the increase in the area colonized by mistletoe is most likely the result of a more accurate recognition of the mistletoe area by foresters (Iszkuło et al. 2020).

Pine stands account for 58% of forests in Poland (Rozkrut 2020). Traditional methods of crown monitoring from ground level (also using binoculars) are often inadequate because of the vast inventory area and the difficulty of identifying the parasite at early development stages in coniferous stands (Iszkuło et al. 2020). This condition is the sum of several factors: young mistletoe bushes are almost invisible at high altitudes because of their small size and colour, similar to pine needles.

Early detection of mistletoe in a stand is crucial to prevent its further spread (Iszkuło et al. 2020). Many methods to control the amount of mistletoe in stands can be found in the literature (Kędziora et al. 2020). However, to proceed with measures to reduce the degree of tree infestation, it is necessary to know in advance the area and severity of mistletoe occurrence. The present study investigated the current degree of infestation of a selected pine stand fragment by common mistletoe at the Rogów Forest Experimental Station.

The aim of this study was: to determine the number of mistletoe bushes visible from ground level on standing trees and to compare this with the actual number of bushes determined after felling the tree; to determine the percentage of mistletoe in the crown volume; to determine the crown parts that are most frequently colonized by mistletoe; and to determine the age structure of mistletoe colonizing a pine stand inside a forest.

Materials and methods

The surveys were conducted on two different dates. The first part of the measurements (*standing trees survey*) took place in February and the second part (*felled trees survey*) in September and October 2020. The surveys were conducted in the Głuchów Forestry in the Rogów Forest Experimental Station, forest sub-compartment 212c (Figure 1). These forests are part of the Forest Experimental Station in Rogów, which is a field unit at Warsaw University of Life Sciences.

The studied stand grows in the habitat of fresh mixed coniferous forests on rusty soil. The dominant species was Scots pine (*Pinus sylvestris* L.), with a single occurrence of pedunculate oak (*Quercus robur* L.), both aged 110. The crown canopy density was assessed at 60%, and the

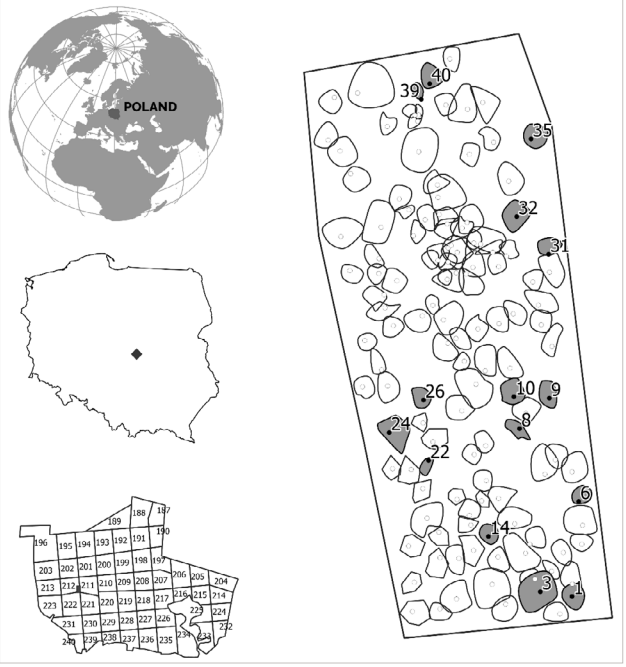

Figure 1. Location of the study area. On the right, the exact locations of each tree and its crown in the stand are shown

Trees fallen for detailed survey are marked with grey.

average height was measured to be 27 m. The site index class for the main species was IA with a volume of approximately 323 m^3/ha (Bank Danych o Lasach 2022). In this stand, common mistletoe (*V. album* subsp. *austriacum*) on Scots pine has been observed to be widespread in recent years.

As part of the *standing tree survey*, all standing Scots pine trees found in the sub-compartment were analysed. Kraft's class was determined for each tree (Włoczewski 1968). Using a diameter gauge at a height of 1.30 m, the breast height of the trees was measured with an accuracy of 1 cm. Using the FieldMap tool (www.field-map.cz), the position and height of the trees, as well as the length and horizontal projection of the crown, were measured. Subsequently, using binoculars from the ground level, the number of mistletoe bushes on individual trees was counted based on the methodology proposed by Wójcik et al. (2021). If there were more than five shrubs on an individual tree and, in addition, it was difficult to distinguish between individual mistletoe specimens, the count was recorded as (6+), i.e. 6 shrubs and more. Another factor was to determine the percentage of mistletoe in relation to the total crown volume to the nearest 10%. The location of the mistletoe within the crown was also recorded, considering the division into the top of the crown (T), middle of the crown (M), and bottom of the crown (B).

The *felled trees survey* was conducted on 15 trees randomly selected from *standing trees survey* that were cut down. The precise number of mistletoe shrubs was determined through detailed vetting of each branch and trunk of the felled tree, considering their location in the tree crown – top (T), middle (M), or bottom of the crown (B). The age of mistletoe individuals was determined according to the number of internodes observed on their shoots, with one year of hypocotyl development (Nierhaus-Wunderwald and Lawrenz 1997, Stypiński 1997). If two or more shrubs grew from the same thickening, they were treated as adventitious shoots and recorded as a single organism (the so-called cluster). Additionally, the diameter of each mistletoe shrub (or cluster) was measured.

Results

Standing trees survey

A total of 193 pine trees were analysed as part of the *standing trees survey*. By conducting observations at the ground level, mistletoe bushes were observed on 172 trees (89%), with varying degrees of infestation. Six or more mistletoe bushes were observed in 97 pines (approximately 50%) (Figure 2). Between one and five mistletoe bushes were observed on 85 trees at ground level, with the number of trees in each mistletoe abundance category ranging from 10 to 22 (average 15 trees). There were 21 (11%) trees on which no mistletoe was observed.

Mistletoe bushes on infected trees accounted for 10–60% of the total crown volume. Most commonly (104 trees, 53% of occurrences) occupied between 10 and 20% of the crown (Figure 3), whereas 60% crown fill was found on two trees (1%).

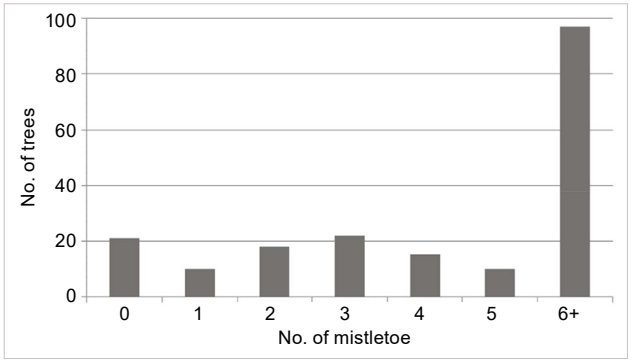

Figure 2. The proportion of standing trees with a given number of *Viscum album* (L.)

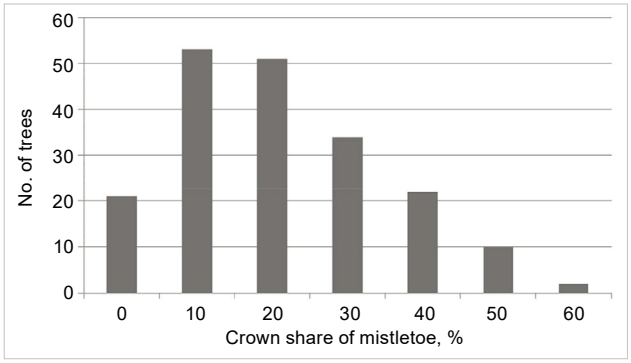

Figure 3. Share of *Viscum album* (L.) in crown volume of standing trees

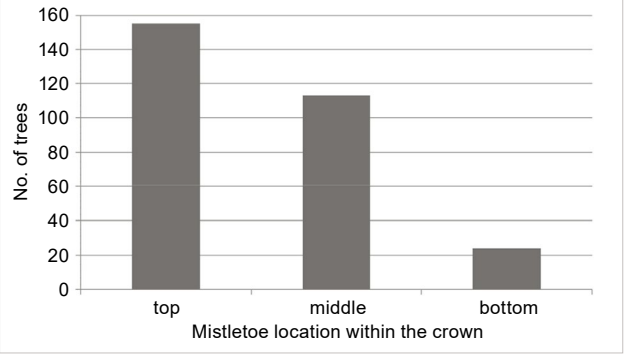

Figure 4. Occurrence of *Viscum album* (L.) shrubs in particular parts of the crown of standing trees

The location of mistletoe shrubs within the crown of each analysed tree was also determined (Figure 4). The sum of records was greater than the number of the surveyed trees, because of multiple shrubs occurring simultaneously in the top, middle and bottom parts of specific pines. The distribution of mistletoe within the canopy was uneven. The largest number of shrubs (53%) was located in the apical part of the crown, whereas the smallest number was located in the lower part (8%).

Felled trees survey

In the *felled trees survey*, the precise number of mistletoe shrubs was counted on each of the analysed felled trees. The results obtained were compared with the number of shrubs observed from ground level in the first round of measurements (Table 1).

The actual infestation rate of pines ranged from 4 to 289 mistletoe bushes per tree. A total of 844 individuals of *Viscum album* were observed on the 15 pines surveyed, with an average of 56 mistletoe bushes per tree. The ages of the surveyed individuals ranged from 2 to 26 years. The average was 7.3 years. The mistletoe bush diameter ranged from 1 to 150 cm, with an average of 23.9 cm.

Table 1. Comparison of the number of *Viscum album* (L.) shrubs observed on trees before and after felling

Tree No.	No. of shrubs observed		
	standing tree (estimate)	felled trees (precise)	difference
1	4	23	19
3	5	37	32
6	2	4	2
8	0	8	8
9	6+	289	~283
10	6+	63	~57
14	6+	67	~61
22	6+	55	~49
24	6+	115	~109
26	0	10	10
31	2	18	16
32	2	33	31
35	6+	35	~29
39	2	5	3
40	6+	82	~76

Of the 844 mistletoe measurements, 221 formed clusters (two or more shrubs growing the same thickness). These were where between 2 and 13 pieces of mistletoe were growing (an average of 4.5 pieces), but for logistical reasons, were measured as one plant.

The largest recorded difference in the number of mistletoe shrubs noticed on a growing tree after it was cut down was about 283 *Viscum album* individuals, while the smallest was only three individuals. On an average, the difference in shrubs on the surveyed trees was approximately 52 individuals.

An in-depth visibility analysis showed that in some cases, an operator assessing mistletoe bush abundance from the ground level may miss a very large number of juvenile individuals, but also mistletoe shrubs with diameters in the range of 42–58 cm, and in extreme cases, even 95 (!) cm. This is probably related to the clumping of perennial individuals growing in the immediate vicinity into a single shrub that is visible from the ground.

The age of each shrub was determined. On this basis, it can be seen that the most numerous shrubs were young, aged between three and six years. Shrubs aged 11 years also appear to be numerous. The crown location of each shrub was also determined (Figure 5). The results differed from the initial work, as the colonization of the top of the tree was 21%, that of the middle 55%, and that of the bottom 24%. Older shrubs were most abundant at the apex of the crown, while younger shrubs were most likely to colonize the middle and bottom of the host crown.

A correlation was observed between mistletoe age and shrub diameter. Statistical calculations were performed, and an R^2 coefficient of determination of 0.73 was obtained. It was lower for shrubs from the top and middle of the crown (0.71 in both cases), whereas it was higher for individuals growing at the bottom of the crown (0.77).

The analysis of the age of each shrub made it possible to create a graph showing the changes in *Viscum album* shrubs in previous years on the pine trees studied

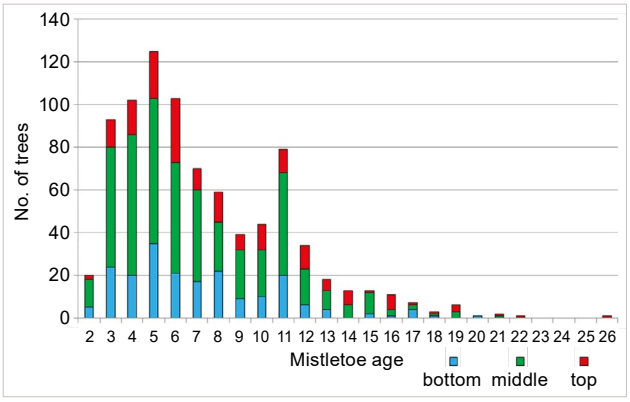

Figure 5. Occurrence of shrubs *Viscum album* L. by age and place in the crown of felled trees

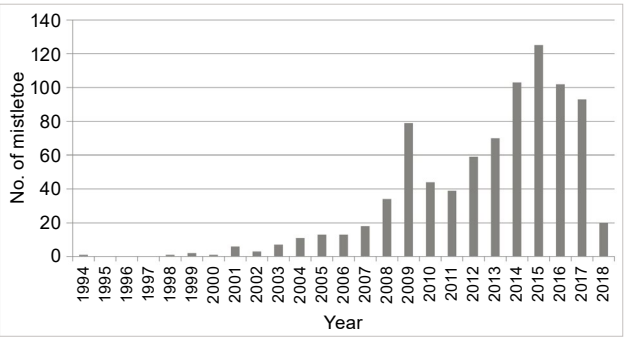

Figure 6. Abundance of *Viscum album* L. shrubs on felled trees by year

(Figure 6). Analysis of the variation in mistletoe abundance between years showed an increasing trend. The oldest individuals appeared in 1994. Dead mistletoe individuals were also observed, for which it was not possible to determine their age or moment of death. Two maxima of *Viscum album* appearances were recorded in 2009 and 2015.

The relationships between mistletoe occurrence and breast height, tree height, Kraft's class, crown length, and horizontal crown projection were not confirmed, both for standing and felled trees assessment

Discussion and conclusions

In recent years, mistletoe has significantly affected the pine trees in Poland. The greatest impact of the semi-parasite was noticeable on trees weakened by drought or water shortage. The presence of *Viscum album* is a major problem that is likely to increase, which may even lead to changes in the species structure of Polish forests (Szmidla et al. 2019).

In a study of a pine stand in the Rogów Forest Experimental Station, mistletoe bushes were observed on 172 trees (89%) out of 193 surveyed, with varying degrees of infestation and the proportion of *Viscum album* in the crown volume. Analyzing the 15 felled trees in detail, the actual degree of infestation on the pines ranged from 4 to 289 mistletoe bushes on a single tree. A total of 844 individuals of *Viscum album* were observed, with an average of 56 mistletoe bushes per tree. The age of the specimens ranged from 2 to 26 years. Such large differences in the same trees (Table 1) are because the observer from the ground level is unable to see the youngest individuals and treats several shrubs growing next to each other (even of significant size) as one object. Stypiński (1997) reported that in the first year of *Viscum album*'s life, the hypocotyl elongates, and the primary sucker develops, whereas in the second year, the first internode is formed. These individuals were small and barely visible in the host crown.

Out of the 844 mistletoe measurements in the surveyed pine stands, 221 formed clusters. These were sites of 2–13 mistletoe growing in the same cluster (average 4.5 units). Zuber (2004) reported that mistletoe can produce adventitious shoots. These are the so-called ramets, that is, the parts of the individual that have been formed by the multiplication of the structural units of the organisms (Falińska 2004).

The negative impact of mistletoe on both coniferous and deciduous trees and stands is a well-documented phenomenon in the literature. Owing to its ability to assimilate nutrients, mistletoe is a semi-parasite, which means that its impact on the host is often underestimated. The loss of water and mineral salts that mistletoe takes up from the tree significantly weakens the host condition. During periods of water deficit, trees try to defend themselves against water loss by closing their stomata. However, this does not reduce the intensity of gas exchange in mistletoe (Zweifel et al. 2012). The intensive transpiration of mistletoe makes periods of drought particularly dangerous for trees growing under water-scarce conditions (Mutlu et al. 2016). Intensive mistletoe transpiration occurs between May and September. On sunny days in July, the total daily transpiration of *Viscum album* can be three times higher than that of the host *Pinus sylvestris*, with a similar rate of CO_2 assimilation. A faster transpiration rate is presumably necessary to take up sufficient nitrogen from the host xylem to produce certain organ biomass (stem, leaves, and fruit) (Schulze et al. 1984). Observations of pine stands infested by mistletoe show that infested trees respond much less well to water deficiency, which manifests itself in the loss of the assimilation apparatus, slowed growth, or even death of part or all of the trees (Kollas et al. 2017). Progressive climate change contributes to the increasing incidence of drought in stands, and this can lead to increased tree mortality (Griebel et al. 2017).

Contrary to popular opinion, mistletoe takes up not only water and mineral salts from trees, but also assimilates from the host. Richter and Popp (1992) demonstrated that 23–45% of the carbon in mistletoe organs can come from the host plant. Furthermore, sites where mistletoe penetrates host organs are exposed to co-infections caused primarily by fungi (Zuber 2004). The uptake of water, mineral salts, and assimilates from the host and co-infections affects the impaired functioning of three basic areas of plant life: reproduction, growth, and defence mechanisms (Iszkuło et al. 2020).

In the surveyed stand, mistletoe shrubs on infected pines accounted for 10–60% of the total crown volume, with the most common (53% of occurrences) occupying 10–20% of the crown. Many authors have reported that the presence of mistletoe negatively affects tree development, including radial growth of infected trees. A study on Scots pine conducted in Poland showed a reduction in the average ring width of infected trees by up to 37% compared to the annual rings of trees without mistletoe (Pilichowski et al. 2018). A significant reduction in growth was also observed in Spain, where the decrease in growth reached 65% (Camarero et al. 2019). In Turkey, a 40% decline in pine growth has been reported (Sönmez 2014), and in Ger-

many, a 32% decline (Kollas et al. 2017). In addition, mistletoe has a negative impact on radial growth of black pine and even North American pine species (Geils et al. 2002, Catal and Carus 2011). Moreover, mistletoe significantly reduced the reproductive capacity of the host tree. Studies on American conifer species have shown the negative effects of mistletoe on the number and size of cones and seeds produced, germination, and seedling quality (Singh and Carew 1989). Similar studies conducted in Poland in an excluded pine seed stand in the Bolewice Forest District showed smaller cones, fewer seeds per cone, lower seed weight, and lower height and weight of seedlings from trees infested with mistletoe compared to trees free of the semiparasite (Jasiczek et al. 2017). Pine mistletoe infestation results in a decline in the crown health by diminishing its size, radial growth, needle length, and the number of year's needles persist on the affected branches (Rigling et al. 2010). Wójcik et al. (2021) have found strong correlation between crown defoliation and mistletoe abundance in Central Poland.

The presence of mistletoe is a weakening factor for trees, which favours the gradation of secondary pests. Fir trees infested with mistletoe are particularly vulnerable to attacks by *Phaenops knoteki* E. Reitter beetle larvae feeding under the bark, which contributes to their dieback in Greece (Tsopelas et al. 2004). Thus, a similar relationship may exist for pine infested by mistletoe and sharp-toothed bark beetle (*Ips acuminatus* Gyllenhal) (Iszkuło et al. 2020).

Mistletoe directly affects plant communities by shortening the lifespan of the host plant. However, its indirect effect on habitat-related features was also noticeable. Changes in the ecosystem caused by mistletoe manifest themselves gradually, and the weakening of the host results in thinning of its crown and even the death of the tree, leading to an increase in the availability of light in the lower parts of the forest and enabling the natural regeneration of many plant species (Mellado and Zamora 2017). In addition, mistletoe affects the presence of birds that eat its fruit, which, in turn, increases the number of plant species carried by endo- and exozoochory (Hódar et al. 2018). Birds also have increased mineral-rich excreta, which increase habitat abundance (Mellado et al. 2016). Trees without mistletoe were occasionally visited by fruit-bearing birds. Once a tree is parasitized by mistletoe, it has a greater chance of re-infestation owing to the increased activity of birds spreading seeds. In addition to mistletoe seeds, birds also spread the seeds of other plants under the tree. Over time, the numerous mistletoe bushes, combined with increased bird activity, not only increase the number of seed deposited, but also increase the amount of organic matter under the host canopy, causing local soil enrichment that promotes subsequent plant growth. This cycle intensifies as more mistletoe bushes mature, culminating in the death of the host, which is eventually replaced by zoochoric undergrowth vegetation (Mellado and Zamora 2017).

In research conducted at the Forest Experimental Station in Rogów, it was found that mistletoe mainly colonizes the apical zone (observations from the ground) and middle zone of the host crown (detailed analysis of mistletoe abundance on felled trees). Similar results were obtained for *Viscum album* subsp. *austriacum* (Wiesb.) Vollm. were obtained in the Bielinek nad Odrą reserve (Ciaciura et al. 1999) and *Viscum album* L. subsp. *album* in the Strzelce Krajeńskie commune (Ciaciura et al. 2008). Jurzyk and Kluczyński (2000) stated that such a location of mistletoe is due to the more frequent residence of birds that bear fruit in the mentioned parts of the tree crown.

It is worth mentioning that spreading of mistletoe by gravity on surrounding branches is unlikely because if the epicarp remains intact, the hypocotyl develops within the fruit, which remains on the trees until May and June (Frochot and Salle 1980). The epicarp remains an effective barrier for the germinating seed (Stopp 1961), birds are primarily responsible for effectively removing this barrier, and the fruit falling on its own is unlikely to eliminate it (Frochot and Sallé 1980).

Investigations carried out in a pine stand in sub-compartment 212c in the Głuchów Forestry at the Rogów Forest Experimental Station led to the following conclusions:

- The number of mistletoe bushes recorded on trees standing from the ground level was significantly lower than that found on trees after felling;
- On average, 56 mistletoe bushes were found on the felled trees, while 289 mistletoe specimens were recorded on the most infested tree;
- Surveys conducted on the ground show that mistletoe is most often found in the apical part of the tree crown. In contrast, a detailed analysis of felled trees has shown that they predominate in the middle part of the tree crown, with shrubs that are mainly a few years old;
- Mistletoe accounted for more than half of the percentage volume of the tree crown in the study area;
- The largest number of mistletoe individuals appeared in the last decade.

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