



Does *Hartigiola annulipes* (Diptera: Cecidomyiidae) distribute its galls randomly?

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Abstract. It is expected that environmental conditions impact the distribution of galls on host plants. Moreover, insects may induce their galls randomly or choose certain parts of a host to induce such growths. This study aimed to determine whether or not the gall midge, *Hartigiola annulipes* (Diptera: Cecidomyiidae), which induces galls on leaves of the European beech (*Fagus sylvatica*), prefers leaves facing a particular cardinal direction. In addition, we wanted to ascertain whether the galls are evenly spread across three leaf zones: proximal, median and distal, distinguished by dividing leaf area along the midrib. The results show that *H. annulipes* chose leaves facing various directions in different studied locations, and leaf choice is not restricted to the specific leaf area, a parameter that accurately reflects the light conditions of leaf growth. Moreover, the medial leaf zone was preferred, while the distal zone was avoided. The choice of the leaf zone modified the distance between the mid-rib and a gall. Gall distribution in the crown of trees is probably random, while at the leaf level, it is determined by leaf morphology.

INTRODUCTION

The search for factors influencing the distribution of insect galls within the crowns of trees and on plant organs has led to several hypotheses suggesting that herbivores choose plant modules to feed on depending on the vigour or size of these modules. One of the most popular is the Plant Vigour Hypothesis (PVH) (Price, 1991), which claims that herbivores choose the most vigorously growing modules to feed on. It appears that the distribution of the eastern spruce adelgid (*Adelges abietis* Linnaeus) reflects PVH, but small galls develop on lateral shoots of Norway spruce (*Picea abies* (L.) H. Karst.) and large galls on main shoots (Pilichowski et al., 2014). The main shoots of spruce are usually larger than lateral shoots, but there are fewer of them, thereby limiting the available space for galls. The aphid *Kaltenbachiella japonica* Matsumura galls the most vigorously growing region of a leaf (Aoyama et al., 2012). Gall-inducers may depend on high vigour and reduce it at the same time by deforming or hindering the growth and development of infested modules (Mendel et al., 2004; Cuevas-Reyes et al., 2008; Urban, 2010).

In addition to different hypotheses (such as PVH, optimal module size hypothesis – OMSH), it is suggested that different abiotic factors may affect gall development, survival and oviposition behaviour, due to their direct effect on a gall-inducer or plant host. Among these factors, insolation,

which is associated with the cardinal direction, should be mentioned. East-facing shoots of *P. abies* may be preferred by *A. abietis*; however, this pattern can differ between years (Pilichowski et al., 2014). South-facing galls of the asexual generation of *Cynips quercusfolii* Linnaeus, on *Quercus infectoria* G. Olivier, are the largest when compared with other directions (Zargaran et al., 2011). *Mikiola fagi* Hartig prefers sites within a tree crown that are most exposed to the sun (Kampichler & Teschner, 2002; Skrzypczyńska & Lorenc, 2005), while *Hartigiola annulipes* Hartig prefers shaded sites (Skrzypczyńska & Lorenc, 2005; Wrzesińska 2012). Both species develop their galls on the adaxial surface of leaves of the European beech (*Fagus sylvatica* L.). Galls exposed to the sun are thicker, and thus suffer fewer parasitoid attacks (Zargaran et al., 2011).

Determining the degree of insolation of leaves at the time and location of a gall when the eggs are laid is often impossible. Therefore, it is useful to use the specific leaf area (SLA) to estimate light conditions, which is a simple and very useful factor calculated as the ratio between leaf area and dry mass. The analysis of SLA indicates differences in the light conditions in which plants grow (Fellner et al., 2016; Konôpka et al., 2016), thus allowing a comparison between plants of different leaf quality (thickness) and of leaves facing different cardinal directions. Intensity of herbivory increases with SLA (Poorter et al., 2004; Par-

thasarathy & Anil, 2016). This may vary, however, with successional stage, and the outcome might be that food availability, not quality, determines herbivory (Poorter et al., 2004). Generally, SLA (and other morphological leaf traits) values are associated with herbivory and fungal damage, which effect the growth rates of trees (Schuldt et al., 2017). Thus, it is worth taking SLA into consideration when studying the distribution of galls on leaves.

Previous studies on the distribution of galls relative to light conditions and/or cardinal directions are mainly at the tree crown level (Kampichler & Teschner, 2002; Skrzypczyńska & Lorenc, 2005; Wrzeńska, 2012; Pilichowski et al., 2014).

In this study, we aimed to evaluate the preference of *H. annulipes* (Cecidomyiidae) to gall leaves facing a particular cardinal direction and determine what factors (location, leaf zone, leaf blade area, number of galls) influence the distribution of galls on leaves. Based on the results of previous studies we hypothesized that (1) light conditions for leaf growth (expressed as SLA) will affect the distribution of galls in the crowns of trees, with a preference for galling shaded leaves (high SLA), (2) *H. annulipes* avoids south-facing leaves, those that receive the most sunlight, and (3) *H. annulipes* prefers to gall the proximal zone of a leaf.

MATERIALS AND METHODS

Beech gall midges, *Hartigiola annulipes* Hartig (Cecidomyiidae), produce one generation each year and spend most of their life within single-chambered, hairy galls that develop on leaves of European beech (*Fagus sylvatica* L.) (Rohfritsch, 1971). The galls detach from the leaves during late summer and early autumn and overwinter in the leaf litter. Imagines emerge from galls in spring, and after mating, females lay eggs on the bottom side of leaves at the beginning of May (Rohfritsch, 1971). In this study, the number of galls counted on leaves included galls that failed to develop and survive, galls that matured and detach from the leaves, and galls still attached to leaves. The purpose was to study the preferences of *H. annulipes* for inducing galls on different parts of a leaf. Whether the galls successfully matured was not measured in this study. Due to the possible effect of microclimatic conditions on the distribution of galls at tree crown and leaf

levels, the locations chosen for this study were different types of forests. Leaves collected were free of signs of herbivory and pathogens as far as it was possible to determine with the naked eye.

This study is based on two sets of data, the first was used to determine preferences for galling leaves facing particular cardinal directions and for an initial study of the distribution of galls on these leaves. The second was used to model the relationship between the factors affecting the distribution of galls on leaves. The permission to collect leaves in nature reserves was provided by the Regional Directorate for Environmental Protection in Gorzów Wielkopolski, Poland (document ID: WPN-1-6205.28.2015.AI). To better interpret the results, each location was assigned a label (two beech forests: BF-01 and BF-02 – both are nature reserves, two Scots Pine forests: PF-01 and PF-02 – both are forestry managed forests, one park: P-01 and one urban forest: UF-01) (Table 1).

Distribution: cardinal directions and the preliminary study of the distribution of galls on leaves

To determine whether there is a preference for galling leaves facing a particular cardinal direction, three thousand leaves were randomly collected in October 2015 from three locations in western Poland (Table 1).

At each location (BF-01, BF-02 and PF-01), one thousand leaves (with and without galls) were gathered from five randomly chosen beech trees (several years old) growing in the understory: 200 leaves per tree and 50 per cardinal direction (N, S, E, W). The maximum height at which the leaves were collected was 2 m. Galls of *H. annulipes* were counted in three zones on the leaf lamina: proximal, medial and distal. Each zone was determined by dividing the leaf perpendicularly to the midrib. The proximal zone was defined as the zone at the base of the leaf (Appendix 1).

To determine whether there were differences in the quality of leaves facing each of the four cardinal directions, their specific leaf areas (SLA [cm²/g]) were determined as follows: 100 leaves were collected from five understory beech trees during late summer, including 20 per tree and 5 per cardinal direction (N, S, E, W) in each stand. The leaves were scanned using an Epson Perfection V700 scanner (Seiko Epson Corporation). The images were processed in ImageJ software version 1.48 by Wayne Rasband, National Institutes of Health, USA, to determine leaf areas. Then, the leaves were oven-dried (60°C, two days) and weighed using an OHAUS Corp. Adventurer Pro scale Model AV2102CM with d = 0.01 grams (precision of scale division).

Table 1. Description of study sites included in the two data sets, with the numbers of trees chosen for collecting leaves and the numbers of leaves collected at each location.

Location	Symbol	Coordinates	Characteristics of the location	No. of leaves	No. of trees
Data set I					
“Bukowa Góra” reserve	BF-01	51°52'17"N, 15°44'25"E	Natural beech forest located on a hill near the Odra river (Bobrowniki in Otyń community)	200	5
“Nad Jeziorem Trześniowskim” (NJT) reserve	BF-02	52°20'41"N, 15°17'22"E	Natural beech forest at the Trześniowskie (Ciecz) Lake in Łągów	200	5
Słupsk district in Zielona Góra	PF-01	51°55'55"N, 15°34'25"E	Cultivated Scots pine (<i>Pinus sylvestris</i> L.) forest (57 years old) in which young beech trees occur in the understory	200	5
Data set II					
“Bukowa Góra” reserve	BF-01	51°52'17"N, 15°44'25"E	Natural beech forest located on a hill near the Odra river (Bobrowniki in Otyń community)	265	5
Nowy Kisielin	P-01	51°55'51"N 15°36'46"E	A park with a mixed canopy, consisting of mainly broad-leaved trees	110	1
Słupsk	UF-01	54°27'12"N, 17°02'28"E	Mixed, urban forest in Słupsk	263	5
Zielona Góra	PF-02	51°53'58"N, 15°29'22"E	Cultivated Scots pine forest near Dzika Ochla pond in Zielona Góra	454	5

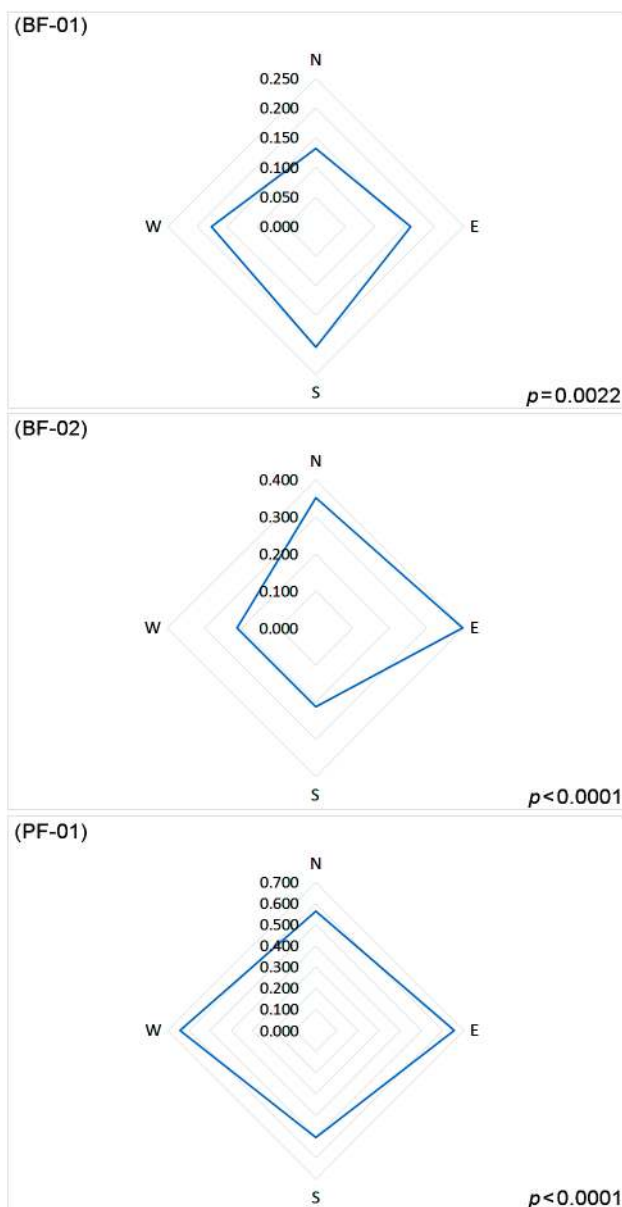


Fig. 1. Radar charts based on χ^2 tests, presenting frequency of occurrence of *H. annulipes* galls on leaves facing each of the four cardinal directions (N, E, W, S) at each study site. BF-01 (“Bukowa Góra” reserve) and BF-02 (“Nad Jeziorem Trześniowskim” reserve) are natural beech forests. PF-01 (Stary Kisielin – Zielona Góra) – a managed Scots pine forest with beech trees occurring in the understory. Probabilities shown in each chart represent p values obtained from the χ^2 tests.

Distribution of galls on leaves – a model

To determine the frequency of occurrence of the galls on different parts of a leaf, 1092 leaves of the common beech, each bearing at least one gall of *H. annulipes*, were collected at four locations (BF-01, P-01, UF-01, PF-02) in Poland from trees growing in the understory (up to 3 m tall) between September and October 2015 (Table 1). Locations of the material collected differed in terms of type of forest, which is expected to be an important factor affecting the results. The number of leaves collected at each location was different due to differences in the availability of beech trees, abundance of galled leaves and forest management. In the park in Nowy Kisielin (P-01), there was only a single European beech tree growing in the understory sufficient for material

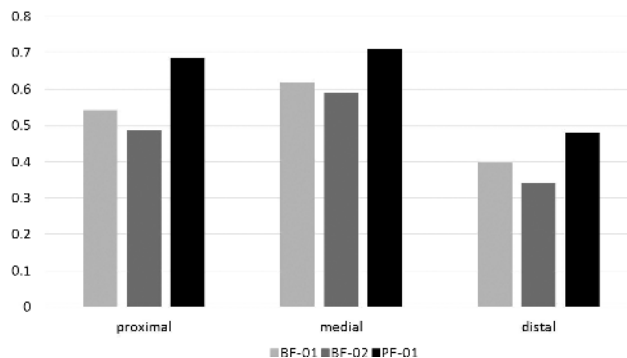


Fig. 2. Differences in the frequency of the occurrence of galls in different leaf zones at three study sites based on a contingency analysis (χ^2). Differences between locations were significant (BF-01: $p = 0.0002$, BF-02 and PF-01: $p < 0.0001$). BF-01 (“Bukowa Góra” reserve) and BF-02 (“Nad Jeziorem Trześniowskim” reserve) are natural beech forests. PF-01 (Stary Kisielin – Zielona Góra) – a managed Scots pine forest with beech trees occurring in the understory.

collection, while five trees were chosen for collection at the other three locations. In Zielona Góra (PF-02), the infestation level was very high, and thus, the number of leaves collected there was the highest.

The area [mm²] and length [mm] along the midrib of each leaf was measured using ImageJ software. ImageJ was also used to measure the perpendicular distance of *H. annulipes* galls from the midrib [mm]. The total number of *H. annulipes* galls was recorded.

Statistical analyses

The χ^2 test was used to check differences between the presence and absence of galls in each leaf zone, as well as the preference for particular cardinal directions (data set 1). An ANOVA was used to estimate the effect of individual beech trees, cardinal direction and interaction on SLA. One gall was randomly chosen out of 1092 galls for each leaf analyzed. We used log-transformed values of the distance between the midrib and galls to normalize the distribution of the data. Then, a linear mixed model (LMM) was used to determine the effect of the factors (leaf zone, leaf blade area, number of galls, location and number of beech trees) on the distance between the midrib and galls (dependent variable) (data set 2). The numbers of beech trees and locations were treated as random factors and beech trees were nested in the locations (the script is shown in Appendix 2).

Statistical analyses were performed using JMP PRO 13 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Hartigiola annulipes galls were very common as they were found on more than 35% of beech leaves. A total of 4512 *H. annulipes* galls were recorded on 3000 leaves. The abundance of galls differed at the different locations. Most galls (3375) were found at PF-01; and 655 and 482 were recorded in the nature reserves BF-02 and BF-01, respectively. Of the collected leaves, 1949 were not galled and 346 had only one gall. The highest number of galls per leaf was 76 (in PF-01). The numbers of galls and galled leaves are shown in Appendix 3.

The χ^2 analysis of gall presence (0 or 1) and the cardinal direction revealed different frequencies at each location studied, which are presented in the form of a radar chart

Table 2. ANOVA results of relationships between specific leaf area (SLA) of beech leaves and the following three factors: individual beech tree, cardinal direction and interaction. Abbreviations: DF – degrees of freedom, DFE – degrees of freedom for error, F – result of the F-test, p – probability.

Source of variation	DF	DFE	F	p
Location	2	12	1.5877	0.2445
Tree (Location)	12			
Cardinal direction	3	36	1.7374	0.1767
Location x Cardinal Direction	6	36	0.5550	0.7628
Cardinal direction (Tree(Location))	36			
Residual	240			

(Fig. 1). At BF-01, *H. annulipes* preferred south-facing and avoided north-facing leaves ($\chi^2_{(3, N=3000)} = 14.595$, $p = 0.0022$). At BF-02, they preferred east-facing leaves and strongly avoided west- and south-facing leaves ($\chi^2_{(3, N=3000)} = 98.522$, $p < 0.0001$). At PF-01, the insects mainly galled east- and west-facing leaves, while avoiding south-facing ones ($\chi^2_{(3, N=3000)} = 44.696$, $p < 0.0001$).

The presence of galls in different leaf zones differed significantly at each location (BF-01: $p = 0.0002$, BF-02 reserve and PF-01: $p < 0.0001$). The medial zone was galled the most, while the distal zone was avoided (Fig. 2).

The influences of location, cardinal direction and interaction factors on the SLA were not significant (Table 2).

The distance between the mid-rib and galls was significantly associated with two factors: leaf blade area and leaf zone (Table 3). The distance between the mid-rib and galls increases with leaf area, and the distance between the mid-rib and galls in the distal zone was shorter than in the proximal zone ($p < 0.0001$). The analyses were done for the proximal zone. The model equation obtained was $Y = 0.3273 - 0.2334DZ + 0.0002A$, where (Y) was the distance between the mid-rib and galls, (DZ) was the distal leaf zone and (A) was the leaf area.

DISCUSSION

Our results show that *H. annulipes* chooses leaves facing different cardinal directions. Skrzypczyńska & Lorenc (2005) and Wrzesińska (2012) claim that *H. annulipes* galls are more common on shaded leaves. The amount of light available for plants in a forest depends on the type and age of the forest (Peters, 1997; Guo et al., 2017) and differs in forests composed of conifers and broad-leaved species (which may shed their leaves seasonally) due to variations in the densities of their canopies, species of trees present, their growth rates and requirements. Older trees, which are usually taller than those growing in the understory, as well as fast growing individuals, compete more efficiently for light than smaller or slow-growing trees (Peters, 1997; Kobayashi et al., 2000; Schulte et al., 2013). These facts may be an important issue affecting the results of this study since the trees studied were growing in the understory of different types of forest. However, since gall midges are considered to be poor flyers (Urban, 2000), studies of gall distribution in mature tree crowns may be pointless. In contrast, Kampichler & Teschner (2002) do not agree that gall midges are poor flyers. The overstorey tree species

Table 3. Linear mixed model relating distance between the mid-rib and galls on different parts of a leaf (zone), leaf area and number of *H. annulipes* galls. Model includes locations and trees nested in location as random effects. SE – standard error.

Term	Estimate	SE	p
Intercept	0.3273	0.0911	0.0157
Zone [distal]	-0.2334	0.0453	<0.0001
Zone [medial]	0.0616	0.0356	0.0840
Leaf area	0.0002	0.00002	<0.0001
Number of galls	0.0010	0.0041	0.8030

composition in forests may influence the amount of light reaching the understory when the leaves are mature (Sercu et al., 2017); moreover, natural and managed forests with different species compositions may influence understory plants differently (Thomsen et al., 2005), and various types of managed forests influence the biodiversity (Gao et al., 2014). These findings indicate it is likely that the stands studied differed in terms of environmental conditions (e.g., light transmittance), which may have influenced the preferences for leaves facing in particular directions.

There were no differences in SLA, which was assumed to be an indirect measure of light conditions. The expectation was that SLA would be important in determining preferences for leaves facing different directions since there are reports of relationships between SLA and herbivory (Poorter et al., 2004; Parthasarathy & Anil, 2016). The results presented do not support the first hypothesis that SLA depends on the direction that leaves face. At BF-01, the galls were mainly on south-facing leaves and north-facing leaves were avoided. At the BF-02 reserve, where there is a natural beech forest by a lake, and at PF-01, where we collected leaves from understory trees growing in a managed conifer forest, the first leaves to be galled were east-facing (which are the first leaves to be exposed to sun in the morning). The comparison of the frequency of *H. annulipes* galls at BF-02 and PF-01 indicates that even though insects prefer east-facing leaves, environmental conditions modify preferences towards other cardinal directions. By environmental conditions, we specifically mean microclimatic differences (abiotic factor) between beech and pine forests and the availability of trees for infestation (biotic factor). At BF-02, where leaves were collected from beeches growing on the western side of a lake, the east-facing leaves may receive more solar radiation due to openings in the canopy and distribution of the trees. However, at PF-01, a managed pine forest with a few beech trees, similar preferences were recorded for west- and east-facing leaves. It seems that in this case, *H. annulipes* is more opportunistic and uses the resources randomly. It is likely that females choose oviposition sites based on the abundance of food rather than its quality (vigour). Statistical analyses indicated no influence of the factors studied on SLA. Even though the trees chosen for SLA analyses were solitary and in the understory (not in contact with other trees) in order to avoid the effect of shading, it was not possible to determine the effect of cardinal direction on leaf quality (expressed as SLA). These results led to the assumption that

the light conditions were similar in each of the four main cardinal directions.

We could not find any evidence in the literature that females of *H. annulipes* were attracted to light, which could account for their oviposition behaviour. Do females become active in the morning after sunrise and lay their eggs on young, east-facing leaves? We speculate that they do, and later during the day, they may randomly lay their eggs on leaves, due to an increase in the activity of the insects in response to rising temperatures during the day. Skrzypczyńska & Lorenc (2005) and Wrzesińska (2012), who claim that *H. annulipes* galls were more common on shaded leaves, did not support this thesis with a statistical analyses, and the aim of their studies was not to determine the preferences of *H. annulipes*. Yukawa et al. (1988) show that *Mycophila* sp. (Cecidomyiidae) exhibits phototaxis. Similar conclusions come from the study of *Dasineura oxycoccana* Johnson (Cecidomyiidae) by Roubos & Liburd (2010) as it has the same preference for light and a southerly direction, whereas females of *Stenodiplosis sorghicola* Coquillett (Cecidomyiidae) are colour- and odour-attracted (Sharma & Franzmann, 2001). If females of *H. annulipes* show the same pattern of behaviour, they may also react to the different light wavelengths reflected by or passing through young leaves during the day according to the position of the sun. The optical properties of leaves and bark of common beech change during the plant's lifetime (Pilarski et al., 2008). Leite et al. (2011) report that an unidentified galling species belonging to the Eurytomidae (Hymenoptera) prefers the middle zones of *Caryocar brasiliense* Camb. north-facing leaflets. The positioning of galls may be strongly influenced by inter-specific competition within a tree crown (Leite et al., 2011), with fungal endophytes being a threat to the survival of gall insects (Wilson & Carroll, 1997) and leaf-miners (Wilson & Faeth, 2001), or as McGeoch & Price (2004) show, positioning may be weakly affected by inter-specific competition. For *H. annulipes*, such a competitor may be *Mikiola fagi* Hartig (Diptera: Cecidomyiidae), which also galls the leaves of *F. sylvatica*. Nonetheless, *M. fagi* galls were absent at PF-01, while they occur at BF-01 and BF-02, but *H. annulipes* galls were by far the most abundant (personal observation). Thus, a comparison of distributions of the galls of both species occurring in the areas studied is difficult. This raises a need for further studies. *Aphidoletes aphidimyza* Rondani (Cecidomyiidae), a predatory gall midge, does not avoid ovipositing at sites already occupied by conspecific females and their eggs (Kohandani et al., 2017). Females of *A. aphidimyza* tend to clutch their eggs at a single oviposition site (Kohandani et al., 2017). Perhaps females react to a site that is acceptable for other females as an optimal site. However, intraspecific competition cannot be avoided, as the food resources for larvae are limited. This would account for the occurrence of 76 *H. annulipes* galls on a single leaf (Appendix 3) at PF-01, where beech trees are rare.

Food resources and natural enemies are the most important biotic factors influencing the success and survival

of herbivores (Whitham, 1978; Kaplan et al., 2014) along with host plant phenology (Urban, 2000; Aoyama et al., 2012). In addition, ontogeny and microclimatic conditions modify the degree of herbivory (Stiegel & Mantilla-Contreras, 2018). Since galling animals are specialists, it is expected that they are favoured or limited by these two factors. Hence, galls should occur in a place where it can be well-supplied by the host plant and where the risk of predation or parasitism is low. Furthermore, the cardinal direction in which a leaf faces and on which galls are located could determine the likelihood of it being attacked by herbivores, predators (Evans & Gregoire, 2006) and/or parasitoids (Zargaran et al., 2011). In contrast, some studies indicate no preference in the occurrence of galls facing any of the four cardinal directions, e.g., *Forda riccobonii* Stefani (Hemiptera: Pemphigidae) forming galls on the *Pistacia atlantic* Desf. (Martinez, 2009). The random distribution of galls according to cardinal directions minimizes parasitoid attacks at some sites since there are studies indicating that light conditions may modify the behaviour of parasitoid wasps (Luo & Chen 2016; Cochard et al., 2017; Kelch et al., 2017). Consequently, such a distribution of galls might be considered as a trade-off between avoiding parasitoids and occupying the most beneficial galling sites.

Moreover, there are reports that herbivores prefer particular parts of plants. Various chemical compounds protect plants against herbivores and pathogens. For example, phenolic compounds, the quantities and concentrations of which differ in different plant species and their parts (Abrahamson et al., 2003; Oliveira Ribeiro et al., 2014). Furthermore, they change seasonally (Bernal et al., 2013; Oliveira Ribeiro et al., 2014) and with age (Bhakta & Ganjewala, 2009) and may be influenced by environmental factors (Estiarte et al., 1994; Loponen et al., 2001; Borges et al., 2013). Petrakis et al. (2011) report phenols in the leaves and bark of *F. sylvatica* in Greece and that infested beech leaves have higher concentrations of total phenolic compounds and that it increases with the number of insects attacking beech trees. The concentration of phenolics in host organs can vary, which affects the distribution of galls (Zucker, 1982; Bergvinson, 1997; Giertych & Karolewski, 2000; Gutterman & Chauser-Volfson, 2000; Ferreira et al., 2014). Thus, we expected that *H. annulipes* would avoid galling the distal zone and prefer the proximal third of leaves. Indeed, the results show that the frequency of galls occurring in the distal zone (Fig. 2) is the lowest when compared to the median and proximal zones. However, they also show that *H. annulipes* slightly prefers the median zone to the proximal zone of leaves, but only in natural beech forests. In the managed Scots pine forest, with a few beech trees in the understory (PF-01), the frequency of galling of the proximal and median zone is much lower. This may be due to the rarity of the host plant, which results in a high number of galls per leaf, the highest recorded number of *H. annulipes* galls on one leaf in PF-01 reached 76 (Appendix 3), while in the BF-01 and BF-02 reserves, it reached 20 and 12, respectively (Appendix 3).

The position of a gall on a leaf may also be important if the distance from the midrib is taken into consideration. The LMM model incorporates only two significant factors (zone of occurrence and leaf area) that influence the distance between a gall and the midrib. Relative to the proximal zone, the distance of a gall in the distal zone is significantly shorter, and galls that developed on the largest leaves tend to be further from the mid-rib. These results correspond with the obvious presumptions that as the distal zone narrows towards the tip, galls have to develop closer to the mid-rib, which also becomes thinner than in the proximal zone. There is still too little knowledge about the distributional patterns of galls on leaves. Giertych et al. (2013) report that leaf size and distance from the petiole to the gall (*C. quercusfolii*) are positively correlated, but they did not discuss the results for the distance of the gall to the midrib as they were not significant. Leite et al. (2011) show that the central parts of *C. brasiliense* leaflets are more frequently galled than the borders and midrib. We suppose that large leaves allow the establishment of galls further from the mid-rib than small leaves, because a larger area and thicker lateral veins provide galls and larvae with enough resources to ensure their growth and development. Furthermore, galls may not be affected by the high concentrations of harmful secondary metabolites flowing in the mid-rib. In this way, *H. annulipes* can optimize the location of its galls. Stiegel & Mantilla-Contreras (2018) studied herbivory rates of common beech at three different heights. The authors assume that it is impossible to establish a herbivory-pattern for a single species. Instead, they propose to study such patterns at the guild level.

It must be mentioned that the material used in this study was collected from understory trees, and thus, the results may be limited to explaining the distributional pattern of galls of *H. annulipes* on young trees. If this gall-midge is sensitive to concentrations of various chemical compounds, especially secondary metabolites, then it may respond with different distributional patterns at different heights and in different parts of the canopy. Dudt & Shure (1994) report that the shade-tolerant plant, *Cornus florida*, L. contains more phenolics than shade-intolerant *Liriodendron tulipifera* L. *F. sylvatica* is also considered to be a shade-tolerant species, and Stiegel et al. (2017) suggest that the high carbon content of *F. sylvatica* leaves in upper canopies reflect high concentrations of phenols. Numerous studies report a connection between the chemical quality of leaves and light availability (Crone & Jones, 1999; Roberts & Paul, 2006; Oliveira Ribeiro et al., 2014), with shaded leaves having high concentrations of phenols (Dudt & Shure, 1994); however, the latter is not a general rule, as proven by Karolewski et al. (2013) and suggested by Stiegel et al. (2017) in the case of *F. sylvatica*.

CONCLUSIONS

This study shows that *Hartigiola annulipes* galls the leaves of common beech randomly at the crown level and leaves facing different cardinal directions.

H. annulipes exhibits some preference for inducing galls on east-facing leaves and tends to avoid south-facing leaves.

H. annulipes prefers to induce galls in the medial zone of a leaf and avoids the distal zone.

SLA values did not explain the distribution of galls on leaves.

The choice of leaf zone can influence the distance between a gall and the mid-rib.

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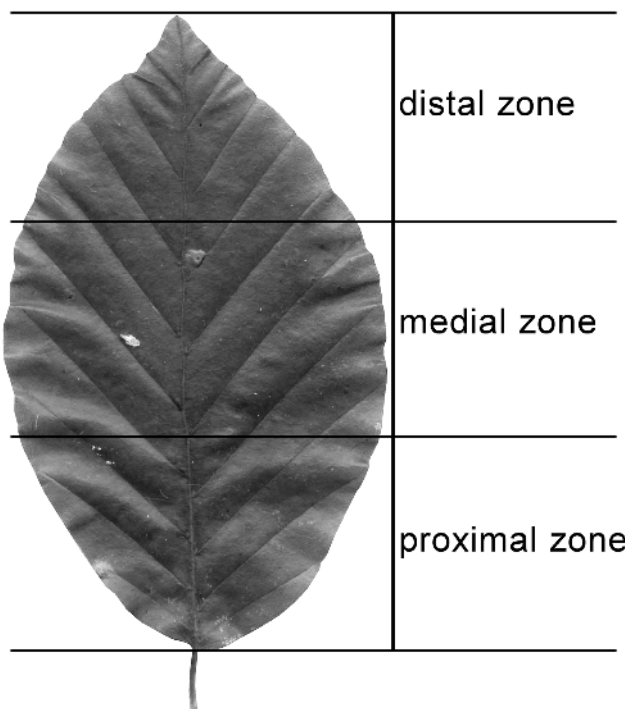
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Appendix 2. The script used in the linear mixed model (LMM). Statistical analyses were performed in the JMP PRO 13 (SAS Institute Inc., Cary, NC, USA).

Fit Model (Y:(Name(“Log[distance between the mid-rib and galls [mm]]”))),
Effects (:zone, :leaf area, :number of galls),
Random Effects (:location, :tree[:location]),
Personality (“Mixed Model”));

Appendix 3. Number of leaves with a particular number of *Harmandiola annulipes* galls recorded at each location.

Number of galls	Number of leaves at each location			Total
	Bukowa Góra (BF-01)	Nad Jeziorem Trześcińskim (BF-02)	Stary Kisielin (PF-01)	
0	832	707	410	1949
1	66	150	130	346
2	37	67	103	207
3	22	25	72	119
4	16	20	62	98
5	5	9	40	54
6	6	7	28	41
7	4	5	25	34
8	5	5	25	35
9	1	1	15	17
10	2	0	12	14
11	2	3	11	16
12	1	1	12	14
13	0	0	1	1
14	0	0	7	7
15	0	0	4	4
16	0	0	4	4
17	0	0	4	4
18	0	0	5	5
19	0	0	4	4
20	1	0	2	3
21	0	0	1	1
22	0	0	2	2
23	0	0	2	2
24	0	0	2	2
25	0	0	3	3
26	0	0	2	2
28	0	0	1	1
29	0	0	1	1
30	0	0	1	1
35	0	0	2	2
36	0	0	1	1
37	0	0	1	1
49	0	0	1	1
52	0	0	1	1
54	0	0	1	1
56	0	0	1	1
76	0	0	1	1
Total	1000	1000	1000	3000



Appendix 1. The figure shows the way the leaf was divided into three zones (proximal, medial and distal) along the mid-rib. Each zone is of equal length along the mid-rib. The proximal zone is the nearest to the petiole.