

Gall Abundance and Leaf Size as Factors Affecting the Hypersensitive Reaction in the Common Beech (*Fagus sylvatica*)

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Abstract

Plant galls being abnormal growths can alter the hypersensitive reaction (HR), a defensive mechanism that is supposed to kill pathogens, parasites and all other alien organisms, including gall-inducers. The aim of the study was to find an answer to the question, whether leaves of different size react similarly to a various number of galls of *Mikiola fagi* and *Hartigiola annulipes*. Leaves (3,179 with 7,011 galls of *H. annulipes* and 482 with 637 galls of *M. fagi*) from seven localities, including two nature reserves, were collected in western Poland in 2014. Analyses conducted as the logistic regression test showed that the HR in large leaves as reaction against *H. annulipes* was more often (but not statistically significant) than in small leaves. This trend was not observed in the case of *M. fagi* while there was no relationship between the HR and the leaf size for *M. fagi*. However, the linear regression test for both species showed a relationship between HR occurrence and the number of galls on the leaf blade.

Keywords: hypersensitive reaction, galls, *Hartigiola annulipes*, *Mikiola fagi*, beech

Introduction

Plants as the living organisms may act as hosts for development and growth of pathogens and parasites. Thus, they use various defensive mechanisms, precluding further distribution and development of alien organisms. Amongst them, the hypersensitive reaction (HR) should be mentioned. As its result, cells closest to a pathogen or parasite die, leading to the local necrotic spots (Bentur and Kalode 1996, Fernandes et al. 2003, Iakimova 2005).

Such necroses also surround the place of induction of galls (Bentur and Kalode 1996, Fernandes et al. 2003). Among galling insects, the representatives of the gall wasps (Hymenoptera: Cynipidae), adelgids (Hemiptera: Adelgidae) and gall midges (Diptera: Cecidomyiidae) may be mentioned. These groups are often characterised by galls with complex structure and geometric shape, what is a subject of intensive studies. Among gall midges, there are two species: *Mikiola fagi* (Hartig 1839) and *Hartigiola annulipes* (Hartig 1839), that are native to Poland and develop galls on leaves of the common beech *Fagus sylvatica* L. (Skuhrava and Skrzypczynska 1983). Both species develop single-chambered galls on the upper side of the leaf lamina. They produce only one generation during a year: their larvae pupate after wintering within galls in the litter, then imagines leave

their galls and mate during spring (Rohfritsch 1971, Urban 2000, Redfern 2011, Akkuzu 2015). Fernandes et al. (2003) showed that the HR mechanism can successfully work against both species mentioned above.

As the galling insects exhibit the phytophagous and parasitic character, they can elicit the HR, meant to fight them. We tried to find a relationship between the leaf size, number of galls of respective galling species, and occurrence of HR. As there are hypotheses connected to the plant vigour, we hoped to determine whether the short and long leaves would react in the same manner to the similar number of galls. There are two most popular hypotheses: Plant Vigour Hypothesis (PVH), which concludes that phytophagous insects prefer large leaves and other large parts of the plant due to the highest availability of nutrients and other compounds (Price 1991). On the other hand, Optimal Module Size Hypothesis (OMSH) implies that the medium-sized plant modules are mostly chosen (McKinnon et al. 1999, Flaherty and Quiring 2008) because they do not raise risk that they will overpower the gall-inducing process, unlike the large modules, with high vigour. Secondly, the medium-sized modules provide much more nutrients than the small modules. As such hypotheses state that insects choose plant parts of different size parameters, we expected that *H. annulipes* and *M. fagi* galls alter the HR differently in regard to the leaf

size and number of galls. Thus, we decided to find an answer to the question:

Does the HR intensity change with the leaf size in regard to a various number of galls?

Materials and Methods

Leaves with galls of *H. annulipes* and *M. fagi* were collected in seven localities (Table 1) in Lubuskie Voivodeship (western Poland) in 2014 from different common beech *F. sylvatica* trees. The seventh location, Łuk Mużakowa, is represented by a small probe since field studies conducted there drew the interest to the subject of this paper. Collecting the leaves in the reserves: “Bukowa Góra” (Bobrowniki, the Community of Otyń) and “Nad Jeziorem Trześniowskim” (Łagów, the Community of Łagów) was possible due to the permission received from the Regional Directorate for Environmental Protection in Gorzów Wielkopolski (document ID: WPN-1-6205.51.2014.AI), and after informing the respective forestry management about the field studies.

Table 1. Localities where the leaves were collected or studied in field with respective numbers of *H. annulipes* (N-Ha) and *M. fagi* galls (N-Mf) and total collected number of leaves (NL). Number of galls that were surrounded by chlorotic or necrotic spots is shown in parentheses

Location	Approximate coordinates	NL	N-Ha	N-Mf
„Bukowa Góra” reserve	N51° 52' 29.47"; E15° 44' 36.9159"	342	476 (196)	123 (49)
„Nad jeziorem Trześniowskim” reserve	N52° 20' 33.856"; E15° 17' 18.6484"	929	1249 (651)	289 (106)
„Stara Dąbrowa w Korytach”	N52° 17' 13.4411"; E15° 10' 41.9047"	631	1493 (767)	40 (17)
„Las Żarski”	N51° 36' 6.9261"; E15° 7' 45.7801"	559	930 (390)	112 (55)
„Buczyna Szprotawsko-Piotrowicka” - Szprotawka	N51° 31' 44.7445"; E15° 38' 6.661"	591	1517 (761)	8 (0)
„Lasy Bierzwnickie” - Osiek	N52° 58' 44.5453"; E15° 42' 55.5671"	553	1203 (736)	55 (27)
„Łuk Mużakowa” Landscape Park	N51° 32' 51.3621"; E14° 44' 12.3804"	56	141 (61)	11 (3)

The leaves were collected from twigs growing in range 0.5-2.5 m above the ground, from trees that were at least 3 m tall, including mature trees with branches that could be reached. About 100 leaves with galls were collected from every tree. In some cases, the number was considerably lower. That was determined by a number of leaves with galls. In total, 3,179 leaves with 7,011 galls of *H. annulipes* and 482 leaves with 637 galls of *M. fagi* were collected from 37 beech individuals. After collecting, leaves were studied in respect of the local colour changes occurring nearby galls. The galls were classified in three categories: 1) without colour changes, 2) yellowish – chlorotic changes, 3) brownish – necrotic

changes. The analyses were conducted for both types of reaction (2 and 3) together due to a long collection period (June-September) because chlorotic changes gain necrotic character after some time.

The leaf size was determined as a length of the leaf blade, measured along the mid-rib by an electronic caliper (0.01 mm accuracy).

To determine the relationship between the HR and number of galls in respect of the leaf size, the logistic regression was used. The presence of the HR (chlorotic or necrotic changes) was labelled as “1” and the absence of the HR was treated as “0”. Secondly, to check the simple relationship between the number of galls and leaf size, the linear regression test was used. The statistical analyses were conducted in JMP 11.2.0 software, the product of SAS Institute Inc.

Results

Among gathered material, 3562 galls of *H. annulipes* induced the HR (51% of analyzed galls), and 257 galls of *M. fagi* among 637 collected galls induced the HR (40%). Analyses conducted as a linear regression test (Figures 1 and 2) showed

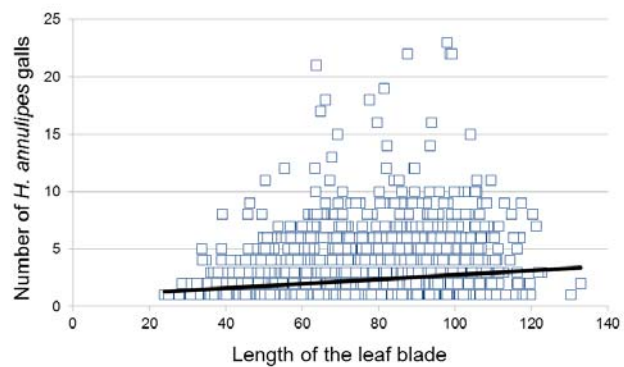


Figure 1. Regression test for the number of *H. annulipes* galls and the length of the leaf blade; $r = 0.172308$, $N_{(galls)} = 0.7948666 + 0.0194847 * Leaf_size$, $P < 0.0001$

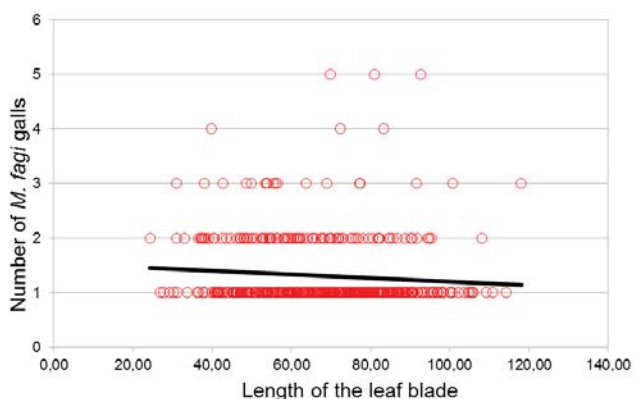


Figure 2. Regression test for the number of *M. fagi* galls and the length of the leaf blade; $r = - 0.088402$, $N_{(galls)} = 1.5271589 - 0.0032243 * Leaf_size$, $P = 0.0429$

a very weak negative relationship between number of galls and the leaf size for *M. fagi*: ($r = -0.088402, N_{(galls)} = 1.5271589 - 0.0032243 \times Leaf_size, P = 0.0429$) and a very weak positive relationship between such parameters for *H. annulipes* ($r = 0.172308, N_{(galls)} = 0.7948666 + 0.0194847 \times Leaf_size, P < 0.0001$). Nominal logistic regression (Table 2) showed that occurrence of the HR is not related with the leaf size for *M. fagi* and for *H. annulipes*, while there is a positive relationship between occurrence of the HR and number of galls for both species (Figure 3). The odds ratio indicates that the chance of occurrence of the HR is eight for *H. annulipes* and five *M. fagi* greater when the leaf has more than one gall.

Table 2. The logistic regression model describing the occurrence of the hypersensitive reaction on beech leaves as the result of occurrence of *Hartigiola annulipes* and *Mikiola fagi* galls

Species	Effect	Estimates	Std Error	ChiSquare	P
<i>Hartigiola annulipes</i>	Intercept	2.022	0.1794	127.06	<.0001
	Numbers of galls	-1.082	0.0520	432.09	<.0001
	Leaf length	-0.0039	0.0022	3.04	0.0812
<i>Mikiola fagi</i>	Intercept	1.9028	0.5054	14.17	0.0002
	Numbers of galls	-1.404	0.2093	45.03	<.0001
	Leaf length	0.0038	0.0058	0.44	0.5059

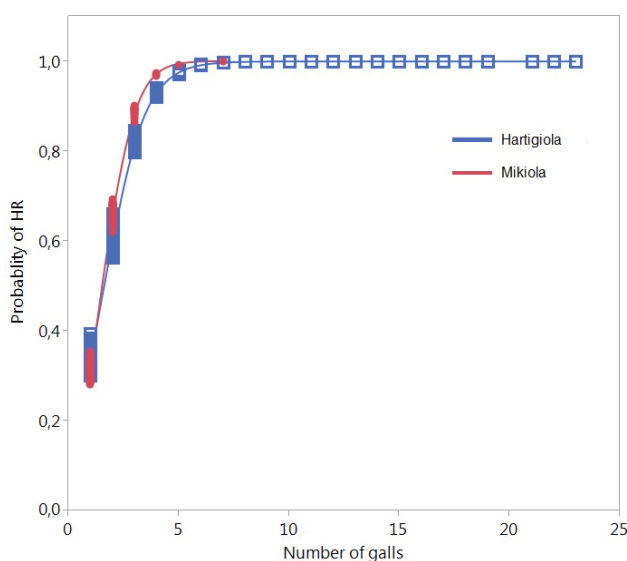


Figure 3. Probability functions of the hypersensitive reactions (HR) associated with number of galls occurring on a single leaf. HR (0 for a lack, 1 for noted) probabilities are shown for *Hartigiola annulipes* (the blue curve) and *Mikiola fagi* (the red curve)

Discussion and Conclusions

The number of *H. annulipes* galls is weakly and positively related with length of the leaf. It may show that females choose large leaves for laying their eggs more often than medium-sized leaves. However, since the relationship is weak, the *H. annulipes* females may also exhibit meaning-

ful interest in the medium-sized leaves. The *H. annulipes* galls are small and seem not to stress the plant when occur individually on the leaves, however they rarely occur singly on the leaf lamina. *H. annulipes* can lead to defoliation of the young beech trees if the galls occur in high numbers (Skrzypczyńska 2008).

Urban (2000) concluded that 82% of mortality of the *M. fagi* larvae is due to the activity of parasitoids. It is interesting that, on the contrary, Fernandes *et al.* (2003) also showed that the HR can be the reason of nearly 80% of deaths of *H. annulipes* and *M. fagi* larvae without species discrimination. It seems that there is a need for data from localities where *M. fagi* is much more abundant to find out whether the relationship between number of galls and the HR exist. Eventually, the material collected in years of mass gradation may be helpful. As the *M. fagi* galls are larger and need more nutrients to develop, we expected them to be more stressful for plant which would try to defend using the HR. Indirectly, however, weak negative relationship between number of galls and the leaf size may suggest that large leaves defend more efficiently against the gall-induction and prevent the galls from developing. Moreover, *M. fagi* might not prefer large leaves for egg-laying for some reason and thus avoid the HR.

The statistical analyses showed that the leaf length (indicating vigour) is not related with occurrence of the HR for leaves with galls of *H. annulipes* and *M. fagi*. It was expected that large leaves would defend against galling process more effectively according to the PVH. However, the results showed that between two studied factors the number of galls plays a decisive role in triggering the HR. It must be noted that the intensity of the HR can be genetic-related (Bentur and Kalode 1996) and different beech populations may differently depend on the HR.

M. fagi is reported to lay eggs in the end of March and beginning of April (Urban 2000), while *H. annulipes* oviposits on the first days of May (Rohfritsch 1971). Such difference leads females to lay eggs on twigs and leaf-buds (*M. fagi*) (Urban 2000) or on the abaxial side of the leaf blade (*H. annulipes*) (Rohfritsch 1971). This is reflected in well-developed *Mikiola* galls seen in June, while *Hartigiola* galls are then starting to develop on the adaxial side of the leaf blade. Since *H. annulipes* infests beech leaves of the later stage of development than *M. fagi*, they may be fought against more successfully by means of HR. Nowadays, however, when the climate is changing and winters in Poland are much milder, *H. annulipes* life cycle seems to be strongly affected (self-observation; not published data). In 2015/2016 flying imagines were seen during the winter months from December to March and thus, probably, destined to die without offspring. As the presence of the HR is related to the number of developing galls, the climatic changes can significantly decrease the number of galls and weaken the HR response of trees.

We conclude that:

1) the hypersensitive reaction is positively related with the number of galls.

2) the length of the leaf blade has no influence on triggering the hypersensitive reaction.

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References

- Akkuzu, E., Dingiloglu, E., Unal, S.** 2015. Edge effects on gall-inducing insect *Mikiola fagi* (Diptera: Cecidomyiidae) in the oriental beach forests. *Pakistan Journal of Zoology* 47(3): 685-690.
- Bentur, J. S., Kalode, M. B.** 1996. Hypersensitive reaction and induced resistance in rice against the Asian ricegall midge *Orseolia oryzae*. *Entomologia Experimentalis et Applicata* 78: 77-81.
- Fernandes, G. W., Duarte, H. and Lüttge, U.** 2003. Hypersensitivity of *Fagus sylvatica* L. against leaf galling insects. *Trees* 17: 407-411.
- Flaherty, L. and Quiring, D.** 2008. Plant module size and dose of gall induction stimulus influence gall induction and galler performance. *Oikos* 117: 1601-1608.
- Iakimova, E. T., Michalczyk, L. and Woltering, E. J.** 2005. Hypersensitive cell death in plants – its mechanisms and role in plant defence against pathogens. *Journal of Fruit and Ornamental Plant Research* 13: 135-138.
- Mckinnon, M., Quiring, D. T. and Bauce, E.** 1999. Influence of tree growth rate, shoot size and foliar chemistry on the abundance and performance of a galling adelgid. *Functional Ecology* 13: 859-867.
- Price, P. W.** 2011. The plant vigor hypothesis and herbivore attack. *Oikos* 62: 244-251.
- Redfern, M.** 2011. Plant galls. Collins, London, 576 pp.
- Rohfritsch, O.** 1971. Développement cécidien et rôle du parasite dans quelques galles d'arthropodes (Development of galls and the role of parasites of different galls of arthropods). *Marcelia* 37(4): 233-339.
- Skrzypczyńska, M.** 2008. Masowy pojaw hartigiółki bukowej *Hartigiola annulipes* (Hartig) (Diptera: Cecidomyiidae) na liściach buka zwyczajnego *Fagus sylvatica* L. w Ojcowskim Parku Narodowym (The mass appearance of *Hartigiola annulipes* (Hartig) (Diptera: Cecidomyiidae) on the leaves of beech *Fagus sylvatica* L. in the Ojców National Park). *Sylvan* 2: 26-29.
- Skuhřavá, M. and Skrzypczyńska, M.** Przegląd pryszczarków (Cecidomyiidae, Diptera) Polski (A review of gallmidges (Cecidomyiidae, Diptera) of Poland). *Acta Zoologica Cracoviensia* 26(12): 387-420.
- Urban, J.** 2000. Beech Gall Midge (*Mikiola fagi* Htg.) and its natural enemies. *Journal of Forest Science* 46 (12): 543-568.

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