Plant-mediated interaction: a first record of thrips feeding on *Hartigiola annulipes* (Diptera: Cecidomyiidae) galls

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ABSTRACT

Galls formed by insects can act as sinks for nutrients and attract other herbivores to feed on gall tissues, which initiates interspecific competition, sometimes nurturing the herbivorous insects and restraining the gall-inducing insect, particularly when this competition is plant-mediated. Here, to our knowledge, we provide the first evidence of a close relationship between a gall insect, *Hartigiola annulipes* (Diptera: Cecidomyiidae), and a sap-sucking, *Liothrips setinodis* (Thysanoptera: Phlaeothripidae). The thrips were observed feeding on young *H. annulipes* galls, formed on the common beech (*Fagus sylvatica*) leaves during spring. Among randomly chosen beech trees, 100 current-year shoots were surveyed to determine the number of *H. annulipes* galls and the presence of thrips on the leaves. Our results show that *L. setinodis* specimens were found significantly more frequently on leaves infested by the galler than on uninfested leaves. The consequences of feeding thrips at the site of gall formation are not known yet, but it can be supposed that they are unfavourable to the gall insects and therefore could be beneficial for the host plant.

The influence of galling insects on the host's biochemical properties has been shown in numerous studies concerning distribution of galls and defensive mechanisms of the host plants (Petersen and Sandstrom 2001, Koyama *et al.* 2004, Pascual-Alvarado *et al.* 2008,). Since galls may act as sinks for nutrients (Koyama *et al*. 2004, Compson *et al.* 2011, Castro *et al.* 2012), they may attract other herbivores. In contrast, the galling process may also increase the defensive response of the host plant and hence decrease the nutrient availability, thus discouraging other herbivores to feed (Koricheva *et al.* 1998, Pascual-Alvarado *et al*. 2008), and this can lead to a state of stand-off that promotes the suboptimal development of both the host and the herbivore (Furstenberg-Hagg *et al.* 2013). Interspecific competition between herbivore insects appears to be plant-mediated (Inbar *et al.* 1995, Petersen and Sandstrom 2001, Qureshi and Michaud 2005) and can be affected by environmental conditions (Foote *et al.* 2017).

Hartigiola annulipes (Hartig, 1839) (Diptera: Cecidomyiidae) is a gall midge species producing one generation per year. Females lay eggs in April/May on the abaxial side of the common beech (*Fagus sylvatica* L.) leaves, and the larvae develop in hairy, single chambered galls occurring on the adaxial leaf side (Rohfritsch 1971).

During field studies, it was noticed that leaves bearing galls of *H. annulipes* in an early stage of development were frequently visited by adult phlaeothripids (Thysanoptera: Phlaeothripidae). These thrips later on were identified as *Liothrips setinodis* (O. M. Reuter, 1880), a common European species found on the bark and leaves of various trees (Gauss 1960, Moritz 2006, Kucharczyk and Stanisławek 2010). The fact that adults of *L. setinodis* had been observed feeding on young-gall tissues (approximately 4 weeks old, Fig. 1) raised questions about a potential relationship between these thrips and the gall midge. To our knowledge, this is the first time such a relationship between *L. setinodis* and *H. annulipes* is described. Our observation led to the following hypothesis: the thrips are attracted by young *H. annulipes* galls and mainly feed on galled leaves. This hypothesis was tested by confronting the number of galls and thrips on randomly chosen beech leaves in the place where *H. annulipes* are common.

To determine whether beech leaves with young galls of *H. annulipes* are more often chosen by the thrips, thrips were counted on leaves without and with galls. All leaves (up to 2 m above the ground) from one hundred randomly chosen current-year shoots (10 per each of 10 beech trees) were observed regarding the occurrence of thrips. If thrips were noticed, their presence on the adaxial and abaxial leaf surface was noted, as well as any noticeable feeding actions. These observations were carried out on 27-06-2016 between 12 p.m. and 13:30 p.m. in western Poland (Zielona Góra-Stary Kisielin, 51°55'57.0"N, 15°34'20.2"E) in a managed Scots pine forest with several years old common beech trees growing in the understory. Secondly, on 15-09-2016, 15 understory beech trees in the same area were randomly chosen to observe whether the galled leaves bear living galls.

To determine the thrips species, 17 specimens were collected. This material, preserved in ethanol, was macerated in KOH (5%) and slide-mounted in Canada balsam according to Ulitzka (2018). Species was determined according to Schliephake and Klimt (1979).

A generalized linear model with mixed effects (GLMM, function glmer.nb (in lme4 package), was used for modelling the relationship between the presence of thrips and galls on leaves (Bates *et al.* 2015). The presence of thrips was approved as a dependent variable with a binomial response (0 – absent, 1 – present), and the presence of galls was modelled as a fixed factor (nominal with two levels, 0 – absent, 1 – present). Tree and shoot nested within trees were included as random factors. The model was fit by maximum likelihood (Laplace approximation) and binomial distribution with the *logit* link function. The full GLMM model was compared with the null model containing only intercept and McFadden ρ^2 calculated (Domencich and

Fig. 1. Adult *Liothrips setinodis* individuals. A, C – thrips feeding on young *Hartigiola annulipes* galls; B – a female and mating pair on a beech leaf, D – an adult thrips feeding on a gall developing on a purple common beech tree.

McFadden 1975). The analysis of deviance was conducted to test for significance (Wald Chi-square test) of the fixed effect (function Anova(car), (Fox and Weisberg 2011). Calculations of the GLMM were conducted in the R environment (R Development Core Team 2019), and the functions and packages of their origin are given in brackets.

The linear regression was used to test the relationship between the mean number of galls and thrips occurring on leaves of each tree. The linear regression was performed in SAS JMP® 11.2.0.

The maximal number of leaves per shoot ranged from 3 to 8, and the mean number of leaves per shoot was 4.14. A total of 277 thrips and 713 galls were counted, and 160 leaves of 414 (39%) were infested by *H. annulipes*. On three studied beech trees, no galls were noticed. The highest number of thrips (69) was counted on the tree with the highest number of galls (227). Thrips were found on both the adaxial and abaxial surface of the leaves, 42.6 and 57.4%, respectively, and 44% of the thrips were observed feeding on gall tissues.

The generalized linear model with mixed effects showed that the presence of thrips and *H. annulipes* galls is positively related ($\rho^2 = 0.30$). The results indicate a significantly (Wald $\chi^2 = 39.4$, $P < 0.0001$) higher probability for the occurrence of at least one thrips on an infested leaf (estimate = 3.46 ± 0.55 SE for presence of galls – fixed effect) (Fig. 2).

The linear regression also showed a positive relationship between the number of galls and the number of thrips (Fig. 3; Mean (*N thrips*) = 0.068 + 0.356 × *Mean* (*N galls*), $P = 0.0002$, $r^2 = 0.844$).

Moreover, during late-summer field observations in Zielona Góra-Stary Kisielin in 2016, no living *H. annulipes* galls were found on 15 randomly chosen beech trees growing in the understory. In September, the leaves initially bearing *H. annulipes* galls exhibited brown necrotic spots instead of galls.

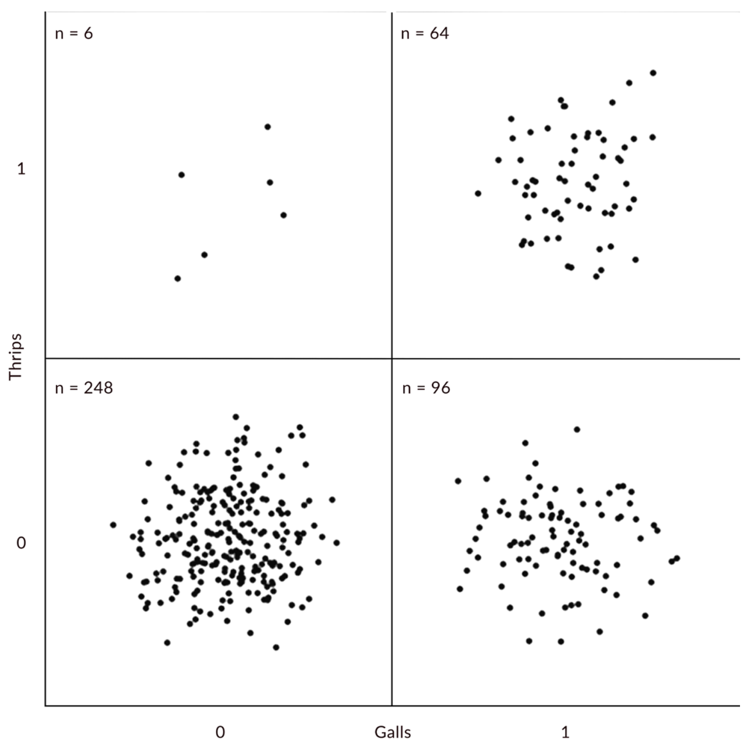

Fig. 2. Frequencies of observed absence (0) or presence (1) of thrips (*Liothrips setinodis*) and galls (*Hartigiola annulipes*) on leaves. Each point represents observation from an individual leaf, n is equal to the sum of surveyed leaves with and without examined herbivores. Generally, model estimate for intercept indicated very low probability for thrips occurrence (-4.08 ± 0.52 SE), but when gall was present, this probability increased significantly (estimate 3.46 ± 0.55 SE).

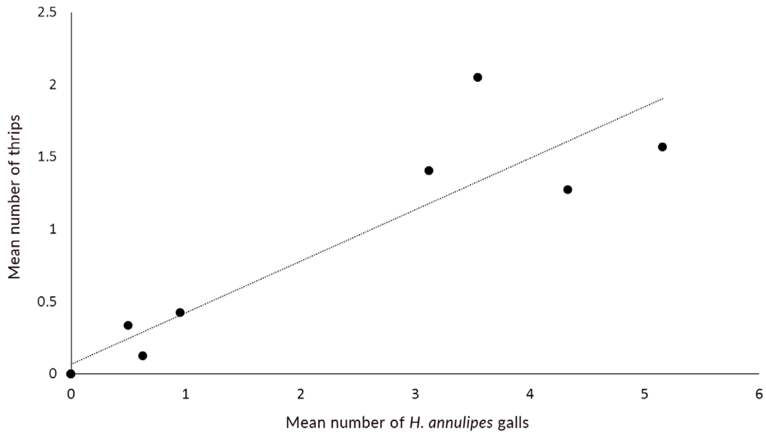

Fig. 3. The linear regression test showing relationship between mean number of *Hartigiola annulipes* galls on the common beech leaves and mean number of *Liothrips setinodis* thrips found on leaves. *Mean (N thrips) = 0.06784 + 0.3562 × Mean (N galls)*, $P = 0.0002$, $r^2 = 0.8442$.

Liothrips setinodis is a sap-sucking thrips frequently found in Europe on common beech trees (Kucharczyk and Stanisławek 2010). The observations discussed here indicate that this species is attracted to leaves infested by *H. annulipes*. Adult thrips were observed while feeding on gall tissues on the adaxial leaf surface. According to Rohfritsch (1971), *H. annulipes* lays eggs on abaxial leaf surface, but the galls are created on adaxial surface; thus, the nutritive tissue forms above the larva. In June, the *H. annulipes* galls are in an early stage of development and look similar to rings emerging from the upper leaf surface. Plant galls may act as sinks by storing more nutrients and assimilates than do the surrounding plant tissues (Koyama *et al.* 2004, Kaplan *et al.* 2011, Castro *et al.* 2012); however, the content of various chemical compounds can change during the maturation of a gall (Castro *et al.* 2012). It seems possible that the thrips are attracted by nutrients flowing to the gall tissues, supplying the gall midge larva. However, it remains unclear whether the thrips and galling species compete or enhance the sink effect. According to Ferrière (1958), the population of *L. setinodis* mainly seems to be regulated by parasitoids such as *Entedonastichus gaussi* (Loomans and Lenteren 1995). We did not study such relationship, thus we can only take it into consideration as one of possibilities which might explain why thrips had not been found on beech leaves during summer observations. The common beech trees are known to fight with gall-inducers by means of hypersensitive reaction (Fernandes *et al.* 2003, Pilichowski and Giertych 2017) leading to the death of a high percentage of galls and larvae living inside them. Although the reason of failure of development of the *H. annulipes* galls where we studied them remains unclear, it might have been connected with 1) the hypersensitive reaction of the host plant triggered by two herbivore species stressing the host plant; 2) hypothetical transition of an endophytic fungi by *L. setinodis* between beech leaves, what led to death of *H. annulipes*; or 3) the asymmetrical interspecific competition. Herbivores like galling-insects modify the nutrients flow in the host plant leading to local increase of nutrients what benefits the gall tissues and developing insects inside. There might occur a competition between insects and their galls due to activity of more than one galler. For example, Kaplan *et al.* (2011) studied potential sink competition between gall-inducing nematodes and an aphid species. These authors did not find any negative impact of one species on the other. However, this result could be connected to the different distant organs they fed on: the nematodes galled the root, and the aphid species is a vascular-feeding herbivore. On the other hand, Inbar *et al.* (1995) proved the existence of a plant-mediated interspecific competition occurring between two aphid species galling leaves of *Pistacia palaestina* Boiss. Furthermore, the relationship found between those two galling aphids was assymetrical – one of them was more successful in the influence on the phloem-transport of nutrients what can lead to a high rate of mortality of the second species. Similarly, two aphid species (*Monellia caryella* Fitch, 1855 and *Melanocallis caryaefoliae* Davis, 1910) feeding on the pecan *Carya illinoensis* (Wangenh.) K. Koch) compete indirectly: *M. caryaefoliae* is able to increase amino acid concentrations in infested leaves, while *M. caryella* inhibits this ability (Petersen and Sandstrom 2001). The competition between sap-sucking insects representing different species can affect their development. Nonetheless, the occurrence of certain species together on the same plant organ can give various outcomes, depending primarily on the species composition and stage of the development, not their population density (Qureshi and Michaud 2005). Furthermore, such relationships can be also influenced by the plant's water-stress so they may be modified by the environmental factors (Foote *et al.* 2017). Larson and Whitham (1997) proposed that the competition between organs acting as natural sinks (buds) and those produced by galling insects should be examined to explain distributional patterns of phloem-feeding herbivores on the host plant. Moreover, these authors showed that such competition has an important impact on the success of gall-forming aphids – less competing sinks ensure development of the remaining sinks, including galls. Koyama *et al.* (2004) found that leaves with galls can contain a five-fold increase of amino acids compared to ungalled leaves. Since in our study there were more thrips feeding on leaves with *H. annulipes* galls than on ungalled leaves, we can suppose that young developing galls change the chemical content of the beech leaves, making them more attractive for other herbivores such as thrips. Nonetheless, we suspect that the thrips may trigger defensive mechanisms of the host plant which results in mortality of *H. annulipes* larvae. Our paper describes a previously unknown observations of thrips feeding on developing *H. annulipes* galls. Although this relationship is statistically confirmed, this needs further research using various methods: ecological study of the co-occurrence of both insect species, chemical analysis of leaf components and field observations of the gall development.

It is worth noting that the relationship between *L. setinodis* and *H. annulipes* seems to be advantageous for the thrips reproduction success. Specimens attracted by galls share common leaves, and therefore, the chance of finding mating partners increases.

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