Transpiration effects and inquilines in a lepidopteran stem gall

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Abstract

I measured transpiration rate and cross-sectional xylem area of *Conostegia oerstediana* branches with and without stem galls induced by *Mompha* sp. Xylem area was reduced from an average of 2.61mm2 in an ungalled stem to an average of 0.31mm2 at the widest point of the gall, but transpiration did not differ significantly between galled (0.11 mL/30min/100cm2 leaf area) and ungalled (0.13 mL/30min/100cm2 leaf area) branches. This could be due to contributions to transpiration rate by gall lenticels. Many of the galls (46%), including those still occupied by *Mompha* sp., had peripheral cavities likely made by other insects; I found the arboreal ant *Procryptocerus batesi* nesting in one of these.

Introduction

Galls are common but very specialized structures: atypical plant growths induced by organisms that then live inside and feed on the gall. They arise on all plant organs and can be induced by a variety of organisms including microbes, nematodes, and insects (1). In most insect-induced galls, an insect oviposits on plant tissue and the plant's growth response is dependent on the feeding of the hatched larva (1). The gall is a defense by the plant, in the sense that it isolates an herbivore, but it is also a protective and nutrient-rich environment for the galling insect. In addition to the galling insect, other insects can be found living in galls as parasitoids or inquilines (2).

The effect of galls on the flow of water and mineral nutrients in the xylem is not clear. Fay *et al.* (3) found that galls on apical meristems increase water movement and photosynthesis, perhaps as a compensatory response to the loss of nutrients to the galls. However, results in general have not been conclusive (4) and galls that occur on xylary tissues such as stems could actually have the potential to disrupt and slow conduction of water in the plant.

The stems of the Central American melastome *Conostegia oerstediana* are galled by *Mompha* sp. moths. My study aims to answer this question: what effect do insect galls have on the flow of water through plants? *Mompha* galls have numerous lenticels and my initial hypothesis was that these would cause an increase in water loss by *C. oerstediana*. I was also interested in the route of the xylem through or around the gall.

Materials and Methods

I studied stem galls on the tree *Conostegia oerstediana* (Melastomataceae) induced by *Mompha* sp. (Lepidoptera, Momphidae). The galls are round, 11-29mm in diameter and have a green surface with numerous lenticels. My field site for collections was premontane wet forest adjacent to the Estación Biologica Monteverde in Monteverde, Puntarenas, Costa Rica.

I collected 20 pairs of branches with and without stem galls, each branch structurally similar and cut from the same plant; in total I sampled from eight individual trees. After cutting I immediately placed the branches in water to maintain the xylem water column. Upon returning to the lab, I cut the stems again under water and put each stem into a watertight potometer consisting of rubber tubing and a calibrated pipette. The potometers were filled with a 0.5% acid fluorescein solution to dye the xylem tissue for later sectioning (A.H. Sanjuan, pers. comm.). After a 1-minute equilibration, I tracked the movement of both pipettes' water columns for 30 minutes to measure transpiration. The pairing of galled and ungalled branches was necessary to control for other variables that influence transpiration rate such as light intensity, humidity, and wind speed (5); both branches in the pair experienced the same conditions so their transpiration rates could be directly compared.

To further standardize my transpiration data, I traced each leaf of each branch on paper and used the weight of the cutouts divided by the weight of a square centimeter of paper to determine total leaf area of each branch (6).

Next I cut thin stem sections, sampling both galled and ungalled portions if the stem had a gall. I measured the cross-sectional area of the dyed xylem tissue for each stem section, as well as the maximal diameter of each gall, under a dissecting microscope. I used a paired t-test to analyze differences in transpiration rate and xylem area between the branches in each pair, and a binomial test to check for effects of the time of day on transpiration rates.

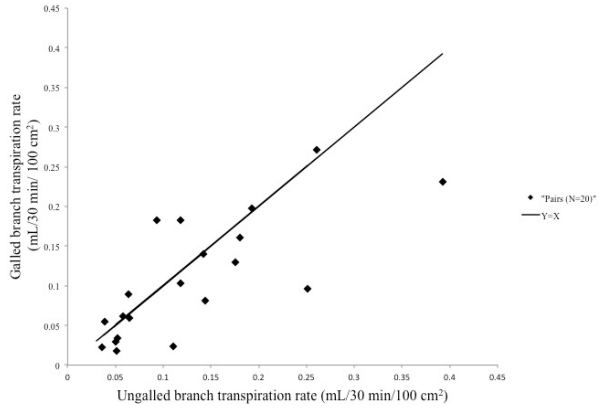


Figure 1. Transpiration rates for pairs of ungalled and galled C. oerstediana branches in mL/30min/100 cm2 leaf area.

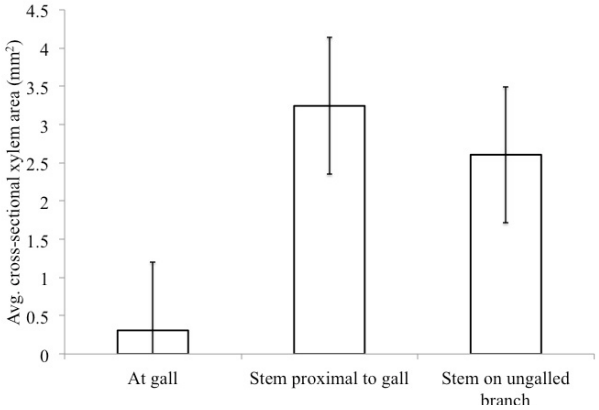


Figure 2. Average cross-sectional xylem area, in mm2, in sections made at the widest point of the gall, in the stem proximal to the gall, and in the stem of an ungalled branch (N=15).

Results

The transpiration rate of *C. oerstediana* branches with stem galls did not differ significantly from that of branches without galls. However, in any pair the ungalled branch was more likely than the galled branch to have the higher transpiration rate (binomial test: N=17, k=5, p=0.07). In 30 minutes, galled branches transpired an average of 0.11 mL per 100 cm2 of leaf area and ungalled branches transpired an average of 0.13 mL per 100 cm2 of leaf area (Fig. 1).

The xylem in a galled *C. oerstediana* stem does not go through the gall, but in fact flattens out and skirts around the gall mass, with the *Mompha* sp. moth larva or pupa occupying the center of the gall (Fig. 6). There was no significant difference between the cross-sectional xylem area of a galled stem immediately before the gall, which averages 3.24 mm2, and that of an ungalled stem, averaging 2.61 mm2. However, the xylem area at the widest point of the gall was always significantly reduced compared to the stem before the gall ($t(14)=9.98$, $p<0.0001$) and averaged only 0.31 mm2 (Figs. 2-3).

The time of day at which data collection occurred ranged from 09:00 to 14:00 and did not significantly affect transpiration rate differences within pairs; however, variation between pairs was greater earlier in the day (Fig. 4).

Twenty-three of the 26 galls collected (88%) were occupied by a living Mompha sp. larva or pupa. Twelve galls (46%) had some sort of cavity surrounding the moth's central chamber. Nine of the 12 galls with cavities (75%) were occupied by a moth larva or pupa.

Most of the cavities were filled with frass but no insects (Fig. 5); however, one contained a small colony of the arboreal ant Procryptocerus batesi (Hymenoptera: Formicidae, 7) including worker adults, eggs, larvae, and pupae (Fig. 6). Of the galls with cavities, all had a hole in them; every gall without a cavity except one did not have a hole like this. Essentially, galls with holes had cavities dug out of them and galls without holes did not ($\gamma^2(1, N=21)=17.36$, $p<0.001$).

Discussion

In this study galled branches transpired at the same rate as their ungalled counterparts (Fig.1). Galled branches were more likely to be the slower-transpiring branch in a given pair, but there was a lot of variation in the transpiration rate differences between galled and ungalled branches so the overall effect was not significant (Fig. 4). The highest amount of variation occurred in pairs sampled during the morning,

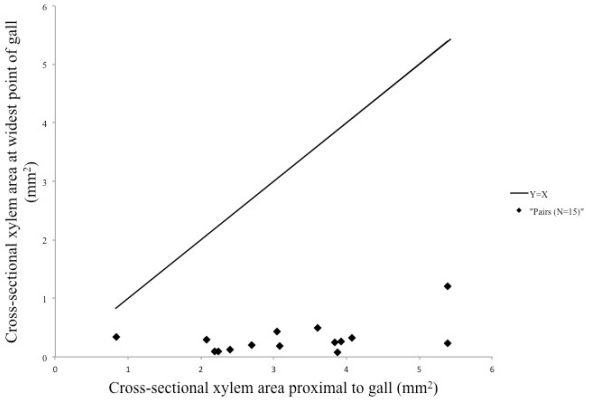


Figure 3. Paired cross-sectional xylem areas in mm2 of galls and stems proximal to galls.

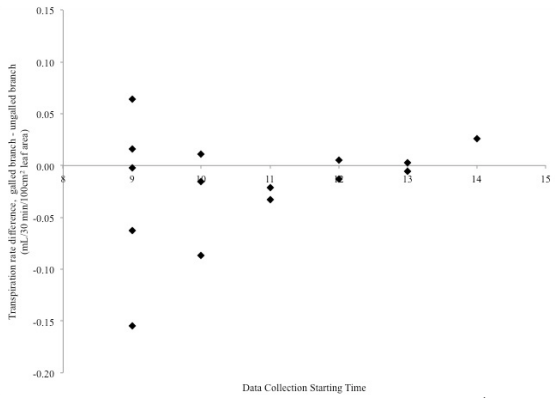
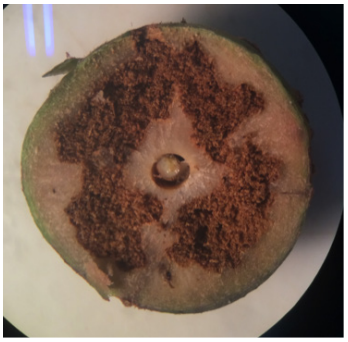
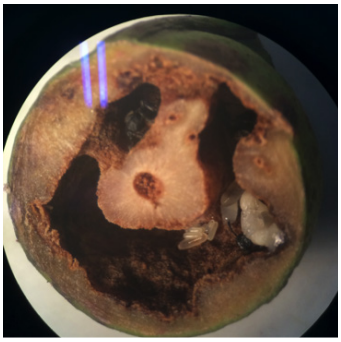


Figure 4. Difference in transpiration rate (mL/30min/100 cm2) between galled and ungalled branches in pairs tested at different times of day. Data collection start times range from 09:00 to 14:00. Negative rate differences represent pairs in which the galled branch had a lower transpiration rate than the ungalled branch.

Figures 5 and 6. Cross-sections of stem galls induced by Mompha sp. on C. oerstediana. 5: Gall with a frass-filled cavity and Mompha sp. larva at center. 6: Gall with P. batesi nesting in an open cavity and Mompha sp. larva at center. The flattened xylem column is visible at the top of the gall (see arrow).

when stomatal openness is peaking; this variation could have been caused by differing rates of change in stomatal water conductance between paired branches (8).

Because they use the plant's resources and have lenticels through which water is lost (5), I had expected the *Mompha* sp. galls to increase the amount of water transpired by C. oerstediana branches. However, there was an unexpected and significant reduction in xylem area due to displacement by the gall tissue (Figs. 2-3). Reduction in xylem area likely reduced flow (9) and offset or trumped any increase in transpiration caused by the gall and its lenticels. The largely neutral effect of the stem galls on transpiration reflects a gall morphology that maximizes the gall's growth while minimizing its effects on plant water needs. This benefits *Mompha* sp. because it reduces the selective pressure on *C. oerstediana* to evolve defenses against the gall. It also suggests that morphological diversity among galls may preclude a universal theory about galling and water use in plants.

Similar methods to those used in this study could be used to determine whether the lenticels on the gall surface are indeed offsetting reduced xylem conductance by releasing more water. Transpiration rates could be compared between branches without galls and branches with their galls sealed with wax. Sealed galls would not be able to transpire through their lenticels and would show the effects of reduced xylem in isolation.

Another outcome of this study was the discovery of a colony of *P. batesi* ants living in the hollowed-out periphery of a gall still occupied by its inducer. *P. batesi* often nest in live, ungalled stems (7) and other ants are known to colonize abandoned galls of similar size (10), but this species has not been reported as a gall inquiline.

I only found one gall with ants nesting inside it, but almost half of the galls collected had a similar cavity. The cavity was generally filled with frass and always had an exit hole too small for the moth but large enough for other insects. P. batesi do not leave frass-filled nest sites (J.T. Longino, pers. comm.) and are opportunistic stem nesters. This leads me to believe that the ants did not dig out that gall cavity themselves but instead moved in after the cavity-making inquiline, whose identity is unknown, had eaten out the gall pith and departed. The cavity appears to be dug in avoidance of the moth's chamber (Fig. 5). Though not typical of ants (11), using the gall while it is still occupied by a moth may be nutritionally and structurally beneficial for both *P. batesi* and the cavity-making inquiline because the gall's tissue growth is most likely maintained by its inducer (1). Further study could reveal the identity of the cavity-making inquilines and the frequency of subsequent ant colonization.

Conclusion

The relationships between gall-inducing insects, their host plants, and other gall-associated fauna are complex. Resultant transpiration rates in this system were not significantly different in galled branches, even though galls had a profound effect on stem vascular anatomy and anecdotally resulted in herbivory by secondary gall users. This combination of heavy use by the inducing insect and mild effects on the specific host plant's fitness gives insight into the success of galling as a life history strategy.

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