Willow Regrowth After Galling Increases Bud Production Through Increased Shoot Survival

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ABSTRACT Insect herbivory can negatively or positively affect plant performance. We examined how a stem gall midge *Rabdophaga rigidae* affects the survival, growth, and bud production of current year shoots of the willow *Salix eriocarpa*. In mid-May, the gall midge initiates stem galls on the apical regions of shoots. The following spring, galled shoots had thicker basal diameters and more lateral shoots than ungalled shoots. Although galled shoots were on average 1.6 times longer than ungalled shoots, there were no significant differences in shoot length or in the numbers of reproductive, vegetative, and dormant buds per shoot. However, the subsequent survival of galled shoots was significantly higher than that of ungalled shoots, probably because of the thicker basal diameter. This increased shoot survival resulted in approximately two times greater reproductive, vegetative, and dormant bud production on galled shoots compared with ungalled shoots in the following spring. These results suggest that the willow regrowth induced by galling can lead to an increase in bud production through increased shoot survival.

KEY WORDS apical dominance, basal diameter, lateral shoots, sink-source relations, stem galls

The effects of herbivory on plant growth and reproduction have been examined for many plant–herbivore interactions (Paige and Whitham 1987, Sacchi et al. 1988, DeClerck-Floate and Price 1994, Nozawa and Ohgushi 2002a). For example, Sacchi et al. (1988) reported that shoot with galls suffered a loss of 43% of their reproductive buds relative to shoots without galls. Herbivory is well documented to be detrimental to plants as inferred from plant defensive responses, including physical defenses involving trichomes and tissue hardness (Hoffman and McEvoy 1985, Gross and Price 1988, Tuberville et al. 1996) and chemical defenses using phenolic compounds (Schultz and Baldwin 1982, Karban and Myers 1989). One reason that the hypothesis that herbivory is generally detrimental to plants is accepted is the evidence that host plants respond in ways that limit further herbivory. However, there is increasing evidence that plants can benefit from herbivory because they have the ability to partially or fully compensate for lost tissues by increasing plant biomass (Bergström and Danell 1987, Mopper et al. 1991, Whitham et al. 1991). Although several studies have shown an increase in vegetative plant parts, little attention has been given to how the increased plant biomass relates to reproductive success under natural conditions (Belsky 1986).

Modular demography can provide insights into how herbivores affect host plants. In this context, plants are considered as a population of reiterated architectural units or modules, such as shoots that undergo birth and death events (White 1979); changes in the numbers of modules reflect plant growth and fitness (Maillette 1982). Tuomi and Vuorisalo (1989) suggested that modular demography may be a more biologically meaningful measure than measurements of individual plants. Because galls directly alter the growth of modules (e.g., shoots) or portions of modules (e.g., leaves; Larson and Whitham 1991, Koyama et al. 2004), the modular approach is particularly useful to examine the effect of galling on host plants (DeClerck-Floate and Price 1994).

In central Japan, the willow *Salix eriocarpa* Franch is commonly attacked by the gall midge *Rabdophaga rigidae* Shinji, which initiates stem galls on the apical regions of current year shoots (Yukawa and Masuda 1997). Adults emerge in April, and females lay eggs on the surface of leaves and stems of growing current year shoots. Newly hatched larvae crawl to the apical region of current year shoots and initiate stem galls in mid-May. After gall initiation in mid-May, apical regions of shoots stop growing and lateral shoots develop vigorously from the leaf axils below the stem galls (Nakamura et al. 2003).

In this system, there are two effects of galling on plant reproduction. First, an increase in plant biomass after regrowth may increase shoot bud production. Paige and Whitham (1987) reported that scarlet gilia

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Ipomopsis aggregata Pursh that was heavily grazed by mammals produced twice as much leaf biomass and three times as many flowers and fruits as unbrowsed plants. Second, an increase in subsequent shoot survival after regrowth may increase bud production. Shoots with higher biomass generally have a larger basal diameter, leading to better support of shoots to branches, resulting in increased resistance against a wide variety of environmental threats, including wind, rain, and snow, until the following spring (Roininen et al. 1994, Nozawa and Ohgushi 2002b). Therefore, to measure the reproductive success of *S. eriocarpa* shoots after gall initiation, we should consider both plant biomass and shoot survival after regrowth.

Our objective was to examine how the gall midge subsequently affects bud production in *S. eriocarpa.* We conducted a shoot-level census as a measure of the plant response to galling. In particular, we addressed the following three questions. Does the gall midge increase the shoot length, shoot diameter, and number of lateral shoots (components of shoot biomass), and number of buds per shoot? Does the gall midge increase shoot survival until the following spring? How do shoot biomass and subsequent shoot survival after galling affect overall bud production?

Materials and Methods

Study Site and Host Plant. This study was performed on a floodplain along the Yasu River (35° N, 136° E), Shiga Prefecture, central Japan, in 2002–2003. At least seven willow species grow naturally in and around the study site: *Salix eriocarpa* Franch. et Savant, *S. gilgiana* Seemen, *S. serissaefolia* Kimura, *S. subfraglis* Andersson, *S. chaenomeloides* Kimura, *S. gracilistyla* Miquel, and *S. integra* Thunb. Field observations were conducted on *S. eriocarpa* because it was one of the dominant willow species in the area. The willow is a deciduous woody plant; shoot elongation occurs rapidly from early April to late May.

Effect of Galls on Shoot Survival. To examine whether the gall midge *R. rigidae* affects the survival of current year shoots until the following spring, 10 *S. eriocarpa* trees were randomly selected just after gall initiation in mid-May 2002. On each tree, five pairs of shoots were labeled using plastic bands; each shoot pair contained one shoot with and one without galls. To control shoot size, we paired shoots with similar basal diameters (Mann–Whitney U test: $U = 1226$, $P = 0.87$; Table 1) and shoot lengths ($U = 1239$, $P = 0.94$). The following spring (i.e., mid-April 2003), we counted the number of surviving labeled shoots on each tree. The survival rate was calculated as the number of labeled shoots that survived, divided by the five that were labeled on each tree in mid-May 2002. Data were compared using the Wilcoxon paired rank test because of non-normal distribution. Individual willow trees were used as replicates in the analysis.

Effect of Galls on Shoot Growth. To determine whether the gall midge affects shoot growth, we measured the basal diameter and total shoot length on galled and ungalled shoots that survived to the following spring (i.e., mid-April 2003). These shoot parameters are components of shoot biomass. We also counted the number of lateral shoots that emerged in 2002 and survived until mid-April 2003 as a measure of regrowth in response to galling because the number of lateral shoots is related to the net increase in shoot biomass. Lateral branching is a typical response to damage on shoot apical regions (Mopper et al. 1991). These data were analyzed using the Mann–Whitney U test because of non-normal distribution. Individual willow shoots were used as replicates in the analysis.

Table 1. Mean ± SE shoot length and basal diam of galled (n = 50) and ungalled (n = 50) shoots just after gall initiation (mid-May 2002)

	Galled shoot	Ungalled shoot
Basal diameter (cm)	2.84 ± 0.05a	2.86 ± 0.06a
Shoot length (cm)	12.7 ± 0.6a	12.7 ± 0.6a

Different letters indicate a significant difference (Mann-Whitney U test: $P < 0.05$).

Effect of Galls on Bud Population. To determine whether the gall midge affects bud production in the following spring, we conducted a census of the number of buds per shoot. In the census, we counted the number of buds not only on the main (labeled shoots) but also lateral shoots arising from labeled shoots. Because bud availability limits future plant growth and reproduction, buds are a meaningful currency for measuring the effects of herbivory over time (Maillette 1982). Willow buds can be classified into three types, reproductive, vegetative, and dormant, with different functions in plant reproduction and survival. Reproductive buds contribute to seed production, vegetative buds contribute to clonal reproduction through branch propagation (Rood et al. 2003), and dormant buds contribute to tree survival by sprouting after physical disturbance such as floods (Bond and Midgley 2001, Barsoum 2002). Each type of bud directly and indirectly contributes to plant reproductive success in different ways. Thus, we counted the numbers of reproductive, vegetative, and dormant buds on surviving shoots in mid-April 2003.

We considered not only changes in plant biomass, but also subsequent shoot survival as factors affecting bud production in the following spring. To examine the effect of an increase in plant biomass, the number of buds per shoot that survived to the following spring was compared between galled and ungalled shoots. These data were analyzed using the Mann–Whitney U test because of non-normal distribution. Individual willow shoots were used as replicates in the analysis. Furthermore, to examine the effects of both plant biomass and subsequent shoot survival, we calculated the net number of buds as the total number of buds of surviving labeled shoots per tree divided by the five that were labeled at the start of the census. The net number of buds was defined as the overall bud production after changes in shoot biomass and subsequent survival. Data were compared using the Wilcoxon paired rank test because of non-normal

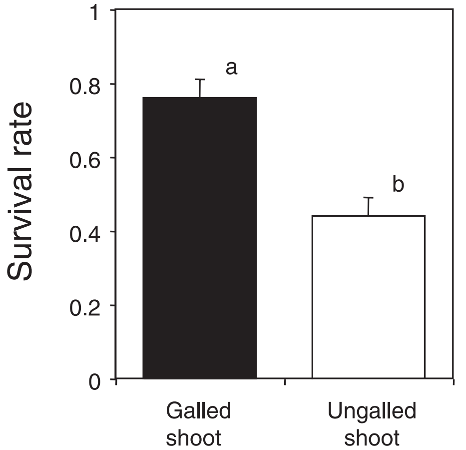

Fig. 1. Mean (±SE) survival rate of galled ($n = 10$) and ungalled shoots ($n = 10$) the following spring. Different letters indicate a significant difference (Wilcoxon paired rank test: $P < 0.05$).

distribution. Individual willow trees were used as replicates in these analyses.

Results

The gall midge affected the survival rate of *S. eriocarpa* shoots. The survival rate of galled shoots was significantly higher than that of ungalled shoots (Wilcoxon rank paired test: $Z = -2.724$, $P < 0.01$; Fig. 1).

The following spring (i.e., mid-April 2003), galled shoots had a thicker basal diameter and produced more lateral shoots than ungalled shoots (basal diameter, $U = 277$, $P = 0.03$; lateral shoots, $U = 260.5$, $P = 0.01$; Table 2). Furthermore, the mean length of galled shoots was 1.6 times longer than that of ungalled shoots, although this difference was not significant ($U = 314$, $P = 0.11$; Table 2).

However, there were no significant differences in the numbers of reproductive, vegetative, and dormant buds per shoot between galled and ungalled shoots that survived until the following spring (Mann–Whitney U test: reproductive, $U = 523$, $P = 0.29$; vegetative, $U = 526$, $P = 0.33$; dormant, $U = 559$, $P = 0.55$; Table 3). However, the net numbers of reproductive, vegetative, and dormant buds, which were expressed as overall bud production caused by changes in shoot biomass and subsequent survival, were approximately two times greater on galled shoots than on ungalled shoots (Wilcoxon paired rank test: reproductive, $Z = -2.192$, $P = 0.03$; vegetative, $Z = -1.957$, $P = 0.05$; dormant, $Z = -2.091$, $P = 0.04$; Table 4).

Table 2. Mean ± SE shoot length, basal diameter, and no. of lateral shoots of galled ($n = 38$) and ungalled ($n = 22$) shoots that survived to the following spring (mid-April 2003)

	Galled shoot	Ungalled shoot
Basal diameter (cm)	4.76 ± 0.23a	3.99 ± 0.26b
Shoot length (cm)	74.5 ± 12.6a	46.5 ± 11.9a
Number of lateral shoots	3.82 ± 0.64a	1.77 ± 0.61b

Different letters indicate a significant difference (Mann-Whitney U test: $P < 0.05$).

Table 3. Mean ± SE no. of reproductive, vegetative, and dormant buds on galled ($n = 38$) and ungalled ($n = 22$) shoots that survived until the following spring

	Galled	Ungalled
No. of reproductive buds	18.3 ± 4.6a	10.7 ± 4.9a
No. of vegitative buds	13.3 ± 1.9a	13.4 ± 1.5a
No. of dormant buds	8.5 ± 1.6a	6.4 ± 1.2a

Different letters indicate a significant difference (Mann-Whitney U test: $P < 0.05$).

Discussion

Our results clearly showed that the gall midge stimulates the development of lateral shoots from the leaf axils below stem galls. Galled shoots were thicker in basal diameter and showed increased shoot survival until the following spring than ungalled shoots. The increased shoot survival resulted in approximately two times greater overall production of reproductive, vegetative, and dormant buds on galled shoots than on ungalled shoots.

Mechanisms of Regrowth Response. There are two possible mechanisms that explain the regrowth response to galling in the apical region of current year shoots. First, the loss of apical dominance may stimulate lateral branching (Whitham and Mopper 1985, Mopper et al. 1991, Whitham et al. 1991, Pilson 1992, Nozawa and Ohgushi 2002a). Apical dominance is defined as control exerted by the shoot apex over the outgrowth of lateral buds and is controlled by the ratio of cytokinin to auxin phytohormones (Cline et al. 1997). Destruction of the apical meristem can release the lateral buds from apical dominance (Mooby and Wareing 1963). Second, stem galls may alter the sink-source relations within the plant (Whitham et al. 1991, Honkanen et al. 1994). Galls function as strong physiological sinks (Larson and Whitham 1991, Inbar et al. 1995, Koyama et al. 2004) that draw assimilates from surrounding plant sources (e.g., storage tissues) to improve their nutritional status, resulting in an increase in plant biomass.

Nozawa and Ohgushi (2002a) reported that oviposition by the spittlebug *Aphrophora pectoralis,* which

Table 4. Mean ± SE net no. of reproductive, vegetative, dormant buds was defined as overall bud production as a result of increases in shoot biomass and survival

	Galled	Ungalled
Net no. of reproductive buds	29.7 ± 14.1a	11.2 ± 6.0b
Net no. of vegitative buds	19.0 ± 9.2a	10.6 ± 5.1b
Net no. of dormant buds	7.0 ± 1.6a	3.2 ± 0.7b

Net no. of buds was expressed by total bud no. of survived shoots on a tree divided by five, which was the no. of labeled shoots. Different letters indicate a significant difference (Wilcoxon paired rank test: $P < 0.05$).

destroyed the apical region of 1-yr-old shoots of *S. miyabeana* and *S. sachalinensis,* could greatly increase the growth of current year shoots in the following spring, probably because of the removal of apical dominance. Consequently, the regrowth was fully compensating at the 1-yr-old shoot level, although the apical buds of the 1-yr-old shoots died in the previous year. However, our results showed that galled shoots had thicker basal diameters and more lateral shoots but same shoot length compared with ungalled shoots. Similarly, galling led to full compensation for future shoot growth, which stopped because of gall initiation in mid-May 2002. This compensation may have been caused by the mechanisms of the removal of apical dominance and alteration of sink-source relations. However, the mortality of smaller shoots on ungalled shoots occurred more frequently, resulting in decrease in the difference in shoot growth between galled and ungalled shoots. Thus, our measurements on the remaining shoots may underestimate the regrowth response to galling because smaller shoots were not included.

Shoot Survival After Gall Initiation. A wide variety of adverse environmental conditions (e.g., wind, rain, snow) are important causes of mortality for current year shoots. However, there is increasing evidence that increased shoot length can improve shoot survival because larger shoots are stronger and more able to withstand damage (Craig et al. 1989, Nozawa and Ohgushi 2002b). Similarly, the gall midge significantly increased both shoot basal diameter and survival, probably because the greater basal diameter of galled shoots may have enhanced resistance to environmental threats.

Furthermore, because the larval development of the gall midge is completed within the stem gall until the following spring, the survival of galled shoots would greatly affect larval mortality. Several authors have reported that premature leaf abscission exerts high mortality on some endophagous insects (e.g., leaf miners and leaf gallers; Williams and Whitham 1986, Simberloff and Stiling 1987, Stiling et al. 1991, Preszler and Price 1993). Therefore, the attraction of assimilates by larvae in stem galls probably serves not only to acquire food resources but also to decrease larval mortality. This larval behavior may result in the high survival rates of galled shoots.

Effect of Galls on Bud Production. *Salix* and *Populus* species are early successional floodplain species capable of both sexual and asexual reproduction. The relative contribution of each bud type to plant growth and reproduction depends on the environmental conditions (Barsoum 2002). In relatively undisturbed environments, reproductive buds are important contributors to reproduction through seed production. However, in highly disturbed environments (e.g., by flooding or fire), vegetative and dormant buds are more important contributors to reproduction through clonal reproduction and sprouting. In our study area, the higher shoot survival (likely increased by the greater basal diameter) resulted in approximately two times greater overall production of reproductive, vegetative, and dormant buds on galled shoots than ungalled shoots. This implies that the gall midge enhanced plant fitness, even if future disturbances occur. In contrast, DeClerck-Floate and Price (1994) reported that the majority of lateral shoots arising from galled shoots on *S. exigua* had abscised until the following growing season, resulting in significant loss of buds and sexual modules. However, our study showed that galled shoots still had more lateral shoots than ungalled shoot on *S. eriocarpa* in the following spring. The difference in survival of lateral shoots is more likely to cause the difference in reproductive outcome between two willow species.

It could be argued that abscised shoots on the ground may reproduce new shoots in the following spring. However, this is unlikely because their buds were undeveloped and the biomass was small (M.N., unpublished data).

Previous studies focused only on changes in plant biomass as a measure of plant fitness after herbivory (Nozawa and Ohgushi 2002a). However, we found that the gall midge increased overall bud production in galled *S. eriocarpa* shoots by increasing shoot survival, probably because of the thicker basal diameter than in ungalled shoots. We emphasize that to understand precisely how herbivores affect the reproductive success of plants, it is important to consider plant resistance against environmental threats (e.g., wind, rain, snow) until the following growing season. Currently, little attention is given to this process for a wide variety of plant–herbivore interactions (DeClerk-Floate and Price 1994). Furthermore, although Tuomi and Vuorisalo (1989) suggested that modular demography may be a more biologically meaningful measure, the increase in shoot production on galled shoots may be caused by a decrease in bud production on other shoots so that the overall change of the total bud production of the plant may be negative, positive, or neutral. To solve this problem, further research should measure total bud production on galled and ungalled plants to reveal the overall impact of galling on the plant.

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