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Stem galling of *Ageratina adenophora* (Asterales: Asteraceae) by a biocontrol agent *Procecidochares utilis* (Diptera: Tephritidae) is elevation dependent in central Nepal

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**ABSTRACT**

Gall inducers are widely used as biocontrol agents to suppress the vegetative and reproductive growth of invasive weeds. *Procecidochares utilis* Stone (Diptera: Tephritidae) is a gall forming fly that was released as a biocontrol agent against the noxious invasive weed *Ageratina adenophora* (Sprengel) R. King and H. Robinson (Asterales: Asteraceae). However, the effectiveness of *P. utilis* in controlling *A. adenophora* has been reported to vary according to geographic regions, with very low effectiveness in the Himalaya. In this study, we measured the abundance of *P. utilis* stem galling on *A. adenophora* along the elevation gradient (240–2965 m asl) in central Nepal. We found that elevation had a significant effect on gall abundance as well as gall size. Gall abundance and size peaked at mid-elevation (1940–2000 m asl). Stem galling by the fly reduced the stem diameter of the weed and all the three nutrients (N, P and K) in galls were significantly higher than in ungalled stems. Our study indicates that elevation is an important abiotic factor that strongly influences the *P. utilis* stem gall abundance, as well as gall size, and galls formed by *P. utilis* act as nutrient sinks. These findings provide insights in insect gall ecology, which may help the successful biocontrol of *A. adenophora*.

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**KEYWORDS**
Biological control; galling insects; gall abundance; gall morphology; nutrient sinks

**Introduction**

Control of invasive weeds using biocontrol agents is a growing strategy worldwide (Schwarzlander et al., 2018). Among different biocontrol agents, herbivorous especially gall-inducing insects are used in weed biocontrol since they retard the vegetative growth and reproduction of invasive weeds (Bitume et al., 2019; Erasmus et al., 1992; Flor-entine et al., 2005). Gall inducers are valuable biocontrol agents because they often have a narrow host range, and low probability of non-target impacts (Harris & Shorthouse, 1996). Interactions among gall-inducing insects used as a biocontrol agent, the host plant, and the environment determine the population dynamics of the gall inducers.
Therefore, understanding the factors influencing population dynamics of gall inducers is central to the successful management of weeds (Clerck-Floate & Bourchier, 2000; McEvoy, 2018). Infestation by galling insects results in the formation of plant galls that provide nutrition, protection, and shelter to the residing insects (Raman, 2007; Shorthouse et al., 2005). An estimated 132,930 species of gall-inducing insects have been reported globally (Espírito-Santo & Fernandes, 2007). Among these, Prorcedochares utilis Stone (Diptera: Tephritidae) is a stem galling fly on one of the noxious invasive weed Ageratina adenophora (Sprengel) R. King and H. Robinson (Asterales: Asteraceae). The P. utilis fly is one of the seven biocontrol agents against A. adenophora that is established in eight countries outside of its native range (Poudel et al., 2019; Table S1). In Hawaii, P. utilis had significant negative impact on the plant but was not successful in high rainfall areas (Bess & Haramoto, 1959, 1972). Partial success in the control of A. adenophora infestation was achieved in New Zealand (Fowler et al., 2000). In Australia, P. utilis reduced the vegetative and reproductive vigour of A. adenophora, halting its rapid spread (Page & Lacey, 2006; Winston et al., 2014). Studies indicate that persistent and high population of galls are necessary to suppress the plants (Bess & Haramoto, 1959; Page & Lacey, 2006). Therefore, knowledge on P. utilis stem gall abundance is central to the successful management of the weed.

In Nepal Himalaya, A. adenophora is distributed across a wide elevation range (164–3280 m asl) (GBIF, 2019; Siwakoti et al., 2016). Environmental change along the elevation gradient produces variation in the suitability of the host plant, which affects the growth and development of insects residing inside it (Hodkinson, 2005; Korner, 2007). This ultimately shapes the distribution and size of gall-inducing insects (Hodkinson, 2005). Interactions among plant genotype, insect genotype, and the environment determine the gall size (Weis & Abrahamson, 1986; Weis & Gorman, 1990). There is evidence that galling insect survival and fitness depends upon gall size (Egan et al., 2011; Marchosky & Craig, 2004; Sopow & Quiring, 2001). Generally, maggots in small-sized galls are more susceptible to parasitism than in the large-sized galls (Marini-Filho & Fernandes, 2012; Weis et al., 1985). Also, the number of galling insects present inside the gall is positively correlated with the gall size (Sopow & Quiring, 2001; Tabuchi & Amano, 2004). However, knowledge of the role of elevation on gall inducers is poor. Thus, studies on the effect of environmental factors on gall abundance and size are of great ecological importance and will be useful for understanding the survival and fitness of galling insects.

The complex relationship between gall inducers and host plants has been well recognised and a number of hypotheses have proposed the adaptive nature of gall induction (Hartley & Lawton, 1992; Price et al., 1987; Stone & Schonrogge, 2003). The nutrition hypothesis states that plant galls are adaptations that provide insects with higher nutritional food sources in comparison to ungalled plant tissue (Price et al., 1986; Price et al., 1987; Stone & Schonrogge, 2003). The ability of the gall inducers as a biocontrol agent to harm host plants mainly depends upon the efficiency of galls to act as nutrient sinks (Harris & Shorthouse, 1996). Thus, establishment of sinks in galls deprives the host plant of resources that could have been otherwise utilised by the plant in growth and reproduction, exerting negative impacts upon the host plant fitness (Abrahamson & Weis, 1997; Marini-Filho & Fernandes, 2012; McCrea et al., 1985).

To our knowledge, the effects of elevation on the performance of P. utilis has not been reported. Some studies have indicated that galling by P. utilis causes reduction in shoot
height, biomass, and reproductive potential of the weed (Buccellato et al., 2012; Buccellato et al., 2019; Erasmus et al., 1992), while its effect on stem diameter of the weed has not been explored. Furthermore, the ability of galls to act as nutrient sinks has not been examined in *A. adenophora*. The aim of the present study was therefore to examine the effect of elevation on gall abundance and gall size, investigate the nutrient sink efficiency of galls, and assess the impact of the gall fly on stem diameter of the weed. An increased understanding on insect gall ecology will assist in the use of this biocontrol agent against *A. adenophora*.

**Materials and methods**

**Field sampling**

A distribution survey of galls was carried out during November–December 2018 along the elevation gradients in two sites: Eklefaant-Simbhanjyang (hereafter called Simbhanjyang) (Dhading and Makwanpur districts) and Chandragiri (Kathmandu district) (Figure 1; Table S2). Two additional sites (Rasuwa and Chitwan districts) were also surveyed that represented the highest and lowest elevational range of *A. adenophora* distribution in Nepal Himalaya.

For data collection, three quadrats (10 m × 10 m, and 50 m apart from each other) were sampled in each elevation belt. One hundred ramets were counted in each quadrat and the gall abundance was noted as the number of ramets with galls (Smith et al., 2011). From

![Figure 1. Map of central Nepal showing elevation zones and sampling sites.](image)
each quadrat, 20 ramets having fresh galls without the emergence hole were selected. The length and breadth of each gall were measured using digital vernier calipers (WT 4171). The diameter of stems with galls was measured 1 cm below the gall (Marini-Filho & Fernandes, 2012). The ramet of the same bunch (i.e. genet) without a gall was considered to be a normal stem. The diameter of the normal stem was also measured at the same height as that of the galled stem. Galls and stem section of 5 cm in length (just 1 cm below the gall) were excised from the galled ramets. A similar stem section (5 cm) was also cut out from the normal stem at the same height as that of the galled stem (Tooker et al., 2008). In this way, from one genet, three samples were collected: gall, stem below gall (galled stem), and normal stem (ungalled stem). The samples were kept in a paper bag and allowed to dry in the shade until taken to the laboratory. From each elevation, altogether 60 (20 ramets × 3 sites) samples of gall, stem below gall, and normal stem were collected.

**Laboratory analysis**

From each gall sample, the gall flies in different stages were counted and removed carefully. Then the galls, galled, and ungalled samples were oven-dried at 80° C for 24 h (Florentine et al., 2005). The dried samples were ground into powder with an electric grinder. The samples from three different quadrats of the same elevation belt were mixed so as to have an adequate amount of plant materials for nutrients analysis. Then they were kept in the fridge (4° C) until further analysis. The samples were analysed for three essential nutrients: nitrogen (N), phosphorus (P), and potassium (K). Nitrogen was analysed using the Micro-Kjeldahl method (Horneck & Miller, 1998). Wet method using sulphuric acid and hydrogen peroxide was used for digestion of plant samples for the determination of phosphorus and potassium concentration (Campbell & Plank, 1998). Then, phosphorus was estimated calorimetrically using the vanado-molybdate method (Juo, 1978) and potassium by using a flame photometer (Horneck & Hanson, 1998). Nitrogen estimation was done in Regional Soil Testing Laboratory at Hetauda, whereas phosphorus and potassium were estimated in the Agricultural Technology Centre in Kathmandu.

**Statistical analysis**

All the statistical analyses were carried out in R version 3.4.4 (R Core Team, 2019). Values of gall abundance (number of galls per 100 ramets), nutrient concentrations, gall diameter and diameter of the stem with and without galls were evaluated for normality using Shapiro–Wilk test and for homogeneity of variance using Bartlett test. To examine the variation in gall abundance (response variable) with elevation (predictor variable), a generalised linear model with linear as well as quadratic terms was fitted using the Poisson distribution with logit function in R. We used Akaike Information criteria (AIC) to select the best model. For this, the data of galls from all sites were used to get a full distributional range of the fly ($n = 48$). The Pearson’s correlation and a simple linear regression were used to analyse the relationship between number of larvae per gall and gall diameter. Similar analyses were used to examine the relationship between gall diameter and diameter of the stem with gall. Differences in gall diameter across different elevations were compared using analysis of variance (ANOVA) followed by post hoc Tukey test. To assess the impact of galling on stem diameter of the weed, Kruskal–
Wallis test was used. The nutrient data of samples from Simbhanjyang and Rasuwa was normal; therefore, nutrient data of these sites were compared using analysis of variance (ANOVA) followed by post hoc Tukey test. However, nutrient data of samples from Chandragiri were not normal; therefore, was subjected to Kruskal–Wallis test with Bonferroni’s correction. The relationship between the three nutrients was analysed by using Pearson’s correlation test.

Results

Effect of elevation on P. utilis gall abundance

The relationship between elevation and gall abundance ($n = 48$) was unimodal ($R^2 = 0.78$, $P < .0001$; Figure 2). Elevation explained about 78% of the variance in abundance of galls. The abundance of galls per 100 ramets were the highest in plots between 1940–2000 m asl,
which is close to the mid-elevation of the entire elevation range of the distribution of *A. adenophora* (240–2965 m asl) in Nepal. Galls were absent at the highest (Dhimsha of Rasuwa district, 2965 m asl) and lowest (Jutpaani of Chitwan district, 240 m asl) elevational distribution range of *A. adenophora*.

**Effect of elevation on *P. utilis* gall size**

The gall diameter in Simbhanjyang was 13.33 ± 2.38 mm, whereas it was 14.50 ± 2.14 mm in Chandragiri. Change in gall diameter with elevation was different between the two sites. The gall diameter increased with the increasing elevation up to 2000 m asl and then decreased after that in Simbhanjyang ($F_{1,236} = 57.31, P < .001$), where the elevation difference between the lowest and the highest sites was 1920 m asl (Figure 3(A)). However, in Chandragiri, with narrow elevation difference (980 m asl), the gall diameter did not vary significantly along the elevation gradient ($F_{1,344} = 5.134, P = .07$; Figure 3(B)).

The number of larvae per gall in the study area ranged from one to six. The number of larvae per gall increased with increasing gall diameter ($R^2 = 0.43, P < .001$ (Simbhanjyang); $R^2 = 0.67, P < .001$ (Chandragiri); Figure 4). Furthermore, the gall diameter was also significantly and positively correlated with stem diameter ($R^2 = 0.03, P < .001$ (Simbhanjyang); $R^2 = 0.08, P < .001$ (Chandragiri); Figure S1).

**Effect of gall on stem diameter**

The mean diameter of galled stems was significantly lower than stems without galls ($\chi^2 = 7.4, \text{ d.f.} = 1, P < .006$ (Simbhanjyang); $\chi^2 = 24.2, \text{ d.f.} = 1, P < .001$ (Chandragiri); Figure 5) in both of the sampling sites. The diameter of galled stem was 1.1 times smaller than the stems without galls (ungalled stem) in both the sites.

**Nutrient concentrations**

In Simbhanjyang, nitrogen concentration was higher in gall samples than in stems below galls and the normal stems without galls ($F_{2,42} = 61.69, P < .001$), whereas phosphorus concentration was significantly higher in gall samples than in normal stems without galls ($F_{2,42} = 3.958, P < .001$). However, potassium concentration did not differ among the samples ($F_{2,42} = 2.597, P = .0864$; Figure 6(A)). In contrast, all three nutrients (N, P$_2$O$_5$ and K$_2$O) were higher in galls than in stem below galls and normal stem without galls in Chandragiri ($\chi^2 = 40.913, \text{ d.f.} = 2, P < .001$ (N); $\chi^2 = 33.811, \text{ d.f.} = 2, P < .001$ (P$_2$O$_5$); $\chi^2 = 37.375, \text{ d.f.} = 2, P < .001$ (K$_2$O); Figure 6(B)). In Rasuwa (the highest elevation sampled), all the three nutrients were significantly higher in galls than in normal stem ($F_{2,6} = 483.7, P < .001$ (N); $F_{2,6} = 15.56, P < .001$ (P$_2$O$_5$); $F_{2,6} = 12.42, P < .001$ (K$_2$O); Figure 6(C)). However, the potassium concentration in gall samples did not differ with that of stem below gall while the difference was significant for nitrogen and phosphorus.

The concentration of all these three nutrients, i.e. nitrogen, phosphorus and potassium were significantly and positively correlated with each other in both sites (Table 1).
Figure 3. Gall diameter along the elevation gradient in central Nepal. (A) Simbhanjyang and (B) Chandragiri. Different letters above the bar denote significant differences among the samples from different elevation belts ($P < .001$, Tukey-HSD test).

Table 1. Correlation between mineral nutrients.

<table>
<thead>
<tr>
<th>Gradients</th>
<th>Nutrients</th>
<th>$P$-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chandragiri</td>
<td>P$_2$O$_5$</td>
<td>0.54***</td>
</tr>
<tr>
<td></td>
<td>K$_2$O</td>
<td>0.6***</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.44***</td>
</tr>
<tr>
<td>Simbhanjyang</td>
<td>P$_2$O$_5$</td>
<td>0.73***</td>
</tr>
<tr>
<td></td>
<td>K$_2$O</td>
<td>0.64***</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.41***</td>
</tr>
</tbody>
</table>
Discussion

To, the best of our knowledge, this is the first study that examined the effect of elevation on *P. utilis* gall abundance. We found that gall abundance varied with elevation in a unimodal pattern, with the highest gall abundance at a mid-elevation range along the elevation gradient in central Nepal (240–2965 m asl). A similar pattern was reported for dung beetles (Coleoptera: Scarabaeidae) (Escobar et al., 2005), *Palgiometriona* species (Coleoptera: Chrysomelidae) (Flinte et al., 2011), and *Rhopalomyia solidaginis* (Diptera: Cecidomyiidae) (Crutsinger et al., 2013). This mid-domain effect can be explained by the ‘Abundant-centre hypothesis,’ which posits that species abundance is the highest at the centre of its range where the most favourable biotic and abiotic conditions occur, and gradually

![Figure 4. Relationship between number of larvae per gall and gall diameter in two sites in central Nepal. (A) Simbhanjyang and (B) Chandragiri.](image-url)
decline toward the edges (Brown, 1984; Hengeveld & Haeck, 1982). However, these assumptions have been frequently questioned because there are complex biogeographical processes like interspecific interactions, changing environmental conditions, temporal variation in abundance as well as life history traits of species acting together that determine the spatial abundance of the species along the environmental gradients (Dallas, Decker, & Hastings, Hastings, 2017; Santini et al., 2019). Nevertheless, these studies did not include gall inducers; therefore, we argue that the pattern of distribution of species abundance may be contextual and differ with taxa. The survey of distribution pattern of A. adenophora

**Figure 5.** Diameter (mean ± S.E) of normal stem and galled stem in two sites of central Nepal. (A) Simbhanjyang, (B) Chandragiri. Means denoting different letter are significantly different ($P < .05$, Kruskal Wallis Test)
Figure 6. Nutrient concentrations (mean ± S.E) within gall samples, stem below gall and normal stem without gall in three sites of central Nepal. (A) Simbhanjyang, (B) Chandragiri, (C) Rasuwa. Means followed by different letter are significantly different ($P < .001$, Tukey-HSD test for normal data (Simbhanjyang and Rasuwa), whereas Kruskal–Wallis test with bonferroni’s correction for non-normal data).
along the elevation gradient of 300–2500 m asl in western Himalaya also revealed a unimodal pattern of distribution, its probability of occurrence peaking at 1320 m asl (Datta et al., 2017). Although our study did not take weed abundance into account, previous research suggests that increased host plant abundance increases the gall abundance (Boaventura et al., 2018). This suggests the possibility of greater host plant abundance at the mid-elevation range in central Nepal.

Our results indicated that gall diameter varied along the elevation gradient, peaking at mid-elevation range when the elevation gradient of the study area is sufficiently wide. This is consistent with previous works (Hartmann, 1984: Smith et al., 2011). Marchosky and Craig (2004) reported a strong influence of environmental variation on the size of gall. The tritrophic interaction between plant genotype, insect genotype and the environment determines the gall size (Weis & Abrahamson, 1986; Weis & Gorman, 1990). Variation in abiotic factors like temperature, humidity, soil factors etc. along with the elevation gradient act as bottom-up forces that cascade upward, creating differences in the evolution of interaction of plants, galling insects, and their natural enemies (Craig et al., 2007; Rasmann et al., 2014). These differences in interaction among different trophic levels might have led to the variation in gall size. The largest gall diameter (15.05 ± 0.29 mm) measured in the present study is similar to that observed in South Africa (15.05 mm, Bennett & van Staden, 1986). As both gall abundance and gall size peaked at mid-elevation, this suggests that mid-elevation sites may have an optimal condition for the growth of *P. utilis*. Our observation that gall diameter increased with the increasing number of larvae per gall is also consistent with previous findings (Marchosky & Craig, 2004; Tabuchi & Amano, 2004). Furthermore, while gall size is positively related to survival and fitness of the galling insects (Marchosky & Craig, 2004; Sopow & Quiring, 2001), it can be assumed that larvae of *P. utilis* might have the greatest fitness at mid-elevation. However, this assertion needs further evaluation.

Galling by *P. utilis* reduces the vegetative and reproductive growth of *A. adenophora* (Buccellato et al., 2012, 2019; Erasmus et al., 1992). We also found that stem diameter, a growth-related variable, was reduced by the gallfly. Gall development alters the sink-source relationship in their host plants, which can reduce the photosynthetic capacity in the remaining ungalled tissues, placing overall metabolism of the host plant in stress (Raman & Abrahamson, 1995; Florentine et al., 2005). Reduction in stem diameter from galling has also been reported in other plants like *Solidago altissima* L. (Asterales: Asteraceae) by *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) (Civettini et al., 1999), *Milicia excelsa* (Welw) C.C. Berg (Moraceae) by *Phytolyma lata* Scott. (Hemiptera: Psylidae) (Iroko gallfly) (Agyeman et al., 2009), *Genista monspessulana* L. (Fabales: Fabaceae) (invasive French broom) by *Lepidapion argentatum* Gerstaecker (Coleoptera: Brentidae) (weevil) (Bitume et al., 2019).

Gall inducers are able to intercept or redirect nutrients from other parts of the host plant to galls, making galls metabolic sinks (Abrahamson & McCrea, 1986; Bagatto & Shorthouse, 1991; Li et al., 2017; Marini-Filho & Fernandes, 2012; McCrea et al., 1985). Our result supports this assertion. The concentration of all three nutrients (N, P and K) in galls were higher than in stem section below the gall and normal stem without gall. This implies that galls acted as mobilising sinks by drawing nutrients from other parts of the host plant as reported by previous studies (Larson & Whitham, 1991; Marini-Filho & Fernandes, 2012; McCrea et al., 1985). Furthermore, an anatomical study
reveals the fact that during gall formation, the innermost layer of the galls formed by *P. utilis* is transformed into a layer of nutritive tissue containing six to seven layers of cells with dense cytoplasm, large nuclei, and is highly mitotic (Bennett & van Staden, 1986). Besides, nutrient sink, a radiochemical experiment demonstrates that *P. utilis* gall also acts as strong sinks of photoassimilates (van Staden & Bennett, 1991). Formation of gall with strong sink capacity is often associated with significant harm to the host plants (Harris & Shorthouse, 1996). Therefore, our data and previous studies suggest that stem galling by *P. utilis* can have significant negative impact on *A. adenophora*. Our results corroborate the nutrition hypothesis which states that gall tissues have higher concentration of nutrients than the tissues without gall (Price et al., 1986, 1987).

Nitrogen is one of the limiting nutrients that plays a vital role in growth, development, reproduction, and survival of herbivores (Awmack & Leather, 2002; Mattson, 1980). Our result that gall tissues had higher nitrogen concentration in comparison to normal stem tissues is consistent with the previous findings (Cuevas-Reyes et al., 2011; Li et al., 2017). Similarly, phosphorus is another essential nutrient for the growth and development of herbivores (Cease et al., 2016; Perkins et al., 2004). We found that phosphorus concentration was also higher in gall tissues in comparison to ungalled tissues and this pattern is similar to the previous studies (Abrahamson & McCrea, 1986; Cuevas-Reyes et al., 2011; Tsao & Whaley, 1950). A high concentration of potassium in gall cells as we reported helps to maintain high negative osmotic potential, thereby increasing the turgor pressure, which helps in spontaneous bursting of the exit hole of the gall so that the insect can easily come out of the gall (Bagatto & Shorthouse, 1994).

Although this study considered elevation gradients as the major influential factor for the abundance and size of galls, there are other factors such as host plant density, age, architectural complexity, soil nutrients, and seasonality that are reported to affect the insect gall distribution (Boaventura et al., 2018; Cuevas-Reyes et al., 2011; Silva et al., 2015; Stokes & Stiling, 2013). Similarly, gall size is also determined by the interaction among plant genotype, insect genotype, and environment, and evidence also suggests that gall size (diameter) has evolved in response to selection by natural enemies (Weis & Abrahamson, 1985, 1986; Weis & Gorman, 1990). Therefore, incorporating all the bottom-up effects (e.g. host plant quality, quantity, and morphology), top-down effects (e.g. natural enemies like parasitoids), and the interaction between them would provide valuable knowledge on insect gall distribution, gall size, and population dynamics of galling insects, which ultimately determines the success of a galling insect as a biocontrol agent against weeds.

**Conclusion**

Using the data collected from sites covering the entire elevation range of distribution of *Ageratina adenophora* and its galling insect in Nepal, we showed that the abundance of galls peaked at the mid-elevation and declined towards higher and lower elevation regions, exhibiting a unimodal pattern. Overall, our results suggest that mid-elevation represents the optimal condition for the gall insect growth within the elevation range studied and galls induced by *P. utilis* on *A. adenophora* act as strong nutrient sinks. Since our study considered only one abiotic factor (elevation) in shaping the distribution of galls and their size, future studies that incorporate the effect of other abiotic factors like soil nutrition, seasonality, and biotic factors like natural enemies (i.e. parasitoids) on gall
abundance and size will improve our understanding of the gall dynamics. Besides biotic and abiotic factors, future investigations should also consider how the interaction among these factors determines the distribution and size of galls, which determines the success of a galling insect as a biocontrol agent.

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Disclosure statement

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