

Establishment of *Cecidochares connexa* (Diptera: Tephritidae) in Guam and Its Effect on the Growth of *Chromolaena odorata* (Asteraceae)

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ABSTRACT The gall fly *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) was imported from Indonesia into Guam in 1998. It was field-established at one location in north central Guam, and its spatial and temporal spread in Guam was monitored. The biology of the gall fly is presented. No relationship between the number of larvae in a gall and the dimensions of the gall (length and width) existed. Three pupae per gall was the most frequent density with a range of 1–7. The sex ratio of flies emerging from galls was 1:1 irrespective of number of larvae per gall. *C. connexa*-induced galls established a “nutrient sink” as a result of the formation of a nutritive tissue along the walls of the larval chamber. Younger galls contained more mineral nutrients, namely, calcium, iron, potassium, manganese, and zinc, which were depleted as the galls matured, but the reverse trend was noted for sodium. Galls developing on terminal meristems significantly reduced the length of the shoots of *Chromolaena odorata* (Asteraceae).

KEY WORDS *Chromolaena*, gall, nutrient sink, biocontrol, *Cecidochares*

Chromolaena odorata (L.) R. M. King and H. Robinson (Asteraceae) is a Neotropical shrub introduced as an ornamental plant to India in 1845 (Voigt 1845), and has spread throughout humid tropical Asia, Africa and Micronesia. It has become a serious weed in plantations, pastures, disturbed land, and forests (Muniappan et al. 2005). *C. odorata* is perennial, forms thickets and grows to a height of three meters. It is a problem mostly in plantations, pastures, vacant lots, disturbed forests, and wildlife reserves, but not in annual-cropping fields (Muniappan et al. 2005). Attempts to control this weed by mechanical, cultural, and chemical means have proved either uneconomical or ineffective (Muniappan et al. 2005). Biological control is considered as the main option available to manage this weed and it has been adapted in several countries, where this weed is a problem.

The gall fly *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) is considered an effective biological control agent, because it induces galls both on the terminal and axillary vegetative meristems of *C. odorata* (McFadyen et al. 2003). *C. connexa* was imported into Indonesia in 1993 from Colombia (McFadyen et al. 2003) and imported from Indonesia into Guam in 1998 (Muniappan and Bamba 2002). *C. connexa* has been tested for host specificity and was field-established in Guam in 2003 (Horner 2002, Muniappan et al. 2005). Gall inducers are frequently preferred in the biological control of weeds because of their nar-

row host range. The galls also act as nutrient sinks, reducing vegetative and reproductive growth (Harris and Shorthouse 1996, Muniappan and McFadyen 2005). Because their effects are indirect, the effectiveness of gall inducers is frequently underrated compared with shoot and root borers, leafminers, and defoliators (Dennill 1988). In many instances, the efficiency of gall inducers is reduced by local parasitoids within the introduced countries (Julien and Griffiths 1998, McFadyen et al. 2003). Absence of such parasitoids in Guam (Z.T.C., personal observation) makes *C. connexa* a desirable agent. Other factors known to reduce the effectiveness of gall inducers as biocontrol agents include plant vigor determining gall insect abundance, plant response to galling through overcompensation, and possible competitive displacement by other agents with overlapping niche (Harris and Shorthouse 1996, McFadyen et al. 2003).

In this article, we present the spatial and temporal distribution of *C. connexa* in Guam. We describe its biology, the characteristics of the nutrient sink induced by the feeding action of *C. connexa* larvae, and the reduction in vegetative growth of the host plant due to gall induction.

Materials and Methods

Spatial and Temporal Distribution. *C. connexa* pupae received from Indonesia in 1998 were cultured in the quarantine laboratory at the University of Guam for host specificity tests. Flies were field-released at

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Tiyan in north central Guam in late May 2002; however, a powerful typhoon hit Guam in July 2002, destroying the plants in the released site. A second release of *C. connexa* was made in Tiyan in 2003, and the spatial and temporal spread of the gall fly was monitored periodically by surveying galls along the outer margins of the establishment.

Biology of the Gall Fly. A pair (male and female) of flies were maintained in independent test tubes (20 by 3 cm) for egg laying; each test tube was stoppered with a muslin cloth-wrapped plug. Flies were fed with 50% aqueous honey solution. Eggs laid in the test tube were measured under a binocular microscope by using a calibrated ocular micrometer. One pair of mated flies was released onto a single, potted *C. odorata* plant in the laboratory, which remained within a muslin cloth cage, for a period of 24 h for oviposition by the gravid female. Shoots were examined daily for 3 wk after the release of the flies. Before pupation, mature larvae cut a tunnel up to the epidermis, and in this article, the context of the tunnel remaining closed by the epidermis is referred as the "window." The galls were removed from the plant when the window formed and the larvae were in prepupal stage. The galls were dissected and the prepupae were transferred to a petri dish. Pupae were monitored for adult emergence to calculate the pupal duration, which was done by subtracting the egg duration time from the time of egg laying to prepupal period. To determine the relationship between the number of pupae and the gall size, 100 galls with windows were harvested from *C. odorata* plants in the field at Tiyan. The width and length of the galls were measured using vernier calipers (Wiha Werkzeuge GmbH, Schonach, Germany). Galls were dissected, and numbers of pupae were counted. Forty mature galls with unopened windows were harvested from the field, dissected, and the pupae counted to determine the greatest number of pupae per gall. One hundred mature galls with unopened windows were harvested from the field, dissected, and the pupae were sorted according to the number per gall. Emerged adults were sexed and recorded to determine the sex ratio of the individuals in a gall.

Nutrient Sink within Galls. Young, medium, and mature galls \approx 30, 40, and 50 d old respectively, were harvested from *C. odorata* plants in the field at Tiyan using sterile scissors. These galls were sorted by age and transferred to Ziploc plastic bags. Larvae and pupae were removed by dissection of the galls. One gram of gall tissue was placed in an ashing beaker (50 ml), and ashed in a muffle furnace at 550–660°C for 2 h and allowed to cool. Ten milliliters of 0.2 N HCl was then added and transferred into a 50-ml volumetric flask. The beaker was rinsed with 0.1 N HCl and was added to make a total volume of 50 ml. The solution was left undisturbed overnight at a room temperature of 25°C. The solution was filtered through Whatman no. 5 filter paper and diluted 10 times. One milliliter of the filtrate was added to 9 ml of lanthanum solution. The resulting solution was analyzed on a 220 Fast Sequential of Atomic Absorption Flame Spectrophotometer (Varian, Walnut Creek, CA). Galls of differ-

ent ages were analyzed separately for different nutrient elements. Each analysis was replicated six times.

Effect of Galling on the Height of the Plant. Sixty *C. odorata* seedlings (15–18 cm in height) were uprooted from the field and placed in plastic buckets (37 cm in height by 30 cm in diameter) with tap water to prevent wilting until they were transported to the laboratory. Seedlings were then individually transplanted in 3.0-liter pots with soil. All potted plants were placed in a nylon screen cage (183 by 183 by 183 cm) and watered daily. One tablespoon of Miracle-Gro (15-30-15, Scotts Miracle-Gro Products Inc., Marysville, OH) was dissolved in 3.7 liters of water, and 250 ml was applied per plant per week. Ten plants were randomly selected as the control and placed in a separate nylon screen cage. Fifty plants were exposed to 19 females and 15 males of *C. connexa* for 24 h. When signs of gall development became visible, plant height was recorded weekly until the windows formed on galls.

Statistical Analysis. All the statistical analyses were carried out in SPSS for Windows 12.0 (SPSS Inc., Chicago, IL). The data were analyzed using one-way analysis of variance (ANOVA) at $P = 0.05$, and significant differences between means were separated using Tukey honestly significant difference (HSD) test at $P = 0.05$. The data from the effect of galls on the plant growth of *C. odorata* were analyzed using one-sample *t*-test.

Results

Spatial and Temporal Distribution of *C. connexa*. The gall fly has spread \approx 3,800 ha since its introduction in June 2003 (Fig. 1). The prevailing winds on Guam are mostly northeasterly. Initially, the spread was slow, the fly was found only 2 km downwind 1 yr after the release. However, in the second year, the fly was found over 10 km downwind and \approx 2 km upwind from the release point.

Biology of the Gall Fly. Males and females of *C. connexa* can be easily distinguished using external characteristics. Duration of different stages in the life cycle is shown in Table 1. Larvae remained in individual gall chambers within a gall and the wall between the larvae coalesced rarely. The most frequent number of pupae found in a gall collected in the field was three (42% of the total population) (Fig. 2). The number of pupae in a gall varied from one to seven. The length ($r^2 = 0.05$, $P \geq 0.05$) and width ($r^2 = 0.31$, $P < 0.05$) of the galls did not correlate significantly with the number of pupae in them. The sex ratio was 1:1, and the males emerged earlier than the females.

Gall as a Nutrient Sink. Iron (Fe), zinc (Zn), manganese (Mn), potassium (K), calcium (Ca), and magnesium (Mg) were significantly ($P < 0.05$; Fig. 3) higher in young galls than in the medium-aged galls. However, Fe, Zn, K, Ca, and Mg were significantly lower in the mature galls than the young and medium-aged galls (Fig. 3). No significant difference was found between medium and mature galls in Mn ($P < 0.05$; Fig. 3). A reverse trend was noted in the value of

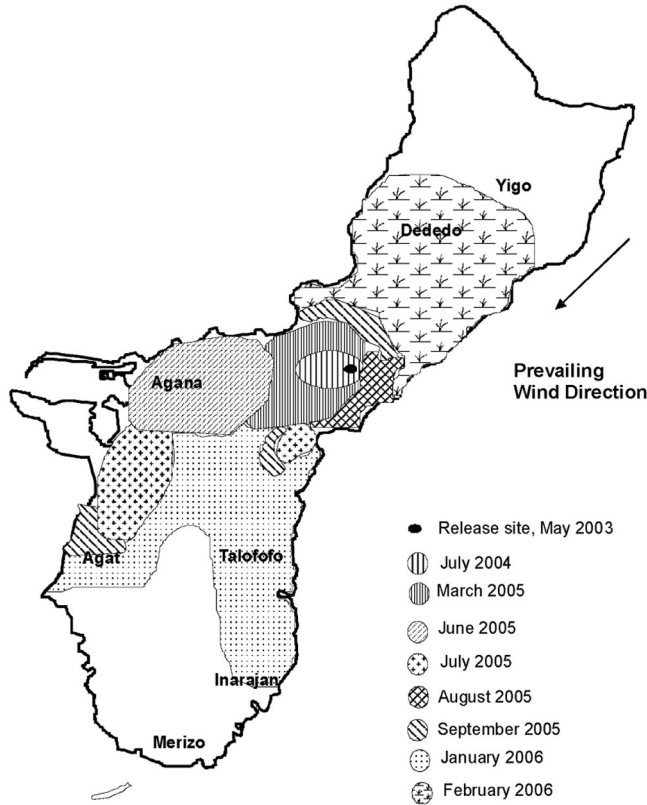


Fig. 1. Spatial and temporal distribution of *C. connexa*.

sodium (Na). There was significantly less Na in young galls than in medium aged and matured galls.

Effect of Galls on the Growth of the Plants. The height of plants with and without galls is shown in Fig. 4. The plants bearing galls were significantly shorter than the control throughout the 8 wk of observations.

Discussion

Gall inducers are favored as biocontrol agents of weeds because they usually have a narrow host range (Harris and Shorthouse 1996). *C. connexa* is highly host specific to the genotype of *C. odorata* that has invaded Asia, West Africa, and Micronesia, whereas it does not survive on the genotype that has invaded southern Africa (Muniappan et al. 2005).

The spatial and temporal spread shows a relatively rapid movement and establishment of the flies in the

areas downwind and southwest from the release point (Fig. 1). Movement in the upwind and northeasterly direction was restricted. The spread toward southwest was 8 times more than in the northeasterly direction. A similar trend was observed with the spread of another biocontrol agent, *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) of *C. odorata* in Guam (Seibert 1989).

McFadyen et al. (2003) made some observations on biology of *C. connexa* in Indonesia. Adult females and males lived on the average 7.6 and 6.4 d, respectively. Both males and females mated immediately after emergence and mated repeatedly. Eggs were laid

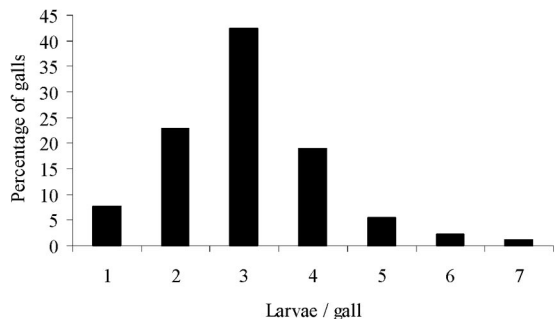


Fig. 2. Number of *C. connexa* larvae per in gall in the field (n = 92).

Table 1. Duration of different stages in the life cycle of *C. connexa*

Stage	n	Days ± SE
Egg	11	5.8 ± 1.3
Larva	11	41.6 ± 3.6
Pupa	11	21.6 ± 3.1
Female life span	13	7.0 ± 2.7
Male life span	13	6.2 ± 3.3
Egg to adult	11	69.0 ± 3.5

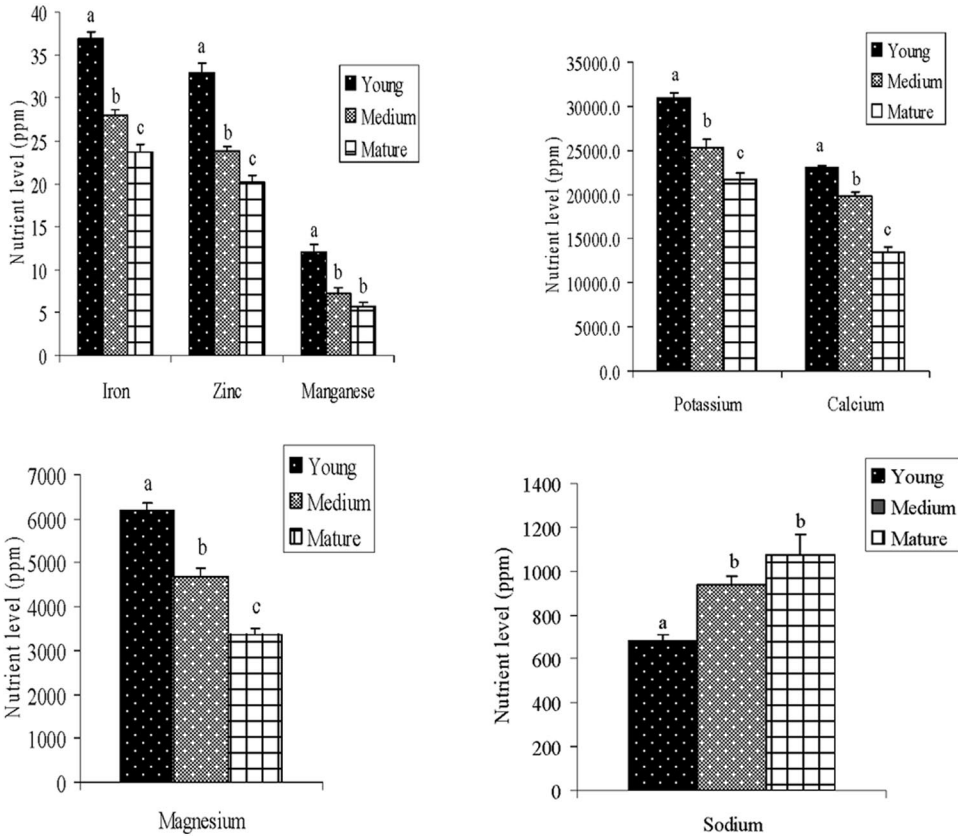


Fig. 3. Minerals in *C. odorata* galls at different stages of development. Bars (mean of six replicates per treatment) with the same letter within each nutrient level are not significantly different ($P < 0.05$; one-way ANOVA and Tukey HSD test).

within a day after mating. Elongated-spindle shaped creamy white eggs were laid in patches in the terminal or axillary buds. Eggs were 0.6 mm in length and 0.12 mm at maximum width. Egg duration averaged 5.7 d. Emerged larvae burrowed into the tips of *C. odorata*, inducing galls. The life cycle averaged 69.0 ± 3.5 d. Because our studies were conducted in the laboratory and under caged conditions during the nonflowering season, no diapause of the larva was observed, as

mentioned by McFadyen et al. (2003) in Indonesia. The most frequent number of pupae found in a gall found was three. The size of the gall and the number of pupae in it depended on the vigor of the plant (McFadyen et al. 2003). We observed one to seven pupae per gall in this study. McFadyen et al. (2003) found *C. connexa* to lay eggs in packed clusters of two to 16. Our finding of a maximum of 10 pupae per gall is probably due to competition for nutrients and space in the gall and possible mortality of some maggots.

The larva that induces the gall derives its nourishment from the specialized tissue in the gall chamber, which is made of proliferating parenchyma cells on the walls of the chamber (Meyer and Maresquelle 1983, Bronner 1992). Such a specialized nutritive tissue becomes a metabolic sink for nutrients from adjacent plant tissues or other parts of the plant (Raman 1994, Raman and Abrahamson 1995, Harris and Shorthouse 1996). Galls arise through the modification of the structure and metabolism of plants (Raman et al. 2005). Gall induction reduces several vital metabolic functions of the host plant, e.g., photosynthetic and transpiration efficiencies, stomatal conductance, and water potential (Abrahamson and Weis 1987; Raman 1994; Florentine et al. 2001, 2005). Galls also accumulate minerals, such as Ca, copper (Cu), Fe, Mg, Mn,

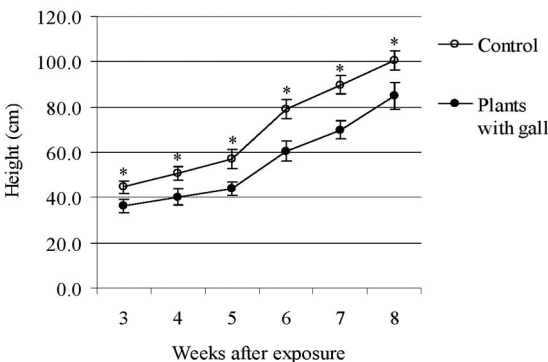


Fig. 4. Effect of galls on growth of *C. odorata*. Asterisks indicate significance at $P < 0.05$ (one-sample *t*-test).

nickel (Ni), and Zn (Abrahamson and Weis 1987, Bagatto and Shorthouse 1991, 1994, 1997; Florentine et al. 2005; Paquette et al. 1992). In some plants, gall induction reduces main shoot height, flower and leaf production, and shoot and root biomass (Raman and Abrahamson 1995, Navie et al. 1998, Dhileepan and McFadyen 2001), and it also causes temporary cessation of growth resulting in death of stems above the gall (Erasmus et al. 1992). Insect-induced galls become nutrient sinks (Kirst and Rapp 1974, Larson and Whitham 1991, Fay et al. 1993), which would otherwise be used by the plants for their growth and reproduction (Lalonde and Shorthouse 1984, Raman and Abrahamson 1995). Young galls of *C. connexa* accumulated Fe, Zn, Mn, K, Ca, and Mg. The larvae used these mineral nutrients during their development (Fig. 3). However, a reverse trend was noted in Na. Further studies are required on this aspect.

The metabolic sink that the galls formed by drawing the nutrients from the adjacent tissues or other parts of the plant distinguishes gall inducers from nongalling organisms in terms of their effectiveness as weed control agents (Harris and Shorthouse 1996). Lalonde and Shorthouse (1984) and Raman and Abrahamson (1995) stated that the nutrient sinks affect the growth and reproduction of the plant. There was significant difference in the height of the plants with and without galls in the terminal shoots (Fig. 4).

The gall flies *Procecidochares utilis* Stone (Diptera: Tephritidae) in Hawaii, New Zealand, Australia, South Africa, India, Nepal, China, and Thailand and *Procecidochares alani* Steyskell (Diptera: Tephritidae) in Hawaii and Australia have been released for control of the weeds *Ageratina adenophora* and *Ageratina riparia*, respectively (Julien and Griffiths 1998). The effectiveness of these gall flies was greatly reduced by the local natural enemies of the tephritid flies (McFadyen et al. 2003). In spite of 50% parasitism observed in some parts of Indonesia, McFadyen et al. (2003) stated that *C. connexa* has given successful control of *C. odorata*. No parasitism of *C. connexa* has been recorded in Guam. We expect the impact of the gall fly to complement the effect of the already established natural enemy, *P. pseudoinsulata*, in controlling *C. odorata* in Guam. *P. pseudoinsulata*, introduced into Guam \approx 20 yr ago, has reduced *C. odorata* population from thickets to scattered patches. The induced-defenses in *C. odorata* have minimized the effectiveness of *P. pseudoinsulata* in suppressing the scattered plants (Marutani and Muniappan 1991). The impact of the gall fly on growth and reproduction in addition to the defoliation by *P. pseudoinsulata* should provide a successful control of *C. odorata* in Guam.

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