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Distribution and Frequency of Galls Induced by *Anisodiplosis waltheriae* Maia (Diptera: Cecidomyiidae) on the Invasive Plant *Waltheria indica* L. (Sterculiaceae)

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Distribuição e Freqüência de Galhas Induzidas por *Anisodiplosis waltheriae* Maia (Diptera: Cecidomyiidae) na Planta Invasora *Waltheria indica* L. (Sterculiaceae)

RESUMO - Estudou-se a freqüência de galhas induzidas por *Anisodiplosis waltheriae* Maia*,* espécie recentemente descrita, em *Waltheria indica* L. *W. indica* é uma erva invasora em áreas de regeneração de Floresta Atlântica no Sudeste do Brasil. As plantas foram coletadas em maio de 2004 e a biomassa aérea, comprimento do caule, número de folhas, número de galhas por folha e área foliar de cada indivíduo foram registrados. Quase 90% das plantas e 25% das folhas foram atacadas, com a média de 0,67 galhas/folha. A área foliar pouco influenciou o número de galhas, porém o número de folhas não afetou a abundância de galhas (P > 0,05). Apenas 31% da variação no número de galhas foi explicada pela biomassa da planta. Um terço das galhas foi morta por inimigos naturais. A predação explicou 22,9% da mortalidade, fatores desconhecidos mataram 7,6%, microhimenópteros parasitóides mataram 2,5% e fungos apenas 1%. Os fatores de mortalidade não foram influenciados pela área foliar nem densidade de galhas.

PALAVRAS-CHAVE: Aimorés, fator de mortalidade, Floresta Atlântica, herbivoria, galha de inseto, invasão biológica

ABSTRACT - The frequency of galls induced by *Anisodiplosis waltheriae* Maia, a recently described species, on *Waltheria indica* L. was studied. *W. indica* is an invasive weed in regeneration areas of Atlantic Forest in southeastern Brazil. Plants were collected in May 2004 and above-ground biomass, main stem length, number of leaves, number of galls per leaf and leaf area of each individual were recorded. Nearly 90% of all plants and 25% of all leaves were attacked by the gall midge, with an average of 0.67 galls/leaf. Leaf area had a weak effect on gall abundance while the number of leaves had no effect on gall abundance. Only 31% of the variation in gall abundance was explained by plant biomass. Natural enemies killed one third of the sampled galls. Predation accounted for 22.9% of gall mortality, unknown factors killed 7.6%, microhymenopteran parasitoids killed 2.5% and fungi only 1%. Mortality factors were not influenced by leaf area or gall density.

KEY WORDS: Aimorés, Atlantic Forest, insect gall, herbivory, mortality factor, biological invasion

Biological invasion is the second largest cause of biodiversity loss after habitat destruction (Vitousek *et al.* 1997). Invasive plants adversely affect ecosystem structure and function in habitats across the world, altering water and fire regimes, changing the soil nutrient status and altering geomorphological processes (Rose & Fairweather 1997). They also diminish native species richness through competition, usually won by the invaders. The success of exotic plants especially weeds, relates to particular attributes, such as rapid vegetative growth and flowering, large quantities of seeds produced, vegetative propagation, lack of a specialized pollination system and germination requirements (Lake & Leishman 2004). One of the processes believed to allow the greatest success of exotic plants is the absence of herbivores, or the Enemy Release Hypothesis (ERH). This hypothesis states that a plant species introduced into a new region should experience a decrease in the regulation by herbivores and other natural enemies, therefore resulting in an increase in distribution and abundance (Keane & Crawley 2002).

Natural ecosystem invasion by exotic species is disturbing-dependent and the kind of disturbance is critical on the success of the invasive plants and impact on the native species (Lake & Leishman 2004). Besides, soil nutritional enrichment would be a critical requirement to invasive success (Li & Norland 2001). The ideal scenario for biological invasion described by Lake & Leishman (2004) is similar to many regeneration areas found in the Atlantic Forest in southeastern Brazil. In rehabilitation programs of the Atlantic forests, invasive plant species may encounter better conditions to grow as both plant and animal communities are disturbed. Furthermore, soils are enriched by the application of fertilizers and elimination of some important herbivores such as leaf cutting ants. This scenario may favor the success of invasive species.

Attempts to control invasive plants have centered primarily on aggressive management options, including the use of herbicides (Rice & Toney 1998), though biological control methods have been also considered (Rieder *et al*. 2000, Florentine *et al*. 2001, Hoffman *et al*. 2002, Tewksbury *et al*. 2002). Therefore, detailed knowledge is needed on the natural history and ecology of invasive plants. For instance, the biological control of such species with insects should be preceded with detailed studies as the controlling agents might cause deleterious effects to non-target species (Pearson *et al*. 2000, Jordon-Thaden *et al*. 2003, Ortega *et al*. 2004).

The use of herbivorous insects with a close association with their host plant, such as galling insects, is of extreme relevance when considering invasive plant species control. Many species of galling insects have been used in weed biological control because they may cause strong losses on growth and fitness of their host plants (Fernandes 1987, Silva *et al*. 1996, Rieder *et al*. 2000, Florentine *et al*. 2001, Hoffman *et al*. 2002) and because they are highly tunned to their host plants' physiology, genetics, chemistry and phenology. In a recent review on the biology and ecology of gall-inducing arthropods employed in the biological control of weeds, Muniappan & McFadyen (2005) argue that galling insects are excellent candidates for biological control practices though their use have been underestimated (see also Fernandes 1987). Few examples from Brazilian species are available. *Psidium cattleianum* Sabine (Myrtaceae) is a Brazilian native species that forms dense monotypic stands excluding all other vegetation in Hawaii. It has been ranked as the primary weed of forests and has been the subject of control efforts for a number of years, which employ the use of five galling species (Pedrosa-Macedo *et al*. 2000).

In a study of galling insects that attack plant species used in land reclamation in an Atlantic Forest in southeastern Brazil, we found large numbers of galls induced by *Anisodiplosis waltheriae* Maia on an important invasive weed, *Waltheria indica* L. This weed is widespread in Brazil, and represents a species of much concern (Lorenzi & Matos 2002). Although at this stage the species does not appear to represent a major ecological problem to the reclamation program, we present the first data on the interaction of the galling insect and the host plant, providing basic information useful for further studies of biological control. Four questions were addressed: i) What is the local frequency of attacked plants in the field and what is the frequency of attacked leaves per plant?; ii) Is gall abundance influenced by leaf area?; iii) How does plant architecture influence gall abundance?; iv) What are the mortality factors that act upon the galling larva and how are the mortality factors influenced by leaf area and gall density?

Material and Methods

The host plant *W. indica* is a perennial weed present mainly in the cerrado vegetation and grassland of Brazil. It occurs from Minas Gerais, in southeast Brazil, to Rio Grande do Sul, in the extreme south (Lorenzi & Matos 2002). *W. indica* is an autochorous species that grows spontaneously in perennial agriculture forest plantations, along road sides, abandoned pastures, limestone mining quarries and unused plots of land (Rodrigues & Carvalho 2001, Frenedozo 2004), therefore being considered a weed of high economic importance in early-established communities (Frenedozo-Soave 2003). Otherwise, *W. indica* may represent a rich food source to wasps which predate upon crop insect plagues (Macedo & Martins 1998, 1999).

The galls are induced by a recently described species, *A. waltheriae* (Maia & Fernandes 2005). Galls are induced on leaves, leaf buds and even inflorescences of *W. indica*. They are spherical to conical, covered with short light yellow to light brown trichomes, have one chamber where a single larva can be found (Fig. 1).

Study area. The study was conducted in May 2004, in Fazenda Bulcão, located in Aimorés, state of Minas Gerais, southeast Brazil (19º 26' 42'' S, 41º 3'51'' W). The farm has an area of 676 ha and is recognized as a Natural Patrimony Reserve, where a large land reclamation program was initiated in 2001. The Aimorés mountain ridge was originally covered by Atlantic Forest vegetation, but nowadays the region is in an accelerated degradation stage due to the colonization process, wood logging, generalized deforestation and the Vitória - Belo Horizonte railroad construction. Intensive and inconsequent agriculture and cattle farming activities provoked further forest fragmentation and degradation. In the land reclamation project, native species have been propagated and planted in large forest stands.

Sampling. We randomly sampled 70 *W. indica* plants in a roadside area that contour the various native rehabilitation plots in Fazenda Bulcão. Sampled individuals were cut at the soil level, stored in plastic bags and immediately taken to the laboratory, where main stem length, total weight, number of leaves and number of galls per leaf were recorded. We also recorded leaf area of galled leaves. Galls were later dissected to determine the larvae mortality factors according to Fernandes & Price (1992): parasitoids of the galling larvae, predation on the gall tissue and/or galling larvae, fungus-caused diseases and other factors probably plant resistance and death caused by parasitoids during feeding or oviposition.

To test the correlation between total number of galls and plant length, biomass and number of leaves, we used a Stepwise Multiple Regression. To analyze the correlation

Figure 1. (A) External morphology of the leaf gall of *A. waltheriae* on *W. indica*. (B) High abundance of galls on the host plant may cause death of the attacked organs.

between leaf area and number of galls we used a simple linear regression.

Results and Discussion

Along the roadside studied we found several species of African and native grass species, many shrubs and herbaceous species, including *W. indica*. We did not evaluate the relative abundance of *W. indica* but it was visually one of the most common species. We surveyed 1943 galls on the 70 individuals of *W. indica*. Out of the 70 individuals of *W. indica* sampled, 62 were attacked by the gall midge, representing 88.6% of all plants sampled. Overall, 734 leaves were galled while 2,156 remained free of galls, indicating that approximately 25.4% of leaves in the population studied supported at least one gall. On a plant basis, one individual had an average of 27.8 galls per leaf while the overall average was 0.67. These data indicate that the cecidomyiid galls are very frequent on this host species. It may represent an important finding as W. *indica* is a weed of high invasive potential (Lorenzi & Matos 2002). We also found some heavily attacked plants whose leaf blades were no longer visible because of the large number of galls present (Fig. 1-B). This kind of attack may be of a major impact to the plant since any decrease in leaf area should represent a decrease in photosynthesis rate. Furthermore, because galls act as sinks to nutrients and photosynthate (Kirst 1974, Kirst & Rapp 1974, Larson & Whitham 1991), heavily attacked individuals could have a diminished performance.

Leaf area was not a good predictor of gall abundance (F = 3.662; P = 0.056; r^2 = 0.004). This may not be viewed as a surprise as galls are known to strongly influence the host organ attacked (e.g., Larson & Whitham 1991, Fay *et al*. 1993, Fernandes *et al.* 1993). Attack was very intense on many leaves, and we argue that attacked leaves may have the normal growth pattern altered resulting in the weak observed trend. Otherwise, future studies of the galling insect during the gall inducing process could aid in the solution of this question.

Nor stem length neither number of leaves contributed to

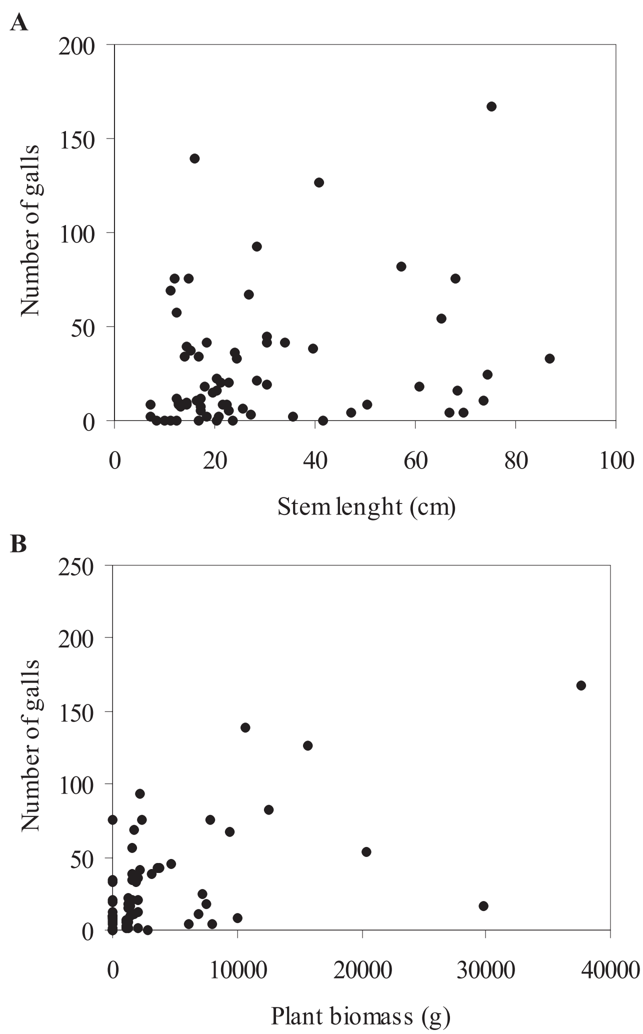

Figure 2. Regression analysis between total number of galls on *W. indica* and main stem length (A) and total plant biomass (B).

explain gall abundance ($P > 0.05$). Thirty-one per cent of the variation in gall abundance per plant was explained by plant biomass, i.e., larger plants supported more galls ($P < 0.001$; Fig. 2). However, when the number of galls was divided by plant biomass, this relationship no longer exists. Larger plants supported fewer galls per unit of weight than smaller plants ($F = 4.72$; $P = 0.03$; $r^2 = 0.05$) meaning that females oviposit on plants regardless of host size.

Approximately one third (33.6%, n = 648) of the sampled galls died due the action of natural enemies (top-down effects). Predation by lepidopteran catterpillars that consumed the gall tissue from outside was responsible for most of the mortality acting upon the gall-inducers (22.9%). Unknown factors killed 7.6% of the gall population, while parasitism by microhymenopteran insects killed another with 2.5%. Mortality induced by pathogens (unidentified fungi) was very low (1%) during the study (Fig. 3). Therefore, in nearly 66% of the surveyed galls, insects survived after successfully inducing galls. This is a rather high survivorship rate for galling insects (see Mani 1964, Fernandes & Price 1992) and also an attribute for candidates for weed biological control (Muniappan & McFadyen 2005). Other factors such as plant resistance may play an important role in gall abundance (see Fernandes *et al*. 2000), but bottom-up effects were not evaluated in this study. Neither leaf area nor gall density influenced the mortality factors. The correlation of these factors with the percent of total mortality per plant was insignificant in both cases.

With exception of Homoptera, all gall-inducing taxa were employed in biological control, but the effectiveness in controlling the weeds by gall midges is reduced by high levels of parasitoids, which are regarded as the main reason for failure of weed biological control (Muniappan & McFadyen 2005). The high gall survival rate of *A. waltheriae,* due to low parasitism, suggests that this species may be employed for biological control of *W. indica*. However, future studies should be performed to better address the viability of biological control of *W. indica* using *A. waltheriae*, particularly in the Atlantic forest where biological invasions are completely undesired (Myers *et al*. 2000).

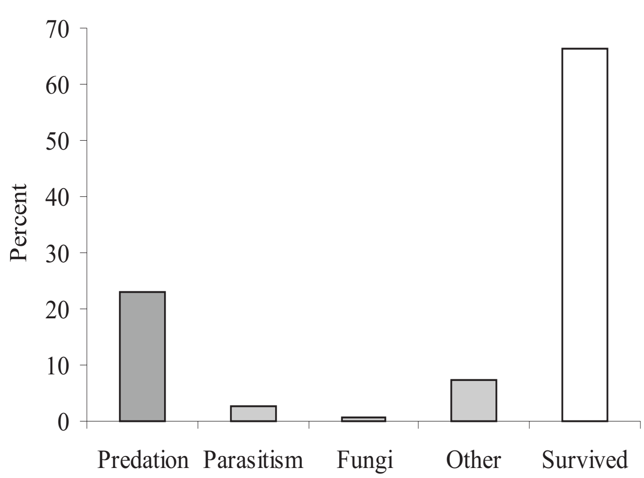

Figure 3. Mortality factors acting upon the galls on the host plant *W. indica*. Black bars represent the mortality agents acting upon the gall while the white bar represents survivorship of the galling larvae. The mortality factor indicated by others represents mortality factors that could not be identified.

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