The occurrence and effectiveness of hypersensitive reaction against galling herbivores across host taxa

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Abstract. 1. Hypersensitive reaction is an important type of induced defence by which the plant elicits a defence response to pathogens and insects. Hypersensitive reaction has been argued to be the most common plant resistance mechanism against insect herbivores that have intimate associations with their host plants.
2. The work reported here attempted to establish how important and widespread hypersensitive reaction might be against gall-forming species across host taxa.
3. Hypersensitive reaction was the most important mortality factor against gall formation across host plant taxa in seven out of eight cases.
4. The number of insect galls correlated with the size of the leaves but module (leaf) size was a weak factor influencing the incidence of plant hypersensitive reaction to galling.
5. Insect galls and hypersensitive reactions occurred in genetically distant as well as geographically widespread host plant taxa.

Key words. Bottom-up effect, hypersensitive reaction, induced defences, insect galls, insect–plant interactions, plant module size.

Introduction

Hypersensitive reaction is an important type of induced defence whereby the plant elicits a response to fungi, bacteria, virus, nematode, or insect attack (Maclean et al., 1974; Agrios, 1988; Fernandes, 1990, 1998; Beckman & Ingram, 1994; Gopalan et al., 1996; Low & Merida, 1996; Fritig et al., 1998). Hypersensitive reaction was recently argued to be the most common plant resistance mechanism against insect herbivores that have intimate associations with their host plants, such as gall formers, bark beetles, and wood borers (reviewed by Fernandes, 1990). These guilds represent a large number of species, and/or include species that cause serious economic damage to crop plants (see Coulson & Witter, 1984; Fernandes, 1987).

The physiological changes and, at least in part, the induced gene expression driving hypersensitive reaction are well characterised. In response to the recognition of pathogens, the plant triggers a cascade of events, such as oxidative burst, the expression of defence-related genes in neighbouring cells (Somssich et al., 1988; Schmelzer et al., 1989), the deposition of callose and lignin (Dixon & Lamb, 1990), and the biosynthesis of complex antimicrobial secondary metabolites (phytoalexins) (Bayley & Mansfield, 1982; Smith & Banks, 1986; Dixon & Lamb, 1990; Dangl et al., 1996), which culminate in the hypersensitive response (Dixon & Harrison, 1994; Jones, 1994; Staskawicz et al., 1995; Baker et al., 1997). The host’s responses are achieved by a massive and rapid redirection of the plant’s metabolism, including morphological, histological, and biochemical changes that ultimately cause the death of the attacked tissue. In this way, the invading organism is localised, contained, inactivated, and eventually dies (Müller, 1959; Wong & Berryman, 1977; Baker et al., 1997; Fritig et al., 1998; Fernandes et al., 2000).

The necrotic response around the attacked site is the result of a disturbance in the balance between oxidative and reductive processes, which leads to excessive oxidation of polyphenolic compounds and a breakdown of cellular and subcellular structures (Cruickshank, 1980; Király, 1980; Staskawicz et al., 1995). Hypersensitive reaction is a localised induced resistance that can be detected only in the area immediately adjacent to the site of attack. It is seen as a circular, brown, necrotic spot. The same symptoms of localised necrosis have been observed in response to insect herbivore attack, leading to death of the eggs and/or the youngest instar larvae, however plant induced defences have rarely been considered as mortality factors in studies on the population.
dynamics of insect herbivores (see Price et al., 1990). Fernandes (1990) argued that studies may have missed hypersensitivity because necrotic spots frequently seen on attacked leaves and stems may have been treated erroneously as spots caused by pathogens, possibly because they affect the youngest, most inconspicuous stages of the insects. If these reactions go undetected, studies of population dynamics and life-table analysis for galling insects may underestimate the value of the hosts’ induced defence by misinterpreting the nature of plant–herbivore interactions.

The work reported here attempted for the first time to establish how important and widespread hypersensitive reaction might be against a wide range of gall-forming species. The goal was to find out how effective hypersensitive reaction is against galling herbivores across host plant taxa and whether hypersensitive reaction is influenced by host organ size.

Materials and methods

Study areas

The study was conducted in three distinct areas in tropical Brazil between August 1997 and July 1998. The first area, the Parque Nacional da Serra do Cipó, is located in the southernmost portion of the Espinhaço Mountains (19°15’–19°40’S, 43°30’–43°55’W), south-eastern Brazil. The region is situated within the central Brazilian plateau, which is 850 m a.s.l. on average (Freitas, 1951) and covered predominantly by cerrado (savanna) vegetation. The soil is sandy, shallow, has a low capacity to retain water and is nutrient-poor, with high concentrations of aluminium (Giulietti et al., 1987; Fernandes, 1994).

The second area is in the Estação Ecológica de Pirapitinga, southern Brazil. The biological station is a man-made island, created in 1965 in the Três Marias Reservoir (18°23’S, 45°20’W) at an altitude of 560 m a.s.l. The vegetation is primarily cerrado and the soil is sandy, shallow, and also nutrient-poor, with high concentrations of aluminium (Azevedo et al., 1987).

The third area is in the Estação Ecológica da Universidade Federal de Minas Gerais in Belo Horizonte, Brazil (19°52’S, 43°58’W). The area has been protected since 1969, and has several types of vegetation, including secondary mesophytic forest, cerrado, and swamps at an altitude of 830 m a.s.l. (Martins & Almeida, 1994).

Studied species

Plant species and individuals were selected with sufficient galls (at least 25 attacked leaves per individual) for statistical analysis. Despite a high species diversity of galls in the study areas (Fernandes et al., 1988; Lara & Fernandes, 1996; Gonçalves-Alvim & Fernandes, in press), the abundance of galling species and host species was low during the study period. Samples of the host plant leaves and galls were taken to the laboratory for dissection, counts of galls, measurements of leaf size, and evaluation of gall mortality factors. Most plant species were hosts to only one species of galling insect at all study sites, except Davilla rugosa, which had leaf and terminal shoot galls. Only the leaf gall was included in this study. Each plant species was studied in a single area although most occur in all three areas.

Bowdichia virgilioides Kunth. (Leguminosae) is a widely distributed tree in open areas of South America. Byssorina verbascifolia Rich. (Malpighiaceae) is a tree, about 3 m tall, with a tortuous and thick trunk, which is found all over Brazil. Davilla rugosa Poir. (Dilleniaceae) is a shrub composed of several sprouting stems that also occurs throughout Brazil. Oonatea floribunda Engl. (Ochnaceae) is a small shrub found in south-east and west-central Brazil. Serjania lethalis St. Hil. (Sapindaceae) is a liana that occurs in south-eastern and southern Brazil. Strychnos pseudoquina St. Hil. (Loganiaceae) is a tall and tortuous tree found in south-eastern and southern Brazil. Terminalia brasiliensis Camb. (Combretaceae) is a tree 12 m tall found in south-eastern, west-central, and north-eastern Brazil. Vatairea macrocarpa Benth. (Logumiaceae) is a tall (=9 m) tree found in north-eastern and south-eastern Brazil. Details of the host plants can be found in Bentham (1859), Ferri (1969), and Corrêa (1978).

All galls studied were located on leaves and were glabrous (Figs 1a–8a). They varied in shape from discoid to spherical and elliptical galls, had one larva per chamber, and one to three chambers per gall. Galls also varied in colour from yellowish-brown to green. They were all caused by Cecidomyiidae (Diptera), the most common galling taxon (see Mani, 1964; Gagné, 1989).

Methods

The first 15 individuals found of each plant species were selected for the study. Twenty-five mature leaves containing galls and/or hypersensitive reaction were collected haphazardly around the canopy of each individual. Leaves were placed in numbered plastic bags and taken to the laboratory. All collected leaves were numbered, their area (length × width, to the nearest mm) was estimated, and galls were counted and opened for identification of mortality factors (see Fernandes & Price, 1992). Data were averaged and presented on a per plant basis. Mortality was caused by parasitoids of the galling larva, predation on the gall tissue and/or galling larva, fungus-caused diseases, hypersensitive reaction, and other (unknown) factors. Deaths of galling larvae in galls opened by predators, but with fungus invasion, were considered as death by predation. Deaths of galling larvae in closed galls with fungus invasion were considered as death by fungal disease. Potential galls killed by the hypersensitivity of the plant could not be opened because galls never begin to develop or, in those that develop a few millimetres, the necrotic reaction fuses their walls (Fernandes, 1998). Hypersensitive reaction to galling can easily be distinguished from other insect herbivore and pathogen damage by a depression surrounded by the circular-shaped necrotic tissue that confines the attacking larva (see Maclean et al., 1974; Balbyshev & Lorenzen, 1997; Fernandes, 1998; Cornelissen & Fernandes, 1999; Fernandes et al., 2000).
Furthermore, the shape of the reaction is clearly maintained across host plant taxa (see Figs 1–8). Overall, 19,992 galls were dissected. Per cent data were arcsin transformed for statistical analysis. The data were then analysed by regression, using leaf area as the independent variable (Zar, 1984; Kleinbaum, 1996).

Results

The phenotypic reaction to gall attack

All hypersensitive reaction to gall induction was easily observed as a round, red-brownish spot around the gall

Fig. 9. Frequency of mortality factors and survivorship of galls on several host plants. Each bar represents the average (mean ± 1 SE) mortality factors and survivorship by plant species. HR = hypersensitive reaction.

Figs 1–8. Insect galls and hypersensitive reaction on several host plants. (1a) Cecidomyiidae leaf galls, (1b) hypersensitive reaction to galling on Bowdichia virgilioides (Leguminosae). (2a) Cecidomyiidae leaf galls, (2b) hypersensitive reaction to galling on Byrsonima verbascifolia (Malpighiaceae). (3a) Cecidomyiidae leaf galls, (3b) hypersensitive reaction to galling on Terminalia brasiliensis (Combretaceae). (4a) Cecidomyiidae leaf galls, (4b) hypersensitive reaction to galling on Duratea floribunda (Ochnaceae). (5a) Cecidomyiidae leaf galls, (5b) hypersensitive reaction to galling on Vatairea macrocarpa (Leguminosae). (6a) Cecidomyiidae leaf galls, (6b) hypersensitive reaction to galling on Strychnos pseudoquina (Loganiaceae). (7a) Cecidomyiidae leaf galls, (7b) hypersensitive reaction to galling on Davilla rugosa (Dilleniaceae). (8a) Cecidomyiidae leaf galls, (8b) hypersensitive reaction to galling on Serjania lethalis (Sapindaceae).
Fig. 10. Regression of the average number of galls per leaf against average leaf area in *Bowdichia virgilioides* ($r^2 = 0.53, F_{1,13} = 13.248, P < 0.01, y = -13.308 + 0.939x$), *Byrsonima verbascifolia* ($r^2 = 0.58, F_{1,13} = 18.005, P < 0.001, y = -0.751 + 0.028x$), *Terminalia brasiliensis* ($r^2 = 0.20, F_{1,13} = 3.194, P = NS, y = 0.976 + 0.063x$), *Ouratea floribunda* ($r^2 = 0.62, F_{1,13} = 21.101, P < 0.001, y = -14.629 + 0.613x$), *Vatairea macrocarpa* ($r^2 = 0.57, F_{1,13} = 16.892, P < 0.001, y = -0.713 + 0.142x$), *Strychnos pseudoquina* ($r^2 = 0.13, F_{1,11} = 1.676, P = NS, y = 1.242 + 0.089x$), *Davilla rugosa* ($r^2 = 0.58, F_{1,9} = 12.346, P < 0.01, y = -3.198 + 0.5x$), and *Serjania lethalis* ($r^2 = 0.01, F_{1,13} = 0.155, P = NS, y = 2.677 + 0.108x$).
initiation site (Figs 1b-8b). In these reactions, morphological and histological changes caused the death of the attacked tissue, which culminated in the localisation, containment, and death of the galling larvae (see Fernandes et al., 2000). The circular area was observed on both leaf surfaces but was darker on the adaxial surface where the galls would develop.

Fig. 11. Relationship between average percentage of hypersensitive reaction per leaf and average leaf area on several host plants. For statistical analysis see Table 1.
Microscopical observation of some galls indicated that the mesophyll structures were necrotic, lacked transport tissues, and the corky dead tissue was filled with polyphenolic compounds (G. W. Fernandes, unpublished).

The major mechanism of plant resistance to galling

Hypersensitive reaction was the most important mortality factor against gall formation across host plant taxa (Fig. 9). In four species, this plant resistance mechanism killed more than two-thirds of the galling population, leaving few larvae to survive and be found by natural enemies. In three out of eight species, hypersensitive reaction rates were approximately the same as the survivorship rates, while in one species, *Serjania lethalis*, the most important mortality factor was caused by pathogens and unknown factors (Fig. 9).

Module size and hypersensitive reaction against galling

The average number of galls per leaf was correlated with the average size of the leaves on a per plant basis (Fig. 10). In *B. virgilioides*, 53% of the variation in the number of galls per leaf was explained by leaf area. In *B. verbascifolia*, 58% of the variation in the number of galls per leaf was explained by leaf area, while in *T. brasiliensis*, no significant relationship was found between the variation in the number of galls per leaf and leaf area. In *O. floribunda*, 62% of the variation in the number of galls per leaf was explained by leaf area while in *V. macrocarpa* leaf area explained 57% of the variation in the number of galls per leaf. In *D. rugosa*, 58% of the variation in the number of galls per leaf was explained by leaf area. In *S. pseudoquina* and *S. lethalis*, no significant relationship was found between the variation in the number of galls per leaf and leaf area. Thus, there must be other factors influencing the variation in the number of galls per leaf (Cornelissen et al., 1997).

The hypersensitive reaction against the galling herbivore was common in leaves of all sizes and across plant taxa (Table 1, Fig. 11). Nevertheless, module (leaf) size was a weak factor influencing the incidence of plant hypersensitive reaction to galling. In *O. floribunda*, 27% of variation in hypersensitive reaction per leaf was explained by leaf area, while in *S. pseudoquina*, leaf area explained 31% of the variation in hypersensitive reaction per leaf. No influence at all was observed in *B. virgilioides*, *B. verbascifolia*, *D. rugosa*, *S. lethalis*, *T. brasiliensis*, or *V. macrocarpa*.

### Hypersensitive reaction and gall occurrence in the plant kingdom

Insect galls and hypersensitive reactions occurred in genetically distant as well as geographically widespread host plant taxa. They were found on Combretaceae, Dilleniaceae, Leguminosae, Loganiaceae, Malpighiaceae, Ochnaceae, and Sapindaceae in a short survey in tropical Brazil. The studied hosts also varied in growth form from liana (one species) to shrubs (three species) and trees (five species). Therefore, hypersensitive reaction was found to be common in the plant kingdom, irrespective of plant phylogeny and growth form.

### Discussion

Questions currently debated in ecology relate to the selective pressure of natural enemies on the pattern of attack by herbivores and the relative strengths of bottom-up (plant-driven) vs. top-down (enemy-driven) effects in three-trophic-level interactions (Clancy & Price, 1986; Hunter & Price, 1992; Preszler et al., 1996). In this study, effects driven by the host plant (the hypersensitive reaction) represented a strong mortality factor against gall formation while natural enemies of the galling insects represented weak mortality factors, except in one case. Nevertheless, trophic relationships vary both within and among systems and can be affected strongly by abiotic heterogeneity (Whittaker & Feeny, 1971; Hunter & Price, 1992; Preszler & Boecklen, 1994). In spite of the need for more studies to evaluate the population-level effects of hypersensitive reaction against galling insects, a long-term, detailed population study in a single system, *Bauhinia brevipes* (Leguminosae–*Contarinia* sp. (Diptera: Cecidomyiidae), has shown repeatedly the importance of this localised induced reaction (Fernandes, 1998; Cornelissen & Fernandes, 1999; see also Anderson et al., 1989; Bentur & Kalode, 1996).

Although the data presented are from a single survey, many life tables of galling insects may have overestimated the effects of top-down factors. The inclusion of plant resistance mechanisms (hypersensitive reaction for instance) in the life tables of galling insects may offer an interesting opportunity to observe the strength of this induced response to insect herbivores and possibly to unravel the dynamic interplay between bottom-up and top-down effects under various environmental conditions.

Overall, no significant relationship was found between plant module size and hypersensitive reaction induction against galling. Fernandes (1998) and Cornelissen and Fernandes...
(1999) also noted the lack of relationship between module size and rates of hypersensitive reaction in a system formed by a galling Contarinia and the host B. brevipes. Other factors such as plant genetics and host plant physiology might play a more important role in this induced resistance mechanism (Cornelissen & Fernandes, 1999).

In this study, hypersensitivity was the most important mechanism whereby host plants terminated attacks by their ceccidomyiid herbivores. Few studies have documented the occurrence and effectiveness of hypersensitive reactions against insects. It is possible that the lack of documentation of hypersensitivity is not due to the scarcity of its occurrence in nature but to the fact that studies on plant resistance to herbivory may have misinterpreted some of the host reactions, including hypersensitivity (Fernandes, 1990, 1998). Necrotic spots frequently seen on attacked leaves and stems may have been identified erroneously as being caused by pathogens and not recognised as a successful plant defence against the eggs or young instars of herbivorous insects.

Authors of recent studies on insect-plant relationships have argued that many galling herbivores select the largest and fastest growing host individuals or host modules (leaves and stems) (e.g. Price et al., 1990; Price, 1991; Preszler & Price, 1995) and that the mechanism for such preference is an increased performance of offspring on these individuals or modules. As hypersensitive reaction has not previously been included in such studies, it is necessary to understand and broaden the view of how such plant-induced defence relates to module size within and across plant taxa. Although it was not possible to show clearly that hypersensitive reaction can affect a large proportion of the herbivorous insect population, future studies should investigate in detail whether physiological differences between plant modules of different sizes affect hypersensitive reaction.

This study provides the first evidence of the widespread occurrence of hypersensitive reaction in the plant kingdom. This induced defence mechanism occurred in plant taxa unrelated genetically and distributed widely (see Cronquist, 1981). Several other host plant taxa studied independently also exhibited an induced host response to gall formation: *Vitis* spp. (Vitaceae) (Börner & Schilder, 1934), *Picea excelsa* (Pinaceae) (Thalenhorst, 1972; Rohfritsch, 1981), Solidago altissima (Asteraceae) (Anderson et al., 1989), Chrysothamnus nauseosus (Asteraceae) (G. W. Fernandes et al., in prep.), Bauhinia brevipes (Leguminosae) (Fernandes, 1998; Cornelissen & Fernandes, 1999), Solanum dulcamara (Solanaceae) (Westphal, 1980; Westphal et al., 1981), Triticum aestivum (Poaceae) (Shukle et al., 1992), and Orzya sp. (Poaceae) (Bentur & Kalode, 1996).

This study described an interesting yet seldom studied induced defence mechanism of plants to insect herbivores. Although preliminary, the results indicate some interesting and intriguing questions that can be pursued. The primary goal of this study will have been met if students pursue and enlarge these questions in an attempt to unravel the relationships involving this host-driven mortality factor and galling insect population dynamics.

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