



IMPACT OF NEMATODE - INDUCED GALLS ON *MICONIA PRASINA* (MELASTOMATACEAE) TRAITS IN ATLANTIC FOREST OF NORTHEASTERN BRAZIL

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INTRODUCTION

Many phytopathogenic nematodes decrease host plant performance by dramatic morphological and physiological alterations (e.g., Agrios 2005) and in the reproduction of their host plants (e.g., Sturhan & Brzeski 1991). Since nematode species induce changes in their host, plant - parasitic nematodes can impose serious damage to economically important crops worldwide (Jasmer *et al.*, 2003) and many efforts have been directed to pest - control of nematode - induced crop damage (e.g., Chitwood 2003). Furthermore, several nematode species can be considered potential vectors of plant viruses (Sturhan *et al.*, 1997).

The major part of the studies about nematodes is focused on nematodes that infect humans, animals, and plants causing serious diseases that are deleterious to human health and agricultural productivity (see Jasmer *et al.*, 2003). So nowadays, little is known about the ecological patterns of nematode attack above - ground on native plants (e.g., Viana *et al.*, 2008). The ecological importance of pathogenic nematodes on host plants is still unclear, mainly in tropical ecosystems, where few studies were conducted regarding plant - nematode interactions (e.g., Seixas *et al.*, 2004, Viana *et al.*, 2008). Despite the fact that the Brazilian Atlantic forest is considered one of the most important areas for biodiversity conservation (Myers *et al.*, 2000), there is a chance that many of those non - studied interactions are being lost as a consequence of the destruction of tropical ecosystems or even modified in function of the global warming.

Miconia prasina (Melastomataceae) is a native tropical tree that grows in secondary and remnant of Brazilian Atlantic Forest. Based on a priori observations, we found *M. prasina* individuals heavily attacked by a plant parasitic nematode, *Ditylenchus* sp and according to previous arguments, we investigated the potential negative effects of the plant parasitic nematode *Ditylenchus* sp. on this vegetal species.

OBJECTIVES

This research aims on the development of better comprehension of the ecological aspects found in the interaction of nematode - induced galls and *M. prasina*. In order to achieve this, we raised the hypothesis that nematode colonization affects negatively the host plant performance based on the deleterious effect of plant parasitic nematodes on important crops and addressed the following questions: (1) how is the intensity of nematode infection on plant traits? (2) does nematode infection increase susceptibility to the attack of herbivores? and (3) does aggregate host spatial distribution correlates positively to nematode colonization? More specifically, we tested the prediction that nematode infection decrease plant performance through: (a) a lower number of leaves, shoots and plant height; (b) a higher herbivory damage because nematode alterations leave plants more susceptibility by herbivores and (c) a higher nematode colonization in aggregate plants.

MATERIAL AND METHODS

Study site and species

This study was conducted between February and June of 2007 at Parque Estadual de Dois Irmãos. A fragment of 387.4 ha of the highly - threatened Atlantic forest, located in an urban area of Recife, Pernambuco, Northeastern Brazil. *Miconia prasina* (Sw.) DC (Melastomataceae), known as camasey blanco, is a shrub or small tree, reaching between one to 12 meters high, commonly found in the Caribbean, Central America and South America. This species is a shade intolerant pioneer species that grows in secondary and remnant forests, and disturbed areas (e.g., Silva *et al.*, 2007) and is attacked by at least five species of gall inducing insects in four remnants of Atlantic forest of northeastern Brazil: three unidentified species of leaf gall midges (Diptera: Cecidomyiidae), one insect species induce bud gall

(Lepidoptera) and one coleopteran species *Prospoliata* cf. *bicolorata* (Curculionidae) (Almeida - Cortez *et al.*, 2006, Silva & Almeida - Cortez 2006). This host plant is also attacked by a diversity of free - feeding herbivores (Braga *et al.*, 2007). At the moment of this study, no record of nematode galls in *M. prasina* was available.

Nematode extraction, specimen preparation and identification

The nematodes were extracted from shoot and leaf galls of *M. prasina*, according to the methodology describe in Viana *et al.*, (2008). A gall suspension obtained with a home blender was sieved through a 60 mesh metal screen, and the nematodes with fine plant residue were recovered in a 400 mesh screen. For clarification, the nematode suspension was submitted to the sugar floatation technique according to Jenkins (1964). After centrifugation for five minutes at 3,000 rpm, the nematodes and plant debris were concentrated in the bottom of centrifuge tubes, then the supernatant was discarded and the pellet re - suspended with sucrose solution (456 g/l). After a second round of centrifugation for one minute at 1000 rpm, the floating nematodes were recovered and washed on the 400 mm mesh screen.

Non - galled parts of *M. prasina* leaves were processed as described above. The nematodes were heat killed by immersion in water at 50 °C for one minute, then fixed in 3 percent formaldehyde (Flegg & Hooper 1970). Fixed nematodes were glycerin - infiltrated following Seinhorst method (1959). For nematode identification, permanent slides were mounted with adults of both sexes. Nematode identification was based on specimen morphology and morphometry with a compound light microscope according to specific literature such as Fortuner (1982) and Sturhan & Brzeski (1991).

Quantitative assessment of potential impact on host plant
To test the impact on host - plant, in February, we determined two groups of 30 plants each based on nematode gall symptoms: healthy (absence of symptoms due to nematodes) and attacked (presence symptoms due to nematodes). For each plant, we measured the plant height, number of shoots, number of leaves and number of attacked leaves by nematodes. In order to evaluate whether plants attacked by nematodes were more susceptible to herbivory damage we also measured the number of insect galls and leaf miners for each plant in both groups.

Patterns of nematode and herbivore attack were determined randomly collecting 10 leaves around the canopy of the same selected *M. prasina* individuals. These leaves were bagged, numbered and taken to the laboratory where their IP (index of pathogen damage) and IH (index of herbivore damage) were recorded per plant. We defined six levels of leaf damage by nematodes and herbivores: (0) no damage; (1) 1-5%; (2) 6 - 12%; (3) 13 - 25%; (4) 26 - 50%; (5) 51 - 100%. Then, the index of nematode damage (IP) and index of herbivore damage per plant were calculated according to Dirzo & Domínguez (1995) and García - Guzmán & Dirzo (2001).

Miconia prasina spatial distribution and *Ditylenchus* sp. attack

To test whether nematode galls are affected by *M. prasina* density, we quantified the IP and the host density for each plant. The IP for each plant was obtained by analyzing ten

selected leaves of all marked plants (see methods above). To assess host density, we counted the mean distance of five randomly *M. prasina* individuals with a height equal to or greater than 1m (see Viana *et al.*, 2008).

Statistical analysis

All the variables analyzed were first submitted to Kolmogorov-Smirnov test for data normality and a log - transformation was employed to stabilize variances and normalize the IP data (Zar 1996, STATISTICA 6.0, StatSoft 2001). Differences in variables of attacked and unattacked plants by nematodes were analyzed by One - way ANOVA (parametric data) or Kruskal - Wallis test (nonparametric data). A linear regression analysis was used to examine the relation between the mean distance between plants and IP; and between IP and FA (STATISTICA 6.0, StatSoft 2001).

RESULTS AND DISCUSSION

Plant - pathogens species exert selective pressures on host plants populations reducing the plant fitness (see Gilbert 2002). In fact, several plant - parasitic nematodes are recognized in literature to cause negative damages on their host plants, as ornamentals and crops plants (see review Jasmer *et al.*, 2003).

Only one nematode species was associated with galls in *M. prasina*. Abundant females, males and juveniles were recovered from gall tissues, but not from non - galled leaf blades. The nematode was identified as a species of *Ditylenchus*, with characteristics of an amphymitic population, and morphologically similar to *Ditylenchus* sp. commonly found in leaf galls of *Miconia* spp., such as *Miconia albicans* (Viana *et al.*, 2008). Alike other galls in the genus *Miconia*, the nematode - induced were green - brown spherical galls and were found on all side of leaf, on the meristem region, and on reproductive parts as inflorescences and infrutescences.

Nematodes colonized all aerial part of plants; leaves, shoots, flowers and fruits. Casual observations showed that nematode - galls are so massive that some of the attacked plant parts disappear. Due to the high abundance of galls, the terminal stems and reproductive parts died back on many plant individuals. Under attack conditions, *Ditylenchus* sp. galls were commonly found on *M. prasina* leaves. The average number of attacked leaves per plant was 20.33 ± 20.83 (mean \pm SD, n = 30 plants), which represented approximately 15% of the total available leaves (145.57 ± 90.75 , mean \pm SD, n = 30 plants).

Contradicting our hypothesis, healthy and attacked plants did not differ in relation to plant height, and number of shoots and leaves (ANOVA: p > 0.05). Probably, *M. prasina* can tolerate the nematode impact by compensation. Many plant species may minimize nematode damage. Compensation is used to refer to the degree of tolerance exhibited by plants, where damaged and undamaged plants have the same fitness (see Strauss & Agrawal, 1999). Besides, plants species respond to the presence of plant parasitic nematodes by employing various generic defense strategies, as nematocides or by a highly specific defense system, as resistance genes (see Jasmer *et al.*, 2003).

The hypothesis that nematode colonization increases host susceptibility to the attack of herbivores was partially corroborated. Apparently, the nematode attack can facilitate and inhibit herbivore damage on *M. prasina*. The IH of healthy plants was higher compared to attacked plants (ANOVA: $F_{1,58} = 7.93$, $p < 0.01$). However, the number of insect galls was higher in attacked than in healthy plants (Kruskal - Wallis test: $H_{1,60} = 7.60$, $p < 0.01$), and the number of leaf miners did not present any variation between plant category (Kruskal - Wallis test: $p > 0.05$). We expected a higher herbivory damage or number of galls or leaf miners in attacked plants because plant - feeding nematode could cause changes in host plant quality, thus leaving plants more susceptible to herbivores. This result showed that herbivore guilds can have different strategies to select plants because of facilitative and inhibitory interactions mediated by plant - parasitic nematode. Our speculations suggests that probably galling species showed a tendency to oviposit on stressed plants with low plant defenses, while free feeding herbivores prefer to consume plants with more resource availability, although these had not been tested.

In plant - pathogen systems, the increase of plant - density usually increases the plant - pathogen attack ratios (see Gilbert 2002), but our results showed that healthy plants were more grouped than plants that suffer higher nematode attack rates (ANOVA, $F_{1,58} = 6.23$, $p < 0.05$). Within attacked plants, the mean plant distance and IP were positively related ($r^2 = 0.16$, $p < 0.05$, $n = 30$ plants). Thereby, the density - dependency was not consistent with the model for plant - pathogen systems. In spite of that, in *M. albicans*, Viana *et al.*, (2008) found that plants at higher densities had more area lost to nematode - induced galls than isolated plants. Our expectation was that selection should favor *M. prasina* plants in low densities, nevertheless, the isolated plants supported more nematode - galls and nematode - infection was positively related with the mean distance of host plant. More explanations are necessary regarding the spatial dispersion pattern of *Ditylenchus* sp. to know why healthy plant are more grouped than attacked ones.

CONCLUSION

This study represents one more contribution to knowledge on ecology of interaction between native host plants and parasitic - nematodes. Many other *Miconia* and *Melastomataceae* species of several ecosystems present nematode - galls and therefore constitute a potential in further future studies about the impact of parasitic - nematodes on native plant species. Future researches should also focus in the multi - trophic interactions between plant, parasitic nematodes and herbivores in order to obtain a more comprehensive knowledge on the ecology and evolution of these interactions.

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