

Ecophysiological effects of psyllid-induced galls on chlorophyll content in host plants of South Goa

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Abstract

This study investigates the interactions between gall-inducing insects and their host plants in South Goa, India, focusing on the morphological and biochemical impacts of gall formation. Three gall-inducing insect species—*Pauropsylla depressa*, *Pauropsylla tuberculata*, and *Phacopteron lentiginosum*—were identified infesting *Ficus racemosa*, *Alstonia scholaris*, and *Garuga pinnata*, respectively. Fieldwork conducted from November to February identified *Ficus racemosa* as the most heavily infested host, particularly in urban areas, suggesting a potential correlation with heightened environmental stress. Gall formation resulted in distinct leaf morphological changes, such as curling, and significant biochemical alterations, as evidenced by a reduction in chlorophyll content in galled leaves. Chlorophyll analysis revealed that galled leaves had substantially lower concentrations of chlorophyll a, b, and total chlorophyll compared to unaffected leaves. Specifically, *Ficus racemosa* exhibited a decrease from 6.34 mg/L to 2.77 mg/L in total chlorophyll, *Alstonia scholaris* from 5.40 mg/L to 3.18 mg/L, and *Garuga pinnata* from 6.45 mg/L to 4.75 mg/L. These results highlight the complex ecological dynamics of gall formation and its potential impact on the physiological health of host plants, emphasizing the need for further investigation into the broader ecological consequences and implications for biodiversity conservation in Goa. This study provides valuable insights into the effects of insect-plant interactions and the role of gall-inducing insects in ecosystem functioning.

Keywords: Gall-inducing insects, ficus racemosa, alstonia scholaris, garuga pinnata, chlorophyll content, environmental stress, south goa, biodiversity conservation

Introduction

The state of Goa, located on the western coast of India and part of the biodiversity hotspot of the Western Ghats, is home to a remarkable diversity of flora and fauna. Despite its relatively small size, Goa's rich ecosystems support a wide range of ecological interactions that are essential for maintaining population dynamics. These interactions include positive relationships, such as mutualism and commensalism, as well as negative ones like predation, parasitism, and ammensalism. This research focuses on one such interaction between plants and insects: the formation of plant galls.

Goa's unique location in the Western Ghats, a region known for its high floral and insect diversity, provides a fertile ground for studying these complex plant-insect interactions. Plant galls are specialized growths induced by the interaction between plants and arthropods, most commonly insects. Although fungi, bacteria, and nematodes can also trigger gall formation, insect-induced galls are by far the most prevalent. Gall formation typically begins during the winter season (Harley, 1998) [13], when insects, particularly aphids, induce abnormal tissue growth by feeding or laying eggs in plant tissues. These galls offer both shelter and a nutrient-rich environment for the developing insects. Aphids, for instance, reproduce parthenogenetically within these galls, thriving in conditions that favor rapid population growth as long as nutritional resources remain abundant.

According to the nutrition hypothesis (Koyama, Yao, & Akimoto, 2004) [23, 25], galls accumulate higher concentrations of nutrients than surrounding plant tissue, which enhances the survival and performance of gall-forming insects. Aphid-induced galls, in particular, are expected to contain higher nitrogen levels than galls formed by other insects, as they support multiple generations of

aphids. This process underscores the intricate relationship between the diverse plant species of the Western Ghats and the gall-inducing insect populations that inhabit them.

This research explores the diversity of galls in Goa and the impact of gall-inducing insects on their host plants, specifically how these insects provoke physiological and chemical changes in plants. Galls form when insects or mites feed on plant tissues or lay eggs, triggering the plant to release growth hormones in response to the insect's saliva. The increased hormone production leads to abnormal cell growth, resulting in the formation of galls. Once formed, galls continue to grow even after the insect has died. Typically, galls measure 0.5 to 1 inch in length and house one or more maggots (larvae of tiny flies called midges), which feed on the tissue inside. Galls caused by aphids, for example, are about an inch long, with irregular edges and a red color when mature. Over time, the galls harden, dry out, and turn brown, with aphids sometimes visible through small slits in the gall (Koyama, Yao, & Akimoto, 2004) [23, 25].

Although gall formation is often viewed as a parasitic interaction, some gall-inducing insects engage in mutualistic relationships with other organisms. Among the primary gall-makers are gall wasps, gall midges, gall psyllids, and phylloxerans. Some studies suggest that the diversity and frequency of insect herbivores vary with the age of the host plant, with different insect guilds favoring saplings or mature plants (Ananthkrishnan, 1978) [3].

In addition to serving as shelters and food sources for gall-inducing insects, galls provide refuge for other organisms once the insects mature or leave. Abandoned galls become homes for beneficial insects and spiders, which prey on the former inhabitants. Moreover, galls are rich in resins and tannic acid, substances used in the production of permanent

inks and astringent ointments. Some galls are even believed to possess medicinal properties (Merchant, 2019) [29]. The intricate web of interactions between plants, insects, and other organisms underscores the ecological complexity of gall formation, particularly in a biodiverse region like Goa. Here, parasitism, mutualism, and human utility intersect, making galls an especially fascinating subject for studying plant-insect dynamics in the context of the Western Ghats' extraordinary biodiversity. Here's a more streamlined and easy-to-follow version of the methodology, while maintaining the original sequence and structure:

Methodology

Selection of Study Site and Gall Collection

- **Study Area:** The research was conducted in the southern region of Goa, specifically within various urban and suburban areas of South Goa.

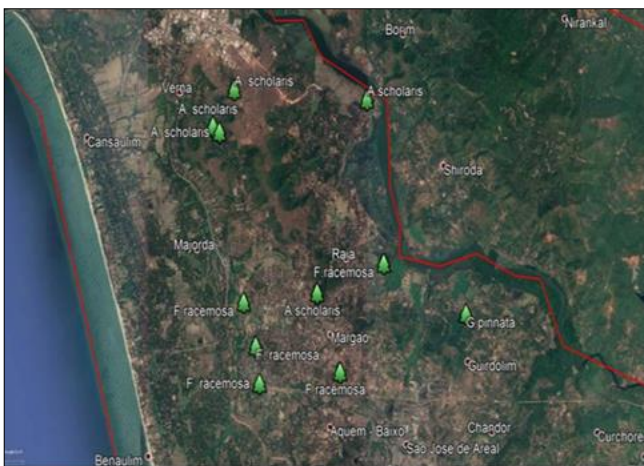


Fig 1: the above map depicts the areas where the labelled samples were collected

- **Site Selection Process**

- The process involved identifying areas with gall-infested plants based on specific criteria.
- The selected sites were visited for fieldwork, which took place from
- **November to February. Logistics:** Site visits were organized based on accessibility and the presence of gall-infested plants.
- **Gall Collection:**
 - The GPS coordinates of infested plants were recorded.
 - Leaves from infested plants were collected and stored in air-tight plastic bags to prevent contamination for further analysis
- **Insect Dissection and Observation:**
 - Galls were carefully dissected using a sterile blade.
 - Insects at different developmental stages were isolated on clean cavity slides.
 - The morphology of the insects was analyzed to aid in identification.
 - Live insects, along with their nymphal stages, were observed under a light microscope at 10x magnification.
 - Insect identification was confirmed by comparing the data with available literatures.
 - Plant species identification was conducted by comparing data with the available literature and with the help of plant taxonomist.

1. Biochemical Analysis

- **Chlorophyll Quantification:** The concentrations of chlorophyll a, b, and total chlorophyll were measured by reading the optical density (OD) at 663 nm and 645 nm.
- The methodology for chlorophyll analysis followed the protocol outlined in Kamble, (2015) [21], and Johan *et al.* (2014) [20].



Fig 2: Dissection of *G. pinnata* leaf gall



Fig 3: Dissection of *F. racemose* leaf gall



Fig 4: finely cut leaf material



Fig 5: Mixture after grinding

2. Statistical Analysis:

- **Chlorophyll Calculations:** The concentration of chlorophylls was calculated using the following equations:
- **Chlorophyll a** ($\mu\text{g/g/ml}$) = $(12.7 \times \text{O.D. at } 663 \text{ nm}) - (2.69 \times \text{O.D. at } 645 \text{ nm})$

- **Chlorophyll b** ($\mu\text{g/g/ml}$) = $(22.9 \times \text{O.D. at } 645 \text{ nm}) - (4.08 \times \text{O.D. at } 663 \text{ nm})$
- **Total Chlorophyll** ($\mu\text{g/g/ml}$) = $(20.2 \times \text{O.D. at } 645 \text{ nm}) + (8.02 \times \text{O.D. at } 663 \text{ nm})$
- **Expression of Chlorophyll Content:** The chlorophyll content was expressed in mg/L as per the methods in Johan *et al.* (2014)^[20].



Fig 6: Mixture stored in the refrigerator



Fig 7: Centrifuged samples



Fig 8: Supernatant diluted in acetone

3. Results

The photo plate provides detailed information about the gall-inducing insects and the associated gall characteristics found on three different plant species: *Ficus racemosa*, *Alstonia scholaris*, and *Garuga pinnata*.

Plant-Insect Interaction: All three-plant species in the study exhibit insect-induced galls, caused by species from the Psyllidae family (order Hemiptera), which are known to induce abnormal growths in plants. The specific insects involved are *Pauropsylla depressa*, *Pauropsylla tuberculata*, and *Phacopteron lentiginosum*, each affecting different host plants.

1. Gall Morphology and Coloration

- The galls show a variety of shapes: ovate for *Ficus racemosa*, spherical for *Alstonia scholaris*, and

elongated for *Garuga pinnata*.

- All galls undergo color changes throughout their developmental stages:
- *Ficus racemosa* galls change from green to brown and eventually reddish-brown as they senesce.
- *Alstonia scholaris* galls are yellowish during development, becoming brown at maturity.
- *Garuga pinnata* galls transition from bright red to brown as they mature.

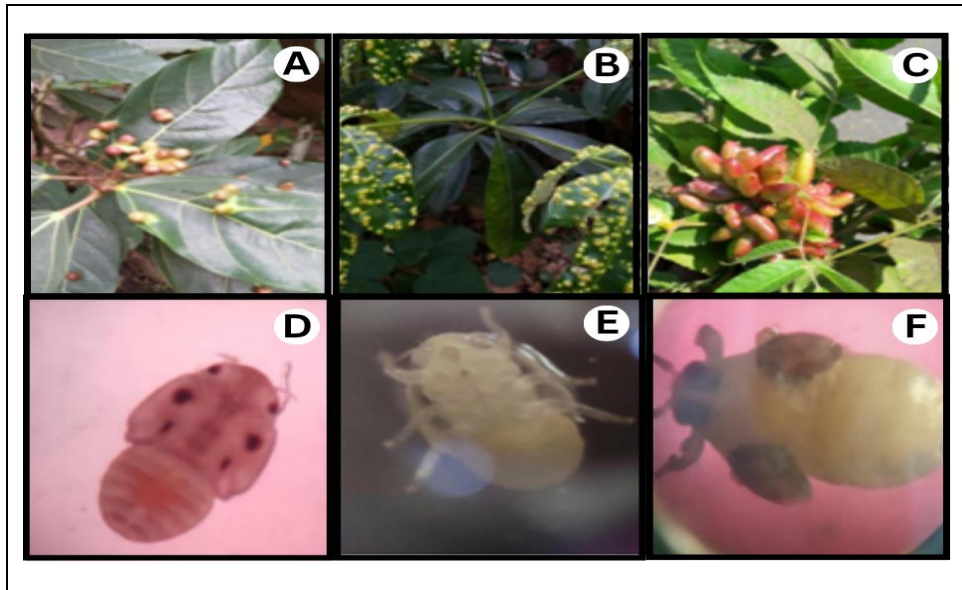
2. Insect Development

The presence of nymphal stages in the galls across all plant species indicates that these galls serve as developmental habitats for the insects. In the case of *Garuga pinnata*, both nymphal and adult stages of the insect were found, suggesting that the galls not only provide shelter but also enable the insects to complete their entire life cycle within

the plant tissue.

This Photo plate highlights the host specificity of gall-inducing psyllids and underscores the morphological diversity of the galls they produce. It also reveals, how these

galls function as crucial habitats for insect larvae, influencing the plant-insect interaction dynamics and the overall physiological changes in the host plants.



Gall Formation & Associated Insect Morphotypes on Host Plants. Top A-C: (A) *Ficus racemosa* (B) *Alstonia scholaris* (C) *Garuga pinnata*. Below: D-F: (D) *Pauropsylla depressa* (E) *Pauropsylla tuberculata* (F) *Phacopteron lentiginosum*

Analysis of Chlorophyll Concentrations in Unaffected and Affected Leaf Samples

The data presented shows the chlorophyll concentrations (Chlorophyll A, B, and Total Chlorophyll) in the leaves of three plant species (*Ficus racemosa*, *Alstonia scholaris*, and *Garuga pinnata*) both before and after gall infestation (Table no.- 2).

3. *Ficus racemosa*

- **Chlorophyll A:** There is a significant decrease from 4.04 mg/l in unaffected leaves to 1.31 mg/l in affected leaves, representing a 67% reduction. This suggests a major impairment in photosynthesis due to gall formation.
- **Chlorophyll B:** Chlorophyll B drops from 2.51 mg/l (unaffected) to 1.10 mg/l (affected), a 56% decrease, indicating a notable decline in chlorophyll production.
- **Total Chlorophyll:** The total chlorophyll content declines by 56% from 6.34 mg/l in unaffected leaves to 2.77 mg/l in affected leaves, reflecting a significant reduction in the plant's photosynthetic capacity.

4. *Alstonia scholaris*:

- **Chlorophyll A:** The reduction in Chlorophyll A is 45%, from 3.71 mg/l (unaffected) to 2.02 mg/l (affected), indicating a moderate impact of gall infestation on photosynthesis.
- **Chlorophyll B:** Chlorophyll B decreases from 2.22 mg/l (unaffected) to 1.26 mg/l (affected), showing a 43% decline, further demonstrating the negative effect of gall formation.
- **Total Chlorophyll:** The total chlorophyll concentration drops by 41%, from 5.40 mg/l in unaffected leaves to 3.18 mg/l in affected leaves, suggesting a moderate

reduction in photosynthetic efficiency.

5. *Garuga pinnata*

- **Chlorophyll A:** Chlorophyll A shows the smallest decline of 23%, from 3.91 mg/l (unaffected) to 3.01 mg/l (affected), indicating a lesser impact of gall infestation on this species.
- **Chlorophyll B:** The concentration of Chlorophyll B drops by 31%, from 2.75 mg/l (unaffected) to 1.89 mg/l (affected), suggesting that gall infestation still affects chlorophyll synthesis, but to a lesser degree than in the other two species.
- **Total Chlorophyll:** The total chlorophyll content decreases by 26%, from 6.45 mg/l (unaffected) to 4.75 mg/l (affected), indicating that while there is a reduction, the overall impact on *Garuga pinnata* is milder compared to the other two species.

All three-plant species show a reduction in chlorophyll content in response to gall infestation, indicating a negative impact on their photosynthetic capacity.

- ***Ficus racemosa*:** exhibits the most substantial decrease in chlorophyll levels, with a 67% reduction in Chlorophyll A and 56% in total chlorophyll, suggesting this species is particularly sensitive to the effects of gall formation.
- ***Alstonia scholaris*:** experiences moderate reductions in chlorophyll content, with 45% less Chlorophyll A and a 41% drop in total chlorophyll, indicating a moderate physiological impact from gall infestation.
- ***Garuga pinnata*:** demonstrates the least decline in chlorophyll concentrations, with a 23% reduction in Chlorophyll A and a 26% decrease in total chlorophyll, suggesting that this species is more resilient to the

physiological effects of gall formation compared to the other two species. The data is given in the table no. 2.

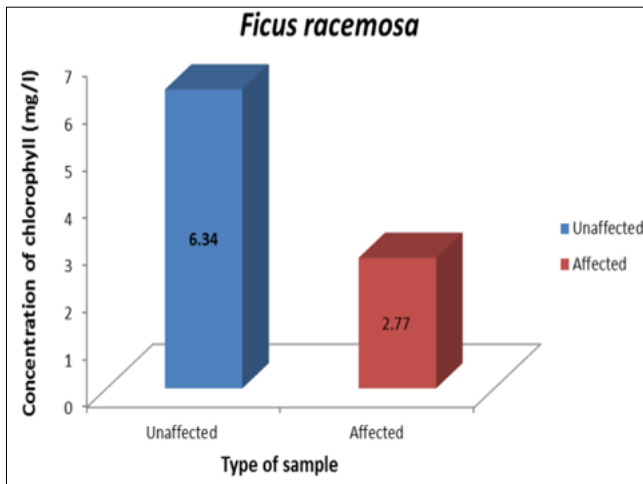
Overall, the findings highlight the varying degrees to which gall infestation impacts the photosynthetic capacity of

different plant species, with some species (like *Ficus racemosa*) being more severely affected than others (like *Garuga pinnata*). This variation underscores the importance of species-specific responses to biotic stress factors like gall formation.

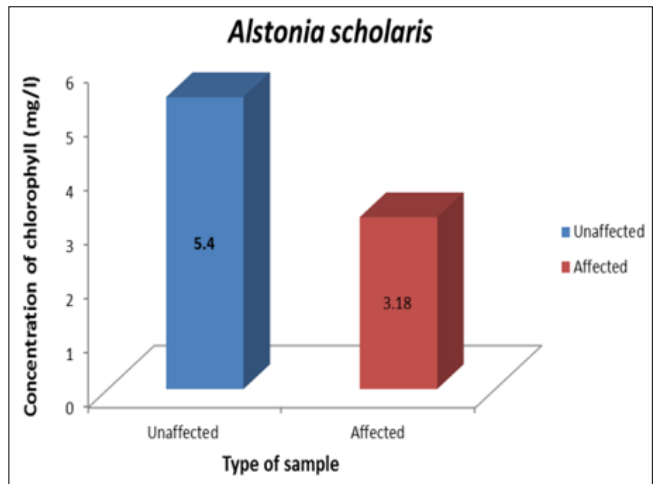
Table 2: Chlorophyll Concentrations in Unaffected and Affected Leaf Samples of Different Plant Species

Plant	<i>Ficus racemosa</i>		<i>Alstonia scholaris</i>		<i>Garuga pinnata</i>	
Type of sample	Unaffected	Affected	Unaffected	Affected	Unaffected	Affected
Chlorophyll A	4.04	1.31	3.71	2.02	3.91	3.01
Chlorophyll B	2.51	1.10	2.22	1.26	2.75	1.89
Total chlorophyll	6.34	2.77	5.40	3.18	6.45	4.75

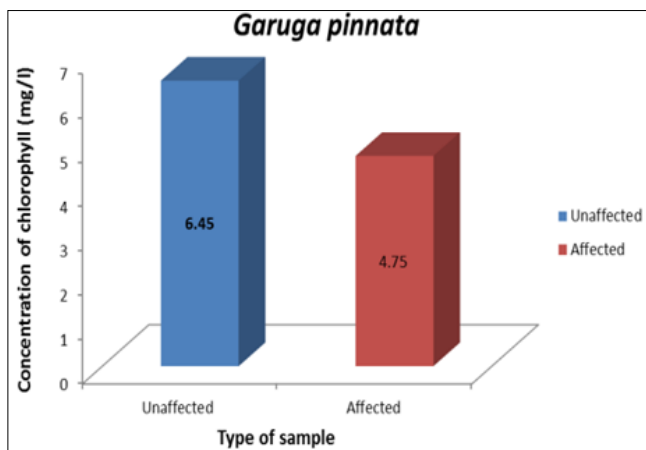
Effect of Stress on Chlorophyll Concentration in (1) *Ficus racemosa* (2) *Alstonia scholaris* (3) *Garuga pinnata*



(1)



(2)



(3)

Discussion

Gall formation represents a complex and fascinating interaction between herbivorous insects and their host plants. The Indian subcontinent, with its rich biodiversity, is home to an impressive diversity of gall-inducing species, with approximately 2000 gall types and an equal number of associated insect species (Raman, 2007). Several studies have been conducted on gall formation across various Indian states; there remains a significant gap in research on this phenomenon in the state of Goa. This study aims to fill this gap by exploring the diversity of gall formation and its effects on host plants in South Goa, with a particular focus on the biochemical and morphological changes induced by galling insects.

Apart from the parasitic relationships between gall-forming insects and their host plants (Ronquist & Liljeblad, 2001)^[41], mutualistic interactions have also been documented. For instance, the galls induced by *Leptocybe invasa* (Eucalyptus gall wasp) on *Eucalyptus camaldulensis* have been shown to provide frost resistance to the host plant (Washburn, 1984)^[52]. Furthermore, various hymenopteran parasitoids, especially Braconid wasps, have been found associated with plant galls. Notably, a Braconid species reared from the galls of *Garuga pinnata* induced by *Phacopteron lentiginosum* was recently described as a new species (Saleem, 2015)^[42, 43].

Biochemical studies have also extensively documented changes in the nutritional composition of galled plant tissues. Studies have shown that galls induce significant alterations in the levels of chlorophyll (Albert *et al.*, 2011)^[1, 2], and amino acids (Koyama, Yao, & Akimoto, 2004)^[23, 25]. The current study focused on the diversity of gall-inducing insects and their impact on the biochemical and morphological aspects of the host plants.

Ficus racemosa, a large evergreen tree, is one of the most common host plants in South Goa. The leaf galls of *F. racemosa* were induced by *Pauropsylla depressa*, a species of psyllid (Body, David, Elisabeth, & Graham, 2015)^[5]. This finding is consistent with a study conducted in the Nanded district of Maharashtra (Gawate & Papdiwal, 2011)^[11]. Interestingly, during the dissection of the galls, an adult insect belonging to the parasitoid wasp species *Bracon heteron sp.* (Family: Braconidae) was observed in one of the samples, corroborating previous reports by Sheeba & Narendran (2013)^[45], on parasitoid associations with galled plants.

Similarly, leaf galls on *Alstonia scholaris* were observed to be induced by *Pauropsylla tuberculata*, a psyllid species. This observation aligns with findings reported by Albert *et al.*, (2011)^[1, 2], in Gujarat, India. However, no parasitoids were observed in the galls of *A. scholaris* in this study, indicating that this plant species may have a different parasitoid complex or a lower susceptibility to parasitoid infestation.

Garuga pinnata, although a less frequently observed gall-infested plant in this study, exhibited elongated galls induced by *Phacopteron lentiginosum*. This result supports the observations of Saleem (2015)^[42, 43], who identified this psyllid species as the gall inducer for *G. pinnata*.

The current study revealed that the gall formation process is highly species-specific. All the identified gall-inducing insects belong to the family *Psyllidae*, with the majority of insect stages being nymphal, except for *Phacopteron lentiginosum*, which also exhibited adult forms.

A striking observation was the prevalence of gall-infested plants in urban areas, which could be attributed to environmental stress factors such as elevated pollution levels and poor soil fertility. According to Larsson (1989)^[27], plants experiencing higher levels of stress are more susceptible to herbivore infestations, including gall-inducing insects. Morphologically, the galls led to noticeable leaf deformations, including crinkling and curling of the leaves, which is indicative of the plant's response to insect feeding and the formation of abnormal growths.

Biochemically, the study found that chlorophyll concentrations were significantly lower in the galled leaves of all three-plant species compared to unaffected leaves. This reduction in chlorophyll content is likely due to the disruption of palisade tissue, the loss of chloroplasts, and modifications to the spongy mesophyll, as described by Kamble, (2015)^[21]. Additionally, elevated amino acid concentrations in galled tissues further support the hypothesis that herbivory-induced galls alter the metabolic processes of the host plant (Koyama, Yao, & Akimoto, 2004)^[23, 25].

Conclusion

This study highlights the gall formation process in three plant species - *Ficus racemosa*, *Alstonia scholaris*, and *Garuga pinnata* in South Goa. The gall-inducing insects identified in this study include *Pauropsylla depressa*, *Pauropsylla tuberculata*, and *Phacopteron lentiginosum*. Of these, *Ficus racemosa* was found to be the most frequently gall-infested plant. The majority of these galls were found in urban areas, which could be linked to environmental stress factors, including pollution and reduced soil fertility.

Gall formation appears to be a highly species-specific phenomenon, with each gall-inducing insect associated with a specific host plant species. The study revealed two primary impacts of gall formation on the host plants: morphological and biochemical changes. Morphologically, gall formation resulted in abnormal leaf development, including crinkling and curling of leaves. Biochemically, galled leaves exhibited significantly lower chlorophyll concentrations and higher levels of amino acids compared to unaffected leaves.

This research provides valuable insights into the ecological interactions between gall-forming insects and their host plants, contributing to the understanding of plant-herbivore dynamics in the understudied region of Goa. By

investigating these interactions, this study helps fill an important gap in knowledge, shedding light on the complexity of ecological relationships and the impact of environmental factors on species coexistence in tropical ecosystems.

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