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PATTERNS OF LEAF-PATHOGEN INFECTION IN THE UNDERSTORY OF A MEXICAN RAIN FOREST: INCIDENCE, SPATIOTEMPORAL VARIATION, AND MECHANISMS OF INFECTION[1]

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This study assessed the levels of damage by leaf pathogens and their variability in terms of host species, space (four mature forest sites) and season of the year (dry and rainy), and the mechanisms of infection in the understory of the Los Tuxtlas tropical rain forest. Sixty-five percent of the species surveyed in the dry season ($N = 49$) and 64.9% of those surveyed in the rainy season ($N = 57$) were damaged by fungi. Leaf area damaged per plant, on average, was <1% (range: 0.25–20.52%). There was considerable variation in the degree of infection among species, but not among sites and seasons. The survey showed that 43% of the leaves were damaged by herbivores and pathogens concurrently, 16% showed damage by insect herbivory alone, and only 1.4% of the sampled leaves showed damage by pathogens alone. Pathogenicity assays experimentally confirmed that the predominant mechanism of fungal establishment was wounding, such as that caused by herbivory (or other similar sources), and only rarely did infection occur through direct contact (without wounds). The results revealed the omnipresence of leaf fungal infection, although with low damage per plant, and the importance of herbivorous insects in the facilitation of fungal infection in tropical understory plants.

Key words: herbivory; infection; leaf-pathogens; Los Tuxtlas; tropical rain forest; understory.

The study of several diseases of tropical and temperate crops has provided valuable means of understanding how pathogenic infection is influenced by environmental factors and how diseases affect individual plants in crop systems (Burdon, 1987). In contrast, information on plant–pathogen interactions in natural systems, particularly in tropical rain forests, is very scarce and only a handful of studies is available (see Coley and Barone, 1996; Gilbert and Hubbell, 1996; Lodge, Hawksworth, and Ritchie, 1996). Available data suggest that the incidence of diseases is higher in tropical crops than in temperate ones, and it is argued that this is partly due to the more suitable conditions of tropical zones for pathogens (Waller, 1976; Shivas and Hyde, 1997). No available evidence exists to assess whether such tropical–temperate differences in agronomic systems are applicable to natural ecosystems (see Coley and Barone, 1996).

The mesic and shady environment, together with the density of foliage coverage found in the understory of tropical rain forests (cf. Dirzo et al., 1992), are expected to be particularly appropriate for the development of plant pathogens (see Augspurger, 1983, 1984), including leaf pathogens. Some preliminary observations in the rain forest of Los Tuxtlas, Veracruz, Mexico (Dirzo, 1987; de la Cruz and Dirzo, 1987) support this expectation. However, to our knowledge, no quantitative assessments exist of the incidence of leaf pathogen infection at the plant community level in tropical rain forests. In these communities, incidence of leaf pathogen infection would be expected to vary with time, according to the seasonal variation in rainfall (Soto and Gama, 1997), and with space (García-Guzmán et al., 1996), according to ecological heterogeneity such as that imposed by light availability or microsite floristic composition (e.g., low vs. high local density and species diversity; Dirzo et al., 1992). Overall, it could be expected that the wettest period and the more shaded microsites of the forest should lead to greater pathogen infection (Burdon, 1987).

Another major gap in knowledge about plant–pathogen interactions in natural tropical systems concerns the proximal mechanisms of leaf infection. Studies in agricultural systems and in some temperate forests have shown that leaf damage by pathogens with life-histories such as that of the nonsystemic airborne fungal pathogens appears to be associated in many cases to damage by herbivores, mainly insects (Manners, 1993). Given the enormous diversity and abundance of phytophagous insects (Erwin, 1982) and the prevalence of herbivory in tropical rain forests (Howe and Westley, 1988; Coley and Barone, 1996), it is possible that such animals may play a significant role in the incidence of disease in the plants of these forests. It is known that insects can promote pathogenic infection either by active transportation and inoculation of spores (Manners, 1993) or by causing physical damage that then facilitates penetration of pathogenic inoculum (Dirzo, 1987; García-Guzmán, 1990).

In the present study we address two issues basic to the study of plant–pathogen interactions in tropical forests. The first issue concerns documentation of the incidence of leaf damage by pathogens based on a quantitative survey of the extent of leaf damage in understory plants of the Los Tuxtlas tropical rain forest. Such analysis focuses specifically on the following questions: what is the overall magnitude of damage by pathogens and its variation among species and is there variation in the incidence of pathogen damage in relation to the seasonal and spatial variation of this forest? The second issue addresses the association of damage by pathogens and herbivores, with the aim of exploring the following question: does insect her-

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bivory play a role on the incidence of pathogenic disease symptoms in the forest understory?

MATERIALS AND METHODS

Study area—The study was carried out at the Los Tuxtlas Research Station, located in Southern Veracruz, Mexico (95°04′ and 95°09′ W, 18°34′ and 18°36′ N; Dirzo, González, and Vogt, 1997). The region is hot and humid; mean monthly temperature is 27°C, but it ranges from 17°C (January) to 29°C (June). Annual rainfall averages 4750 mm with a relatively dry season (April–May) during which rainfall drops to ~200 mm and a very wet season from June to February (Soto and Gama, 1997). The vegetation of the area is classified as ''high evergreen rainforest'' (Pennington and Sarukhán, 1998), characterized by evergreen vegetation and trees exceeding 30 m. The forest understory is composed of seedlings and saplings of trees, palms, shrubs, lianas, and herbaceous plants, as well as the young stages of climbing vines. The most abundantly represented plant families include Araceae, Bignononiaceae, Euphorbiaceae, Leguminosae, Moraceae, Orchidaceae, and Piperaceae (Ibarra et al., 1997). Detailed accounts of the natural history of the area are given in González, Dirzo, and Vogt (1997).

Incidence of leaf damage—To assess the levels of damage by leaf pathogens and their occurrence in terms of the species that characterize the understory community and the season of the year, we selected four sites of mature forest (without treefall gaps) on relatively flat areas. Bearing in mind these criteria of forest age and topography, the four sites were chosen on the basis of the local dominance of a particular plant species along each of the four major trails of the field station (see García-Guzmán, 1990). Such local dominance was defined by the clumped distribution and high local density of a given species of tree and a predominance of seedlings and saplings of the same tree (see Dirzo and Miranda, 1991). The local dominant tree species in each of the sites were *Dussia mexicana* (site 1), *Nectandra ambigens* (site 2), and *Omphalea oleifera* together with *Brosimum alicastrum* (sites 3 and 4). In addition, the four sites differed in the degree of shading in the direction of site 2 (most shaded) > site 1 > site 3 > site 4 (least shaded). Degree of shading was estimated by Dirzo et al. (1992, and R. Dirzo, unpublished data) with black-and-white hemispherical (fish-eye)-lens canopy photographs, using a Sigma Fisheye Lens (1:4, F = 8 mm). In each site a sampling plot was established by means of a 50 m north–south straight line. Along the line, ten randomly selected points were chosen. On each of these points we established 5-m long transects, perpendicular to the central line. Their position (right or left of the 50-m line) and distance from the origin were selected at random. The survey was carried out using a variation of the point–quadrat technique for sampling species composition and coverage (Greig-Smith, 1983). Along the 5-m transects we located 25 points by the random positioning of a descending needle; all plants <1 m height touched by the tip of the needle were recorded and all their leaves collected for subsequent analysis of pathogen damage and herbivory in the laboratory (see below). There were 250 sampling points per site. Each sampling point included all plants touched by the descending needle or no plants if the needle did not touch any plant. The survey was carried out during the latter part of the rainy (November) and the peak of the dry (April) seasons, with sampling plots independently established in each of the two seasons.

With this sampling protocol we were able to obtain average levels of pathogen damage per plant and species, as well as a floristic description of the understory of each of the four sites.

Damage per plant and association of damage types—For each of the collected leaves from the understory plants we estimated the percentage of area lost to herbivores and/or pathogens classifying the leaves into six categories of damage: 0 (0% of leaf area damaged), 1 (1–6% of leaf area damaged), 2 (6–12% of leaf area damaged), 3 (12–25% of leaf area damaged), 4 (25–50% of leaf area damaged), and 5 (50–100% of leaf area damaged). In the case of diseased leaves the categories of damage were assigned considering both the necrosed and surrounding area, which might have been discolored or with an otherwise coloration in the lamina adjacent to the necrosed area. This classification system ensured that low levels of damage per leaf (<25%), which were the predominant ones (Dirzo, 1987), would not be lumped into a single category. The proportion of area damaged per leaf was assessed visually and an index of pathogen damage per plant, IP (Kremer and Unterstenhöfer, 1967; Dirzo and Domínguez, 1995) was obtained according to the following formula:

$$\mathrm{IP} = [\Sigma(L_i)(i)]/n$$

where L_i is the number of leaves in each category of damage, i is the category of damage, and n is the total number of leaves sampled per plant.

We described the symptoms of pathogen damage and analyzed the leaves to determine the type of damage association. The leaves from the plants were assigned to any of four damage types: (1) herbivory and pathogen damage on the same leaf, (2) pathogen damage alone, (3) herbivory alone, and (4) intact leaves.

Isolation of the causal agents of damage and inoculation experiments—To isolate the causal agents of the observed symptoms, leaf sections of the affected area (including a marginal healthy section of ~5 mm width) were immersed in sodium hypochlorite (1%) for 2 min. These were then rinsed in sterile distilled water and placed in potato–dextrose–agar plates and incubated at 20°C (see López, 1981). After fungal growth occurred, cultures were purified using single-spore isolates (see Booth, 1971) or by transferring, several times, small portions of the culture medium including the advancing margin of the mycelia (see López, 1981). Although no antibacterial agents were applied to the culture media, no bacteria associated with the leaf portions were detected. Fungal morphotypes were described, and when possible, identified (Barnett and Hunter, 1972; Streets, 1978). To assess whether the isolated fungi were effectively the causal agents of the symptoms and to know the role played by herbivory (experimentally simulated) on the establishment of pathogens, we carried out inoculation experiments, whereby healthy disinfected leaves of 40 plant species were inoculated with the isolated pathogens. These plant species were chosen because they were relatively abundant in the field and showed characteristic symptoms of damage by leaf-fungal pathogens. To carry out the inoculation of leaves with the fungal pathogens we selected 20 plants from each plant species, growing in the field, with at least five healthy leaves (i.e., without any kind of visible damage). The plants and their leaves were marked and each leaf disinfected with alcohol (70%) and inoculated with an inoculum suspension using four treatments: (1) simulated herbivory (by inflicting wounds or scrapes that mimic the characteristic type of damage of each species, followed by the application of inoculum on the damaged lamina) (N = 5 plants), (2) direct contact, consisting of direct application of inoculum suspension, without damage (N = 5 plants), (3) control direct contact consisting of direct application of sterile water instead of the inoculum suspension, without damage (N = 5 plants), and (4) control simulated herbivory consisting of simulated herbivory followed by the application of sterile water instead of the inoculum suspension (N = 5 plants each). Purified fungi for inoculation were grown on 20 mL of potato–dextrose–agar medium in 50-mL flasks. To carry out the inoculations, these flasks were filled with an additional 30 mL of sterile distilled water. The inoculum suspensions were produced by shaking and scraping, with a sterile needle, a mixture of mycelia and spores of the fully developed fungi. To apply the inocula, a sterile cotton ball was immersed in the flasks with the suspension. The cotton balls were then placed on the lamina of the leaves to be inoculated. In cases where more than one fungal morphotype was isolated from a single lesion, we prepared a suspension with sterile distilled water, mycelia, and spores from all the isolated fungal morphotypes. Following this, each plant was covered with a plastic bag for 24 h to increase humidity and to avoid the inoculum from being washed away by rainfall. Plants were inspected daily, up to 15 d, until development of any disease symptom occurred. Once disease symptoms were observed, fungi were isolated and purified, as described above, to compare with those originally inoculated.

RESULTS

The symptoms of pathogen damage and causal agents—Taking into account all four sites and both seasons together,

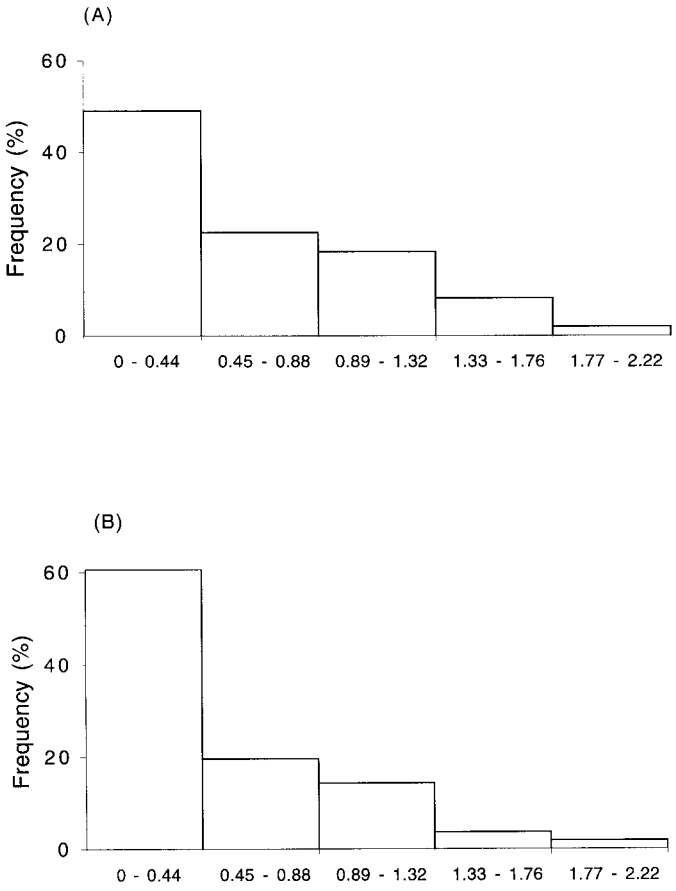

Fig. 1. Frequency distribution of the index of pathogen damage (IP) values for the sampled plant species growing in the (A) dry and (B) rainy seasons. $N = 49$ species and 620 plants in the dry season, and 57 species and 483 plants in the rainy season. Categories of IP are as follows: 1 = 1–6% leaf area damaged; 2 = 6–12%; 3 = 12–25%; 4 = 25–50% and 5 = 50–100%.

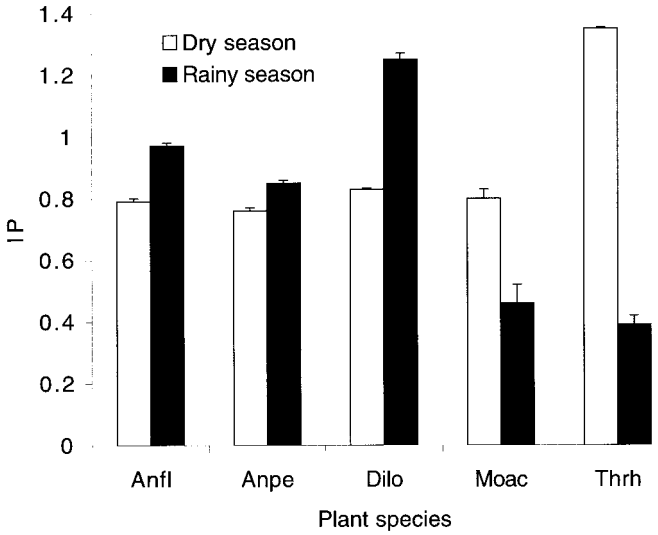

Fig. 2. Mean values of IP (±1 SE) of five plant species during the dry and rainy seasons. Differences between seasons for each plant species are significant (Mann-Whitney *U* tests, $P < 0.05$ in all cases). Anfl = *Anthurium flexile*, Anpe = *Anthurium pentaphyllum*, Dilo = *Diplazium lonchophyllum*, Moac = *Monstera acuminata*, and Thrh = *Thelypteris rhachiflexuosa*.

our survey showed that in addition to damage by herbivorous insects, the only microorganisms causing leaf damage of the understory plants were fungi. No pathogenic bacteria were isolated nor virus symptoms observed. We did not detect attack by rusts, smuts, or mildews. The identification of the isolated fungi is still in progress. Plants were affected by four predominant disease symptoms: blight, and chlorotic, necrotic, and small necrotic spots. Descriptions of disease symptom for each plant species are provided in Appendix 1. Blight was characterized by an extensive necrosis of the leaf tissue. The chlorotic spots had different shapes and sizes but were characterized by their yellow color. The necrotic spots were irregular in shape and commonly light brown to grayish-tan color, and were large (>1 cm in diameter). In contrast, the small necrotic spots were round in shape and small (no more than 2 mm in diameter), black, and commonly surrounded by a chlorotic halo. Generally, each of the symptoms was originated by a single morphotype of fungus (probably a genus), however some symptoms were caused by up to two different fungal morphotypes (morphologically distinguished mycelia and spores) (see Appendix 1).

Temporal variation—During the dry season we surveyed a total of 49 plant species considering all four forest sites (Appendix 2). The analysis showed that 65.3% of the species and 43.3% of their leaves were attacked by leaf-fungal pathogens. The IP values for the sampled species ranged from 0 to 2, but 72% of the infected species had IP values <1.0 (equivalent to <6% of leaf area damaged per plant) (Fig. 1A), including 17 species (i.e., 34.7%) with zero damage. Only 2.0% of the sampled species had IP values ≥2.0 (i.e., 6–12% of leaf area damaged).

The rainy season survey included 57 plant species (Appendix 2). At this time of the year 64.9% of the species and 45.4% of their leaves were attacked by fungi. The frequency distribution of leaf area damaged per species followed a similar pattern to that of the dry season (although the distribution is even more skewed) (Fig. 1B). In this case ~80% of the species had IP values <1.0 (including 20 undamaged species), and only one species (1.8%) had an IP >2.0.

A species-level analysis showed that 38 plant species occurred in samples from both seasons of the year (Appendix 2). Taking into account only these plant species, 10.5% were healthy in the dry season while they were diseased in the rainy season, whereas 13.16% were healthy in the rainy season but diseased during the dry season. Furthermore, 15.79% of the species were healthy in both seasons. For these comparisons, a plant was considered as diseased if having one or more symptomatic leaves. The IP per plant, considering those plant species present and diseased in both seasons, was higher in 15 plant species during the dry season, while in 13 species it was higher in the rainy season. However Mann-Whitney *U* tests indicated that out of these 28 plant species differences were significant in only five of them (Fig. 2). *Anthurium flexile*, *Anthurium penthaphyllum*, and *Diplazium lonchopyllum* had higher IP values during the rainy season, while the IP values in *Monstera acuminata* and *Thelypteris rhachiflexuosa* were higher in the dry season ($P < 0.05$ in all cases).

During the dry season we analyzed 620 individual plants with 3890 leaves, while the corresponding numbers in the rainy season were 483 plants and 4475 leaves, respectively.

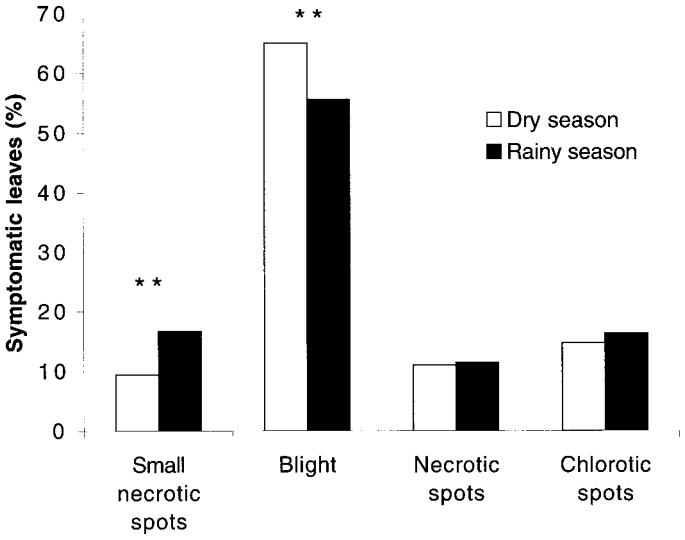

Fig. 3. Percentage of leaves affected by the four predominant disease symptoms during the dry and rainy seasons. Chi-square, ** $P < 0.01$.

The analysis of the incidence of leaf pathogens at the levels of plants and leaves showed that the proportion of diseased plants was higher during the dry season (65.80%) than during the rainy season (55.16%) (contingency test for the number of symptomatic and nonsymptomatic plants in both seasons, $\chi^2 = 13.16$; $P < 0.01$). In contrast, the numbers of symptomatic and nonsymptomatic leaves did not significantly vary between seasons ($\chi^2 = 3.95$; $P > 0.05$).

Considering the four predominant symptoms, at the leaf level (Fig. 3) we found that leaves were significantly more damaged by fungi causing small necrotic spots during the rainy season than during the dry season ($\chi^2 = 45.42$; $P < 0.01$). In contrast, leaves had significantly more blight during the dry season than during the rainy season ($\chi^2 = 33.91$; $P < 0.01$). The other two disease symptoms affected a similar proportion of leaves during both seasons.

Spatial variation in the incidence of pathogen damage—There was considerable variation among sites in both the percentage of symptomatic leaves per species and the IP per species during both seasons of the year (Table 1). In the dry season, the greatest contrast was that of percentage of symptomatic leaves in sites 1 and 3 (a twofold difference). However, there was much variation among species (see Appendix 2). Therefore, a two-way analysis of variance of the arcsine-transformed percentages of infected leaves per plant in the four surveyed sites for each season of the year did not reveal significant differences (overall model, $F = 1.49$, df = 7, 248; $P = 0.17$). The analysis indicates that there was not a significant effect of season ($F = 0.9133$, df = 1; $P = 0.34$) and site ($F = 0.80$, df = 3; $P = 0.5$). The site × season interaction was significant ($F = 2.69$, df = 3; $P = 0.05$), indicating that intersite variation depended on the season of the year. Similarly, the analysis of variance of the IP arcsine-transformed values indicated no significant effects (overall model, $F = 1.20$, df = 7, 248; $P = 0.34$).

In order to determine whether the IP and the percentage of diseased plant species per site were related to site diversity, we carried out Spearman's rank correlation analyses. Neither the correlation between site diversity (the Shannon-Wiener diversity index; Greig-Smith, 1983) and IP (Spearman Rho = 0.40; $P > 0.60$) or the percentage of plant species damaged (Spearman Rho = −0.20; $P > 0.80$) were statistically significant. Finally, our results do not show a consistent spatial variation in the levels of disease associated with the variations in the degree of shading of the studied sites. On the one hand, the percentage of symptomatic leaves did not vary among sites on a given season and, on the other, the highest values of IP corresponded to the site that ranked third in degree of shadiness.

TABLE 1. Spatial variation in the proportion of individual leaves damaged by pathogens, including the mean values of the index of pathogen damage per plant (IP), calculated with all sampled leaves.

Site	Dry season		Rainy season	
	Percentage of damaged leaves	IP	Percentage of damaged leaves	IP
1	25.0	0.73	38.6	0.69
2	44.4	0.81	38.5	0.91
3	54.4	1.14	56.5	1.01
4	42.6	0.72	36.5	0.71

Association of damage types—The analysis of types of damage (Table 2) indicated that a very low proportion (1.4%) of the leaves were damaged by fungi alone. Nevertheless, the percentage of leaves that bore damage of both pathogens and herbivores increased to nearly 43% of the total number of leaves. In contrast, 16% of the leaves were damaged by herbivory alone. This indicates that pathogen attack may be highly dependent on damage by herbivores, while herbivory can occur in the absence of pathogenic infection. A two-by-two contingency analysis of the association of pathogen damage (present/absent) and damage by herbivores (present/absent) using the number of leaves in each category (cf. Table 2) was highly significant ($\chi^2 = 3956.88$; $P < 0.00001$). In this contingency analysis there was a marked overrepresentation of the combination herbivory–pathogen and a marked underrepresentation of pathogen damage alone.

Inoculation experiments—We were able to experimentally infect 12 (33%) of the 40 tested plant species (Table 3). Infection occurred most commonly when the inocula was applied in combination with simulated herbivory (wounds and scrapes) (Table 3). The percentage of inoculated leaves that resulted in positive infection ranged from 40 to 100% when inoculation was combined with simulated herbivory. In contrast infection by direct contact (i.e., in the absence of simulated herbivory) was unsuccessful in all cases but one. In *Diplazium lonchophyllum* (affected by necrotic spots), 60% of

TABLE 2. Number and proportion of leaves affected by the different kinds of damage and their associations ($N = 8365$). IP = index of pathogen damage and IH = index of herbivory.

Type of damage association	No. of leaves	%	IP	IH
Pathogens alone	114	1.4	0.94	0.00
Herbivory alone	1333	15.9	0.00	2.08
Herbivory + pathogens	3577	42.8	0.52	1.41
Intact	3341	39.9	0.00	0.00
Total pathogens	3691	44.4	0.22	0.00
Total herbivory	4910	59.0	0.00	1.72

TABLE 3. List of host-plant species that tested positive within 12 d after experimental inoculation and the percentages of successful infection as a result of the experimental treatments. $N = 5$ plants and 5 leaves for each treatment per species. The table includes the symptom for each species and the number of fungal morphotypes for each symptom. None of the leaves from the control treatments tested positive.

Plant species	Symptom	Treatment		No. of morphotypes
		Simulated herbivory (%)	Direct contact (%)	
A. flexile ssp. *flexile*	Chlorotic spots	80	0	1
	Blight	60	0	1
A. pentaphyllum var. *bombacifolium*	Blight	100	0	1
A. aurantiaca	Necrotic spots	40	0	1
A. mexicanum	Chlorotic spots	100	0	2
D. lonchophyllum	Necrotic spots	100	60	2
D. seguine	Chlorotic spots	80	0	1
M. acuminata	Chlorotic spots	80	0	1
	Necrotic spots	60	0	2
N. ambigens	Necrotic spots	80	0	2
P. guttiferum	Necrotic spots	80	0	1
P. armata	Necrotic spots	100	0	1
P. faxlucens	Necrotic spots	40	0	1
R. aff. *wendlandii*	Chlorotic spots	40	0	1
	Necrotic spots	100	0	1
S. podophyllum	Chlorotic spots	100	0	2
	Small necrotic spots	100	0	2

the leaves were infected through direct contact (Table 3). The symptoms produced in these experimental plants were the same as those caused by the originally isolated fungal morphotypes (cf. Appendix 1). Although we have not identified the isolated fungi, we verified in these experiments that the microorganisms initially inoculated were the same as the ones isolated from the tested leaves. Therefore, we were able to confirm that the isolated microorganisms were the causal agents of the observed symptoms, according to Koch's postulates.

The time for the manifestation of the symptoms caused by pathogens that penetrated through wounds ranged from 2 to 12 d. The average time for the development of the symptoms caused by these pathogens when combined with simulated herbivory was 4.12 ($\pm$1.87) d, while the symptom caused by the pathogen that infected the leaves of *D. lonchophyllum* by direct contact was visible after 5 d. This indicates that infection combined with simulated herbivory occurred relatively quickly, although in the exceptional case of the plant species infected by direct contact, the speed of symptom development was comparable.

DISCUSSION

The results of this study showed that a considerable proportion of the understory plant species (69%) and their leaves (44%) were affected by a variety of disease symptoms caused by fungi. However, the severity of disease was relatively low and never exceeded 12% of leaf area damaged per plant. It is possible that this value may be an underestimate if diseased leaves fall off the plant and are therefore overlooked in one-time censuses like those of our study. In studies of herbivory, it has been found that heavily damaged leaves tend to be dropped by the plant, and thus one-time censuses yield underestimates of herbivory in those plants but not so much in the less damaged plants (Filip et al., 1995). The low incidence of cases of extensive pathogen damage in this forest (see also de la Cruz and Dirzo, 1987; Dirzo, 1987) suggests that our data may not be greatly underestimated. Nevertheless, a study directed to the long-term monitoring of pathogen damage and leaf persistence in individually marked leaves is needed to clarify this aspect. Although we do not know of any study assessing leaf-pathogen damage at the community level in any other tropical rain forest, it has been observed that many tropical plant species show leaf-spots, typically caused by fungi. For example, in Central Amazonian forests, associations between fungi and leaf-spots have been reported in seedlings of several Sapotaceous trees (Benítez-Malvido, García-Guzmán, and Kossmann-Ferraz, 1999). Furthermore, Travers, Gilbert, and Perry (1998) have also reported damage by a rust fungus in flowers and young leaves of *Faramea occidentalis.*

Our results showed that there was considerable variation in the levels of damage by pathogens among plant species. The most affected species included unrelated taxa such as *Abuta panamensis* (Menispermaceae) in the dry season, *Astrocaryum mexicanum* (Arecaceae) in the dry and rainy seasons, and *Psychotria faxlucens* (Rubiaceae) in the rainy season (cf. Appendix 2). On the other hand, many plant species were not attacked by pathogens, including several species represented by a few individuals, with few leaves, as well as a few abundant species (e.g., *Cymbopetalum baillonii*). The group of unattacked species included both unrelated taxa and congeneric species. High levels of damage in some species and the lack of damage in others may be explained by stochastic reasons (the probability of a pathogen of finding a suitable host as a function of its abundance), however, the high interspecific variations could be explained by variation in defensive mechanisms. Plant secondary metabolites have been found to be a determinant of leaf pathogenic infection in many plant species. For example, several phenolic compounds, and others, such as saponines, have been proposed as responsible for the resistance of plant tissues to a variety of pathogenic microorganisms (Agrios, 1997). The role of secondary chemistry in explaining interspecific variation in pathogen damage warrants further study.

The seasonality present at Los Tuxtlas did not affect the incidence of leaf diseases. This suggests that the environmental conditions found in the understory of this forest are favorable for the establishment and growth of several pathogens

throughout the year. Some studies (Dinoor, 1970; Thrall and Jarosz, 1994; García-Guzmán et al., 1996) have shown that the interaction between host and pathogens has a very strong environmental component. Changes in specific physical factors may affect the host, the pathogen, or their interaction, by altering the germination of spores and growth of pathogens, as well as the germination, growth or susceptibility of host plants, disease expression, the survival of infected plants (Colhoun, 1973; Agrios, 1997), or behavior of vectors. One possible explanation for the lack of seasonal variation in disease incidence could be that leaf longevity is so extended that our sampling on a particular season could have included foliage infected in another season. Leaf longevity varies considerably among tropical species in the understory of neotropical forests (Barajas, 1998), so this effect may have occurred in some species. This aspect warrants further investigation.

Temporal variation in disease incidence was only evident in two symptoms in Los Tuxtlas. We found that the percentage of leaves affected by small necrotic spots and blight varied between seasons. However, the pattern of variation of these two symptoms was not consistent. While the percentage of leaves affected by blight was higher during the dry season, the percentage of leaves affected by small necrotic spots was higher during the rainy season. Although this variation might have been the result of more suitable environmental conditions for the development and establishment of the causal agents of these particular symptoms, it is also possible that other factors might explain the seasonal variation observed in these two symptoms. For example, the apparently lower incidence of small necrotic spots in the dry season may have been the result of the extensive development of blight in that season thus obscuring the apparency of small necrotic spots.

Our study shows that there was not a spatial variation in the levels of damage by pathogens. This absence of variation may be explained by the similarity of the four study sites, which were representative of mature forest. Although the four sites represented a gradient of shading, probably the contrast among them was not as marked as that needed to cause significant contrasts in pathogen infection, such as that reported by Augspurger and Kelly (1984) when comparing damping-off between mature forest and gaps. In addition, the diversity and density of host plants have been considered as important factors determining pathogen incidence and disease severity (Burdon and Chilvers, 1982; Burdon, 1987) and a number of examples, mainly in agricultural systems, have shown that the homogeneous spatial distribution and high densities of the host plants favor the spread and establishment of pathogenic microorganisms (Burdon and Chilvers, 1982). In wild systems the number of studies is limited. However, the heterogeneity of these systems may result in pathogens being less likely to spread efficiently. For example, Carlsson and Elmqvist (1992) reported that the occurrence of the systemic smut *Microbotryum violaceum* (= *Ustilago violacea*) was higher in larger and more dense populations of the host plant *Silene dioica*, while less dense populations remained healthy. Nevertheless, in our study the diversity of each site was not significantly related to the levels of damage by pathogens per plant or to the percentage of diseased plants.

Considering that some studies in natural wild systems with systemic (e.g., Augspurger and Kelly, 1984; García-Guzmán et al., 1996) and nonsystemic pathogens (Alexander, 1984; Burdon et al., 1992) have shown a positive relationship between host density and incidence of diseases, we suggest that in the understory of Los Tuxtlas the presence of high density single-species stands (e.g., seedling banks) (see Dirzo and Miranda, 1991; Dirzo et al., 1992), may render plants highly susceptible to infection due to an increased shading, moisture, and proximity of plant tissue available for plant pathogens and herbivorous insects. Thus, disease incidence in a large proportion of plants may take place.

In this forest, besides density, the positive relationship between herbivory and pathogen damage, as well as the high proportion of wound-dependent pathogens (inoculation experiments), suggests the presence of a high proportion of opportunistic or facultative parasitic fungi, unable to cause disease unless previous leaf wounding occurs. These factors could be increasing the chances of a plant becoming infected simply by virtue of the presence of diseased plants in high-density stands as well as herbivorous insects able to feed on different plant species. This could be an additional factor to explain why levels of infection were found to be consistently high among sites.

Plant pathogens can penetrate host tissues by a variety of means (Tarr, 1972; Agrios, 1997). However, in this study we obtained strong evidence suggesting that most of the pathogens causing leaf diseases in this forest required wounds or scrapes to penetrate the host tissues. Moreover, the inoculation experiments carried out in our study strongly suggest that these pathogens largely depend on the mechanical damage inflicted by herbivory and perhaps by other agents of physical damage, such as wind or water. This suggests that leaf pathogens in Los Tuxtlas seem to be dependent on an agent that is relatively common (although not necessarily predictable). Insects are known to be important agents of fungal, bacterial, and viral disease dissemination in many temperate and tropical agricultural systems. For example, *Sphaceloma perseae*, causal agent of scab in leaves of avocado, may be transmitted by insects (Carvalho, 1976), and the blueberry shoestring virus is transmitted by the aphid *Illinoia pepperi* (Terhune et al., 1991). However there is little evidence for the role insects play as pathogen-dispersal agents in tropical natural systems (see Benítez-Malvido, García-Guzmán, and Kossmann-Ferraz, 1999, for an example). Alternatively, it could be argued that if the leaves of the understory plants are poorly defended (and/or have high quality for consumers) they could be attacked simultaneously by both insects and pathogens leading to an association like the one found in this study (P. D. Coley, personal communication, University of Utah). However, our inoculation experiments strongly support the argument of pathogen dependence on herbivore damage.

The pathogens affecting these species were fungi that caused discrete lesions that individually may have little noticeable effect on host fitness. Other studies in temperate forests have shown that nonsystemic leaf pathogens, such as the ones found in our study, have limited or no discernible effect on their hosts (Burdon, 1993). However, when plants are severely diseased, foliar pathogens can reduce survivorship, reproduction, growth, and competitive ability of infected plants (Jarosz and Davleos, 1995). Nevertheless, the combined effects of herbivory and pathogen damage found in a high proportion of the surveyed plants may affect the performance of the host plants in this forest by reducing the photosynthetic area. This three-way interaction is an aspect that warrants further study.

The interplay between biotic and abiotic factors is vital in determining the intensity of host–pathogen interactions and their long-term consequences. It has been suggested that path-

ogens that cause local leaf lesions are characterized by high levels of spatial and temporal variability, often related to habitat, weather, or climatic variables (Jarosz and Davelos, 1995). This has been particularly evident in some associations involving local lesion diseases, such as wild barley infected by the powdery mildew *Erysiphe graminis* (Dinoor and Eshed, 1990) and *Linum marginale* infected by the rust *Melampsora lini* (Jarosz and Burdon, 1992). In these cases it was found that disease incidence was highly variable both spatially and temporally. The need for specific environmental conditions and the susceptibility of hosts seem to be primarily responsible for the observed variability (Jarosz and Davelos, 1995). However, more studies are needed to determine whether this is also the case for tropical systems such as Los Tuxtlas, where climatic conditions remain more or less constant throughout the year.

Disease can affect plant fitness in a myriad of ways, however more detailed studies are needed to determine the impact of leaf-fungal pathogens at the population and community dynamics of tropical systems (see Augspurger and Kelly, 1984). Our results suggest that the study of plant–pathogen interactions constitutes an area of potential interest in the field of the biotic interactions in tropical natural systems and highlights the role of herbivory in the ecology of plant diseases in natural communities. Furthermore, our study places tropical herbivory in another perspective, whereby phytophagous insects are not to be seen only as agents of leaf tissue removal, but as agents of disease facilitation and/or dispersal.

LITERATURE CITED

AGRIOS, G. N. 1997. Plant pathology. Academic Press, New York, New York, USA.

ALEXANDER, H. M. 1984. Spatial patterns of disease induced by *Fusarium moniliforme* var. *subglutinans* in a population of *Plantago lanceolata*. *Oecologia* 62: 141–143.

AUGSPURGER, C. K. 1983. Seed dispersal of tropical tree, *Platypodium elegance*, and the escape of its seedlings from fungal pathogens. *Journal of Ecology* 71: 759–771.

———. 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light gaps, and pathogens. *Ecology* 65: 1705–1712.

———, AND C. K. KELLY. 1984. Pathogen mortality of tropical seedlings: experimental studies of the effect of dispersal distance, seedling density and light conditions. *Oecologia* 61: 211–217.

BARAJAS, A. N. 1998. Respuestas ecológicas y fisiológicas de plantas pioneras y tolerantes a la defolicaión en La Selva Lacandona, Chis., México. Bsc. thesis, Universidad Nacional Autónoma de México, Mexico, DF, Mexico.

BARNETT, H. L., AND B. B. HUNTER. 1972. Illustrated genera of imperfect fungi. Burgess Publishing Company, Minneapolis, Minnesota, USA.

BENÍTEZ-MALVIDO, J., G. GARCÍA-GUZMÁN, AND I. D. KOSSMANN-FERRAZ. 1999. Leaf-fungal incidence and herbivory on tree seedlings in tropical rain forest fragments: an experimental study. *Biological Conservation* 91: 143–150.

BOOTH, C. 1971. Introduction to general methods. *In* C. Booth [ed.], *Methods in Microbiology* 4: 1–47.

BURDON, J. J. 1987. Disease and plant population biology. Cambridge University Press, Cambridge, UK.

———. 1993. The structure of pathogen populations in natural plant communities. *Annual Review of Phytopathology* 31: 305–323.

———, AND G. A. CHILVERS. 1982. Host density as a factor in plant disease ecology. *Annual Review of Phytopathology* 20: 143–166.

———, A. WENNSTRÖM, L. ERICSON, W. J. MÜLLER, AND R. MORTON. 1992. Density-dependent mortality in *Pinus sylvestris* caused by the snow blight pathogen *Phacidium infestans*. *Oecologia* 90: 74–79.

CARLSSON, U., AND T. ELMQVIST. 1992. Epidemiology of anther-smut disease (*Microbotryum violaceum*) and numeric regulation of populations of *Silene dioica*. *Oecologia* 90: 509–517.

CARVALHO, F. 1976. El aguacate. Editorial Ra, Mexico, DF, Mexico.

COLHOUN, J. 1973. Effects of environmental factors on plant disease. *Annual Review of Phytopathology* 11: 343–364.

COLEY, P. D., AND J. A. BARONE. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* 27: 305–335.

DE LA CRUZ, M., AND R. DIRZO. 1987. A survey of the sanding levels of herbivory in seedlings from a Mexican rain forest. *Biotropica* 19: 98–106.

DINOOR, A. 1970. Sources of oat crown rust resistance in hexaploid and tetraploid wild oats in Israel. *Canadian Journal of Botany* 48: 153–161.

———, AND N. ESHED. 1990. Plant disease in natural populations of wild barley (*Hordeum spontaneum*). *In* J. J. Burdon and S. R. Leather [eds.], Pest, pathogens and plant communities, 169–186. Blackwell, Oxford, UK.

DIRZO, R. 1987. Estudios sobre interacciones planta-herbivoro en ''Los Tuxtlas'', Veracruz. *Revista de Biología Tropical* 35: 119–131.

———, AND C. A. DOMÍNGUEZ. 1995. Plant–herbivore interactions in Mesoamerican tropical dry forests. *In* S. H. Bullock, H. A. Mooney, and E. Medina [eds.], Seasonal dry tropical forests, 304–325. Cambridge University Press, Cambridge, UK.

———, AND A. MIRANDA. 1991. Altered patterns of herbivory and diversity in the forest understory: a case of the possible consequences of contemporary defaunation. *In* W. P. Pric, M. Lewinson, G. Wilson, and W. B. Woodruff [eds.], Plant–animal interactions: evolutionary ecology in tropical and template regions, 273–447. John Wiley and Sons, New York, New York, USA.

———, C. C. HORVITZ, H. QUEVEDO, AND M. A. LÓPEZ. 1992. The effects of gap size and age on the understory herb community of tropical Mexican rain forest. *Journal of Ecology* 80: 809–822.

———, S. E. GONZÁLEZ, AND R. C. VOGT. 1997. Introducción general. *In* S. E. González, R. Dirzo, and R. C. Vogt [eds.], Historia Natural de Los Tuxtlas, 3–6. Universidad Nacional Autónoma de México, Mexico, DF, Mexico.

ERWIN, T. L. 1982. Tropical forests: their richness in Coleoptera and other arthropod species. *Coleoptera Bulletin* 36: 74–75.

FILIP, V., R. DIRZO, J. M. MAASS, AND J. SARUKHÁN. 1995. Within- and among-year variation in the levels of herbivory on the foliage of trees from a Mexican tropical deciduous forest. *Biotropica* 27: 78–86.

GARCÍA-GUZMÁN, G. 1990. Estudio sobre ecología de patógenos en el follaje de plantas en la selva de Los Tuxtlas. Msc. thesis, Universidad Nacional Autónoma de México, Mexico, DF, Mexico.

———, J. BURDON, J. ASH, AND R. CUNNINGHAM. 1996. Regional and local patterns of the spatial distribution of the flower infecting smut fungus *Sporisorium amphilophis* (Syd.) Langdon & Fullerton in natural populations of its host *Bothriochloa macra* (Steud) S.T. Blake. *New Phytologist* 132: 459–469.

GILBERT, G. S., AND S. P. HUBBELL. 1996. Plant diseases and the conservation of tropical forests. *BioScience* 46: 98–106.

GONZÁLEZ, S. E., R. DIRZO, AND R. C. VOGT. 1997. Historia Natural de Los Tuxtlas. Universidad Nacional Autónoma de México, Mexico, DF, Mexico.

GREIG-SMITH, P. 1983. Quantitative plant ecology. University of California Press, Berkeley, California, USA.

HOWE, H. F., AND L. C. WESTLEY. 1988. Ecological relationships of plants and animals. Oxford University Press, Oxford, UK.

IBARRA, M. G., M. MARTÍNEZ-RAMOS, R. DIRZO, AND J. NÚÑEZ-FARFÁN. 1997. La vegetación. *In* S. E. González, R. Dirzo, and R. C. Vogt [eds.], Historia Natural de Los Tuxtlas, 72–85. Universidad Nacional Autónoma de México, Mexico, DF, Mexico.

JAROSZ, A. M., AND J. J. BURDON. 1992. Host–pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*. *Oecologia* 89: 53–61.

———, AND A. L. DAVELOS. 1995. Effects of disease in wild plant populations and the evolution of pathogen aggressiveness. *New Phytologist* 129: 371–382.

KREMER, F. W., AND G. UNTERSTENHÖFER. 1967. Valoración de resultados fitosanitarios según el método de Townsend y Heuberger. *Instituto de Biología de Farbenfabriken Bayer A. G., Leverkusen* 20: 657–660.

LODGE, D. J., D. HAWKSWORTH, AND B. J. RITCHIE. 1996. Microbial diversity and tropical forest functioning. *In* G. H. Orians, R. Dirzo, and J. H. Cushman [eds.], Biodiversity and ecosystem processes in tropical forests, 69–100. Springer, Berlin, Germany.

LÓPEZ, A. G. F. 1981. Manejo de hongos fitopatógenos. Universidad Autónoma Chapingo, Chapingo, Mexico, Mexico.
MANNERS, J. G. 1993. Principles of plant pathology. Cambridge University Press, Cambridge, UK.
PENNINGTON, T. D., AND J. SARUKHÁN. 1998. Arboles Tropicales de México. Fondo de Cultura Económica, Mexico, DF, Mexico.
SHIVAS, R. G., AND K. D. HYDE. 1997. Biodiversity of plant pathogenic fungi in tropics. *In* K. D. Hyde [ed.], Biodiversity of tropical microfungi, 47–56. Hong Kong University Press, Hong Kong.
SOTO, E. M., AND L. GAMA. 1997. Climas. *In* S. E. González, R. Dirzo, and R. C. Vogt [eds.], Historia natural de Los Tuxtlas, 7–23. Universidad Nacional Autónoma de México, Mexico, DF, Mexico.
STREETS, R. B. 1978. The diagnosis of plant diseases. University of Arizona Press, Tucson, Arizona, USA.
TARR, S. A. 1972. Principles of plant pathology. Macmillan, London, UK.
TERHUNE, B. T., D. C. RAMSDELL, K. L. KLOMPARENS, AND J. F. HANCOCK. 1991. Quantification of blueberry shoestring virus RNA and antigen in its aphid vector, *Illinoia pepperi*, during acquisition, retention, and transmission. *Phytopathology* 81: 1096–1102.
THRALL, P. H., AND A. M. JAROSZ. 1994. Host–pathogen dynamics in experimental populations of *Silene alba* and *Ustilago violacea*. I. Ecological and genetic determinants of disease spread. *Journal of Ecology* 82: 549–559.
TRAVERS, S. E., G. S. GILBERT, AND E. T. PERRY. 1998. The effect of rust infection on reproduction in a tropical tree (*Faramea occidentalis*). *Biotropica* 30: 438–443.
WALLER, J. M. 1976. The influence of climate on the incidence and severity of some diseases of tropical crops. *Review of Plant Pathology* 55: 185–194.

APPENDIX 1. Description of disease symptoms and the isolated fungal morphotypes for each plant species.

Plant species	Symptom	Fungal morphotype
Abuta panamensis (Standley) Krukoff & Barneby (Menispermaceae)	Very small necrotic spots (1–2 mm in diameter), whitish, with a chlorotic halo.	Mycelium white, septate. Spores hyaline, one-celled, ovoid.
Acalypha diversifolia Jacq. (Euphorbiaceae)	Irregular to round chlorotic, large spots (1–2 cm in diameter) that may coalesce.	Green and septate mycelium with sclerotia.
Adiantum sp. (Polypodiaceae)	Necrotic spots light brown that may reach a diameter of 5 mm.	*Alternaria* sp.
Ampelocera hottlei (Standley) Standley (Ulmaceae)	Chlorotic spots, small, round in shape.	Mycelium hyaline, septate, and branched. Black pycnidia developing on the spots.
Anthurium flexile Schott ssp. *flexile* (Araceae)	Chlorotic spots appear first small, then enlarge rapidly up to 3 cm in diameter and become brown. Pink spore masses appear in the necrotic tissue.	*Colletotrichum* sp.
	Leaf lesions originate near the leaf apex as dry, whitish to light-brown irregular spots that enlarge toward the leaf base.	Dark-green mycelium, septate, and branched. Dark and simple conidiophores, bearing a branched chain of dark and ovoid conidia.
Anthurium pentaphyllum (Aublet) G. Don. var. *bombacifolium* (Schott)	Chlorotic spots as described for *A. flexile.*	Hyaline mycelium septate.
Madison (Araceae)	Blight as described for *A. flexile.*	White septate and abundant mycelium. Globose and multisporic sporangia, with columella.
Aphelandra aurantiaca (Scheidw.) Lundell (Acanthaceae)	First lesions appear as small chlorotic spots, then enlarge and appear as brown necrotic lesions irregular in shape.	Green, septate mycelium. Short, simple, septate conidiophores, appear in clusters in the spots. Green, oval conidia.
Astrocaryum mexicanum Liebm. Ex Mart. (Arecaceae)	Small chlorotic angular spots (3–5 mm in diameter) that may coalesce.	Fungus 1: Green and septate mycelium in culture, with dark, scattered picnidia appear in the spots. Fungus 2: Spores cylindric with truncate ends.
Bolbitis bernoullii (Kuhn ex Christ) Ching (Polypodiaceae)	Grayish-tan colored necrotic spots, more or less circular, up to 5 mm in diameter, with a distinct marginal halo.	Brown and septate mycelium with sclerotes.
Brosimum alicastrum Sw. (Moraceae)	Small, irregular chlorotic spots scattered at random.	Light-pink septate mycelium.
Chamaedorea tepejilote Liebm. In Mart. (Arecaceae)	Small (2–5 mm in diameter), angular chlorotic spots, with red margins.	Mycelium dark-green in culture, brownish in leaf spots, with a netlike growth. Spores dark and ovoid.
	Lesions at the bases and along the margins of pinnae as light-brown dry areas.	Pycnidia black, globose, single, ostiolate, which appear black and scattered in the killed leaf tissue. Spores dark, two-celled. Similar to *Diplodia* spp.
Costus sp. (Zingiberaceae)	Large, irregular chlorotic spots with dark-brown borders.	White, septate mycelum, pycnidia brown, globose. Spores hyaline, several-celled.
Crataeva tapia L. (Capparaceae)	Small, irregular, chlorotic spots.	Brown septate mycelium.
Croton schiedeanus Schldl. (Euphorbiaceae)	Rust colored necrotic spots, more or less round in shape.	Mycelium brownish, septate, and branched. Oval spores produced in chains, gray in mass on the necrotic spots.
Dichapetalum donnell-smithii Engl. (Dichapetalaceae)	Large, irregular, dry whitish spots.	Pycnidia dark, globose, ostiolate. Spores hyaline, elongated.
Dieffenbachia seguine (L.) Schott (Araceae)	Chlorotic, subcircular to angular spots, usually large (up to 5 cm in diameter), and may coalesce.	Pink septate mycelium. Simple and slender conidiophores producing hyaline conidia.
Diplazium lonchophyllum Kunze (Polypodiaceae)	Dark-brown, round necrotic spots up to 5 mm in diameter.	Fungus 1: *Fusarium* sp. Fungus 2: Grayish abundant mycelium, septate, and branched. Globose and multisporic sporangia.

APPENDIX 1. Continued.

Plant species	Symptom	Fungal morphotype
Dussia mexicana (Standley) Harms (Leguminosae)	Chlorotic irregular to round small (up to 1 cm in diameter) spots that may coalesce.	Light-green mycelium. Conidiophores hyaline, branched. Conidia hyaline, one-celled, ovoid.
Eugenia sp. (Myrtaceae)	Irregular, dry, light-brown necrotic spots with margins bordered by yellow halos.	Mycelium gray, septate.
Faramea occidentalis (L.) A. Rich. (Rubiaceae)	Small leaf necrotic spots, no more than 2 mm in diameter, surrounded by a chlorotic halo.	White mycelium, dark pycnidia, globose, ostiolate.
Guarea glabra Vahl raza *glabra* (Meliaceae)	Chlorotic irregular spots, large up to 3 cm in diameter that may coalesce.	Conidiophores hyaline, simple and bent. Conidia hyaline, cylindrical one-called, in short chains.
	Light-brown, dry, necrotic lesions 1–3 cm in diameter that coalesce to form irregular spots.	Brown mycelium with sclerotia.
Inga sp. (Leguminosae)	Mostly irregular chlorotic spots 1–2 cm in diameter.	Dark-green mycelium. Conidiophores tall, dark, branched. Conidia dark, lemon-shaped.
	Dark-red necrotic spots 5–10 mm in diameter.	Dark mycelium, conidiophores single, conidia oval two-celled.
Monstera acuminata G. Koch. (Araceae)	Irregular, large (up to 5 cm in diameter) chlorotic spots with red margins.	White and flat mycelium, conidia ovoid.
	Round, dark, large necrotic spots with dark-brown borders. Lesions may coalesce. Dark pycnidia appear in necrotic tissue.	Fungus 1: Gray and septate mycelia. Conidiophores long, slender, and branched. Conidia in clusters. Fungus 2: Dark-green mycelium. Pycnidia dark, globose, ostiolate.
Nectandra ambigens (Blake) Allen (Lauraceae)	Initially lesions are chlorotic, small (2–3 mm in diameter), then become light-brown, enlarge, and may coalesce to cover most of the leaf area. Necrotic tissue may break and tear away. Pink spore masses and black globose pycnidia appear in the necrotic tissue. Older lesions turn white, desiccate, and crack, leaving holes in the leaves.	Fungus 1: *Colletotrichum* sp. Fungus 2: *Phomopsis* sp.
Ocotea dedrodaphne Mez (Lauraceae)	Round large (1–3 cm in diameter) chlorotic spots with reddish borders.	Gray mycelium, abundant. Spores, one-celled, brown in mass.
	Dry, rust-colored, necrotic irregular spots. Large, 1–2 cm in diameter.	Dark-green mycelium. Dark pycnidia, globose. Spores hyaline, one-celled.
Odontonema callistachyum (Schldl. & Cham.) Kuntze (Acanthaceae)	Large irregular chlorotic spots that may coalesce.	Short, dark conidiophores. Conidia dark, pyriform.
Omphalea oleifera Hemsl. (Euphorbiaceae)	Round 1–2 cm in diameter chlorotic spots that may coalesce.	White mycelium. Dark, globose pycnidia.
Paragonia pyramidata (Rich.) Bur. (Bignoniaceae)	Light-brown, dry necrotic spots, round, small (5–10 mm in diameter).	Dark mycelium. Conidia hyaline, one-celled, ovoid with branched apical appendage.
Paullinia clavigera Schldl. (Sapindaceae)	Roughly circular, brown to black necrotic spots, and up to 5 mm in diameter.	Dark pycnidia, globose, ostiolate. Spores hyaline, filiform, septate.
Philodendron guttiferum Kunth in H.B.K. (Araceae)	Dark-brown to grayish-tan necrotic spots, more or less round, large (1–5 cm in diameter). Lesions may enlarge and coalesce.	*Colletotrichum* sp.
Philodendron inaequilaterum Liebm. (Araceae)	Angular, large chlorotic spots with red margins.	White, abundant, septate mycelium.
	Very small (1–2 mm in diameter) necrotic spots, that may enlarge and coalesce.	*Colletotrichum* sp.
Philodendron scandens G. Koch & Sell (Araceae)	Chlorotic spots very similar to the ones observed in *P. inaequilaterum.*	Light-pink, septate mycelium. Hyaline, lemon-shaped spores.
	Necrotic spots very similar to the ones observed in *P. inaequilaterum.*	*Colletotrichum* sp.
Piper aequale Vahl (Piperaceae)	Round, black necrotic spots. Small (up to 5 mm in diameter), that may coalesce.	Fungus 1: Conidia dark, septate, with apical appendages. Fungus 2: Pycnidia dark, ostiolate, globose. Spores hyaline, elongated.
Pleuranthodendron lindenii (Turcz.) Sleumer (Flacourtiaceae)	Dark-brown, dry necrotic spots, small (5–10 mm in diameter) and irregular in shape.	Two-celled spores, hyaline.
Poulsenia armata (Miq.) Standley (Moraceae)	Roughly circular dark-brown necrotic spots. Large (1–3 cm in diameter).	*Fusarium* sp.
Pouteria durlandii (Standley) Baehni (Sapotaceae)	Round, dark-brown necrotic spots. Small (5–10 mm in diameter).	Fungus 1: *Colletotrichum* sp. Fungus 2: Light brown conidia, slightly curved.
Pseudolmedia oxyphyllaria J.D. Smith (Moraceae)	More or less round rust-colored necrotic spots. Dry and small (5–10 mm in diameter).	Dark-yellow, abundant mycelium, oval spores.
Psychotria faxlucens Lorence & Dwyer (Rubiaceae)	Whitish to light-brown, small and irregular necrotic spots.	Dark mycelia, spores blackish and globose.
Reinhardtia gracilis (H. Wendl. In Otto & Dietr.) Burret var. *gracilior* (Burret) H. Moore (Arecaceae)	Very small (1–2 mm in diameter) chlorotic angular spots that may coalesce.	White and abundant mycelium. Spores hyaline, one-celled, ovoid.

APPENDIX 1. Continued.

Plant species	Symptom	Fungal morphotype
Rhodospatha aff. *wendlandii* Schott (Araceae)	More or less round and large (1–3 cm in diameter) chlorotic spots, with dark-brown margins.	Pink mycelium, slender conidiophores, and hyaline conidia.
	Dark-brown irregular necrotic spots. Large (1–5 cm in diameter).	*Alternaria* sp.
Salacia megistophylla Standley (Hippocrateaceae)	Reddish to dark-brown, dry necrotic spots. Roughly round, 2–6 cm in diameter.	Spores slender, curved, three-celled.
Spathiphyllum cochlearispathum (Liebm.) Engl. (Araceae)	Large, irregular chlorotic spots that may enlarge and coalesce.	Pycnidia dark, globose, ostiolate. Spores hyaline, cylindrical.
	Small (1–2 mm in diameter), dark-brown necrotic spots with a chlorotic halo.	Mycelium septate. Spores two-celled, ovoid.
Syngonium aff. *schottianum* Wendl. Ex Schott (Araceae)	Round and large (up to 3 cm in diameter) chlorotic spots.	*Fusarium* sp.
	Small (up to 1 cm in diameter) black, round necrotic spots.	Dark-green mycelium, Dark, simple conidiophores. Dark, lemon-shaped conidia.
Syngonium podophyllum Schott (Araceae)	Round, large (up to 3 cm in diameter) chlorotic spots. They may enlarge, coalesce, and become dark brown with a chlorotic halo.	Fungus 1: *Pestalotia* sp. Fungus 2: Light-green mycelium. Conidiophores branched. Ovoid conidia.
	Small (up to 2 mm in diameter) black, round necrotic spots with a chlorotic halo.	Fungus 1: *Fusarium* sp. Fungus 2: Brown mycelium, dark sclerotia.
Thelypteris rhachiflexuosa Riba (Polypodiaceae)	Small (up to 1 cm in diameter), dark brown and round necrotic spots with darker margins.	Dark-green mycelium. Pycnidia dark, globose.
Trophis mexicana (Liebm.) Bureau in DC. (Moraceae)	Elliptical to irregular chlorotic spots. Small (5–10 mm in diameter)	Conidiophores dark, septate, slender. Conidia dark with three arms very similar to *Triposporium* spp.
	Roughly circular, dark-brown necrotic spots. Small (5–10 mm in diameter).	Conidiophores dark, simple. Hyaline dark conidia, filiform.

APPENDIX 2. List of the surveyed plant species during the dry and rainy seasons, with the number of sampled leaves, proportion of infected leaves, and the index of pathogen damage (IP) for each species. Rank is based on the index of pathogen damage values.

Plant species	Total no. of leaves	Infected leaves (%)	IP	Rank
Dry season				
A. panamensis	4	25.00	2.00	1
A. diversifolia	17	23.53	1.17	= 5
A. hottlei	38	5.26	1.00	= 8
A. flexile ssp. *flexile*	117	14.53	0.79	12
A. pentaphyllum var. *bombacifolium*	97	16.49	0.76	13
A. aurantiaca	261	1.53	0.31	= 23
A. mexicanum	39	84.62	1.76	2
B. bernoullii	243	33.33	0.64	16
B. alicastrum	11	27.27	0.38	21
C. tepejilote	20	55.00	1.08	7
Costus sp.	4	75	1.50	3
C. tapia	28	3.57	1.00	= 8
C. schiedeanus	53	1.89	0.09	26
Cupania glabra Sw. (Sapindaceae).	2	0.00	0.00	= 27
Cymbopetalum baillonii R.E. Fries (Annonaceae).	17	0.00	0.00	= 27
D. lonchophyllum	1660	61.93	0.83	10
Eugenia sp.	17	0.00	0.00	= 27
F. occidentalis	35	14.29	0.29	24
G. glabra raza *glabra*	4	50.00	0.50	19
Inga sp.	10	20.00	1.00	= 8
Lonchocarpus sp. (Leguminosae)	2	0.00	0.00	= 27
M. acuminata	152	42.11	0.80	11
N. ambigens	179	71.51	1.14	6
Nectandra globosa (Aublet) Mez (Lauraceae)	5	0.00	0.00	= 27
O. dedrodaphne	8	25.00	0.25	25
O. callistrachyum	32	0.00	0.00	= 27
O. oleifera	33	30.30	0.70	14
Orthion oblanceolatum Lundell (Violaceae)	3	0.00	0.00	= 27
P. clavigera	10	0.00	0.00	= 27

APPENDIX 2. Continued.

Plant species	Total no. of leaves	Infected leaves (%)	IP	Rank
P. guttiferum	3	0.00	0.00	= 27
P. inaequilaterum	26	38.46	0.91	9
P. scandens	27	25.93	0.47	20
P. oxyphyllaria	26	11.54	0.31	= 23
P. faxlucens	9	0.00	0.00	= 27
Pterocarpus rohrii Vahl. (Leguminosae)	10	0.00	0.00	= 27
Quararibea sp. (Bombacaceae)	7	0.00	0.00	= 27
Randia pterocarpa Lorence & Dwyer (Rubiaceae)	6	0.00	0.00	= 27
R. gracilis var. *gracilior*	2	100.00	1.00	= 8
Rheedia edulis (Seemann) Triana & Planchón (Guttiferae)	8	0.00	0.00	= 27
R. aff. *wendlandii*	219	21.92	0.58	18
S. megistophylla	25	20.00	1.17	= 5
Schaueria calycobractea Hilsenbeck & Marshall (Acanthaceae)	1	0.00	0.00	= 27
S. cochlearispathum	56	30.36	0.68	15
S. aff. *schottianum*	7	28.57	0.33	22
S. podophyllum	97	67.01	1.35	= 4
T. rhachiflexuosa	92	82.61	1.35	= 4
Tradescantia zanonia (L.) Sw. (Commelinaceae)	16	0.00	0.00	= 27
Trichilia breviflora Blake & Standley (Meliaceae)	3	0.00	0.00	= 27
T. mexicana	149	20.13	0.60	17
Total	3890	43.26	0.85	
Rainy season				
A. panamensis	8	25.00	0.50	= 17
Adiantum sp.	265	63.77	1.15	6
A. hottlei	3	0.00	0.00	= 31
A. flexile ssp. *flexile*	149	47.65	0.97	9
A. pentaphyllum var. *bombacifolium*	72	50.00	0.85	13
A. aurantiaca	187	2.14	0.90	10
A. mexicanum	20	80.00	2.22	1
B. alicastrum	6	0.00	0.00	= 31
C. tepejilote	7	28.57	0.67	= 14
C. tapia	1	0.00	0.00	= 31
C. schiedeanus	50	10.00	0.25	= 26
C. baillonii	23	0.00	0.00	= 31
D. donnell-smithii	13	15.38	0.40	= 20
D. seguine	29	34.48	0.67	= 14
Diospyros digyna Jacq. (Ebenaceae)	4	0.00	0.00	= 31
D. lonchophyllum	2101	60.73	1.25	4
D. mexicana	53	1.89	0.07	30
Eugenia sp.	3	33.33	0.33	22
G. glabra raza *glabra*	21	9.52	0.10	29
Hippocratea celastroides Kunth in H.B.K. (Hippocrateaceae)	37	0.00	0.00	= 31
Inga sp.	11	27.27	0.88	11
Licaria sp. (Lauraceae)	4	0.00	0.00	= 31
M. acuminata	179	43.58	0.46	19
N. ambigens	73	63.01	1.08	7
O. dedrodaphne	4	25.00	0.25	= 26
O. callistachyum	6	83.33	1.50	3
O. oleifera	1	100.00	1.00	= 8
O. oblanceolatum	3	0.00	0.00	= 31
P. pyramidata	13	7.69	0.23	27
Parathesis lenticellata Lundell (Myrsinaceae)	6	0.00	0.00	= 31
P. clavigera	30	40.00	0.50	= 17
P. guttiferum	24	16.67	0.27	25
P inaequilaterum	30	10.00	0.25	= 26
P. scandens	26	38.46	0.48	18
P. aequale	23	8.70	0.13	28
P. lindenii	40	15.00	0.40	= 20
P. armata	10	50.00	0.86	12
P. durlandii	7	57.14	1.00	= 8
P. oxyphyllaria	72	23.61	0.29	24
P. faxlucens	20	20.00	1.60	2
Psychotria simiarum Standley (Rubiaceae)	10	0.00	0.00	= 31
P. rohrii	3	0.00	0.00	= 31
R. gracilis var. *gracilior*	5	0.00	0.00	= 31
R. edulis	16	0.00	0.00	= 31
R. aff. *wendlandii*	259	26.25	0.54	16
Rinorea guatemalensis (S. Watson) Bartlett (Violaceae)	5	0.00	0.00	= 31
Rollinia jimenezii Saff. (Annonaceae)	10	0.00	0.00	= 31

APPENDIX 2. Continued.

Plant species	Total no. of leaves	Infected leaves (%)	IP	Rank
S. megistophylla	8	0.00	0.00	= 31
S. calycobractea	7	0.00	0.00	= 31
S. cochlearispathum	140	21.43	0.31	23
Spigelia palmeri Rose (Loganiaceae)	10	0.00	0.00	= 31
Stemmadenia donnell-smithii (Rose ex J.D. Smith) Woodson (Apocynaceae)	8	0.00	0.00	= 31
S. aff. *schottianum*	5	60.00	0.60	= 15
S. podophyllum	167	52.10	1.24	5
T. rhachiflexuosa	145	30.34	0.39	21
T. breviflora	17	0.00	0.00	= 31
T. mexicana	26	7.69	0.60	= 15
Total	4475	45.43	0.98	