Light environment affects the levels of resistance hormones in *Syngonium podophyllum* leaves and its attack by herbivores and fungi

Graciela García-Guzmán[1], Frida Domínguez-Velázquez[1], Jaime Mendiola-Soto[2] and Martin Heil[2]*

Botanical Sciences
95 (3): 363-373, 2017

DOI: 10.17129/botsci.1213

Abstract

Background: Little is known on factors determining infection of tropical plants by fungal pathogens, although multiple studies on model species show that light conditions affect the two major hormones that control resistance in plants to enemies. This is the first study using a natural population of a wild tropical plant to relate light conditions to resistance hormones and, the beneficial and detrimental interactions with insects and fungi.
Hypothesis: Light conditions affect the endogenous levels of resistance hormones and thereby cause quantitative shifts among herbivores and nectrotrophic, biotrophic and endophytic fungi.
Studied species: The tropical vine *Syngonium podophyllum*.
Study site: Los Tuxtlas tropical rain forest, Mexico.
Methods: We used GC-MS to quantify the concentration of the two resistance hormones in intact leaves and natural levels of herbivory and fungal disease in *S. podophyllum* plants growing naturally in the full sun or in the shade.
Results: The proportion of leaves damaged by herbivores or infected by hemibiotrophic or necrotrophic pathogens was higher in shade than under full-light conditions. Damage caused by biotrophic pathogens was frequently observed in sun but not in shade. Levels of both hormones in phenotypically healthy leaves were higher in sun than in shade.
Conclusions: (i) light has an effect on concentrations of plant resistance hormones in nature; (ii) these differences have consequences for fungi and animals that naturally interact with the plant, and (iii) the described effect can represent a physiological mechanism via which an abiotic factor affects the members of the guilds at higher trophic levels.
Key Words: biotroph, fungal pathogen, endophyte, herbivore, hormone crosstalk, ITS5, jasmonic acid, necrotroph, resistance, salicylic acid

Resumen

Antecedentes: Poco se sabe sobre los factores que determinan la infección de plantas tropicales por patógenos, pero varios estudios con especies modelo muestran que las condiciones lumínicas afectan a las hormonas que controlan la resistencia de las plantas contra sus enemigos. Este es el primer estudio que analiza como las condiciones lumínicas afectan a las hormonas de resistencia y sus interacciones con insectos y hongos en una población silvestre.
Hipótesis: Las condiciones lumínicas afectan los niveles endógenos de las hormonas de resistencia y por lo tanto afectan la incidencia de herbívoros y patógenos necrótrofos, biótrofos y endófitos.
Especie estudiada: *Syngonium podophyllum*.
Sitio de estudio: Los Tuxtlas, Veracruz, México.
Métodos: Se utilizó GC-MS para cuantificar la concentración de hormonas de resistencia en hojas sanas y se evaluaron los niveles de daño por herbívoros y hongos patógenos en plantas de sol y sombra.
Resultados: La proporción de hojas dañadas por herbívoros o infectadas por patógenos hemibiótrofos o necrótrofos fue mayor en la sombra que en luz. El daño causado por patógenos biótrofos se detectó en sol pero no en sombra. Los niveles de las hormonas de resistencia fueron más altos en sol que en sombra.
Conclusiones: (i) la luz afecta la concentración de las hormonas de resistencia en plantas; (ii) estas diferencias afectan a los hongos y animales que interactúan naturalmente con la planta, y (iii) estos efectos pueden representar un mecanismo fisiológico a través del cual un factor abiótico afecta a los miembros de los gremios a niveles tróficos superiores.
Palabras claves: ácido jasmónico, ácido salicílico, biótrofo, crosstalk, endófito, herbívoro, hongo patógeno, ITS5, necrótrofo, resistencia

Author contributions
Graciela García-Guzmán: conceived the idea, analysed data and wrote the paper.
Frida Domínguez-Velázquez: analysed data, and performed the methodology.
Jaime Mendiola-Soto: analysed data, and performed the methodology.
Martin Heil: conceived the idea, analysed data and wrote the paper.

[1] Instituto de Ecología, Universidad Nacional Autónoma de México, México
[2] Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados, Irapuato, Guanajuato, México
* Corresponding author: mheil@ira.cinvestav.mx

Plants in natural as well as agro-ecosystems are continuously under attack by their biological enemies: herbivores and microbial pathogens. However, the ecological research into plant—enemy interactions in natural ecosystems is dominated by studies on insect herbivores, whereas plant pathology is dominated by studies on crops and a few model plants, like *Arabidopsis thaliana* (cf. Figure 3 in García-Guzmán & Heil 2014). The aim of the present study was to provide a first dataset that relates light conditions to the frequency at which a tropical plant is attacked by herbivores or biotrophic vs. necrotrophic fungal pathogens, or colonized by symptomless endophytic fungi, and to propose the control of plant-enemy interactions by plant hormone signalling as a putative physiological explanation of effects of ambient light on the frequencies of herbivores and pathogens of tropical plants. Resistance in plants to insects and microbial enemies is commonly controlled by two hormones: jasmonic acid (JA) controls the resistance to herbivores and necrotrophs, and is consequently induced after the respective attacks, whereas salicylic acid (SA) controls the infection by biotrophic pathogens (Thaler *et al*. 2012). Besides pathogens, plants are also colonized by fungal endophytes, which grow in the intercellular spaces without causing symptoms of disease. Phylogenetic analyses indicate that endophytes are genetically more closely related to necrotrophs, rather than biotrophs (Delaye *et al*. 2013), but it remains unknown how the host ranges of endophytes are determined and how endophytes are kept in the non-symptomatic stage.

Interestingly, changing light conditions can cause a symptomless endophyte to shift to necrotrophy (Álvarez-Loayza *et al*. 2011). Light conditions interact in multiple ways with plant resistance (Ballaré *et al*. 2012). Among others, low red:far-red ratios as they occur in the forest understory can inhibit JA signalling (Ballaré *et al*. 2012), but we do not know to which degree such physiological events determine the more general patterns in the attack of tropical wild plants by enemies with different life histories. A literature survey revealed more reports for biotrophic fungal pathogens on light-demanding herbaceous tropical plants, whereas necrotrophic fungal pathogens more frequently attack shade-tolerant host species (García-Guzmán & Heil 2014). However, the possible explanations for this general pattern comprised both taxonomic (*i.e*., genetic) and environmental factors. With the present study we aim to provide a first dataset that allows to test whether the above-mentioned general patterns also apply at the within-species level. Using leaves of plants of a single species, we related light conditions (full sun *vs*. shade) with the endogenous levels of SA and JA and with the average levels of damage caused by herbivores and biotrophic vs. necrotrophic pathogens as well as the frequency of symptomless endophytes.

Methods and materials

Study species. *Syngonium podophyllum* Schott (Araceae) is an evergreen climbing epiphyte found in tropical humid forests and disturbed areas of Mexico, and other countries from Central and South America. The stems are 10 to 20 metres long, and support the plants on tree trunks by means of adventitious roots. The leaves vary greatly as the plant ages. When young they have the shape of an arrowhead, but as they mature this changes and lobes develop at the base of the leaves. The older the plant is, the more lobes the full-sized leaves will have (Morgan *et al*. 2004). *Syngonium podophyllum* grows both in the forest understory and in the full sun in the tropical rain forests of Mexico and is frequently affected by foliar fungal pathogens (García-Guzmán & Dirzo 2001).

Study site. The study was carried out at the tropical rain forest of Los Tuxtlas (Southern Veracruz, Mexico; 95º 04' and 95º 09' W, 18º34' and 18º 36' N; altitude ca. 530m). The mean annual temperature is 24 °C and the mean annual rainfall is 4,639 mm (Bongers *et al*. 1988). Precipitation is seasonal, with a short dry season between March and May (Soto & Gama 1997). The predominant vegetation in the area is classified as lowland tropical high evergreen rain forest with a canopy height ranging from 30 to 35 m (Bongers *et al*. 1988, Ibarra-Manríquez *et al*. 1997).

Methods. In order to describe the frequency at which fungal pathogens and herbivores affect this species under different light environments, twenty plants were chosen randomly from the

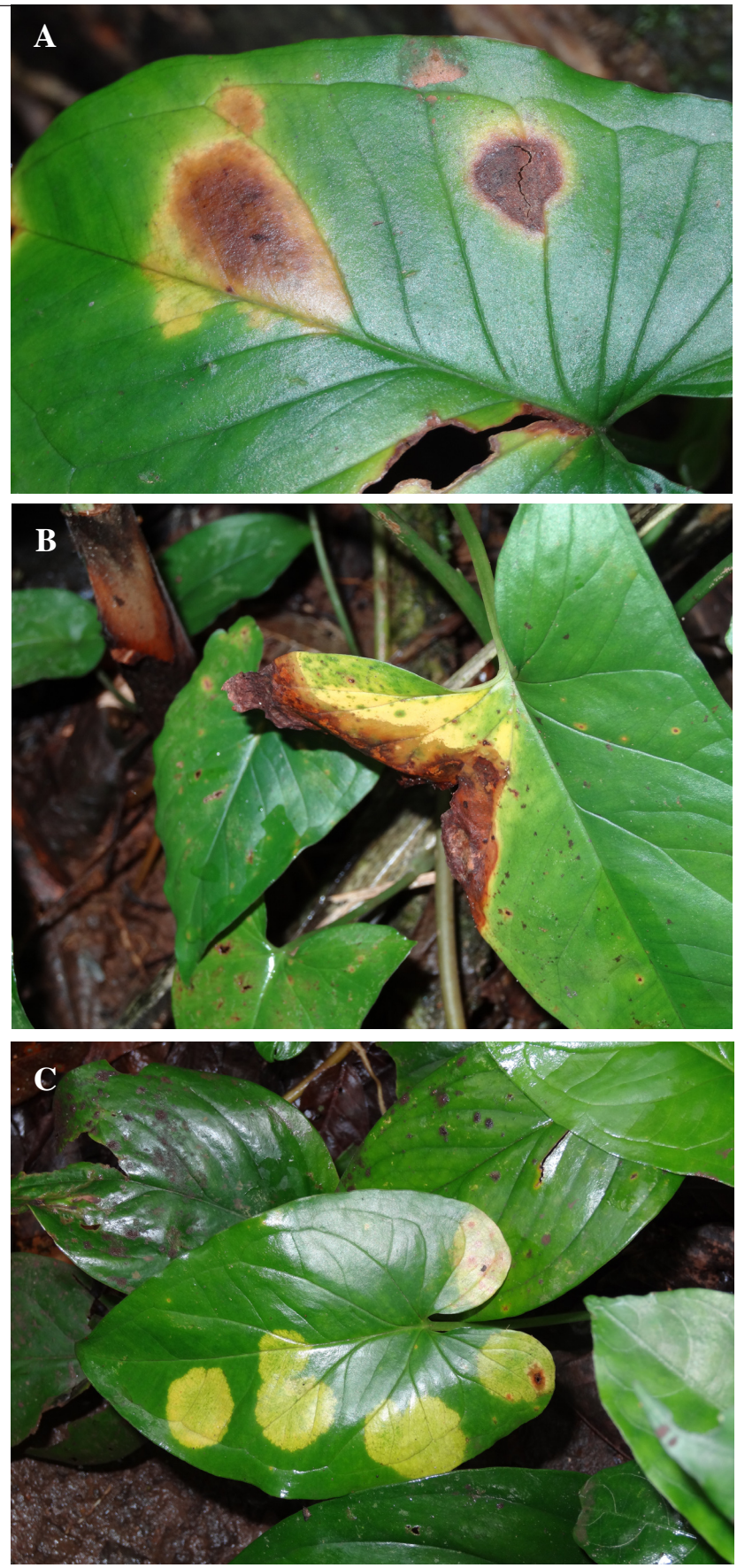

Figure 1. *Syngonium podophyllum* leaves affected by necrotrophic **A)** hemibiotrophic **B)**, and **C)** biotorphic pathogens.

natural population at the study site, with the only restriction that they had two phenotypically undamaged leaves (which were used for the analysis of endogenous JA and SA and for the isolation of fungal endophytes). Half of the plants were growing in the sun (200 ± 3.94 μM/m^2/s) and the other half in the shade (5 ± 0.58 μM/m^2/s). We measured light intensity with a luxometer (Li-COR LI-250, USA).

Two undamaged leaves per plant were selected and cut in half transversally with scissors. One part was shock frozen in liquid nitrogen for the quantification of endogenous levels of defence hormones (Heil *et al*. 2012) and the remaining part was rinsed with sterile water and cut into at least ten sections (of ca. 3 mm^2) per leaf. The segments were surface-sterilized by serial immersion in 95 % ethanol (10 s), 0.525 % sodium hypochlorite (2 min), and 70 % ethanol (2 min) and allowed to surface-dry under sterile conditions. Ten segments per leaf were selected haphazardly and plated individually on 1 ml of potato dextrose agar (PDA) in a sterile 2 ml microcentrifuge tube. The tubes were incubated at room temperature for four months and emerging hyphae were subcultured to 90 mm Petri dishes containing PDA, to obtain axenic strains.

We randomly collected three further leaves from each plant to determine their phenotypic level of damage by herbivores and fungal pathogens. Fungal pathogens were grouped according to the characteristic symptoms caused: necrotic irregular spots (considered to be caused by necrotrophs), necrotic spots with a chlorotic halo (indicating hemibiotrophic pathogens), and circular chlorotic spots (considered as biotrophic lesions) (cf. Figure 1). Five leaves from each, sun and shade areas showing disease symptoms were used to isolate and identify the causal agents of damage: leaf sections of the affected area including a marginal healthy section of ca. 3 mm width were disinfected as mentioned above and placed in safe-lock Eppendorf tubes containing PDA to be further treated as described above.

For the preliminary, sequence-based characterisation of common fungal strains, total genomic DNA was extracted from each isolate and the nuclear ribosomal internal transcribed spacers, the 5.8S gene (ITSrDNA) and ca. 600 bp of the ribosomal large subunit (LSUrDNA) were amplified as a single fragment, using primers ITS5 (GGAAGTAAAAGTCGTAACAAGG) and LR3 (CCGTGTTTCAAGACGGG ; see http://biology.duke.edu/fungi/mycolab/primers.htm). All products yielding single bands were purified, normalized, and sequenced on an ABI 3730-xl DNA Analyser (Applied Biosystems; www.appliedbiosystems.com). Annotation used the top hit in a GenBank BLAST sequence similarity search at NCBI, and UNITE (http://unite.ut.ee/index.php).

Results

The endogenous levels of resistance hormones in phenotypically healthy leaves were significantly correlated with light conditions: leaves of shaded plants contained significantly lower concentrations of both SA and JA than leaves from plants in full sun areas (for SA: t-test: p = 0.011, n = 10; for JA: t-test: $p < 0.001$, n =10; see Figure 2A). Concordantly, the proportion of leaves free of symptoms of fungal infection was lower in the forest understory than in full sun areas, whereas the proportion of leaves damaged by herbivores was significantly higher in

Table 1. Identity of fungi isolated from *Syngonium podophyllum* leaves, including environment from where they were isolated, expressed fungal life style, and GenBank and Unite accession numbers.

Isolate Code	BLAST NCBI	Unite Accession	GenBank Accession	Environment	Fungal life style
FD4	*Hymenochaete* sp.	UDB016365	JQ780066.1	Shade	Endophyte
FD6	*Xylaria* sp.	UDB015373	JX427059.1	Full sun	Hemibiotrophic pathogen
FD8	*Phlebiopsis* sp.	EU118662	JX946673.1	Full sun	Necrotic pathogen
FD9	*Bjerkandera* sp.	UDB017981	KJ140583.1	Full sun	Endophyte
FD10	*Aspergillus* sp.	EF661220	KF938958.1	Shade	Endophyte
FD11	*Hyphodermella* sp.	EU118630	KF638510.1	Shade	Endophyte
FD12	*Aspergillus* sp.	EF661220	KF619560.1	Shade	Endophyte
FD17	*Penicillium* sp.	JF922035	KF880953.1	Shade	Endophyte
FD21	*Penicillium* sp.	AF034450	KF880953.1	Shade	Endophyte
FD23	*Penicillium chrysogenum*	AF034450	KF938433.1	Full sun	Endophyte

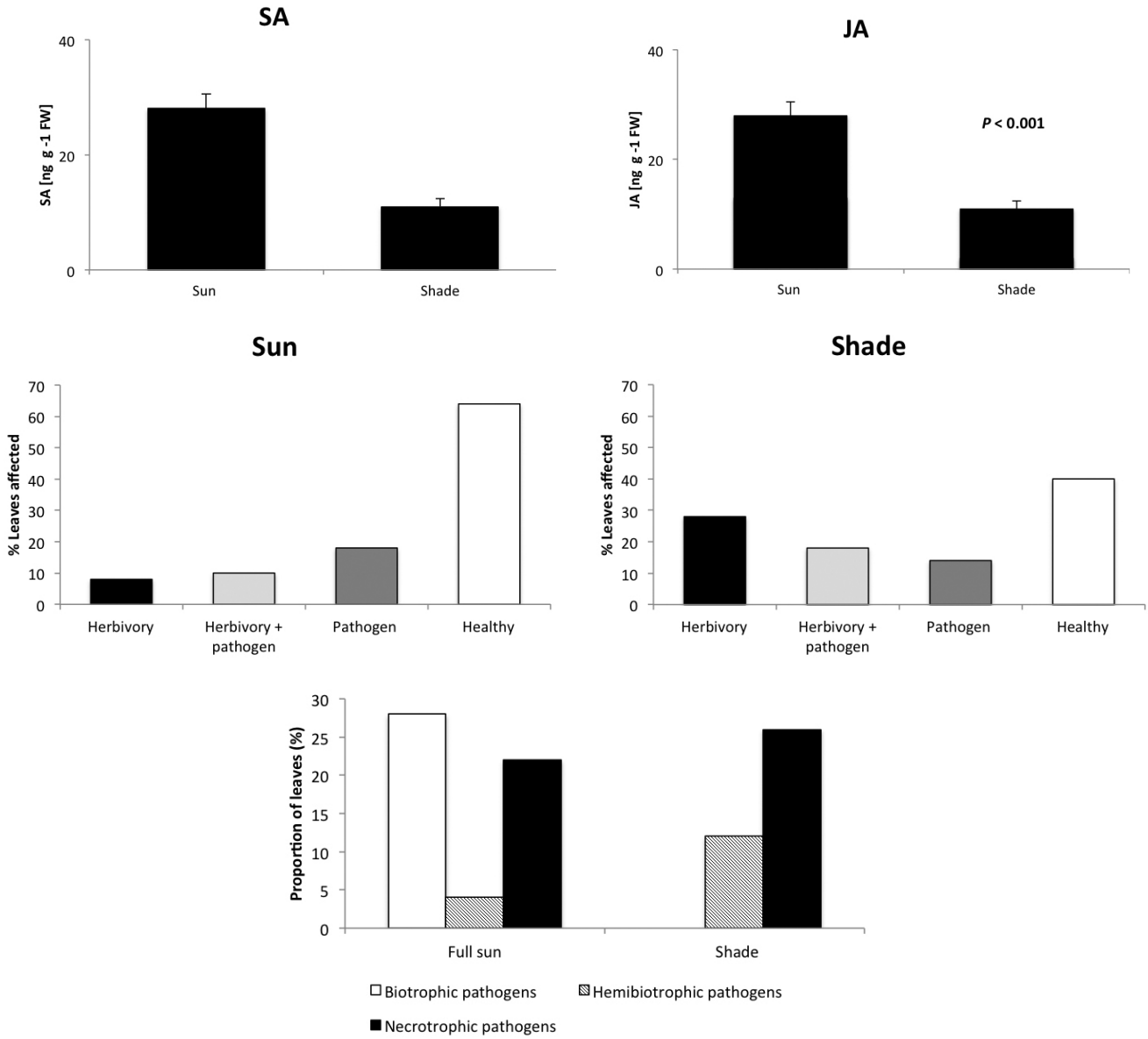

Figure 2. Light dependency of endogenous levels of defence hormones and damage caused by biological enemies with different life histories in wild *Syngonium podophyllum*. Panel **A** depicts the endogenous concentrations [in ng per gram leaf fresh mass] of salicylic acid (SA) and jasmonic acid (JA) in phenotypically healthy leaves (n = 10 samples from different individual plants per condition). Panel **B** indicates the percentages of leaves affected only by herbivores, by both herbivores and fungal pathogens, only by fungal pathogens, or that were phenotypically healthy. Panel **C** depicts the percentage of leaves affected by biotrophic pathogens (live in and consume living plant tissue), necrotrophic pathogens (kill host tissue and consume the dead cells) or hemibiotrophic pathogens (start as biotrophs and the shift to a necrotrophic lifestyle).

shaded than in full sun areas ($\chi^2 = 5.56$; df = 1; $p < 0.05$; see Figure 2B). Separating for different types of fungal pathogens, we found that 28 % of the leaves in the sun were affected by biotrophic pathogens, which were not detected in shaded leaves ($\chi^2 = 15.23$; df = 2; $p < 0.01$; Figure 2C). By contrast, relatively more leaves in the shade were damaged by necrotrophic and hemibiotrophic pathogens.

We could cultivate - and preliminarily annotate - 25 fungal strains, nine of which were isolated from plants growing in full sun areas and 16 from plants in the forest understory (Table 1, Appendix 1 and 2). Endophytes comprised a much higher diversity than pathogens among these cultivable strains. Eight strains cultured from leaves in the sun were symptomless endophytes and

one strain was likely to be a hemibiotrophic pathogen. Among the fungi isolated from shade, 87.5 % corresponded to asymptomatic endophytic fungi and 12.5 % were from necrotrophic lesions. The strains from necrotrophic lesions in most cases had the same phenotype when they were cultivated on PDA agar and even those two strains that appeared to be morphologically distinct were annotated as the same species, *Phlebiopsis flavidoalba* (Appendixes 1 and 2). By contrast, we could cultivate 14 morphologically distinct endophytic strains from symptomless leaves in the shade, which were annotated as nine different species (Table 1, Appendixes 1 and 2)

Discussion

Light conditions significantly affected the levels of defence-related hormones in undamaged leaves of *Syngonium podophyllum* and the frequency of attack by insect herbivores or necrotrophic vs. biotrophic fungal pathogens. The concentration of both JA and SA was higher in plants grown in the sun than in the shade, probably due to the infection of sun plants with biotrophic and/or hemibiotrophic pathogens that were yet in an asymptomatic stage when leaves were collected, but nevertheless causing an induction of plant defences. Concordantly, herbivores and necrotrophic fungal pathogens damaged more leaves in the shade. As we measured defensive hormones in phenotypically undamaged leaves, we conclude that plants in the understory of a tropical rainforest might be limited in their capacity to express hormone-based defences.

Our data stem from one collection event at one site and, thus, provide only correlative evidence for interactions among abiotic factors, plant defence hormones and the relative abundances of different types of attackers. In particular, different levels in standing damage can result from varying plant resistance levels or, rather, from different degrees of enemy pressure. Nevertheless, the quantitative patterns that we observed here for a single plant species resembled patterns in the numbers of reports on fungal pathogens on light demanding vs. shade-tolerant hosts (García-Guzmán & Heil 2014), which makes it tempting to speculate that the general patterns are mainly determined by environmental factors, rather than the genotypes of the hosts.

Our study opens several new questions concerning the ontogeny of plant defences and on the mechanism via which light affects plant defence signalling and, consequently, the life histories of other species. First, *Syngonium podophyllum* starts its life cycle in the shaded understory, which could make the early stage more vulnerable to attack by pathogens and herbivores. Because this study only used adult plants, it remains to be investigated whether the early stages possess any alterative, *i.e.*, hormone-independent defence strategies.

Second, more endophytes were isolated from plants in the shade, which underlines the general similarity among endophytes and necrotrophic fungal pathogens rather than the biotrophic pathogens (Delaye *et al.* 2013). However, strains isolated from necrotic tissue in the shade and from symptomless tissue in the sun were likewise were annotated as *Phlebiopsis flavidoalba* based on their ITS sequence, and strains isolated from a dark brown leaf spot surrounded by a chlorotic halo (which indicates a hemibiotroph) in the sun and from symptomless tissues from both shade and sun were equally annotated as *Xylaria longipes*. Thus, as described earlier (Álvarez-Loayza *et al.* 2011), light conditions can determine whether a fungus acts as symptomless endophyte or as pathogen.

Reduced JA levels under shade conditions had been predicted earlier and were suggested to be a consequence of the far-red mediated inhibition of JA-signalling in the understory (García-Guzmán & Heil 2014). This reduction in the resting level in JA might explain the higher levels of herbivore attack and of infection by necrotrophic pathogens in the leaves of shaded plants. Along the same line, plant exposure to UV-B radiation typically increases resistance to herbivores and pathogens (Ballaré *et al.* 2012). For example, specific leaf mass, which is a correlate of leaf toughness, increased with light intensity in *S. podophyllum* (Ackerly 1992). Additionally, directly damage caused by high irradiation on at least some of the enemies of the plant could contribute to the lower levels of herbivory and fungal infection in the full sun. Furthermore, biotrophs frequently depend on hexoses during the infection process (Solomon *et al.* 2003) whereas necrotrophs can hydrolyse cell walls, a pattern that makes it tempting to speculate that current photosynthetic rates might be more important for biotrophs than necrotrophs. For endophytes, colonization rates study were positively affected by air humidity in the understory (Arnold &

Herre 2003), which might explain the higher colonization rates in shaded plants *vs*. plants in the full sun.

In summary, future research will have to disentangle all the causal relationships that determine the effects of light on plant defence signalling and, consequently, on plant-dependent herbivores and fungi. However, we are confident to conclude that the rate at which biotrophic vs. necrotrophic tropical fungal pathogens attack their hosts depends on the local light conditions and that the capacity of plants to maintain high levels of defence hormones might be impaired in the shaded understory of a topical rainforest

Our results suggest that we should consider light conditions when investigating patterns in the biotic stress of a plant, and that endogenous levels of SA and JA in wild plants are likely to determine the different types of attacker that affect the plants under varying light conditions.

Conclusions and future perspectives

Light conditions were significantly correlated with the frequency of attack by herbivores and biotrophic *vs*. necrotrophic fungal pathogens, and also correlated with the average concentrations of the resistance hormones, JA and SA. Thus, our study indicates that we should consider light conditions when investigating patterns in the biotic stress of a plant, likely because they affect the plant enemies both directly and via their effects on plant resistance traits. Understanding the underlying physiological mechanisms will require the repetition of this study at different sites, identification of all fungal pathogens and symptomless endophytes that regularly infect *S. podophyllum*, and then experiments in which healthy plants under controlled light conditions are challenged with specific pathogens. Such studies would have to be accompanied by screenings using healthy susceptible plants and different culture media exposed to the different environments, to neutrally quantify enemy pressure. Only the combination of both strategies can separate the effects of light on plant hormones and inducible resistance traits from its direct effects on the plant enemies.

Acknowledgements

We thank Rosa María Ádame-Alvarez, Irma Acosta-Calixto, Blanca Hernández-Bautista, Rigoberto Pérez-Ruiz and Mariana Del Olmo-Ruiz for technical assistance. Thanks are also extended to the director of the "Los Tuxtlas Biological Research Station" Rosamond Coates for logistical support. Fieldwork was supported by a Universidad Nacional Autónoma de México – Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (UNAM-PAPIIT IN205012) grant to G. García-Guzmán. We thank Ana Mendoza for providing a UNAM-PAPIIT (IN206515) grant to F. Dominguez-Velazquez for undergraduate studies.

Literature cited

Ackerly DD. 1992. Light, leaf age, and leaf nitrogen concentration in a tropical vine. *Oecologia* **89**:596-600. DOI: 10.1007/BF00317169

Álvarez-Loayza P, White JF, Jr., Torres MS, Balslev H, Kristiansen T, Svenning J-C, Gil N. 2011. Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. *PLoS ONE* **6**: e16386 DOI:e1638610.1371/journal.pone.0016386

Arnold AE, Herre EA. 2003. Canopy cover and leaf age affect colonization by tropical fungal endophytes: Ecological pattern and process in *Theobroma cacao* (Malvaceae). *Mycologia* **95**: 388-398. DOI:10.2307/3761880

Ballaré CL, Mazza CA, Austin AT, Pierik R. 2012. Canopy light and plant health. *Plant Physiology* **160**: 145-155. DOI:10.1104/pp.112.200733

Delaye L, García-Guzmán G, Heil M. 2013. Endophytes versus biotrophic and necrotrophic pathogens - are fungal lifestyles evolutionarily stable traits? *Fungal Diversity* **60**: 125-135. DOI:10.1007/s13225-013-0240-y

García-Guzmán G, Dirzo R. 2001. Patterns of leaf-pathogen infection in the understory of a Mexican rain forest: Incidence, spatiotemporal variation, and mechanisms of infection. *American of Journal of Botany* **88**: 634-645. DOI:10.2307/2657063

Received:
January 18th, 2017

Accepted:
February 2nd, 2017

García-Guzmán G, Heil M. 2014. Life histories of hosts and pathogens predict patterns in tropical fungal plant diseases. *New Phytologist* **201**: 1106-1120. DOI:10.1111/nph.12562

Heil M, Ibarra-Laclette E, Adame-Álvarez RM, Martínez O, Ramirez-Chávez E, Molina-Torres J, Herrera-Estrella L. 2012. How plants sense wounds: damaged-self recognition is based on plant-derived elicitors and induces octadecanoid signaling. *PLoS ONE* **7**: e30537. doi:10.1371/journal.pone.003053

Solomon PS, Tan K-C, Olover RP. 2003. The nutrient supply of pathogenic fungi; a fertile field for study. Molecular Plant Pathology **4**: 203-210. Thaler JS, Humphrey PT, Whiteman NK (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci* **17**: 260-270. DOI: 10.1016/j.tplants.2012.02.010

Appendix 1. Identity of fungi isolated from *Syngonium podophyllum* leaves, environment from where they were isolated, expressed fungal life styleand GenBank accession numbers.

Isolate Code	Sequence analysed	BLAST NCBI	Order	Query cover (Ident)	GenBank accession	SCORE	Light Environment	Expressed fungal life style
FD2	ITS5	Uncultured fungus clone L042881-122-061-A11 internal transcribed spacer 1		394/395(99%)	gb|GU054027.1|	725 bits(392)	Shade	Necrotrophic pathogen
	LR3	Phanerochaetaceae sp. ZLY-2010 isolate M19-2 internal transcribed spacer 2	Polyporales	307/314(98%)	gb|HM595616.1|	542 bits(293)	Shade	Necrotrophic pathogen
FD3	ITS5	Uncultured fungus clone L042881-122-061-A11 internal transcribed spacer 1		402/425(95%)	gb|GU054027.1|	649 bits(351)	Shade	Necrotrophic pathogen
	LR3	Phanerochaetaceae sp. ZLY-2010 isolate M19-2 internal transcribed spacer 2	Polyporales	489/502(97%)	gb|HM595616.1|	856 bits(463)	Shade	Necrotrophic pathogen
FD4	ITS5	*Hymenochaete ustulata* voucher He104 internal transcribed spacer 1	Hymenochaetales	233/249(94%)	gb|JQ780066.1|	366 bits(198)	Shade	Endophyte
	LR3	*Hymenochaete resupinata* partial 28S rRNA gene	Hymenochaetales	254/259(98%)	emb|HE650988.1|	449 bits(243)	Shade	Endophyte
FD6	ITS5	*Xylaria hypoxylon* isolate SACCR 010851 internal transcribed spacer 1	Xylariales	428/428(100%)	gb|JX427059.1|	791 bits(428)	Full sun	Hemibiotrophic pathogen
	LR3	*Xylaria* sp. DIS 258g 28S ribosomal RNA gene, partial sequence	Xylariales	312/312(100%)	gb|DQ327629.1|	577 bits(312)	Full sun	Hemibiotrophic pathogen
FD7	ITS5	*Flavodon flavus* strain xsd08084 18S ribosomal RNA gene	Polyporales	402/402(100%)	gb|FJ478126.1|	743 bits(402)	Shade	Endophyte
	LR3	*Phanerochaete* sp. 2 KUC8361 28S ribosomal RNA gene, partial sequence	Corticiales	360/360(100%)	gb|FJ471549.1|	665 bits(360)	Shade	Endophyte
FD8	ITS5	*Phlebiopsis gigantea* isolate ZS2 18S ribosomal RNA gen	Polyporales	287/301(95%)	gb|JX946673.1|	472 bits(255)	Full sun	Necrotrophic pathogen
	LR3	*Phlebiopsis flavidoalba* voucher KHL 13055 (GB) 18S ribosomal RNA gene	Polyporales	285/289(99%)	gb|EU118662.1|	512 bits(277)	Full sun	Necrotrophic pathogen
FD9	ITS5	*Bjerkandera fumosa* voucher CFMR:DLL2011-062 18S ribosomal RNA gene	Polyporales	398/420(95%)	gb|KJ140583.1|	651 bits(352)	Full sun	Endophyte
	LR3	*Bjerkandera adusta* isolate X-75 18S ribosomal RNA gene	Polyporales	344/349(99%)	gb|KC176354.1|	617 bits(334)	Full sun	Endophyte
FD10	ITS5	*Aspergillus aculeatus* strain HFpk01 internal transcribed spacer 1	Eurotiales	328/328(100%)	gb|KF938958.1|	606 bits(328)	Shade	Endophyte
	LR3	*Aspergillus* sp. MMB62 gene for 28S ribosomal RNA, partial sequence	Eurotiales	301/301(100%)	dbj|AB734808.1|	556 bits(301)	Shade	Endophyte

Appendix 1. Continuation.

Isolate Code	Sequence analysed	BLAST NCBI	Order	Query cover (Ident)	GenBank accession	SCORE	Light Environment	Expressed fungal life style
FD11	ITS5	*Hyphodermella* sp. Achao 41 18S ribosomal RNA gene	Polyporales	267/290(92%)	gb|KF638510.1|	396 bits(214)	Shade	Endophyte
	LR3	*Hyphodermella rosae* strain MA-Fungi 75541 28S ribosomal RNA (LSU) gene	Polyporales	183/190(96%)	gb|JN939595.1|	313 bits(169)	Shade	Endophyte
FD12	ITS5	*Aspergillus aculeatus* isolate 18H6 internal transcribed spacer 1,	Eurotiales	241/241(100%)	gb|KF619560.1|	446 bits(241)	Shade	Endophyte
	LR3	*Aspergillus* sp. MMB62 gene for 28S ribosomal RNA	Eurotiales	205/205(100%)	dbj|AB734808.1|	379 bits(205)	Shade	Endophyte
FD13	ITS5	*Sordariomycetes* sp. genotype 415 isolate FL1442 internal transcribed spacer 1	Xylariales	333/334(99%)	gb|JQ761052.1|	612 bits(331)	Full sun	Endophyte
	LR3	*Sordariomycetes* sp. genotype 415 isolate FL1106 internal transcribed spacer 1	Xylariales	272/273(99%)	gb|JQ760729.1|	499 bits(270)	Full sun	Endophyte
FD14	ITS5	*Tinctoporellus epimiltinus* voucher Dai 11831 internal transcribed spacer 1	Polyporales	391/391(100%)	gb|JQ319492.1|	723 bits(391)	Full sun	Endophyte
	LR3	Fungal endophyte culture-collection STRI:ICBG-Panama: TK375 18S ribosomal RNA gene	Polyporales	327/327(100%)	gb|KF435681.1|	604 bits(327)	Full sun	Endophyte
FD15	ITS5	Homobasidiomycetes sp. DIS 181c 28S ribosomal RNA gene,	Agaricales	253/256(99%)	gb|DQ327647.1|	457 bits(247)	Full sun	Endophyte
	LR3	Fungal sp. MS367b internal transcribed spacer 1		275/277(99%)	gb|JQ919943.1|	501 bits(271)	Full sun	Endophyte
FD16	ITS5	*Sordariomycetes* sp. genotype 438 isolate FL1396 internal transcribed spacer 1,	Xylariales	252/252(100%)	gb|JQ761013.1|	466 bits(252)	Shade	Endophyte
	LR3	*Sordariomycetes* sp. genotype 314 isolate FL1048 internal transcribed spacer 1,	Xylariales	230/230(100%)	gb|JQ760671.1|	425 bits(230)	Shade	Endophyte

Appendix 2. Identity of fungi isolated from *Syngonium podophyllum* leaves using Blast Unite, environment from where they were isolated and expressed life style.

ID	Accession number	Organism	Score	Expect	Identities	Gaps	Environment	Expressed life style
FD2	EU118662	*Phlebiopsis flavidoalba*	1590	0	94%	0%	Shade	Necrotrophic pathogen
FD3	EU118662	*Phlebiopsis flavidoalba*	1590	0	94%	0%	Shade	Necrotrophic pathogen
FD8	EU118662	*Phlebiopsis flavidoalba*	1590	0	94%	0%	Full sun	Endophyte
FD7	EU118654	*Phlebia firma*	761	0	93%	0%	Shade	Endophyte
FD11	EU118630	*Hyphodermella corrugata*	734	0	96%	0%	Shade	Endophyte
FD9	UDB017981	*Bjerkandera adusta*	1390	0	95%	1%	Full sun	Endophyte
FD27	HQ604797	*Phlebia radiata*	1598	0	93%	1%	Full sun	Endophyte
FD20	JN649346	*Hydnopolyporu imbriatus*	1534	0	93%	1%	Shade	Endophyte
FD14	KC581319	*Ganoderma applanatum*	1495	0	94%	0%	Full sun	Endophyte
FD24	KC581319	*Ganoderma applanatum*	1495	0	94%	0%	Shade	Endophyte
FD26	JN649335	*Cotylidia undulata*	795	0	91%	0%	Shade	Endophyte
FD28	UDB016320	*Xylodon rimosissimus*	1405	0	95%	0%	Full sun	Endophyte
FD4	UDB016365	*Hymenochaete acerosa*	856	0	96%	0%	Shade	Endophyte
FD15	AF539711	*Cortinarius cervinus*	930	0	95%	0%	Full sun	Endophyte
FD12	EF661220	*Aspergillus aculeatus*	2060	0	100%	0%	Shade	Hemibiotrophic pathogen
FD10	EF661220	*Aspergillus aculeatus*	2089	0	100%	0%	Shade	Endophyte
FD17	JF922035	*Penicillium chrysogenum*	1007	0	100%	0%	Shade	Endophyte
FD21	AF034450	*Penicillium chrysogenum*	1986	0	100%	0%	Shade	Endophyte
FD23	AF034450	*Penicillium chrysogenum*	988	0	99%	0%	Full sun	Endophyte
FD6	UDB015373	*Xylaria longipes*	1384	0	95%	1%	Full sun	Necrotrophic pathogen
FD29	UDB015373	*Xylaria longipes*	1384	0	95%	1%	Shade	Endophyte
FD13	UDB015373	*Xylaria longipes*	1848	0	97%	0%	Full sun	Endophyte
FD22	UDB015373	*Xylaria longipes*	1425	0	94%	0%	Shade	Endophyte
FD16	UDB015373	*Xylaria longipes*	1572	0	96%	0%	Shade	Endophyte
FD19	UDB013130	*Ramaria* sp.	745	0	92%	0%	Shade	Endophyte