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RAFAEL DE PAIVA FARIAS

HERBIVORIA E DEFESAS DE SAMAMBAIAS EM FLORESTAS TROPICAIS

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Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal, Área de Concentração Ecologia e Conservação, da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de doutor em Biologia Vegetal.

Orientadora: Prof.ª Dra. Iva Carneiro Leão Barros

Co-Orientador: Dr. Klaus Mehltreter

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Dedico

A minha Avó,

Aos meus Pais,

Irmãos e Namorada

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RESUMO

Interações entre herbívoros e plantas é um dos temas centrais da Ecologia. Diversas pesquisas têm reportado novas interações e buscado o entendimento do papel de traços defensivos na seleção de plantas hospedeiras, bem como as explicações para os padrões de ataque dos herbívoros. Informações das interações entre herbívoros e samambaias são limitadas, desde as questões básicas como número e identidade de herbívoros até as mais complexas, como padrões de seleção e expressão de defesas antiherbivoria do grupo. O presente trabalho apresenta novas interações entre samambaias e herbívoros, realiza *insights* ecológicos para compreender como herbívoros selecionam samambaias, analisa a variação intraespecífica da herbivoria, e por fim analisa a expressão de traços de defesas do grupo. A partir da delimitação de 22 parcelas retangulares de 10 x 20 m (200 m^2) em três áreas de Floresta Atlântica no Nordeste do Brasil, 34 espécies de samambaias foram amostradas. Herbívoros coletados foram identificados por especialistas. Espécies tiveram seus níveis de herbivoria estimados visualmente. Folhas de cada espécie foram coletadas para mensuração dos traços de defesas: área específica foliar, conteúdo de água, densidade de tricomas, defesas químicas (alcaloides, compostos fenólicos, saponinas, taninos, terpenoides), fósforo, nitrogênio e potássio. Registrou-se pela primeira vez galhas na samambaia arborescente *Cyathea phalerata* (Cyatheaceae). As galhas ocorrem nas folhas, são globoides, com uma câmara larval, glabras, isoladas ou agrupadas e induzidas por Cecidomyiidae. *Cyathea phalerata* sofre danos herbívoros simultâneos a partir de galhadores e lagartas (Lepidoptera). Danos de ambas as guildas são relacionados positivamente ao tamanho foliar, corroborando a Hipótese de Vigor de Plantas. Uma alta porcentagem (87%) das folhas galhadas foram consumidas por lagartas, que evitaram consumir a estrutura da galha, partes lignificadas, suberizadas e com alta concentração de fenóis. Portanto, herbívoros de vida livre não evitam folhas galhadas, mas não afetam diretamente larvas e/ou parasitas do sistema. Também reportamos que formigas cortadeiras podem causar danos em samambaias, em alguns casos de modo intenso. O ataque de formigas cortadeiras esteve relacionado positivamente ao conteúdo de água foliar. Apresenta-se que algumas samambaias constitui uma dieta alternativa, ocasional e de curta duração para formigas. Por outro lado, aquelas pouco consumidas devem ser bem defendidas, uma vez que são logo rejeitas pelas formigas. As análises para avaliar a combinação de traços de defesas em samambaias demonstraram que a maioria das espécies possui síndrome do tipo nutrição e defesa (i.e. elevada qualidade nutricional e vários traços de defesas, químicos e físicos). Em oposição, poucas espécies exibem síndrome do tipo baixa qualidade nutricional e defesas. Esses estudos demonstraram a potencialidade das samambaias como elementos chaves nas interações com herbívoros, como insetos galhadores, larvas e/ou formigas cortadeiras. Em adição, demonstrou-se que a predominante estratégia de defesa das samambaias é a partir de traços químicos.

Palavras-chave: Herbivoria. Interações Planta-Herbívoro. Síndromes de Defesas.

ABSTRACT

Plant-herbivore interactions is a central topic of ecology. Several studies have reported new interactions and looking for understand the role of defensive traits in selection of host plants, as well as explanations for herbivorous attack patterns. Information about fern-herbivore interactions is limited, since basic questions such as number and identify of herbivores until complex questions, like patterns of selection and expression of the antiherbivore defenses. The present study presents new interactions between ferns and herbivores, performs ecological *insights* to understand how herbivores select ferns, investigate the intraspecific variation on herbivory, and finally analyzes the expression of defensive traits for the group. From the delimitation of 22 rectangular plots of 10 x 20 m (200 m^2) in three areas of Atlantic Forest in Northeast Brazil, 34 fern species were sampled. Herbivores collected were identified by experts. Species had their herbivory levels estimated visually. Leaves of each species were collected to measure the defensive traits: specific leaf area, water content, trichrome density, chemical defenses (alkaloids, phenolic compounds, saponins, tannins, terpenoids), phosphorus, potassium and nitrogen. Galls were first recorded in arborescent fern *Cyathea phalerata* (Cyatheaceae). Galls occurred in leaves, are globoid, with one larval chamber, glabrous, isolated or grouped and induced by Cecidomyiidae. *Cyathea phalerata* suffers simultaneous herbivorous damage from galling and caterpillars (Lepidoptera). Damage from both guilds is positively related with leaf size, corroborating the Plant Vigor Hypothesis. A high percentage (87%) of galled leaves were consumed by caterpillars, which avoided consuming the structure of the gall, lignified parts, suberized and with high concentration of phenols. Therefore, free-living herbivores do not avoid galled leaves, but not affect directly larvae and/or parasites in system. We also report that leaf-cutter ants caused damage to ferns, in some cases intense. The attack of leaf-cutter ants was positively related with leaf water content. Some ferns may constitute an alternative, occasional and short-lived diet for ants. Conversely, species with low damage should be well defended, because the ants soon reject them. Analyzes to evaluate the combination of fern defense traits revealed that the most species shows nutrition and defense syndrome (i.e. high nutritional quality, chemical and physical defense traits). In contrast, few species exhibit the low-nutritional quality syndrome. These studies demonstrated the potentiality of ferns as elements kew in herbivore interactions, such as galling insects, larvae and /or leaf-cutter ants. In addition, it has been shown that the predominant defense strategy of ferns is from chemical traits.

Key-words: Herbivory. Plant-Herbivore Interactions. Defensive Syndromes.

SUMÁRIO

1 INTRODUÇÃO

Como produtores primários, as plantas representam a base da pirâmide trófica dos ecossistemas terrestres ao prover recurso nutricional aos consumidores primários (i.e. herbívoros), representados majoritariamente pelos insetos. Essa transferência de energia constitui alta proporção das interações interespecíficas na natureza (CARMONA et al. 2011). As interações entre plantas e herbívoros têm forte influência em processos ecológicos e evolutivos, bem como no setor econômico, devido aos danos em plantas cultivadas na agricultura. Os herbívoros podem afetar negativamente o crescimento e a reprodução das plantas, e, consequentemente seu *fitness* (MARQUIS, 1984; CRAWLEY, 1985; HAWKES & SULLIVAN, 2001). Todavia, as plantas não são passivas aos ataques de herbívoros e desenvolveram ao longo da evolução um diversificado arsenal de defesas antiherbivoria (AGRAWAL, 2007; FUTUYAMA & AGRAWAL, 2009).

As interações entre plantas e herbívoros são complexas e podem ser mediadas por diferentes fatores. Um dos principais fatores envolvidos nessas interações é o químico. Atribui-se que metabólitos secundários (i.e. substâncias não envolvidas em vias metabólicas primárias) são responsáveis pela diferenciação da palatabilidade para os herbívoros, desempenhando um papel defensivo nas plantas (FRAENKEL, 1959). Efeitos dos metabólitos secundários sobre herbívoros foram aplicados na teoria da coevolução. Essa teoria propõe que a alta diversidade de plantas e insetos é resultado de um processo de mudanças em defesas químicas, pressionando herbívoros a desenvolver habilidades de tolerâncias, que por sua vez causam novas mudanças químicas (EHRLICH & RAVEN, 1964). Dessa forma, os níveis de defesa e de contra-ataque são continuamente crescentes, sem que qualquer grupo "vença", ocasionando a chamada "corrida armamentista".

As plantas também desenvolveram traços físicos de defesas, como tricomas e espinhos. Não obstante, o sistema de defesas das plantas não é definido apenas a partir do contexto químico ou físico. Plantas exibem simultaneamente múltiplos traços defensivos (e.g. químicos, físicos, nutricionais e fenológicos) como prediz a hipótese de síndromes de defesas proposta por AGRAWAL & FISHBEIN (2006). A partir dessa hipótese, plantas são agrupadas em três síndromes de defesas: I – plantas que toleram ou escapam por evasão de herbívoros, II – plantas que possuem baixa qualidade nutricional como defesa e III – plantas que possuem alta qualidade nutricional, sendo fortemente defendidas quimicamente e/ou fisicamente (AGRAWAL & FISHBEIN, 2006).

Ao pensar em interações plantas e herbívoros, logo advém a imagem de animais consumindo frutos, sementes ou folhas do grupo das angiospermas. Raramente, se imagina interações entre animais e samambaias, grupo de plantas vasculares com dispersão via esporos. Entretanto, essa importante linhagem possui uma ampla gama de interações com herbívoros (MEHLTRETER, 2010). Atualmente, samambaias possuem representatividade de 10.758 espécies (PPG I, 2016), amplamente distribuídas nos variados ecossistemas, especialmente os tropicais (SHARPE et al. 2010), onde podem representar até 15% da biomassa total (HARMS et al. 2004). O sucesso evolutivo e ecológico das samambaias está relacionado a eficiência de seus traços de defesas contra herbívoros (PAGE, 2002).

A presente Tese traz dados sobre a herbivoria e defesas da linhagem das samambaias. Aqui, apresenta-se novas interações entre samambaias e herbívoros, realiza-se *insights* ecológicos para conhecer como herbívoros selecionam samambaias, analisa-se a variação intraespecífica de herbivoria das samambaias, e por fim, analisa-se como as samambaias expressam seus traços de defesas antiherbivoria.

2 FUNDAMENTAÇÃO TEÓRICA

As interações entre herbívoros e plantas têm sido extensivamente investigadas há décadas por ecólogos vegetais e/ou botânicos sob diferentes abordagens, como descobertas de interações e espécies, estratégias de defesas, formulação e/ou testes de hipóteses. A maioria do conhecimento de interações entre herbívoros e plantas advém a partir de estudos com angiospermas, linhagem com uma ampla rede de interações, suportada a partir da maior complexidade morfológica (flores, sementes e frutos) e da diversidade dominante nos distintos ecossistemas.

Para as samambaias, o *background* de conhecimento e compreensão das interações com herbívoros ainda é limitado. A literatura do tema embora relativamente abundante, é dispersa, concentrada para espécies de *Pteridium* Gled. ex Scop. e sem continuidade temporal, conforme literatura citada a seguir. Atualmente, poucos pesquisadores estão envolvidos com questões acerca da herbivoria para essa linhagem. Em consequência, a ideia de que as samambaias são subutilizadas por herbívoros formulada desde final século XIX e início do século XX ainda persiste nos dias atuais entre vários investigadores e estudantes.

A escassez de insetos utilizando samambaias foi apresentada a partir de observações locais por autores como SCHNEIDER (1982) e BRUES (1920), mas nunca foram apoiadas com evidências. Esta suposição levou às investigações para explicar a "imunidade" relativa das samambaias aos insetos, que foi atribuída a um forte arsenal de defesas químicas (SOO HOO & FRAENKEL, 1964; SCHNEIDER, 1982). Por outro lado, algumas contribuições começaram a listar um número significativo de insetos associados com as samambaias (BALICK et al. 1978; HENDRIX, 1980). Esses estudos iniciaram o reconhecimento de samambaias como recurso para herbívoros e indicaram que a subutilização especulativa ocorreu em grande parte devido a uma amostragem inadequada. O estado atual da arte demonstra que as samambaias possuem uma ampla variedade de potenciais herbívoros, mesmo que seja proporcionalmente estimado 3-7 vezes menor que nas plantas com sementes (MEHLTRETER, 2010). A seguir agrupa-se a seguinte fundamentação teórica nos subtópicos: taxas de herbivoria em samambaias e principais herbívoros; formigas e plantas: um enfoque para samambaias; galhas e samambaias; defesas químicas, defesas físicas e crescimento e, por fim, síndromes de defesas.

2.1 TAXAS DE HERBIVORIA EM SAMAMBAIAS E PRINCIPAIS HERBÍVOROS

Poucas espécies de samambaias tiveram seus níveis de herbivoria foliar mensurados. Os danos foliares registrados nessa linhagem variam entre 5% a 15%, com valor máximo de 36% (BALICK et al. 1978; HENDRIX & MARQUIS, 1983; MEHLTRETER & TOLOME, 2003; MEHLTRETER et al. 2006). Recentemente, a partir de uma análise comparativa das taxas de herbivoria nas principais linhagens de plantas, Turcotte et al. (2014) reportaram que samambaias sofrem danos similares (5,5%) aos das angiospermas (6,2%). Adicionalmente, samambaias terrestres e epífitas sofrem danos similares, não havendo diferenças entre folhas estéreis e férteis (MEHLTRETER et al. 2006). Partindo do ponto que folhas férteis são consumidas, experimentos foram conduzidos com o objetivo de analisar a viabilidade dos esporos após atravessar o sistema digestivo dos herbívoros. Os resultados indicaram que uma alta porcentagem dos esporos (65-85%) permanece viável e germina. Em consequência, esses herbívoros potencialmente atuam como dispersores de curta distância (BOCH et al. 2013; BOCH et al. 2016).

As informações sobre herbívoros que consomem tecido foliar das samambaias ainda são limitadas. A principal contribuição acerca dos potenciais herbívoros do grupo foi realizada por BALICK et al. (1978), que listaram a ocorrência de 420 espécies de insetos explorando de diferentes modos as samambaias. HENDRIX (1980) atualizou a lista para 465 espécies de insetos, sendo a maioria deles pertencentes a Hemiptera (38,7 %), seguida por Coleoptera (22,2%) e Lepidoptera (19,8%). Essas são as ordens mais antigas, sugerindo uma associação persistente e longa entre samambaias e insetos de acordo com COOPER-DRIVER (1978). Em outro estudo nesse contexto, LAWTON & MACGARVIN (1985) reportaram um alto número de morfotipos de insetos (27) explorando *Pteridium aquilinum* (L.) Kuhn na Grã-Bretanha, embora em baixas densidades, menor que cinco indivíduos por planta. Um estudo similar com a mesma espécie na Nova Zelândia, corroborou os resultados anteriores na Grã-Bretanha (WINTERBOURN, 1987). Adicionalmente, demonstrou-se a ocorrência de poucos herbívoros mastigadores em oposição a maior representação dos sugadores (WINTERBOURN, 1987). No Havaí, SWEZEY (1922) demonstrou uma rica fauna de insetos associados com samambaias, alguns sendo especialistas. Pesquisas como essas demonstraram que a partir de amostragem adequada e contribuições de entomólogos, um número considerável de insetos são registrados explorando samambaias.

2.2 FORMIGAS E PLANTAS: UM ENFOQUE PARA SAMAMBAIAS

As interações plantas e formigas estão entre as mais estudadas, envolvendo tanto relações antagonistas quanto mutualísticas ou oportunistas. Essas interações são numerosas e comuns, com exemplos a partir do nosso cotidiano, como as plantas ornamentais ou cultivadas que são danificas a partir da atividade das formigas cortadeiras. As formigas apresentam alta biomassa entre os animais, estimada em aproximadamente 30% nas Florestas Tropicais (ELLWOOD & FOSTER, 2004), onde são herbívoros chave, consumindo 12% - 17% da produção foliar anual (FOWLEr et al. 1989; WIRTH et al. 2003). As folhas e outros recursos, como sementes e frutos, são utilizadas para cultivar fungos simbióticos "jardins de fungos", que representam o recurso nutricional único da fase larval (QUINLAN & CHERRETT, 1979). Como esperado a partir dessa enorme quantidade de biomassa foliar obtida, o ataque de formigas cortadeiras pode reduzir o *fitness* e até causar a morte de plantas (MARQUIS, 1984).

Formigas cortadeiras forrageiam uma ampla variedade de plantas, especialmente árvores com expressiva biomassa (NORTH et al. 1997). Embora polífagas, as formigas cortadeiras demonstram preferências por algumas espécies, assim como evitam outras (CHERRET, 1972; ROCKWOOD, 1976; BLANTON & EWEL, 1985). A seleção de plantas por formigas nem sempre é clara, mas tem sido explicada em alguns casos a partir da variação de traços foliares, como valor nutricional (BERISH, 1986), densidade de tricomas e presença de látex (HOWARD, 1988), conteúdo de água foliar (BOWERS & PORTER, 1981) e composição química (HUBBELL & WIEMER, 1983; HOWARD, 1987). Plantas evitadas geralmente contêm alta concentração de compostos secundários, como terpenoides e taninos, tóxicos às formigas, ao seu fungo simbiótico ou ambos (PELLOTO & MARTÍNEZ, 2002).

Especificamente para as samambaias há poucos relatos na literatura acerca do ataque de formigas cortadeiras. WETTERER (1994; 1995) reportou que formigas cortadeiras, especialmente colônias pequenas de *Atta cephalotes* (L.) e *Acromyrmex coronatu*s (Fabricius), tinham ação herbívora sob espécies de samambaias (não identificadas). WIRTH et al. (2003) reportaram que colônias grandes de *A. cephalotes* cortavam folhas de representantes de Polypodiaceae. Outras pesquisas, associadas a aspectos fenológicos e da história natural das samambaias, indicaram as formigas como possíveis herbívoros da espécie arborescente *Alsophila setosa* Kaulf., atacada por *Atta sexdens* L. (SILVA, 2008) e *Acromyrmex nigrosetosus* Forel (LUEDERWALDT, 1923 *apud* SCHMITT & WINDISCH, 2005). Em adição, uma pesquisa sobre os efeitos de perturbações na diversidade, utilizando formigas cortadeiras como modelo, reportou-se a atividade herbívora para espécies de Blechnaceae (FALCÃO, 2004).

De fato, o conhecimento ainda é limitado à respeito da frequência de interações entre diferentes espécies de formigas cortadeiras e samambaias, bem como da identidade das espécies atacadas e traços foliares que devem ser preditores de resistência e susceptibilidade. Experimentalmente, MEHLTRETER & VALENZUELA (2012) reportaram que formigas cortadeiras evitam completamente algumas espécies, como *Macrothelypteris torresiana* (Gaudich.) Ching. e *Dicksonia sellowiana* Hook., e exibem preferências por *Diplazium expansum* Willd. e *Nephrolepis cordifolia* (L.) C. Presl, com maior quantidade de água, traço considerado importante para a seleção de plantas por formigas.

Formigas além de interagir com as samambaias de maneira antagônica, interagem de maneira mutualística. Essas associações podem ter papel defensivo e nutritivo. Algumas plantas do grupo possuem estruturas como nectários e domácias (i.e. cavidades presentes nas folhas que abrigam formigas), atrativas às formigas. As interações mutualísticas representam a maior parte dos estudos de interações com samambaias, muitos evidenciando a presença de nectários em *Pteridium*, amplamente distribuído ao redor do mundo (e.g. TRYON, 1941; SANTOS & MAYHÉ-NUNES, 2007). Nectários também ocorrem em outros táxons como *Angiopteris* Hoffm., *Aglaomorpha* Schott, *Cyathea* Sm., *Drynaria* (Bory) J. Sm., *Polybotrya* Humb. & Bonpl. ex Willd., *Pecluma* M.G. Price e *Polypodium* L. (KOPTUR et al. 1982; ARENS & SMITH, 1998; POTES, 2010; KOPTUR et al. 2013). Os nectários das samambaias surgiram em diferentes tempos evolutivos (KOPTUR et al. 2013) e suas funções não foram bem compreendidas (KOPTUR et al. 1998).

Os nectários nas samambaias secretam uma quantidade expressiva de açúcares e aminoácidos (e.g. sucrose, frutose e glucose), em diferentes locais de ocorrência (lobo acroscópico da pina, base da pínula e raque) e com suposta maior atividade em folhas jovens, nas primeiras horas do dia e determinadas épocas do ano (LAWTON & HEADS, 1984; KOPTUR et al. 1982). Esses nectários são partes potenciais da dieta de algumas formigas, que em contrapartida podem proteger as plantas, removendo herbívoros e patógenos. Entretanto, poucas são as evidências a partir de experimentos que demonstraram proteção às samambaias a partir desse sistema. HEADS (1986) demonstrou que determinadas espécies agressivas de formigas exercem influência sobre populações de herbívoros de *Pteridium*; já KOPTUR et al. (1998) reportaram que folhas de samambaias sem nectários (controle) são mais consumidas que folhas de *Polypodium plebeium* Schldl. & Cham. com nectários. Adicionalmente, quando as formigas foram excluídas das folhas em desenvolvimento de *P. plebeium*, a herbivoria por lagartas foi maior que em folhas controle (KOPTUR et al. 1998). Estas foram as primeiras demonstrações desse tipo de defesa para samambaias.

KOPTUR et al. (2013) reportaram a ação de proteção de formigas para algumas espécies, como *Polypodium crassinervata* (Fée) T.Moore, e não para outras como *P. furfuraceum* Schltdl. & Cham. Os autores supuseram que a defesa seja mais evidente quando a interação ocorre com formigas mais agressivas e envolve herbívoros não especialistas. Em outros casos, a hipótese que formigas atraídas por nectários de samambaias (e.g. *Pteridium aquilinum*) protegem as frondes de danos herbívoros não foi corroborada (LAWTON & HEADS, 1984; HEADS & LAWTON, 1985). Nesse caso, nectários também foram ocupados por vespas, aracnídeos e moscas, e as formigas não apresentavam comportamento agressivo. *Pteridium* é hospedeira de um número considerável de herbívoros (WIECZOREK, 1973; LAWTON, 1976) que podem ter se adaptado não apenas para lidar com a matriz de compostos secundários dessa samambaia, mas também para evitar a defesa das formigas. LAWTON (1976) observou que plantas de *Pteridium* com e sem formigas tinham o mesmo número de herbívoros.

SCHREMMER (1969) comentou que *Pteridium* pode não obter benefícios das formigas que visitam seus nectários porque a secreção do néctar e as visitas das formigas cessam antes que os herbívoros iniciem o ataque às frondes. Nesse contexto, KEELER (1981) indicou que essa relação mutualística pode ser mais expressiva em estágios iniciais de desenvolvimento foliar, quando as folhas não possuem outras defesas efetivas (e.g. dureza foliar). Em adição, LAWTON & HEADS (1984) propuseram que a baixa densidade de formigas sobre folhas de *Pteridium* é o principal fator que limita a ocorrência de mutualismo dessa planta com formigas em algumas partes do mundo. Essa baixa densidade de formigas foi atribuída a menor qualidade nutricional dos produtos secretados nos nectários de *Pteridium* (SUDD & SUDD, 1985), aliado a grandes populações dessa planta, sendo improvável sustentar um número de formigas para que os benefícios ocorram. Portanto, o papel dos nectários com traço de defesa antiherbivoria é dependente de vários fatores (e.g. densidade e agressividade das formigas) e nem sempre é identificado.

Outra grande parte das pesquisas acerca das interações em samambaias advém de espécies com domácias, que podem ter alto grau de especificidade (MEHLTRETER, 2010).

Lecanopteris spp., distribuídas no Sudeste Asiático e *Solanopteris* spp., com distribuição Neotropical, são samambaias epífitas que possuem domácias, adquirindo parte de seus nutrientes (Nitrogênio) através do mutualismo com as formigas, como demonstrado por GAY (1991). Esse autor descreveu detalhadamente o sistema das formigas no interior de domácias de *Lecanopteris* spp. e realizou uma série de experimentos em laboratório para demonstrar como essas epífitas adquirem nutrientes a partir de formigas (GAY, 1990; 1993a, 1993b). Ocorre transferência de nutrientes através das paredes internas do rizoma em *Lecanopteris* Reinw, onde há detritos e fezes das formigas, que se movem por galerias e câmaras centrais.

Além do benefício nutricional, as formigas presentes em domácias podem atuar na defesa das samambaias ao deter alguns predadores que ameaçam seus ninhos. Cinco espécies de formigas (gêneros: *Iridomyrmex* Mayr. e *Cramatogaster* Lund.) são regularmente associadas com *Lecanopteris* (GAY, 1993a). As plantas adultas deste gênero podem sobreviver sem formigas (GAY, 1990; GAY & HENSEN, 1992), no entanto, essa ausência pode afetar significativamente a sobrevivência e o vigor durante o estágio juvenil (GAY, 1993a). No caso de *Solanopteris* Copel., as formigas *Azteca* Forel tornam-se muito agressivas quando suas plantas hospedeiras sofrem algum distúrbio, “Caso você toque nessa samambaia, mesmo que seja gentilmente, as formigas correrão para fora dos seus caules, pularão sobre seus dedos e subirão por seus braços, até encontrarem uma região mais tenra onde então cravarão suas presas vingativamente” (MORAN, 2004).

Outras espécies possuem pseudo-domácias ou domácias externas a partir da disposição dorsiventral de rizoma e adensamento de raízes, como *Polytaenium cajennense* (Desv.) Benedict que abrigam de modo casual formigas *Azteca* (LEÓN & YOUNG, 2010). As autoras especularam sobre os benefícios da interação, como o possível corte de outras epífitas potencialmente competidores por recursos na planta hospedeira. TANAKA & ITIOCA (2011) apresentaram evidências que formigas *Crematogaster difformis* F. Smith, associadas a *Lecanopteris* e *Platycerium* Desv., cortam e excluem lianas nos troncos das árvores hospedeiras das samambaias. Consequentemente, esse sistema samambaias e formigas pode regular a distribuição de lianas, ao passo que também beneficiam as árvores hospedeiras, uma vez que, em geral, lianas têm efeitos negativos sobre o crescimento e a sobrevivência das árvores hospedeiras, influenciando a captura de luz, nutrientes e água. Em adição, essa pesquisa demonstrou que as formigas operárias não patrulham apenas as samambaias, mas também a árvore hospedeira. A partir desse mesmo sistema (*C. difformis*, *Lecanopteris* e *Platycerium*), TANAKA et al. (2009) demonstraram experimentalmente a potencialidade das formigas em regular abundância de insetos herbívoros, diminuindo a proporção média de área foliar perdida e a proporção de folhas danificadas das samambaias. Interessantemente, algumas baratas coabitam com essas formigas no rizoma das samambaias, não sendo atacadas (INUI et al. 2009).

Um outro caso de interação samambaias e formigas foi reportado entre *Antrophyum lanceolatum* (L.) Kaulf. e *Pheidole flavens* Roger, respectivamente (WATKINS et al. 2008). Essa samambaia abriga formigas em seus rizomas (mais 100 formigas em plantas maiores), que carrega resíduos, posteriormente decompostos e assimilados na planta. A relação é facultativa (i.e. formigas ausentes em 38% da amostra) e as formigas não apresentam comportamento agressivo. Esse é um exemplo de que plantas mesmo sem estruturas especializadas para atração de formigas podem beneficiar-se da associação com esses insetos.

Há também interações formigas e samambaias que são casos totalmente oportunistas. Exemplos advém a partir de *Acrostichum danaeifolium* Langsd. & Fisch., que tem seus pecíolos e raques perfurados por microlepidópteras, formando galerias posteriormente habitadas por formigas, em especial *Tapinoma sessile* (Say) e *Wasmannia auropunctata* Roger, espécies introduzidas que parecem não ter nenhuma ação para proteger a samambaia de qualquer herbívoro (MEHLTRETER & PALACIOS-RIOS, 2003). Formigas também foram registradas no interior do pecíolo de *Pteris deflexa* Link, sem atividade de proteção para a samambaia (SANTOS & MAYHÉ-NUNEs, 2007).

2.3 GALHAS E SAMAMBAIAS

As galhas ou “tumores de plantas” têm sido definidas como tecidos vegetais anormais, modificados morfoanatomicamente principalmente a partir de mensagens induzidas por insetos (RAMAN, 2007). Insetos galhadores formam uma guilda sofisticada que provavelmente evoluiu a partir de pressões seletivas para induzir tecidos meristemáticos de suas plantas hospedeiras, fornecendo a sua prole recurso nutricional com alta qualidade, proteção contra estresses ambientais e inimigos naturais (PRICE et al. 1987). O desenvolvimento de insetos galhadores tem sido registrado a partir de qualquer estrutura vegetal, desde extremidades das raízes até o ápice do caule, tanto nas partes vegetativas quanto reprodutivas (FERNANDES & MARTINS, 1985), embora, a maioria das galhas ocorrem nas folhas (ISAIAS et al. 2014).

Tipicamente, cada espécie de inseto galhador ocorre em apenas uma espécie de planta hospedeira, ou seja, é uma interação espécie-específico (SHORTHOUSE & ROHFRITSCH, 1992; PRICE et al. 1998). Devido à especificidade em relação ao hospedeiro, a descoberta de novas galhas é frequentemente acompanhada pelo reconhecimento de novas espécies de insetos indutores (e.g. MAIA, 2004; MAIA et al. 2005).

A maioria dos registros entre insetos galhadores e plantas advém das angiospermas. Para as samambaias, houve registros esporádicos para a interação. Entretanto, mediante amostragem adequada houve um aumento significativo do número de registros. Por exemplo, HANSON & GÓMEZ-LAURITO (2005) reportaram 18 novos registros de galhas em samambaias na Costa Rica, número maior do que já se tinha registrado anteriormente em todo o mundo.

Estima-se que mais de 100 registros de galhas serão realizados para as samambaias. Atualmente, não se permite nenhuma conclusão detalhada sobre a distribuição de insetos galhadores em relação a árvore evolutiva das samambaias. Entretanto, as galhas ocorrem em distintas famílias de samambaias, a partir diferentes posições na árvore evolutiva. Galhas ocorrem em plantas pequenas (poucos centímetros), como em representantes de Hymenophyllaceae (GIESENHAGEN, 1909; HOUARD, 1933), até espécies arbóreas de Cyatheaceae (MAIA et al. 2008). Em adição, há registros para representantes epífitos em Polypodiaceae (KRAUS et al. 1993; MAIA & SANTOS, 2011), e terrestres de Blechnaceae (HOAURD, 1933), Nephrolepidaceae (DOCTERS VAN LEEUWEN, 1921) e Dennstaedtiaceae (BALICK et al. 1978). Galhas nessas plantas são induzidas por insetos, principalmente aqueles pertencentes a Cecidomyiidae (e.g. MAIA & SANTOS, 2011). Outros grupos, como vespas (e.g. BERA et al. 1994; PATRA et al. 2010), mariposas (e.g. MAIA &

SANTOS, 2015), moscas (e.g. DOCTERS VAN LEEUWEN, 1938) e ácaros (e.g. BERA & GHORAI, 1997; 1999) também induzem galhas nas samambaias.

As interações plantas e galhadores têm sido utilizadas como sistemas para responder perguntas ecológicas diversas e testar teorias ou hipóteses acerca da seleção de plantas hospedeiras por herbívoros. Dentre as hipóteses, destacam-se às relacionadas ao estresse e ao vigor vegetal (WILLIAMS & CRONIN, 2004). A hipótese do estresse vegetal prevê maior herbivoria em plantas sob estresse, devido a uma maior disponibilidade de nutrientes e uma diminuição de compostos defensivos (WHITE, 1984). A hipótese de vigor prediz que os herbívoros atuam preferencialmente em plantas ou módulos mais vigorosos, garantindo mais nutrientes e consequentemente melhor *performance* a sua prole (PRICE, 1991). Várias medidas de robustez têm sido utilizadas para explicar preferência de oviposição por insetos galhadores, destacando tamanho foliar, altura das plantas, número de folhas, disponibilidade de flores e duração da floração (JUENGER et al. 2005). A hipótese de vigor tem sido amplamente apoiada para explicar a distribuição diferencial de danos por insetos de diferentes guildas. Em uma revisão sobre o tema, CORNELISSEN et al. (2008) demonstraram que os efeitos são mais fortes para herbívoros sugadores, minadores e galhadores.

Insetos galhadores interagem com outros organismos, tais como predadores, parasitoides e inquilinos, muitas vezes de forma específica, tornando as relações ainda mais complexas (MANI, 1964). A ação de predadores e parasitoides pode exercer forte influência negativa aos galhadores, principalmente ao reduzir a taxa de sobrevivência larval. Na literatura há variados exemplos de inimigos dos galhadores, como formigas (MENDONÇA-Jr. & ROMANOWSKI, 2002), insetos da ordem Diptera (MAIA et al. 2008) e larvas de Lepidoptera (Almeida et al. 2006). Em outros casos, a interação galhadores e outros organismos é indireta, como por exemplo, larvas de Lepidoptera que evitam o consumo de galhas, mas cortam tecidos ao lado da estrutura da galha (MENDONÇA-JR., 1996). Esse consumo em tecidos ao redor da galha pode ocorrer devido a um maior fluxo de nutrientes nesses sítios. Por outro lado, os galhadores podem afetar as concentrações de metabólitos secundários, como os compostos fenólicos, que podem afetar indiretamente a incidência e o consumo de herbívoros cortadores (PASCUAL-ALVARADO et al. 2008). Portanto, para consumir tecidos próximos às galhas é necessário que herbívoros sejam tolerantes às defesas químicas.

2.4 DEFESAS QUÍMICAS

As plantas se defendem contra herbívoros a partir de um complexo conjunto de traços de resistência. Pesquisas iniciais focaram no papel dos traços químicos (e.g. FRAENKEL, 1959, EHRLICH & RAVEN, 1964; FEENY, 1976). De modo geral, tem sido demonstrado uma grande variedade de metabólitos potencialmente envolvidos na defesa de plantas (CARMONA et al. 2011). Devido a algumas questões, como dificuldade de individualizar o efeito de metabólitos sob a *performance* de herbívoros ou a dificuldade de montagem do modelo experimental, poucos estudos empregaram abordagens para determinar o papel específico de defesas fitoquímicas. Um dos exemplos, advém a partir do gênero *Pinus* L., linhagem com alta diversidade de terpenoides, tanto constitutivos quanto induzidos, que dissuadem ou matam uma ampla gama de insetos não especializados (MUMM et al. 2003; MUMM & HILKER, 2006).

Algumas abordagens têm demonstrado o papel das defesas fitoquímicas a partir do grau em que são conservadas ou alteradas em uma dada filogenia. Utilizando relógios moleculares no sistema Burseraceae e besouros crisomelídeos (Blepharida), BECERRA (2003) reportou que as defesas fitoquímicas não são estáveis evolutivamente, expressando sincronia a partir dos traços de alimentação do herbívoro. De modo geral, esse resultado tem sido corroborado para outras linhagens de plantas e outras substâncias químicas. Por exemplo, a composição e concentração de compostos fenólicos (AGRAWAL et al. 2009; KURSAR et al. 2009; PEARSE & HIPP, 2009), nicotina (ADLER et al. 2012), cardenolídeos (RASMANN & AGRAWAL, 2011) e terpenos (BECERRA, 1997) são flexíveis entre congêneres, isto é, expressam um sinal filogenético baixo a moderado.

Comparações de defesas fitoquímicas entre as grandes linhagens são escassas. Há um consenso que as angiospermas possuem maior diversidade química que samambaias e gimnospermas (COOPER-DRIVER, 1985). Entretanto, não se tem estimativas da magnitude dessa diferença. Tipicamente, a maioria das samambaias não possuem algumas classes de metabólitos, como alcaloides, acetilenos e glucosinolatos (SWAIN & COOPER-DRIVER, 1981; COOPER-DRIVER & HAUFLER, 1983). A maior parte do conhecimento fitoquímico das samambaias advém de investigações acerca do uso medicinal e alimentar dessas plantas, que ocorreram principalmente em regiões asiáticas. Nestes, tem se demonstrado que substâncias como terpenoides, esteróis, fenóis e sesquiterpenos são amplamente distribuídas nas samambaias (KULANDAIRAJ & BRITTO, 2000). Embora com menor diversidade de metabólitos secundários, samambaias possuem alta resistência contra herbívoros, um dos fatores importantes no sucesso ecológico dessa linhagem, que não possui elaboradas estruturas de defesas físicas (PAGE, 2002).

Algumas substâncias consideradas dissuadoras de herbívoros são reportadas para linhagens específicas de samambaias. Essas substâncias incluem polímeros fenólicos mais complexos, como taninos condensados expressos em *Pteridium* (TEMPEL, 1981), alcaloides em Equisetaceae, sesquiterpenos cancerígenos em *Pteridium* e *Onychium* Kaulf. (Somvanshi et al. 2006), tiaminase em *Nephrolepis exaltata* (L.) Schott e *Pteridium* (HENDRIX, 1977), e fitoecdisonas em espécies de Blechnaceae, Dennstaedtiaceae, Dryopteridaceae, Gleicheniaceae, Osmundaceae, Polypodiaceae, Pteridaceae (KAPLANIS et al. 1967; CARLISLE & ELLIS, 1968; RUSSEL & FENEMORE, 1971; HIKINO et al. 1973). As fitoecdissonas "hormônios de muda de insetos" são bastante comuns em samambaias. Por exemplo, HIKINO et al. (1973) reportaram a presença dessa substância para 170 das 283 samambaias estudadas. Alguns metais pesados que são acumulados nos tecidos das samambaias também podem exercer importante função da defesa do grupo. Por exemplo, RATHINASABAPATHI et al. (2007) evidenciaram que a hiperacumulação de arsênio em *Pteris vittata* L. é uma defesa elementar contra a herbivoria do gafanhoto *Schistocerca americana* (Drury).

Algumas samambaias têm sido consideradas cianogênicas, i.e. capazes de sintetizar compostos que liberam ácido cianídrico, substância que inibe a cadeia respiratória do herbívoro e altera outros processos fisiológicos, resultando em uma alta taxa de mortalidade em curto tempo (SCHREINER, 1980; SCHREINER et al. 1984a; SCHVARTSMAN, 1992). Os glicosídios cianogênicos mais encontrados nas samambaias são as prunassina e vicianina (SWAIN & COOPER-DRIVER, 1973). O conhecimento da amplitude de ocorrência das substâncias cianogênicas em samambaias está limitado por métodos qualitativos imprecisos. HARPER et al. (1976) demonstraram resultados positivos para 5,4% das 298 espécies de samambaias analisadas, enquanto SANTOS et al. (2005) reportaram cianogênese em 73,7% a partir de 19 samambaias. Os glicosídeos cianogênicos tornam-se efetivos após o dano foliar herbívoro. Populações de *Pteridium aquilinum* polimórficas variam em seu conteúdo de cianogênicos, correlacionando negativamente com a quantidade de luz. Samambaias cianogênicas foram atacadas com menor frequência por insetos (COOPER-DRIVER et al. 1977; SCHREINER et al. 1984b).

2.5 DEFESAS FÍSICAS E CRESCIMENTO

Características físicas e de história de vida das plantas são preditoras consistentes de resistência contra herbívoros. Características como tricomas, acúleos, cutícula espessa, epiderme espessa e inclusões minerais constituem algumas das defesas físicas (PEETERS, 2002; BOEGE & MARQUIS, 2005; CORRÊA, 2007; HANLEy et al. 2007). Os tricomas são apêndices epidérmicos uni ou multicelulares, glandulares ou não glandulares, expressos constitutivamente ou induzidos a partir de estímulo abiótico ou danos herbívoros (DALIN et al. 2008). Tricomas representam um bom exemplo para demonstrar a multifunção de traços físicos: resistência contra stress abiótico, tolerância a seca por redução da absorção da radiação solar, facilitação da condensação da umidade do ar na superfície da planta e proteção celular contra danos causados por radiação solar ultravioleta ou baixas temperaturas (DALIN et al. 2008). Independente de outras funções, tricomas potencialmente atuam contra herbívoros, dificultando o acesso, locomoção e/ou oviposição dos insetos, além de liberar componentes tóxicos ou repelentes no caso dos glandulares (DALIN et al. 2008). Os acúleos, geralmente maiores, são mais eficazes contra os herbívoros de maior porte, como os vertebrados (HANLEY et al. 2007).

A cutícula representa uma estrutura química complexa por macromoléculas de cutinas ligadas a lipídios que reveste estrutura vegetais e desempenha numerosas funções na planta, dentre elas, a interferência na interação com herbívoros. A ação da cutícula nas interações plantas e herbívoros se dá principalmente no reconhecimento de superfícies foliares adequadas para alimentação ou oviposição (EIGENBRODE & ESPELIE, 1995). Características anatômicas como epiderme espessa, paredes celulares espessas, sílica nas paredes celulares, parênquima paliçádico estratificado e/ou esclerênquima tornam as folhas mais duras e/ou mais difíceis de serem cortadas e digeridas por herbívoros, portanto, são traços negativamente relacionados com a herbivoria (TANNER & KAPOS, 1982; PEETERS, 2002; CORRÊA, 2007).

Plantas têm sido classificadas como de rápido e lento crescimento. Espécies sob condições de alta disponibilidade de recursos investem mais em crescimento que em defesas antiherbivoria, ao passo que as espécies com baixa disponibilidade de recursos investem mais em defesas que em crescimento (COLEY et al. 1985). Como exemplo, as cicadófitas com lento crescimento investem fortemente em defesas para proteger as poucas folhas que produzem anualmente. Uma das defesas se dá a partir da sincrônica e rápida expansão foliar em *Zamia stevensonii* A.S.Taylor & Holzman. Essa planta possui defesas químicas glicosiladas reduzidas com o desenvolvimento foliar (100 dias), sendo posteriormente defendidas a partir da dureza foliar (PRADO et al. 2014). Entretanto, nem sempre a dureza foliar significa redução de defesas químicas. Em ambientes com déficit nutricional (i.e. limitação de fósforo no solo), pode haver relações positivas entre dureza foliar e compostos fenólicos. Esse investimento em defesas físicas e químicas baseadas em carbono é resultado da abundância de carboidratos em solos inférteis (READ et al. 2009).

A maioria das teorias sobre a evolução das defesas antiherbivoria baseia-se na premissa de que as demandas concorrentes de crescimento, reprodução e defesa restringem padrões de alocação energética, hipótese da disponibilidade de recursos (COLEY et al. 1985). Consequentemente, as pesquisas nesta área nas últimas décadas estabeleceram de modo conclusivo de que os herbívoros direcionam a seleção para a expressão de traços defensivos em plantas e que há custos de *fitness* (SIMMS & RAUSHER, 1989; SIMMS & TRIPLETT, 1994; MAURICIO, 1998; DYER et al. 2004). Qualquer traço que confira benefício a aptidão física a uma planta na presença de herbívoro pode ser considerado como defesa (KARBAN & BALDWIN, 1997). Nesta perspectiva, características de tolerância para minimizar o impacto da herbivoria após a ocorrência (e.g., crescimento compensatório ou maior taxa reprodutiva) foram consideradas estratégias alternativas de defesas (STRAUSS & AGRAWAL, 1999), que se correlacionam negativamente com traços de resistência (VAN DER MEIJDEN et al. 1988). A lógica por trás da expressão de traços de tolerância ou resistência está relacionada aos seus custos de produção. Plantas resistentes dispendem mais energia para uma mínima defesa, enquanto que em caso da expressão de traços de resistência ser mais dispendiosa que o crescimento compensatório (e.g., rebrota), há seleção de traços de tolerância. Há algumas evidências que plantas possuam uma ou outra estratégia: resistência ou tolerância, mas evidências crescentes sugerem a combinação de traços de ambas as estratégias (NUÑEZ-FARFÁN et al. 2007). Isto sugere que as defesas das plantas é melhor vista como multidimensional, surgindo o conceito de síndromes de defesas.

2.6 SÍNDROMES DE DEFESAS

Pesquisas sobre a evolução de traços defensivos das plantas tem focado no conceito de síndromes de defesas (KURSAR & COLEY, 2003; AGRAWAL & FISHBEIN, 2006). A hipótese de síndromes de defesas prediz que a seleção natural molda uma covariação positiva de traços, maximizando a defesa das plantas por um efeito sinérgico. Essa hipótese rejeita redundância entre traços de defesas. Vários estudos têm evidenciado convergência em combinações particulares de traços defensivos, abrangendo principalmente linhagens de angiospermas. Por exemplo, JOHNSON et al. (2014) reportaram três tipos de síndromes para a linhagem da *Oenothera* L. (Onagraceae): I – espécies com baixa diversidade de compostos fenólicos, alta concentração de elagitaninos e folhas duras, II – espécies com alta diversidade de compostos fenólicos e alta concentração de flavonoides glicosilados, e III – alta concentração de ácidos caféicos derivados, glicosídeos de quercetina, pubescência, alto teor de água foliar e área específica foliar (SLA). Em Brassicaceae, TRAVERS-MARTIN & MULLER (2008) encontraram três síndromes: I – espécies com alta quantidade de nitrogênio e defesas químicas, II – baixo teor de nitrogênio e baixos níveis de defesas químicas (i.e. glucosinolatos) e/ou físicas, e III – defesas mecânicas associadas a redução de digestibilidade. Esses trabalhos, têm como referência o triângulo de síndromes de defesas proposto por AGRAWAL & FISHBEIN (2006): baixa qualidade nutricional, alta qualidade nutricional e defesas ou tolerância/fuga.

Alguns estudos abordaram síndromes em nível de comunidades, e nestes casos, os traços de defesas analisados são mais gerais (e.g., compostos fenólicos, saponinas, terpenoides, alcaloides e tricomas). A partir de um estudo com espécies do Cerrado, SILVA & BATALHA (2011), demonstraram as síndromes estruturadas a partir de traços que definem a baixa qualidade nutricional (C:N, menor área específica foliar - SLA, menor conteúdo de água) ou alta defesa (alcaloides, taninos, terpenoides, tricomas). KURSAR & COLEY (2003) reportaram uma convergência de defesas para folhas jovens de espécies em Florestas Tropicais. Essa pesquisa demonstrou duas principais síndromes: escape e defesa. Essa última síndrome é caracterizada pelo expansão foliar lenta, baixo conteúdo de nitrogênio para crescimento, alto investimento em defesas químicas (alcaloides, compostos fenólicos e saponinas) e baixa sincronia de produção foliar. Os autores demonstraram que o tipo de síndrome expresso explica variação interespecífica dos níveis de herbivoria. Espécies com a síndrome do tipo escape sofrem aproximadamente 60% de danos por herbívoros, enquanto espécies com síndrome denominada defesa (i.e. resistência) experimentam menos de 20% de área foliar perdida. PRINGLE et al. (2011) reportaram menores taxas de herbivoria em espécies de árvores perenes que em decíduas. As taxas mais baixas de herbivoria foram explicadas por uma síndrome de defesa formada por maior dureza foliar, menor conteúdo de água e maior taxa de C:N para árvores tropicais perenes.

Uma das exceções às pesquisas que investigaram síndromes em angiospermas advém de um estudo com Pinaceae (MOREIRA et al. 2016). A expressão da síndrome de defesa nessa família está relacionada a taxa de crescimento, não havendo relação filogenética. Espécies de crescimento lento, em hábitats pobres nutricionalmente, possuem elevada expressão de defesas constitutivas (resinas) em oposição a maior expressão de defesas induzidas (ácido jasmônico e ácido salicílico) para aquelas de crescimento rápido.

Embora tenha sido bem suportada, a generalidade das síndromes de defesa precisa ser testada para outras famílias de plantas ou linhagens, identificando a variação dos conjuntos de traços que as estruturam. Adicionalmente, essa hipótese oferece uma maneira de avaliar defesas vegetais englobando traços ecofisiológicos, físicos e estruturais, e como esses interagem com o ambiente das plantas. Desse modo, a mensuração de síndromes representa um meio útil para identificar variações ecológicas relevantes em comunidades florestais. Testes deste modelo, em que as plantas são agrupadas em uma das três possíveis síndromes de defesas requer análises de traços estruturais (AGRAWAL & FISHBEIN, 2006). Tais estudos melhorarão nosso entendimento da evolução das defesas antiherbivoria. Do ponto de vista prático, uma melhor compreensão de como e por que as defesas estruturais são expressadas produzem informações importantes sobre como as interações planta-herbívoro são susceptíveis de responder a uma mudança ambiental (HANLEY et al. 2007).

3 FIRST RECORD OF GALLS IN THE TREE FERN *CYATHEA PHALERATA* (CYATHEACEAE) FROM A TROPICAL RAINFOREST IN BRAZIL

3.1 INTRODUCTION

Galls are abnormal, morphogenetically modified plant tissues that were induced by insects (RAMAN, 2007), and occur especially on angiosperms (e.g. Santos et al., 2011; Maia and Silva, 2016). Comparatively few galls have been reported on ferns (e.g., Houard, 1933; Kraus et al., 1993; Balick et al., 1978; Maia and Santos, 2015), although ferns are the second largest group of vascular plants and are especially abundant in tropical forest understories (Sharpe et al., 2010). Consequently, ferns might have been underestimated as host plants of galling insects, due to the lack of adequate sampling (Mehltreter, 2010).

The galling insects are highly specific to their host plants (Shorthouse and Rohfritsch, 1992; Price et al., 1998), because oviposition and/or larval feeding are capable of inducing specific morphogenetic changes only in the tissues of their specific host plants (Mani, 1992). Because of this high specificity, the discovery of new galls is often accompanied by the recognition of new gall-inducing insect species, as well (e.g. Maia and Santos, 2011; Maia and Araújo, 2016). Moreover, the geographical range of fern-insect interactions can provide further insights into their biology and evolutionary origins, especially in the high diverse Neotropics (Fernandes et al., 2011; Santos et al., 2012).

In this study, we report galls on the Brazilian tree fern *Cyathea phalerata* Mart. (Cyatheaceae) for the first time. In addition, we present morphological characteristics and some field observations.

3.2 MATERIAL AND METHODS

The study was performed in a remnant of the Atlantic Forest, located in the municipality of Bonito, Pernambuco State, northeastern Brazil (08°29′56’’S, 35°41’43’’W; 680 m a.s.l.). The forest fragment is less than 50 ha in size and surrounded by a road and open areas, with other forest remnants nearby. The climate is tropical with a rainy season during autumn and winter. Average annual temperatures are 22.1 ºC and mean annual rainfall is approximately 1,200 mm. A voucher of *C. phalerata* was deposited at the *herbarium* of the Universidade Federal de Pernambuco (UFP, nº 81.373). The identification of the specimen followed Weigand and Lehnert (2016).

During fieldwork from May to June 2015, leaves (croziers, sterile and fertile) of *C. phalerata* were collected and examined for the presence of galls. Descriptions of gall morphology (color, shape, number of larval chambers, pubescence and distribution) follow Isaias et al. (2014). The quantification of galls per pinnule was made from 20 samples, which were obtained from 12 plant individuals at the study site. Leaves were composed of 10-16 pinnae with 40-50 pinnules (leaflets) each, and measured 150-220 cm in length.

3.3 RESULTS AND DISCUSSION

Galls were present on completely expanded sterile and fertile leaves of eight plants, but absent in croziers and young, still expanding leaves. Leaf galls of *C. phalerata* were induced on the upper surface of leaves and may superpose the sori. The galls were glabrous and green, but often black at later developmental stages. Galls were globoid and contained one larval chamber with a solitary larva. The exit hole is on the lower surface of the leaf. Galls occurred individually or in groups. The average number of galls was 10.93 ± 6.39 per leaflet and could be in different developmental stages. Galls might occur near the midvein of the leaflet, extended to the marginal incision between two leaflets (Figure 1), and were induced by a new genus of Cecidomyiidae (Diptera), which belongs to the tribe Cecidomyiini (*paper in preparation*).

The gall of *C. phalerata* represents a new record on tree ferns and the third for genus, previously recorded in *Cyathea squamata* (Klotzsch) Domin (Houard, 1933) and *Cyathea* sp. (Maia et al., 2008). We presume that increasing sampling efforts in the tropics may reveal if the number of galls on ferns has been underestimated. In a revision from Costa Rica, Hanson and Gómez-Laurito (2005) reported 17 species of gall insects on ferns, which doubled the number that has been previously recorded worldwide (Balick et al., 1978). If the ratio of 17 gall-forming insects on 1,200 fern species (1:70.6) and 950 gall-formers on 9,000 angiosperm species (1:9.5) for Costa Rica is representative for the world, ferns would have just seven times fewer galls than angiosperms, but there would be still ca. 140 new gall-inducing species to be discovered for the currently 12,000 fern species worldwide (Mehltreter, 2010). Finally, we emphasize the need for a greater sampling effort of fern galls in the Neotropics to study their phylogenetic distribution and diversity on ferns.

Figure 1. Leaf galls induced by Cecidomyiidae in the tree fern Cyathea phalerata Mart. (Cyatheaceae) from a Tropical Rainforest in Brazil. Galls situated near the vein and between two leaf segments, contained one larval chamber.

4 HOST PLANT-SELECTION AND HERBIVORY ON THE TREE FERN *CYATHEA PHALERATA* (CYATHEACEAE), ATTACKED BY GALLING INSECT AND CATERPILLARS

4.1 ABSTRACT

Herbivorous typically shows preferences patterns among or within host plants, influenced by plant traits and/or direct and indirect effects mediated on the other herbivores exploring angiosperms. None investigations focused on patterns of host plant selection in ferns, an abundant component of tropical forest understories. Here, we investigated patterns of host plant selection on a tree fern *Cyathea phalerata* from a Brazilian Tropical Forest, guidance over from the plant vigor hypotheses (i.e. herbivory favored by size of plants or modules) and indirect interactions between galls insects (Diptera) and caterpillars (Lepidoptera). Our questions were: (1) whether herbivores preferentially selected larger leaves? (2) whether the presence of galls or chewing interfered negatively in the occurrence of the other? We found that both gallers and caterpillars attacked more intensely larger leaves of *C. phalerata*, corroborating the plant vigor hypotheses in the system. We found no relationship between gall abundance and levels of herbivory by caterpillars. However, there was no spatial segregation of the attacks. A high percentage (87%) of all the galled leaves suffered damage by caterpillars, but these avoided to consume galls structures, parts lignified, suberized, and with highest concentration of phenolic compounds. About 64% of non-galled presented damages by caterpillars. Therefore, we conclude that the presence of galls not prevent the feeding of chewing insects in *C. phalerata*, but suggests the negative influence of chewing under galls occurrence.

Keywords: chewing insects, galls, herbivory patterns, indirect interactions, plant–herbivore interactions, plant vigor hypothesis.

4.2 INTRODUCTION

Herbivorous exhibits some degree of selectivity face the variety types of host plant potentially available (Bernays and Chapman 1994). Identifying plant traits involved to host-plant selection by herbivorous is a main focus of investigations about plant-animal interaction (e.g., Coley 1983; Aide 1993; Cardenás et al. 2014). Host plant-selection is usually complex, involving process related to find and recognize the resource, plant quality and tolerance to chemical plant-defense (Price et al. 1991; Eigenbrode and Espelie 1995). The evaluation of plant traits and exploration of herbivores are necessary to understand the mechanisms responsible for feeding preferences among or within host plants (Ya-Ide 2006).

One of the main hypotheses currently recognized to explain patterns of preference for herbivores is the plant vigor hypothesis. This hypotheses incorporate traits of quantify and quality resource, predicting that herbivores acts preferentially on the most vigorous plant and/or plant modules, where resource are better and more abundant (Price 1991). The plant vigor hypotheses was corroborated by a meta-analytical review, with stronger effects in the guilds of suckers, leaf miners, and gall insets (see Cornelissen et al. 2008). However, some investigations reported opposite results about this hypotheses. For instance, leaf gall midge *Asphondylia microcapillata* Maia (Diptera) on *Bauhinia brevipes* Vogel – Fabaceae (Santos et al. 2008), and gall-inducing weevil *Prospoliata bicolorata* Hustache (Coleoptera) on *Miconia prasina* (Sw.) DC. – Melastomataceae (Santos et al. 2011) prefers leaves of intermediate size. Therefore, the plant vigor hypothesis is not idiosyncratic to plant–herbivore interactions.

Often the same host plant may be exploited by different herbivores. Co-occurrence of herbivores, for instance, has been reported in super-host plant species of galling insects (e.g., Costa et al. 2010) or galled plants attacked by free-feeding insects (e.g. Cornelissen and Fernandes 2001; Ribeiro and Basset 2007; Costa et al. 2011). Co-occurring insects (belonging to same or different guild) may exert effects on each other, direct or indirectly, positive or negatively (Faeth 1986; Damman 1993; Nakamura et al. 2003). There are examples demonstrating galling insects (i.e. larvae) death by other herbivores (Ferraz and Monteiro 2003). In other cases, free herbivores (i.e. caterpillars) avoid consuming galls, chewing around the galls (Mendonça 1996). The consumption of tissues around the galls may be due to a good nutritional quality after induction of gallers. In opposition, galling insects may increase leaf phenolic concentrations, affecting indirectly the incidence and consumption of folivorous (Pascual-Alvarado et al. 2008). The interaction between gallers and other herbivores, eating (or not) the galls seems quite prevalent (Toma and Souza Mendonça 2014), but triggers factors and their ecological consequences remain unknown.

For ferns, the second largest group of vascular plants (10.578 species), and especially abundant in tropical forest understories (Sharpe et al. 2010), the framework about insect interactions is yet limited (Balick et al. 1978; Mehltreter 2010). The state of the art demonstrates that ferns have a wide variety of potential herbivores (e.g., ants, gall, caterpillars, miners, and spore feeders), and supposedly experience lower leaf damage than seed plants (see Mehltreter 2010). However, fern-insect interactions are underestimated and not understood in light of theory or plant-herbivore hypotheses. Here, we utilized the system in which a tree fern (i.e. with trunk or caudex) suffers simultaneous damage from two guilds of insects herbivorous to gain further insights on patterns of host plant selection in ferns and to know their herbivory patterns, guidance over from the plant vigor hypotheses and indirect interactions between either guilds. Consequently, we aimed in our study to (1) evaluate whether herbivores preferentially selected larger leaves of *Cyathea phalerata* Mart. (Cyatheaceae), attacked by insect-galling (Diptera), and a larva-chewing, caterpillars (Lepidoptera) from a Brazilian Tropical Forest, (2) determine whether the presence of galls or chewing in leaves of *C. phalerata* interfere negatively in the occurrence of the other, and (3) describe histochemical and anatomical traits of *C. phalerata* to demonstrate barriers overcome or avoided by herbivores.

4.3 MATERIAL AND METHODS

Study site: The system studied occurs in a submontane Tropical Forest, situated in Bonito, Pernambuco State, northeastern Brazil (08°29′56’’S, 35°41’43’’W; 680 m a.s.l.). The forest fragment is less than 50 ha in size and surrounded by a no paved road and open areas, with other forest remnants nearby. The climate is hot and humid (As) with a mean annual temperature of 22.1ºC and annual rainfall of 1200 mm.

Description of system: *Cyathea phalerata* is a tree fern endemic to Brazil with a wide distribution, especially in Atlantic Forest, from sea level to 1500 m. This species has a caudex of 1.0-4.0 m height and leaves up to 3.0 m length with up to 10 leaves per plant. In the study area, the population of *C. phalerata* (Voucher herbarium UFP, nº 81.373) hosts abaxial leaf galls induced by insect belong to Diptera (Farias et al. 2018) and suffers damage from caterpillars, which feed on the abaxial side of leaves. Galls were present on completely expanded leaves, but absent in croziers and young, still expanding leaves (Farias et al. 2018).

Experimental design: We sampled all 17-plant individuals of *C. phalerata* at the study site. We measured the leaf length (i.e. utilized as a proxy to vigor) and number of galls per leaf in all mature leaves. Galled leaves were classified as with active galls, inactive galls or both types. Also, we estimated the levels of herbivore damage by chewing, classifying the leaves in categories established by Mehltreter et al. (2006), with modification (i.e. class 100% excluded), as follows: absent (class 0), 1-5% (class 1), > 5-15% (class 2), > 15-25% (class 3), > 25-50% (class 4), > 50-75% (class 5) and > 75% (class 6) of leaf area removed.

Anatomical and histochemistry description: In laboratory, samples (non-galled and galled leaves, free damage by chewing insects) were fixed in FAA (50% formaldehyde, acetic acid and 50% alcohol) for 72 hours, and then dehydrated in ethanol series (Johansen 1940) and embedded in histological paraffin. Transverse and longitudinal sections were made with a rotary microtome (Reichertt Jung model, Autocut 2040), and stained with astra blue and safranin (Bukatsch 1972). Slides were mounted in Canada balsam (Purvis et al. 1964). Histological slides were photographed under a light microscope (Leica DM500) coupled with a digital camera. Additionally, sections were prepared with ferric chloride test to detect phenolic compounds (Johansen 1940).

Statistical analyses: The analysis regressions simples were performed to evaluate the relation between herbivore damage and and leaf length. We used four manner to evaluate herbivorous damages: (1) gall abundance (i.e. number of galls per leaf), (2) percentage of galled leaves into length classes, (3) average number of galls per leaf into length classes, and (4) % levels damage by caterpillar. The length classes were determined dividing the total length leaf into 10 class of 25 cm, ranging from 75 cm to 300 cm. When divided the number of galled leaves by the number of leaves in each length class, we obtained the percentage of galled leaves into length classes, eliminating the effects of differential resource available (see Cornelissen and Fernandes, 2001). The herbivory by caterpillar was obtained from value median in each class of damage for individual leaf. Statistical analyses were performed with R 3.2.5 software (R Core Team 2016).

4.4 RESULTS

Effects of leaf length on herbivory: we sampled a total of 123 leaves from nine galled plants and eight non-galled plants of *C. phalerata*. In only 16% of the leaves no herbivore damage was found. We recorded 2.197 galls (n = 67 leaves), ranging from 36 to 627 galls per plant, and one to 230 per leaf. All leaves had active and inactive galls. In galled plants, 90% of leaves total were galled, with a high concentration of galls in few leaves (Figure 1A). We found a positive relationship between gall abundance and leaf length classes (Figure 1B). In addition, both the percentage of galled leaves and the average number of galls per leaf showed a positive relation with leaf length of *C. phalerata* (Figure 1C and 1D). The damage by caterpillars also was higher on the largest leaves of *C. phalerata* (Figure 1E).

Herbivory attack from galls insects and caterpillars: most leaves (76%) suffered damage by caterpillars (Figure 2 C). Consequently, about one-half of the leaves showed co-occurrence of damage by both herbivore guilds. In addition, a high percentage (64%) of non-galled presented damages by caterpillars. We found no relationship between gall abundance and damage by caterpillar (Figure 1F). Ever a high percentage (87%) of all the galled leaves suffered damage by caterpillars, these chewing avoided to consume galls structures (Figure 2B).

Histochemical and anatomical traits: histochemical analyses of the intact leaf in *Cyathea phalerata* revealed that phenolic compounds were present of the vascular system, while in galled leaves (Figure 2A), phenolic compounds were concentrated in cell adjacent to the larval chamber (Figure 2E and 2F). Galls promote the breakdown and deformation of the adjacent parenchyma, and generate the process of lignification and suberization, especially those adjacent to the larval chamber (Figure 2D). It is also possible to observe the occurrence of scar tissue in the chamber (Figure 2D). The histochemical and anatomical changes prevented the consumption of caterpillars directly on the galls.

4.5 DISCUSSION

Herbivory on ferns: key species to herbivores: the interactions between ferns and herbivores are poorly understood. However, compilations on the theme already makes it possible to recognize ferns as a group that play important role in trophic interactions in an ecosystem (see Balick et al. 1978, Hendrix, 1980; Mehltreter, 2010). In summary, some ferns exhibit the potential to host many herbivores and/or herbivores from different guilds simultaneously, such as *Pteridium* genus (see Swezey, 1922; Lawton, 1976; Lawton and MarGarvin, 1985) and *C. phalerata* in the present study, respectively, equally many angiosperms species (e.g. Fagundes and Fernandes, 2011). These plants suffers a potential greater impact and represents resources key to herbivores. For ferns, chewing herbivorous seems to be the main predators (Hendrix, 1980), such as caterpillars (Bordner et al. 2010), grasshoppers (Rowell et al. 1983), and ants (*paper under revision*), while galls insects, same with new records recently (e.g. Hanson and Gomez-Laurito, 2005; Maia and Santos, 2015;

Farias et al. 2018), remain inaccurate, being currently considered uncommon. Future studies should look for others key ferns to herbivores and take into consideration approaches on patterns of host plant selection in ferns, as performed for the present study.

Plant Vigor Hypothesis corroborated to *Cyathea phalerata:* This study demonstrates that the herbivore attacks in *C. phalerata* occasioned simultaneously by insects galling and caterpillars are positively related with length leaf, parameter that caused intraspecific variation in leaf damage. Therefore, we corroborated the plant vigor hypotheses predictions proposed by Price (1991). Several studies have demonstrated the importance of plant vigor in selection of host plants by galling herbivores or chewing (see Cornelissen et al. 2008). The plant vigor hypotheses confirmed from leaf length is supported by greater probability of access to the resource (i.e. more apparent) (Faria and Fernandes, 2001) and by offering a better quality resource to herbivorous (Price, 1991). Additionality, the positive relationship between gall abundance and length leaf for the studied system might be also caused by mechanisms of plant tolerance to herbivory exhibit by *C. phalerata*, because there are different generations of galls overlapped, which illustrate subsequent attacks, resulting in a pattern of high gall abundance in some leaves. This result supports that there is no negative effect induced by gallers under their own next generations.

Indirect interactions between galls insects and caterpillars: The high overlap of galls and caterpillars actions in the system studied shows that there is no spatial segregation between guilds exploring *C. phalerata*. However, this does not mean a simultaneous initiation of exploitation. Our data even without a temporal scale allow strong predictions about the interactions between guilds, as well as illustrate scenarios of the possible effects either galls and caterpillars exert starts exploration. First, the occurrence just of galled leaves or cutted leaves demonstrates that the herbivorous attack on the system may initialized by either guild. Second, the co-occurrence pattern demonstrates at first that there is no negative interference in the occurrence under another guild, even some studies reported that herbivorous insects induces increases in secondary chemicals and/or decreases in the nutritional status (Schultz and Baldwin, 1982; Karban and Myers, 1989), changes that potentially negatively affect another guild. For instance, systems from angiosperm species has detained indirectly free-living herbivores from an increase of phenols concentration in galled leaves (Pascual-Alvarado et al. 2007). Like this, as galling insects typically cause a cascade of local and/or systemic changes under the attacked plant (see Isaias et al. 2014), when the exploration of *C. phalerata* is initiated by galls, the high frequency of co-occurrence guilds damages demonstrates a resistance of the caterpillars to any systemic changes. On the other hand, there is still another sense of interaction, when the damage is started by caterpillars, representing our third prevision. From the high proportion of non-galled leaves with damage by caterpillars is possible to predict that at least sometimes occurs a negative influence of chewing under galling herbivores, if caterpillars cause the first damage. The possible explanation for this would be that galling insects avoid leaf damage from cutters, which represent a sign of a higher mortality risk for larvae. The occurrence of natural enemies is one of the most important factors in the ecology of galling insects, influencing selection processes of host plant (Fernandes and Price, 1988; 1992). As consequence, the initial caterpillar's damage causes a reduction of the resources for the gallers in *C. phalerata*. The studied system demonstrated that even galls and caterpillars frequently selecting the same resource spatially, relationships between guilds potentially may influences the selection pattern on *C. phalerata*, depending of the guild that start exploration.

Histochemical and anatomical traits prevented consumption of galls: The caterpillars avoid to consume only the tissue with galls structures. This response can be explained by the anatomical and histochemical alterations showed in the galled leaf tissue of *C. phalerata*. It is not attractive for caterpillars to consume leaf tissue with the highest concentration of phenolic compounds, lignified and suberized around galls. Therefore, avoidance of gall consumption in the studied system demonstrates that caterpillars recognized some barrier during feeding. As an important cause of mortality of larvae of galling insects proposed in some studies is attributed from the death by free-feeding chewing herbivores (Ribeiro and Basset, 2007), the main implication of this avoidance is possibly reflected in the larval survival patterns and/or parasitism on the larvae of galling insects in the system.

The herbivorous damages suffered by *C. phalerata* from galls and caterpillars not segregated temporal and spatially demonstrates the susceptibility of the plant's defensive system. We found that there is no distinction of the relation of the guilds with the length leaf, both preferentially selected larger leaves, as it predicts the plant vigor hypothesis. Our results also indicated that indirect effects mediated to another herbivore may influence (i.e. negatively) on leaf selectivity of *C. phalerata*, and this effect is dependent on the herbivore initializing the damage in the system: galling insects avoid leaves attacked initially by caterpillars.

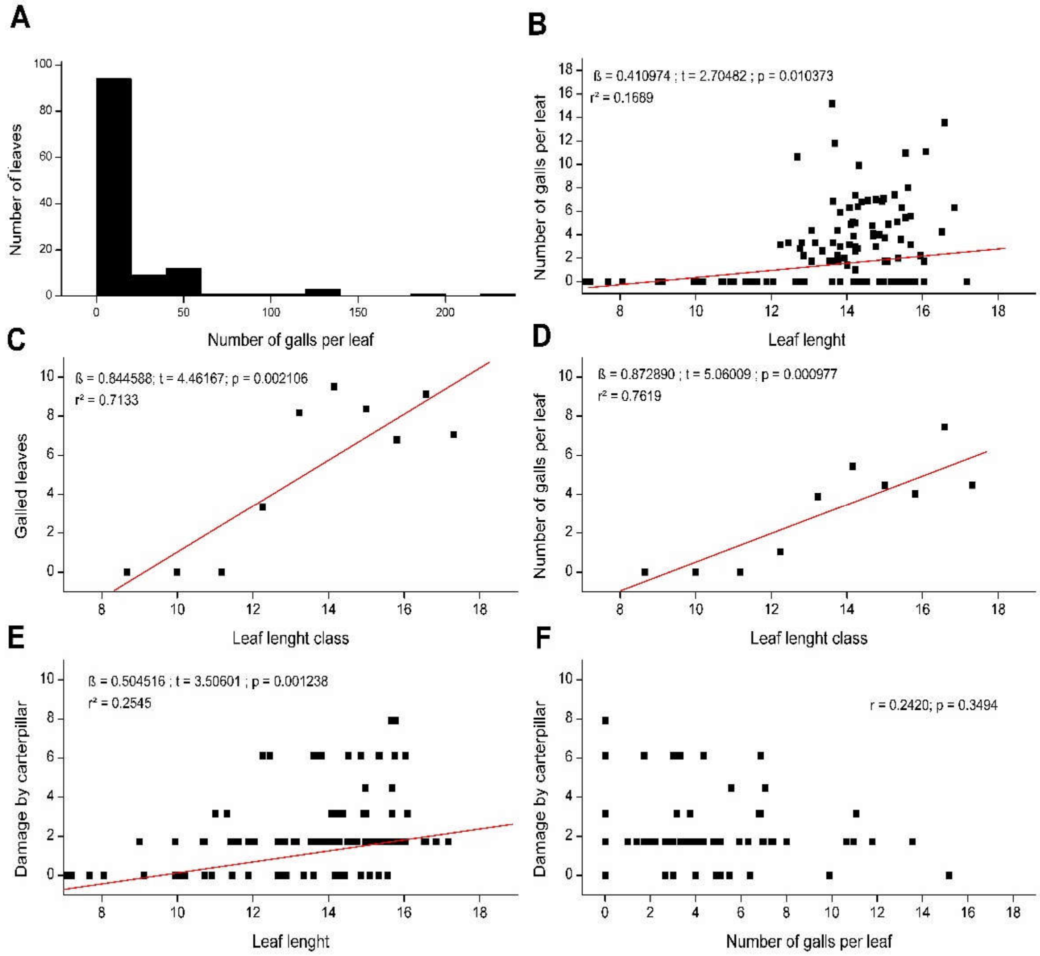

Figure 1. **A** – Distribution of gall abundance in leaves of *Cyathea phalerata* in Brazilian tropical forest. **B** – Relationship between gall abundance and length leaf. **C** – Relationship between percentage of galled leaves and length leaf. **D** – Relationship between average number of galls per length leaf class (cm). **E** – Relationship between damage by caterpillars and length leaf. **F** – Relationship between gall abundance and damage by catterpillars.

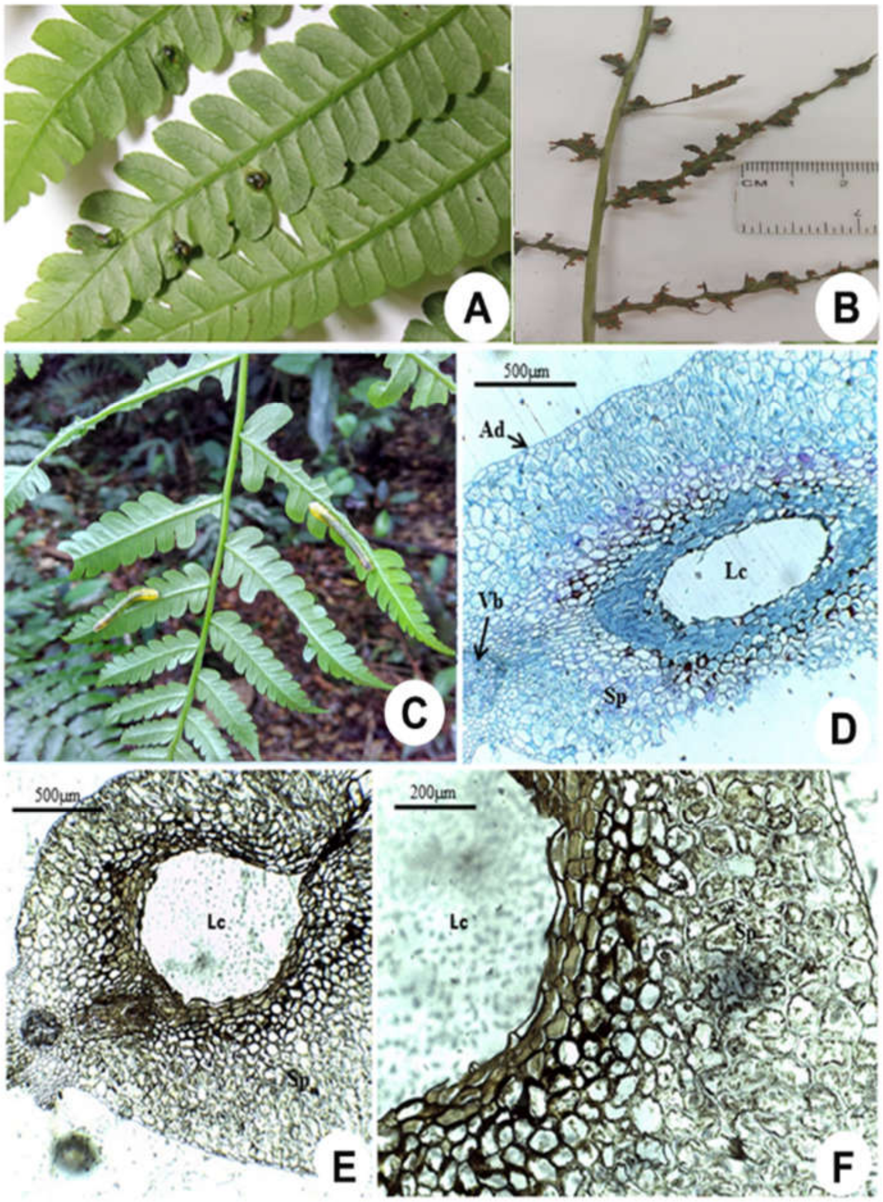

Figure 2. A - Leaf galls induced by Cecidomyiidae in the tree fern *Cyathea phalerata*. B – Galls avoided by catterpillars. C – Catterpillars attacking non-galled leaves. D - D. Gall within parenchymatous tissue. Note the homogenization of the compact and spongy parenchyma (Sp) and vascular bundles adjacent to the larval chamber (Lc). 1E-F. Phenolic compounds (Pc) and starch grains (Sg) in cells adjacent to the larval chamber (Lc).

5 SELECTIVE FERN HERBIVORY BY LEAF-CUTTER ANTS OF *ATTA CEPHALOTES* IN BRAZIL

5.1 ABSTRACT

Although leaf-cutter ants of the genus *Atta* are polyphagous, they discriminate between food plant species. Ferns present the second largest group of vascular plants and are especially abundant in tropical forest understories, but seem to be avoided as food plants. We studied the leaf damage caused by leaf-cutter ants and its relation with water content, trichome density, alkaloids, terpenoids, total phenol and nitrogen content of nine fern species occurring in close vicinity of *Atta cephalotes* (L.) nests. *Meniscium serratum* Cav. presented the highest leaf damage of 32.3%, three other species had a leaf damage of less than 5%, and the remaining five species were undamaged. Food preferences of leaf-cutter ants was related with higher water content (>70%), but independent of any other measured variables. Additional observations from Mexico and Costa Rica provided evidence that a larger number of ferns are regularly attacked by leaf-cutter ants. We suggest that highly damaged fern species might serve them as an occasional, short-term, alternative diet when growing close to ant nests, whereas less damaged fern species might be well-defended, because they were rejected after initial browsing by leaf-cutter ants.

Keywords: *Atta*, Foraging, Herbivores, Phytophagous Insects, Plant-Insect Interactions, Pteridophytes

5.2 INTRODUCTION

Leaf-cutter ants are major herbivores in the Neotropics, particularly in forest ecosystems in which they can remove up to 17% of the annual leaf production (Costa et al. 2008). Heavy attack by leaf-cutter ants may not only reduce plant fitness but even kill plants (Wirth et al. 2003). Harvested leaves are used by the ants to cultivate a symbiotic fungus, which represents the only food source for their larvae (Quinlan and Cherrett 1979). Although leaf-cutter ants are polyphagous and forage on a wide variety of plants, especially angiosperm trees (Wirth et al. 2003), they show clear preferences for some plant species, while avoiding others (Cherrett 1972; Rockwood 1976). Leaf traits, such as nutrient content (Berish 1986), water content (Bowers and Porter 1981), trichome density and latex (Howard 1988), and other chemical components have been considered responsible for food plant selection by leaf-cutter ants (Howard 1987). Avoided plant species, such as ferns, might contain high concentrations of secondary metabolites, such as terpenoids and tannins that are toxic to the ants, their fungus, or both (Pelloto and Martínez 2002).

With about 10.578 species worldwide (PPG I 2016), ferns are the second most diverse group of vascular plants after angiosperms. Although ferns are abundant in tropical forest understories (Sharpe et al. 2010), few mutualistic (see Mehltreter 2010) and antagonistic interactions with ants have been previously reported. For example, only small colonies of *Atta cephalotes* (L.) and *Acromyrmex coronatu*s (Fabricius) (Wetterer 1994, 1995) and a large colony of *Atta colombica* (Guérin-Méneville) (Wirth et al. 2003) have been mentioned to harvest some fern species.

We studied herbivory of leaf-cutter ants on nine terrestrial fern species, occurring next to *A. cephalotes* nests from the understory of tropical forests in Brazil to gain further insights on ant-fern interactions and especially on leaf traits of ferns that may be related with their consumption or rejection by leaf-cutter ants.

5.3 MATERIALS AND METHODS

The study was performed in two tropical rainforest fragments in Brazil, Pernambuco State (site I: 08º35'S, 35º15'W, 700 ha and site II: 08°33'S, 35°10'W, 100 ha). The sites were located on hill tops at less than 100 m above sea level and surrounded by sugar cane plantations. The climate is hot and humid (As) with a mean annual temperature of 24 ºC and annual rainfall of 2100 mm.

In two of the 22 rectangular plots of 10 x 20 m (200 m^2) delimited for the ongoing project "Herbivory and defenses of ferns in Brazilian Tropical Forests", we observed ant-fern interactions, at site I along the forest edge (40 meters from matrix) and at site II along a forest trail, in both cases in close vicinity of ant nests, which were situated inside of the plots. Herbivory was ascertained by direct observations of ants cutting fern leaves or by detection of characteristic leaf damages caused by ants, such as semicircular cuts at the leaf margins (Fig. 1). Leafcutter bees (genus *Megachile* Latreille), which can cause similar leaf damages, do not occur at the study sites (see Raw 2007). Vouchers of fern specimens were collected, identified with specific literature, and deposited at the herbarium UFP. Ants (workers and soldiers) were collected from food plants or ant trails nearby, preserved in 70% alcohol, and sent to experts for identification.

We quantified the frequency (presence/absence) and relative intensity of herbivore damage (0, > 1-5%, > 5-15%, > 15-25%, > 25-50%, > 50-75%, and > 75% of removed leaf area) for each fern species within the plot (Table 1). Only the characteristic semicircular cuts were considered to estimate leaf area loss caused by leaf-cutter ants (Fig. 1).

Leaf traits were evaluated for all nine fern species in the plots. The relative water content of leaves (%) of each species was calculated from the difference between the fresh weight and the dry weight, divided by the initial weight. The trichome density was classified from the coverage of trichomes in grids with area of 4 x 6 mm placed in six points on each side of the leaf, half way between margin and midvein. Values from both sides of the leaf were averaged. The four classes of trichome density were: glabrescent, slightly hairy (1-25% of grids with trichomes), moderately hairy (26-60%) and strongly hairy (above 60%). Alkaloids were determined qualitatively (presence/absence) by use of Dragendorff's test, using extracts of three solvents (hexane, ethyl acetate, and methanol), obtained from 500 mg of leaf material. Qualitative tests (presence/absence) by thin layer chromatography (TLC) were performed for the detection of triterpenoids. For this test, toluene-chloroform-ethanol (40:40:10) was used as mobile phase, lupeol as a standard substance, and anisaldehyde – sulfuric acid for detection. Total phenol content was measured after boiling 100 mg of dry leaf material in 80% methanol, applying a 10% Folin-Ciocalteu reagent (Amorim et al. 2008), and determining absorption of samples at a wavelength of 760 nm (spectrophotometer Genesys 10S UVVIS, Thermo Scientific, Waltham, USA), and compared against a solution of tannic acid (0.1 mg ml^{-1}). Total nitrogen was measured with the adapted Kjeldahl method (Bremmer and Mulvaney 1982). All leaf traits were analyzed for 3-5 replicates per fern species.

Differences between leaf traits of consumed and avoided fern species were compared by t-tests for quantitative traits, after verifying normality (Shapiro-Wilk test, $P < 0.05$). A logistic regression was performed to analyze the relationship between trichome density and the probability of attack by ants. Statistical analyses were carried out with Statistica 7.0 (Statsoft Inc., USA).

5.4 RESULTS AND DISCUSSION

At site I, we observed leaf-cutter ants of *A. cephalotes* harvesting leaves of *Meniscium serratum* Cav. during the day and transporting them to their nest (Fig. 1A). At site II, we observed leaf damages of less than 5% in three fern species: *M. macrophyllum* Kunze (Fig. 1B), *Ctenitis distans* (Brack.) Ching and *Danaea geniculata* Raddi (Table 1), all close to another nest of *A*. *cephalotes*. Five other fern species at the study sites were not damaged by leaf-cutter ants (Table 1).

In *Meniscium serratum*, 80% of the plants were damaged, and ants removed an average of 32.3% of the leaf area (Table 1). Damages on *M*. *serratum* were caused by a single ant colony. In this study, *M. serratum* was the only species with considerably higher damages than the mean herbivory reported for ferns (5.8%; Turcotte et al. 2014) and might lack an effective biochemical defense. Consequently, this fern species might be an alternative food source for leaf-cutter ants to cultivate their fungus or a fast resource of plant sap for ant workers to cover their needs of water, nutrients and energy (Littledyke and Cherrett 1976). The other fern species might be chemically well defended against ant feeding or decomposition by the ant fungus or may even possess fungicidal characteristics. At least, the three fern species with low damages have been initially tasted by the ants, but afterwards found unattractive or repellent.

Water content was the only variable that differed significantly between consumed and avoided fern species ($p < 0.05$, $t = 2.66$). Consumed species had a water content of over 70% with exception of *Cyathea microdonta* (Desv.) Domin (Table 1). In feeding experiments with 13 fern species, laboratory colonies of *Atta mexicana* (F. Smith) also preferred a species (i.e. *Diplazium expansum* Willd.) with high water content (Mehltreter and Valenzuela 2012). In field studies, high water content also increased feeding preferences of leaf-cutter ants (Howard 1987). Because higher water content is also related to younger leaves, softer texture and lower concentrations of tannins and phenols, ants can cut them easier and their fungus benefits from the additional water supply (Cherrett 1972; Bowers and Porter 1981). The other leaf traits varied little among the studied species, with exception of the relatively low N content of *Gleichenella pectinata* (Willd) Ching (Table 1). All species contained terpenoids, but none alkaloids.

Historically, ferns were not considered in the literature as plants that suffer herbivore impact by leaf-cutter ants, even they account for up to 15% of the understory cover in tropical forests (Harms et al. 2004). In our study, we found that leaf-cutter ant damage may be more frequent than previously thought, although only few fern species seem to provide sufficient biomass to sustain repeated heavy consumption of larger colonies of leaf-cutter ants, whose high demand of foliage, estimated in 266 kg year per colony (Herz et al. 2007), drives them mainly to harvest leaves of larger, woody plants (Wirth et al. 2003). Additional observations in forests of Mexico and Costa Rica showed that many more fern species with different growth forms (arborescent, herbaceous, climbers) and at distinct habitats (e.g. terrestrial, epiphytes, epiphytic, riparian) might be consumed by leaf cutter ants (Fig. 1C-F, Table 2, K Mehltreter, unpublished data). Similarly to Brazilian sites, all damaged fern species in Mexico and Costa Rica were situated near nests, demonstrating that the attack on ferns might be conditioned to the proximity of the nests. Plant damages close to ant nests may be consequence of two processes: harvesting or nest management. Ant colonies, especially the smaller ones, prefer short foraging distances and may harvest leaves of appropriate understory fern species for their fungus culture (Wetterer 1994), or they cut plants as part of their environmental nest management to increase temperature and light conditions close to their nests (Farji-Brener and Illes 2000), which are increasingly prevalent in fragmented or edge forests (Wirth et al. 2007). Harvesting is often restricted to the younger, softer leaves and part of the leaves with thick midveins are left intact, whereas cutting for nest management may consist in the complete removal of leaves and leaf pieces are often left at the site rather than transported to the nest (Garrettson et al 1998; Van Gils and Vanderwoude 2012). Because we observed substantial, but partial leaf damage and leaf transport to the nest, we assume that *M. serratum* was indeed harvested for their fungus culture and not attacked for nest management. The leaf damage of less than 5% in three fern species is a typical result of initial trials followed by rejection performed by ant workers to test about 50% to 80% of all available plant species for further selection of appropriate food sources (Cherrett 1989; Wirth et al. 2003). Future studies should investigate the causes of leaf damages and foraging in the vicinity of ant nests and compare the biochemical defense mechanisms and composition of understory fern communities of the Neotropics with the Paleotropics, where leaf-cutter ants are absent.

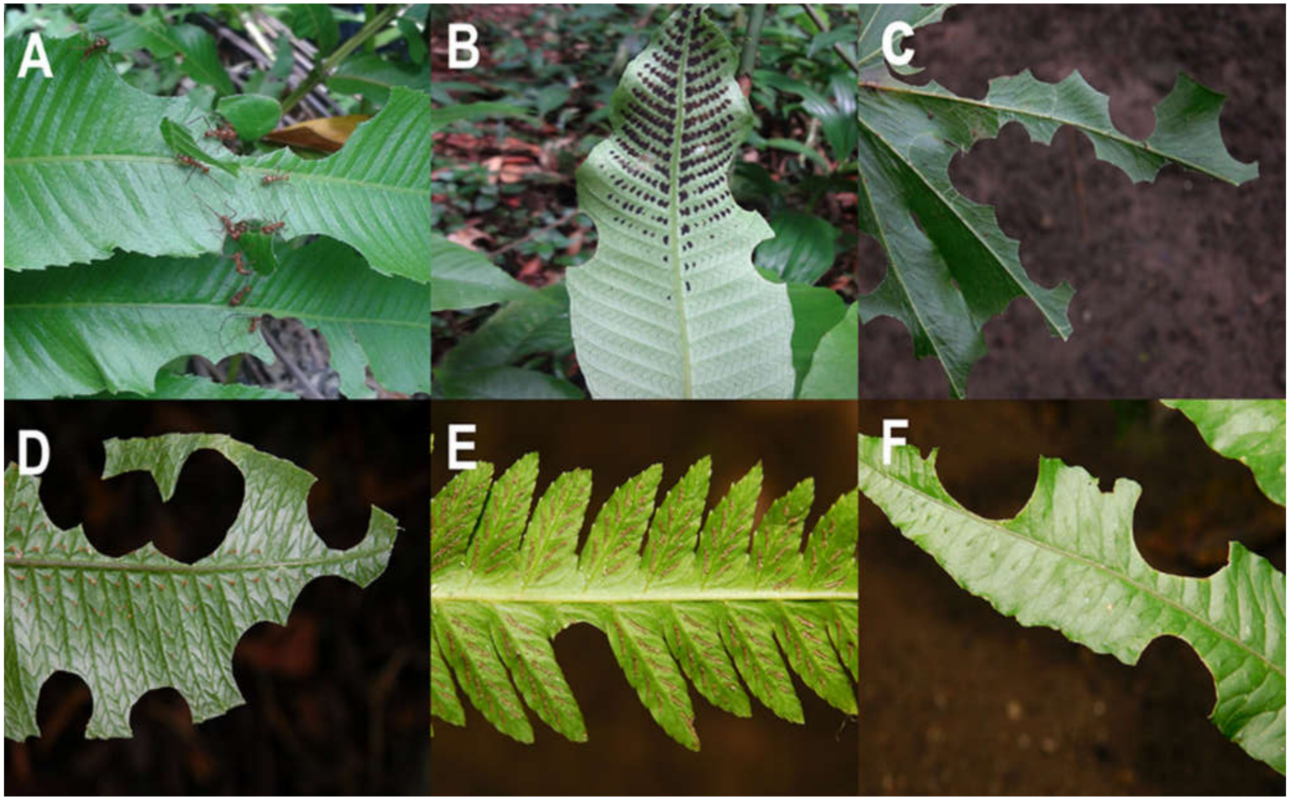

Figure 1. Ferns attacked by leaf-cutting ants from Tropical Forest in Mexico and Brazil. A – *Meniscium serratum*, B – *Meniscium macrophyllum*, C – *Lygodium heterodoxum*, D – *Goniopteris rhachiflexuosa* E – *Diplazium drepanolobium,* F – *Polypodium rhachipterygium*.

Table 1 Leaf damage (%) and leaf traits of nine fern species. In all fern species terpenoids were present and alkaloids absent.

Species	Leaf damage (%)	Water content (%)	Trichome density	Phenols (g kg^{-1} dry matter)	Nitrogen (%)
Blechnum occidentale.	0	69.5	Slightly hairy	30.4	2.2
Ctenitis distans	<5	72.7	Slightly hairy	28.8	2.6
Cyathea microdonta	0	73.3	Slightly hairy	31.1	2.9
Danaea geniculata	<5	78.6	Glabrescent	32.6	2.5
Gleichenella pectinata	0	56.4	Moderately hairy	32.2	1.5
Lindsaea lancea	0	63.8	Glabrescent	26.6	2.4
Meniscium macrophyllum	<5	81.9	Glabrescent	28.5	2.0
Meniscium serratum	32.3	74.0	Moderately hairy	31.2	2.5
Steiropteris polypodioides	0	69.5	Moderately hairy	27.8	2.3

Table 2 List of fern species with observed damages of leafcutter ants. Sites: 1. Montane forest, El Riscal, Mexico (1200 m alt.), 2. Lowland rainforest, Los Tuxtlas, Mexico (200 m alt.), 3. Lowland rainforest, La Selva, Costa Rica (100 m alt.)

Species	Growth form	Habitat	Site
Alsophila firma (Baker) D.S. Conant	Arborescent	Terrestrial	1
Asplenium serratum L.	Herbaceous	Epiphytic/Epipetric	3
Cyathea bicrenata Liebm.	Arborescent	Terrestrial	1
Cyathea divergens Kunze	Arborescent	Terrestrial	1
Cyathea microdonta (Desv.) Domin	Arborescent	Terrestrial	3
Diplazium drepanolobium A.R. Sm.	Herbaceous	Terrestrial	2
Diplazium striatastrum Lellinger	Herbaceous	Terrestrial	3
Goniopteris rhachiflexuosa (Riba) Salino & T.E. Almeida	Herbaceous	Terrestrial	2
Hypolepis blepharochlaena Mickel & Beitel	Herbaceous	Terrestrial	1
Lygodium heterodoxum Kunze	Herbaceous climber	Terrestrial	2
Nephrolepis brownii (Desv.) Hovenk. & Miyam.	Herbaceous	Terrestrial	3
Polypodium rhachipterygium Liebm	Herbaceous	Epipetric/Riparian	2
Serpocaulon triseriale (Sw.) A.R. Sm.	Herbaceous	Epiphytic	3

6 LEAF DEFENSE SYNDROMES IN TROPICAL FERNS

6.1 ABSTRACT

Vascular plants possess defense syndromes, a suite of interdependent defensive leaf traits against herbivores, which may considerably differ between plant groups. Ferns are an abundant component of tropical forest understories and supposedly experience low leaf damage. However, former investigations of relative leaf damages and the number of fern herbivores reported contradictory results and none focused on the underlying defense syndromes. We examined potential defense syndromes of 34 species ferns in Brazilian Tropical Forests, using ten leaf traits. The first three components of a categorical PCA explained 71.74% of the total variation among defensive traits. The first component was related with SLA, water content, nitrogen and phosphorous, the second component with tannins and saponins, but negatively with trichome density, and the third component with phenol concentrations. We identified three groups of fern species with different defensive combination consisting in: (I) high SLA, water content and nitrogen, but all kind of trichome density, (II) lower SLA, water content and nitrogen, high phenol concentrations, and often high trichome density, and (III) glabrous or low trichome density with intermediate values of nutritional quality. Most fern species belonged to group I and III (14 and 16 species, respectively), classified as 'nutritional and defense' syndrome. *Pteridium arachnoideum* is a typical representative of group II, 'low nutritional quality' syndrome, with a powerful defense, including high phenols concentrations and many trichomes. Chemical defenses such as terpenoids, saponins and tannins did not differ among groups. Our results demonstrated that ferns invariably invest heavily in a combination from traits chemical. For most fern species, this chemical expression is the main way to defend their long-lived leaves and with high nutritional quality.

Keywords Defense syndromes, Herbivory, Phenolics, Physical defense, Plant-herbivore interactions, Plant's defensive strategy.

6.2 INTRODUCTION

Herbivores represent one of the main forces of selective pressure on plants (Stamp 2003). Nevertheless, herbivores have to face complex systems of plant defense composed of many variables (Futuyama and Agrawal 2009; Rasman and Agrawal 2009), which may include nutritional quality, regrowth capacity (i.e. tolerance), physical, chemical and phenological traits (Coley and Barone 1996; Ruiz et al. 2008; Lamarre et al. 2014). Traits may serve two or more functions and are considered as defense mechanisms even if this is not their primary function (Strauss and Agrawal 1999). The diversity and combination of distinct defense traits confers plants a generally high partial resistance to herbivores, which restricts annual leaf consumption to an average across all major lineages of vascular plants of 5.3% of the produced leaf tissue (Turcotte et al. 2014). Many studies that have investigated the expression of defense traits and their variation among plant lineages (e.g. Coley et al. 1985; Carmona et al. 2011), have developed evolutionary hypotheses of plant defenses.

The plant defense syndrome hypothesis considers that plants evolved multiple convergent traits simultaneously (i.e. suite of co-varying traits) rather than individual traits (Agrawal and Fishbein 2006), and consequently rejects functional redundancy of any traits, as supported in some studies on trade-off among traits (e.g. Steward and Keeler 1988). The traits are grouped based on palatability and defense status of the plant into three general herbivore syndromes: ‘tolerance or escape’, ‘nutritional and defense’, and ‘low nutritional quality’ (Agrawal and Fishbein 2006). The tolerance or escape syndrome typically occurs in fast-growing plants of resource-rich environments, which are highly palatable and invest little in chemical defenses. In contrast, the nutrition and defense syndrome balances high palatability and an efficient leaf defenses against herbivores, whereas the syndrome of low nutritional quality comprises resource-poor plants that provide little reward for herbivores (Agrawal and Fishbein 2006). The defense syndrome hypothesis has been corroborated for different clades of angiosperms (see Agrawal and Fishbein 2006; Travers-Martin and Müller 2008; Johnson et al. 2014) and gymnosperms (Moreira et al. 2016), as well as within communities from woody species of the Cerrado Brazilian (Silva and Batalha 2011). Nevertheless, the generality and variability of the described defense syndromes has not been tested for ferns.

The lineage of ferns (Polypodiopsida) is supposedly equipped with an array of efficient biochemical defenses, which have been considered the main reason for their high resistance against herbivores (Soeder 1985; Page 2002). There is little information about the role of physical defense traits in ferns. This group is the second-most diverse lineage of vascular plants, with about 10.578 species worldwide (PPG I 2016), and is especially abundant in tropical forests (Sharpe et al. 2010), where they can cover up to 15% of the understory (Harms et al. 2004). In general, ferns have a long leaf lifespan (Mehltreter and Sharpe 2013). Although ferns and angiosperms are sister groups of the same evolutionary age and most modern ferns (e.g. Polypodiales) evolved after the angiosperms (Schneider et al. 2004), ferns have been suggested to support only a small number of interactions with herbivorous insects (Balick et al. 1978; Mehltreter 2010). Ferns have been mainly ignored during the development of theories of plant-herbivore interactions. Studies of fern-herbivore interactions have primarily reported species interactions (e.g. Mehltreter et al. 2003), measured levels of herbivore damage (Mehltreter et al. 2006) and their effects on plants (e.g. Mesipuu et al. 2009), and evaluated the role of nectaries to provide leaf protection by ants (e.g. Koptur et al. 2013). Here, we investigate the defense syndromes of ferns for a better understanding of the combination of their structural, physical and chemical defenses. The main goal of this study was to examine the presence, composition and similarity of potential defense syndromes in tropical ferns.

6.3 METHODS

Study sites and fieldwork: This study was performed on 34 species of ferns at three Tropical Forests located in the State of Pernambuco, Northeastern Brazil. Study sites are classified as Lowland Forest (I – 8º34'S; 35º7'W, below 100 m a.s.l.) and Submontane Forest (II – 8°29´S; 35°41’W, III – 7°35’S; 35°29’S, between 450-550 m a.s.l.). The climate is hot and humid with a mean annual temperature of 22–24ºC and annual rainfall of 1200–1800 mm. The forests have canopy between 15–25 m height and display significant floristic richness, demonstrated from sites II and III that already inventories performed, where are listed 400 and 220 angiosperms, and 90 and 93 ferns, respectively.

A total of 22 rectangular plot of 10 x 20 m (200 m^2) were established, based on the following criteria: presence of ferns, with a minimum distance of 40 m between them. In each plot, all fern species were sampled, independently of abundance. We choose sampling within plots because ferns are plants with restricted distribution to preferred habitats (e.g. shaded environments, moist conditions, semi-shade, wet substrate, well-drained slopes and ravines), so we guarantee to find the species (i.e. sample units), and can define the sampling effort and have some means to randomize of the selected species. For each species, we collected 10–15 fully expanded, undamaged leaves from ten mature (=spore-producing) individuals (Cornelissen et al. 2003). Our decision in sampling fully expanded rather than young developing leaves was made to obtain results of the defensive leaf traits that are acting during most of the time they are exposed to herbivores, and because tropical ferns have on average longer life spans (mean 19.2 months) than seed plants also in tropics (mean 10.0 months) (Wright et al. 2004; Mehltreter and Sharpe 2013; Farias et al. 2015).

Measuring leaf traits: Only the specific leaf area (SLA) was measured on fresh material. Then, leaves were dried at 50ºC for 72 hours, ground, and kept in dry conditions until further chemical analyses. We measured ten leaf traits: SLA, water content, trichome density, nitrogen, phosphorus, alkaloids, phenol concentration, saponins, tannins and terpenoids. These traits have been widely recognized as valuable indicators of plant defense (Hanley et al. 2007).

The SLA was obtained by dividing the one-sided area of a fresh leaf (cm^{-2}) by dry weight (mg^{-1}). The water content (%) was calculated as the difference of fresh weight and dry weight (mg^{-1}), divided by fresh weight, and multiplied by 100 (Pérez-Harguindeguy et al. 2013). The trichome density was determined from the coverage of trichomes in grids with area of 4 x 6 mm placed in six points on each side of the leaf, half way between margin and midvein, analyzed under the stereomicroscope. The four categories of trichome density were: glabrescent, slightly hairy (1-25% of grids with trichomes), moderately hairy (26-50%) and strongly hairy (> 50%).

Leaf nitrogen and phosphorous concentrations were obtained by analyzing samples following the adapted protocols of Kjeldahl (Bremmer and Mulvaney 1982) and methods from Bezerra-Neto and Barreto (2011), respectively.

Initially, extracts of three solvents (hexane, ethyl acetate and methanol) were obtained from 500 mg of leaf material. Then, we performed chemical qualitative tests to alkaloids (Dragendorff's reactions), saponins (boiling distilled water, shaken vigorously and addition HCL), and tannins (ferric reaction), as described by Mattos (1997) from all three types of extracts. Qualitative tests by thin layer chromatography (TLC) were performed for the detection of triterpenoids. For the latter test, we used toluene-chloroform-ethanol (40:40:10) as mobile phase, lupeol as a standard substance, and anisaldehyde – sulfuric acide for triterpenoid detection (Wagner and Bladt 1996). Phenol concentrations were obtained after mid-boiling 100 mg of dry leaves in 80% methanol, using a 10% Folin-Ciocalteu reagent according to Amorim et al. (2008), and interpolation of the samples' absorbance against a calibration curve of tannic acid (0.1 mg/ml^{-1}) in a spectrophotometer (Genesys 10S UVVIS, Thermo Scientific, Waltham, USA) calibrated at a wavelength of 760 nm.

Data analysis: We examined the relationships among all defense traits using a Categorical Principal Components Analysis (CATPCA). This analysis is particularly useful for data sets with a combination of nominal, ordinal, and numeric variables that might be nonlinearly correlated to each other (Jolliffe 2002). We used the variable principal normalization to the dataset as it optimizes the association between variables. The component loadings (i.e. correlations between the quantified variables and the principal components) were used as a descriptor for traits that contributed most to the variation of each axis, reporting values > 0.5. We reported the first three principal components because these axes were associated with eigenvalues >1, according Kaiser's criterion (Legendre and Legendre 2012). The analyses was performed using SPSS software version 22.0 (SPSS Inc. Chicago, USA).

We examined the similarity of traits among species by performing a hierarchical cluster analysis, using Euclidian distances and Ward's method for linkage. Differences of traits between formed clusters were evaluated by means of a t-test. Data were analyzed using R v.3.3.3 (R Development Core Team 2009).

6.4 RESULTS

Leaf traits of ferns were grouped into three principal components, which explained 71.74% of the total variation (CATPCA, Table 1). The first component had positive loadings for SLA, water content, nitrogen and phosphorous, the second component showed negative loadings for trichome density and positive loadings for the presence of tannins and saponins (Table 2, Figure 1), and the third component had positive loadings for phenol concentrations (Table 2). Of the ten defense traits measured (Table 1), two did not showed any variance: alkaloids were absent and terpenoids present in all 34 studied species.

From hierarchical cluster analysis of defense traits, we identified three species groups with different defense combination (Figure 2). The first group included 14 species with higher SLA, water content and nitrogen, but all kind of trichome density (Table 3, Figure 2). The second group accommodated four species with lower SLA, water content and nitrogen, high phenol concentrations, and often high trichome density (Table 3, Figure 2). The third group consisted of 16 glabrous and slightly hairy species with intermediate values of nutritional quality (Table 3, Figure 2). Terpenoids, saponins and tannins did not differ among the three groups (Table 3).

6.5 DISCUSSION

In tropical fern species studied here, leaves are defended by syndromes, invariably formed by the strong expression of chemical traits (i.e. terpenoids, tannins, saponins, phenols content). Some plants besides possessing simultaneously chemical traits expressed morphological defense traits such an abundance of trichomes, potentially combined with lower SLA, water content and nitrogen (low nutritional quality). Our results corroborate that ferns present similar defense syndromes than the ones previously reported for seed plants (see Agrawal and Fishbein 2006; Travers-Martin and Müller 2008; Johnson et al. 2014; Moreira et al. 2016), and that in both plant groups physical traits act in conjunction with chemical defenses (Hanley et al. 2007; Carmona et al. 2011). Therefore, the defense strategy of ferns is not built exclusively on chemical traits, sole defensive type previously studied and that has been considered an efficient barrier against herbivores of the lineage (see Balick et al. 1978; Cooper-Driver 1985).

Based on the possible general defense syndrome triangle proposed by Agrawal and Fishbein (2006), fern species sampled may be grouped into two defense syndromes: ‘low nutritional quality’ (group II, n = 4) and ‘nutritional and defense’ (groups I and III, n = 30). The defense syndrome consisting of ‘low nutritional quality’ exhibited in few ferns has been reported for slow-growing gymnosperms (Moreira et al. 2016), woody species in areas with poor soils (Silva and Batalha 2011) and evergreen trees of dry tropical forests (Pringle et al. 2011). Plants with this strategy typically show low SLA, water content, nutrients (e.g. nitrogen and/or phosphorous) and potentially chemical traits, which together represent predictors of the resistance, because are important in host-plant choice (acceptability) and host-plant suitably for herbivores. In general, insect selection depends on its growth performance, which usually is correlated with physical and chemical traits related to plant quality (Scriber 2009). The low concentrations nitrogen and phosphorus, for example, affects negatively the performance of insects (see Mattson 1980; Stamp and Casey 1993; Sankaran and McNaughton 2005; Pennings and Simpson 2008) due to the high requirements of these nutrients for the bodily tissues them and metabolic process involved in genetic codification, growth and fecundity (Perkins et al. 2004; Huberty and Deno 2006; Lemoine et al. 2014). The ‘low nutritional quality’ as a potential defense against herbivorous insects is especially active if it complemented with chemical traits, because herbivores can compensate for this deficiency by a longer foraging time (Haukioja et al. 1991; Augner 1995). In the ferns there is this complementarity, activing a syndrome ‘low nutritional quality’. On the other hand, plants with syndrome ‘nutritional and defense’ seems depends on the efficiency of chemical traits and some cases combined with trichomes to prevent loss of leaf tissue and other damage caused by herbivorous attack, strategy prevalent in ferns studied.

The syndrome tolerance/escape proposed by Agrawal and Fishbein (2006) cannot be detected using our approach. Few studies have incorporated traits related to growth and tolerance in the context of syndromes, required a long time and experimental approaches (e.g., Strauss and Agrawal 1999; Turley et al. 2013). In fact, our knowledge is still limited to estimating this strategy in ferns. As for the escape strategy in ferns, we have evidence only from the tree fern species *Alsophila firma* (Baker) D. S Conant that show a phenological patters characterized by leaflessness to avoid higher herbivore pressure of the wet season (Mehltheter and García-Franco 2008). This strategy seems require high energetic cost due a total annual leaf turnover and should have resulted from specialist's herbivores pressure. However, an approach on young leaves may reveal the occurrence of tolerance or escape syndrome for many ferns during this ontogenetic stage, because equally to seed plants with this strategy (see Kursar and Coley 2003) tropical ferns expand their leaves rapidly (i.e. proxy to growth) and have high leaf synchrony, finished or concentrated during the first month from the emergency (Sharpe 1997; Farias et al. 2015). Nevertheless, the question to be investigated in the future is how ferns vary chemically during leaf development, because to plants to be incorporated into the syndrome escape/tolerance typically shows lower chemical power, as consequence of the resource displacement for rapid leaf expansion (Kursar and Coley 2003).

The presence of trichomes represents one of the first barriers of defense against insects, making access to the epidermis more difficult for consumption. In addition, trichomes are composed of cellulose (Betancur et al. 2010) and other substances of low nutritional value, being disadvantageous their consume or removal for insects (Dalin et al. 2008). Trichomes also act interfering the movement and oviposition of insects on the leaf surface, and some cases entrap or even puncture small insects (Eisner et al. 1998). Therefore, the lower trichome density can make plants more palatable to herbivores (Hanley et al. 2007; Dalin et al. 2008). Trichomes showed a defensive additive effect for some ferns of both defense syndromes, representing a trait of less importance in the differentiation of species defenses. This additive effect supports a powerful level of defense for group II ferns, which even with low nutritional quality and chemical traits, invest in high amounts of trichomes. The most likely explanation for the expression of this multiple set of defense is that the three fern species of group II (*Anemia villosa*, *Gleichnella pectinata* and *Pteridium arachnoideum*) occur typically in sunny, dry habitats, such as clearings and forest edges (Mickel and Smith 2004), which favors carbon-based physical and chemical traits, as reported in seed plants (Tuomi et al. 1987). Conversely, few species of group I (≅7%) with high nutritional quality, without tannins and saponins also might be strongly covered by trichomes. Therefore, the presence of trichomes in ferns occurs independently of other chemical and physical traits of resistance, equally predict by Moreira and Pearse (2017) from oak species (*Quercus* genus). The expression of trichomes with any combination of defensive traits in ferns may be supported by their multifunctional role (see Dalin et al. 2008). Consequently, the expression of trichomes on plants is influenced by diverse biotic or abiotic variables, as herbivore pressure (Agrawal 2000) and availability of light, causing direct or indirect effects on others (Molina-Montenegro et al. 2006).

The higher concentration of phenols in ferns of group II, those with low nutritional quality, differs from some studies that have reported the investment in chemical defenses declining in many species with high expression in physical defense, as leaf toughness (low SLA) and trichomes (Hanley et al. 2007). However, others plants may shows an opposite covariation, with a positive correlation between leaf toughness and phenolic concentration (Read et al. 2009), similarly ferns of group II. As these ferns are under more light incidence (Mickel and Smith 2004), this result is likely to be attributed to habitat due the phenol production is positively correlated with light (Mole et al. 1998). Regardless of the causes, the higher phenol concentration provide to ferns of group II hard barrier against to herbivorous attack.

Our data corroborate the presence of defense syndromes in tropical ferns. In particular, ferns invariably invest heavily in a combination from traits chemical. For most species, this chemical expression is the main way to defend their long-lived leaves and with high nutritional quality. Nevertheless, trichomes constitute a flexible trait and some ferns species of well-defended chemically also express trichomes. Few ferns, such as the well-know *Pteridium arachnoideum*, exhibited powerful level of defense, because combine chemical traits, many trichomes with low quality nutritional. Future research should explore the relation between defense syndromes and herbivore levels to understand what changes in leaf traits affects activity of herbivorous on ferns.

Table 1. Defense traits from 34 ferns species in Tropical Forest. Groups I, II and III – represented by species with syndromes: high quality nutritional and chemical defense or low quality nutritional and chemical defense, respectively. Mean values for SLA (g/cm^2), Water (%), Nitrogen (%), Phosphorous (g/kg^{-1} MS) and Phenols (g/kg^{-1} MS) (mean ± SD). Ordinal values to Trichomes: 0 – Glabrescent, 1 – Slightly hairy, 2 – Moderately hairy and 3 – Strongly hairy. Presence (1) or absence (0) of Tannins and Saponins.

Species	**Group**	**SLA**	**Water content**	**N**	**P**	**Trichomes**	**Phenols**	**Tannins**	**Saponins**
Anemia hirta	I	303.70	52.49	1.98	0.84	2	33.63	1	0
Christella hispidula	I	364.41	74.63	2.40	0.79	3	26.31	0	0
Cyathea phalerata	I	282.18	78.11	2.77	0.83	0	30.64	1	1
Cyathea microdonta	I	395.06	76.64	2.88	0.93	1	31.15	1	1
Ctenitis distans	I	270.32	66.22	2.57	0.77	1	28.80	1	1
Didymochlaena truncatula	I	262.30	74.41	3.21	0.89	1	28.30	0	1
Diplazium cristatum	I	306.06	79.01	3.50	0.99	0	26.89	0	0
Goniopteris biolleyi	I	368.30	72.70	4.09	0.89	2	28.18	1	1
Meniscium macrophyllum	I	298.681	81.90	2.02	0.78	0	28.52	1	1
Meniscium serratum	I	252.54	73.98	2.47	0.93	2	31.19	1	1
Serpocaulon sp.	I	283.20	75.64	2.49	0.76	3	27.64	0	0
Steiropteris polypodioides	I	251.817	68.72	2.25	0.83	2	27.78	1	1
Tectaria incisa	I	520.50	83.27	3.22	0.99	1	30.83	1	1
Triplophyllum dicksonioides	I	267.06	70.38	3.08	0.78	1	26.29	1	1
Anemia villosa	II	68.79	47.35	2.03	0.83	3	39.79	1	1
Elaphoglossum iguapense	II	107.06	71.42	1.62	0.80	0	28.47	1	1
Gleichenella pectinata	II	85.17	56.44	1.49	0.74	3	32.25	1	1
Pteridium arachnoideum	II	102.76	66.72	1.67	0.85	3	30.00	1	1
Adiantopsis radiata	III	199.28	60.31	3.12	0.79	1	41.71	1	1
Blechnum occidentale	III	223.04	72.56	2.20	0.83	1	30.38	1	1
Cyathea praecincta	III	242.00	76.06	2.13	0.84	0	28.55	1	1
Cyclodium meniscioides	III	195.23	80.43	2.01	0.85	1	28.76	1	0
Danaea geniculata	III	216.90	78.37	2.63	0.82	0	33.03	1	1
Danaea nodosa	III	231.71	76.00	1.58	0.82	0	29.66	0	1
Lindsaea lancea	III	199.87	63.09	2.44	0.75	0	26.55	0	1
Lindsaea quadrangularis	III	193.37	63.52	2.43	0.77	0	28.23	1	1
Metaxya parkeri	III	192.55	69.94	1.88	0.74	1	31.23	1	1
Neoblechnum brasiliense	III	229.29	76.37	2.11	0.84	0	29.83	1	1
Olfersia cervina	III	224.07	78.76	1.98	0.83	0	36.73	1	1
Pecluma robusta	III	145.06	60.40	2.89	0.89	0	50.37	1	1

Polybotrya osmundaceae	III	237.62	71.49	2.32	0.76	0	27.70	1	1
Saccoloma elegans	III	210.91	73.52	3.18	0.83	0	27.95	1	1
Salpichlaena volubilis	III	169.18	73.55	2.02	0.76	0	26.08	1	1
Telmatoblechnum serrulatum	III	220.66	68.85	1.90	0.83	0	32.13	1	1

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Table 2. Relationships among defense traits from 34 ferns species in Tropical Forest, using a Categorical Principal Components Analysis (CATPCA). Variance Accounted of the first three components with eigenvalues >1 and values loadings (i.e. coefficients of correlation between variables and components) to each trait. Values with * symbol were considered significant.

		Component 1	**Component 2**	**Component 3**
Variance Accounted		33.19%	22.47%	16.06%
Eigenvalues		2.65	1.79	1.28
SLA	**Values loadings**	0.86*	0.01	0.05
Water content		0.72*	-0.12	-0.46
Nitrogen		0.68*	0.24	0.37
Phosphorous		0.64*	0.29	0.46
Trichrome		-0.40	-0.62*	0.35
Phenols		-0.38	0.47	0.67*
Tannins		-0.31	0.72*	-0.12
Saponins		-0.27	0.71*	-0.33

Table 3. Trait values for three groups from 34 ferns species in Tropical Forest (mean ± SD). Groups I and III (n = 14 spp, n = 16 spp.) – Syndrome of high nutritional quality and chemical defense and Group II (n = four spp) – Syndrome of low nutritional quality and chemical defense. T-Tests performed to SLA, water content, nitrogen, phosphorous and phenols. Letters indicate significant differences between groups ($p < 0.05$).

Defensive Traits	**Group 1**	**Group II**	**Group III**
SLA	316.15 ± 74.12 ^{a}	90.94 ± 17.55 ^{b}	208.17 ± 25.82 ^{c}
Water content	73.43 ± 7.65 ^{a,c}	60.48 ± 10.76 ^{b}	71.45 ± 6.56 ^{c}
Nitrogen	2.78 ± 0.59 ^{a}	1.70 ± 0.23 ^{b}	2.30 ± 0.45 ^{c}
Phosphorous	0.85 ± 0.08 ^{a}	0.80 ± 0.04 ^{a}	0.81 ± 0.04 ^{a}
Trichomes	Absent, slightly, moderately or strongly hairy	Majority strongly hairy	Absent or slightly hairy
Phenols	29.01 ± 2.16 ^{a}	32.62 ± 5.02 ^{b}	31.80 ± 6.34 ^{a,b}

***The frequency of tannins and saponins did not differ between groups.**

Figures

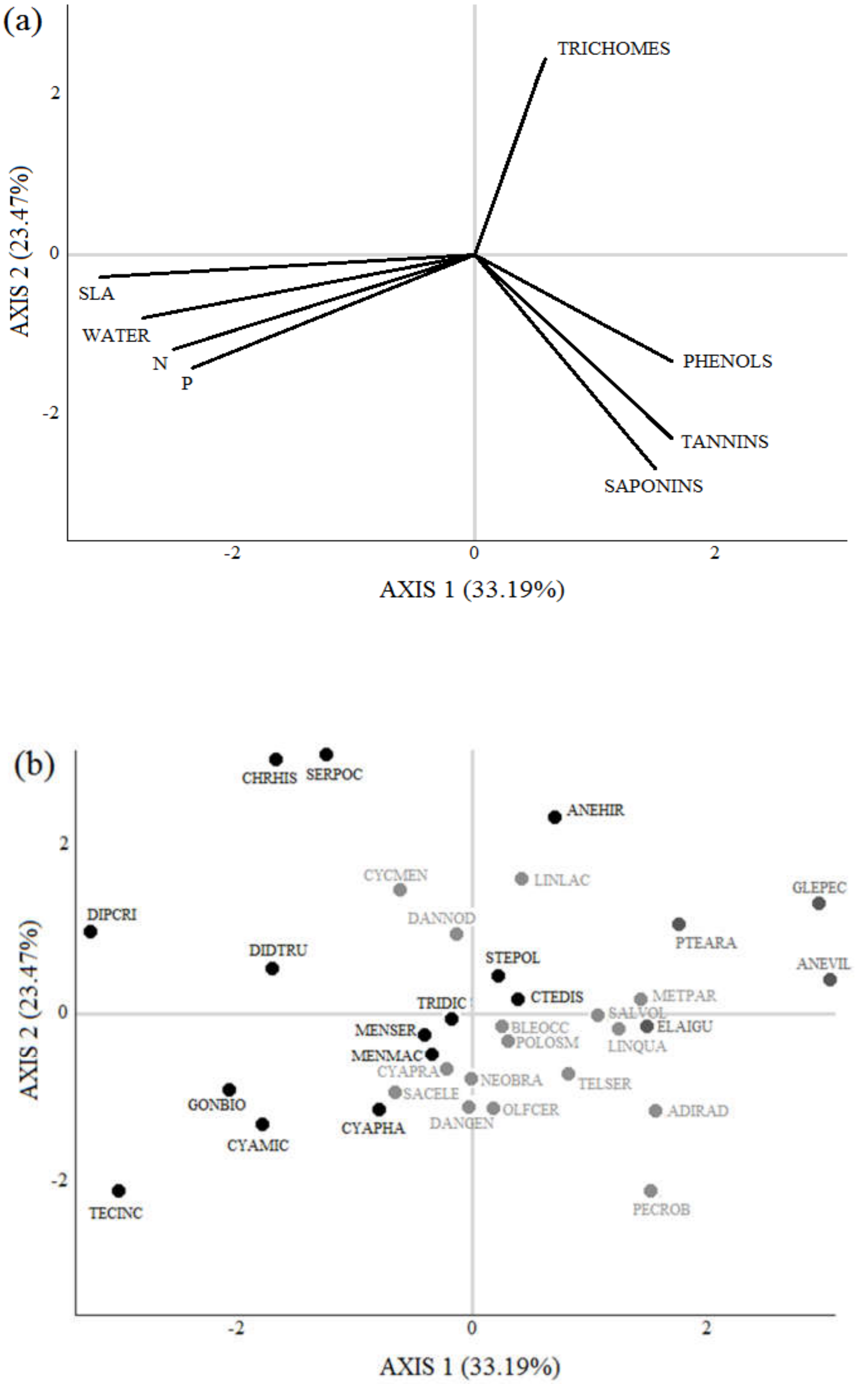

Figure 1. The Categorical Principal Components Analysis (CATPCA) biplot showing (a) the relationships between defensive traits from 34 ferns species in Brazilians Tropical Forest (b) and the species coordinates arrayed on the first two axes. Significate loadings: axis 1 – SLA, water content, nitrogen and phosphorous (+), axis 2 – trichrome (-), tannins and saponins (+), axis 3 – phenol content (-). Species codes are detailed in Table 1.

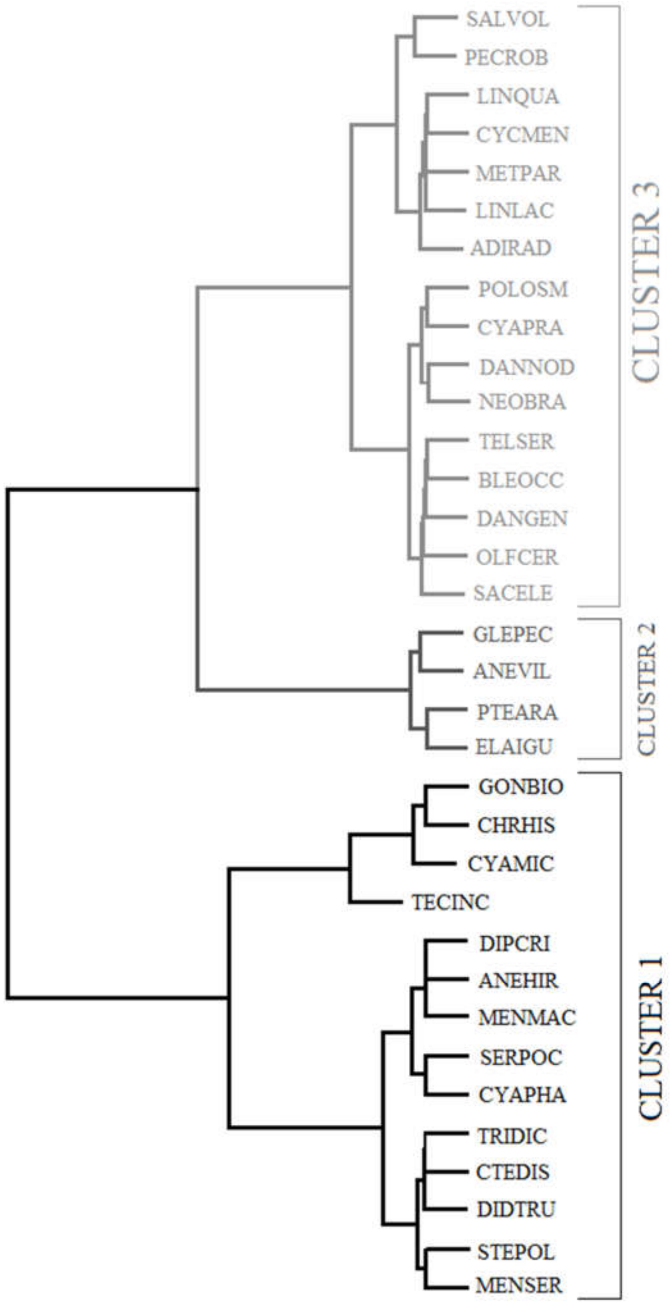

Figure 2. Species clustering for defense traits from 34 ferns species in Brazilians Tropical Forest, based on Ward's grouping linkage method and Euclidian distances. Species codes are detailed in Table 1.

7 CONSIDERAÇÕES FINAIS

Essa Tese demonstrou a importância das samambaias nas interações tróficas dos ecossistemas tropicais. Pesquisas como esta tem modificado o pensamento bastante difundido que samambaias não possuem interações com herbívoros. As contribuições aqui apresentadas atualizam o estado da arte das pesquisas envolvendo samambaias e herbívoros e permitem recomendações para futuros estudos, a seguir:

I – A interação entre samambaias e galhas ainda subestimada na linhagem é incrementada com um novo registro a partir da espécie arborescente *Cyathea phalerata*. Novos registros de galhas em samambaias devem ocorrer com a continuação do esforço de amostragem. Novos registros permitirão uma estimativa da proporção de samambaias que interagem com galhas, análises evolutivas e descrições de novas espécies de galhadores. Adicionalmente, esses novos registros devem ser usados como modelo para estudos ecológicos.

II – Samambaias podem abrigar mais de uma guilda de herbívoros simultaneamente. Nesse caso, efeitos indiretos podem afetar negativamente uma outra guilda. Análises anatômicas e histoquímicas demonstraram mudanças a partir da indução de galhas em tecidos foliares. Essas mudanças atuam como defesas as larvas dos galhadores. Futuras pesquisas nesse contexto devem focar em específicas mudanças químicas causadas após ataque herbívoro.

III – Samambaias interagem de modo antagônico com formigas cortadeiras. Futuras pesquisas devem concentrar nos efeitos pós ataque de formigas nas samambaias.

IV – Samambaias expressam múltiplos traços de defesas contra herbívoros. Algumas espécies possuem defesas físicas e baixa qualidade nutricional. A combinação de traços químicos é invariável na linhagem. Futuras pesquisas devem testar diferenças na efetividade de estratégias defensivas antiherbivoria.

REFERÊNCIAS

ADLER, L. S.; SEIFERT, M. G.; WINK, M.; MORSE, G. E. Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecology Letters*, v. 15, p. 1140–1148, 2012.

AGRAWAL, A. A. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos*, v. 89, p.493–500, 2000.

AGRAWAL, A. A.; FISHBEIN, M. Plant defense syndromes. *Ecology*, v. 87, p. 132–149, 2006.

AGRAWAL, A. A. Macroevolution of plant defense strategies. *Trends in Ecology & Evolution*, v. 22, p. 103–109, 2007.

AGRAWAL, A. A.; SALMINEN, J. P; FISHBEIN, M. Phylogenetic trends in phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation. *Evolution*, v. 63, p. 663–673, 2009.

AIDE, T. M. Patterns of leaf development and herbivory in a tropical understory community. *Ecology*, v. 74, p. 455–466, 1993

ALMEIDA, F. V. M.; SANTOS, J. C.; SILVEIRA, F. A. O; FERNANDES, G. W. Distribution and frequency of galls induced by *Anisodiplosis waltheriae* Maia (Diptera: Cecidomyiidae) on the invasive plant *Walteria indica* L. (Sterculiaceae). *Neotropical Entomology*, v. 35, p. 435–439, 2006.

AMORIM, E. L. C.; NASCIMENTO, J. E.; MONTEIRO, J.M.; SOBRINHO, T.J.S.P.; ARAÚJO, T.A.S.; ALBUQUERQUE, U. P. A simple and accurate procedure for determination of tannin and flavonoid levels and some applications in ethnobotany and ethnopharmacology. *Functional Ecosystems and Communities*, v. 2, p. 88–94, 2008.

ARENS, N.C.; SMITH, A.R. *Cyathea planadae*, a remarkable new creeping tree fern from Colombia, South America. *American Fern Journal*, v. 88, p. 49–59, 1998.

AUGNER, M. 1995. Low nutritive quality as a plant defence: effects of herbivore-mediated interactions. *Evolutionary Ecology*, v. 9, p. 605–616, 1995.

BALICK, J.; FURTH, D.G.; COOPER-DRIVER, G. Biochemical and evolutionary aspects of arthropod predation on ferns. *Oecologia*, v. 35, p. 55–89, 1978.

BECERRA, J. X. Insect on plants: macroevolutionary chemical trends in host use. *Science*, v. 276, p. 253–256, 1997.

BECERRA, J. X. Synchronous coadaptation in an ancient case of herbivory. *Proceedings of the National Academy of Sciences of the United States of America*, v. 100, p. 12804–12807, 2003.

BERA, S.; GHORAI, N. On the occurrence of mite gall caused by *Trioza fletcheri* (Eriophyidae) on *Angiopteris evecta* Hoff. (Angiopteridaceae) from Andaman Islands. *Indian Fern Journal*, v. 16, p. 15–18, 1999.

BERA, S.; GHORAI, N. On the occurrence of mite induced stem gall in *Odontosoria chinensis* (L.) J. Smith (Dennstaedtiaceae) from Darjeeling, West Bengal. *Indian Fern Journal*, v. 14, p. 103–105, 1997.

BERA, S.; GHORAI, N.; RAUT, S.K. Cynipid gall in *Selaginella monospora* Spring. *Indian Fern Journal*, v. 11, p. 146–149, 1994.

BERISH, C. W. Leaf-cutting ants (*Atta cephalotes*) select nitrogen-rich forage. *The American Midland Naturalist*, v. 115, p. 268–276, 1986.

BERNAYS, E. A.; CHAPMAN, R. F. *Host-plant selection by phytophagous insects*. Chapman and Hall, New York, 1994.

BETANCUR, L.; SINGH, B.; RAPP, R. A.; WENDEL, J. F.; MARKS, M. D.; ROBERTS, A. W.; HAIGLER, C. H. Phylogenetically distinct cellulose synthase genes support secondary wall thickening in *Arabidopsis* shoot trichomes and cotton fiber. *Journal of Integrative Plant Biology*, v. 52, p. 205–220, 2010.

BEZERRA NETO, E.; BARRETO, L. P. *Análises químicas e bioquímicas em plantas*. UFRPE, Recife, 2011.

BLANTON, C. M.; EWEL, J. J. Leaf-Cutting ant herbivory in successional and agricultural tropical ecosystems. *Ecology*, v. 66, p. 861–869, 1985.

BOCH, S.; BERLINGER, M.; FISCHER, M.; KNOP, E.; NENTWIG, W.; TURKE, M.; PRATI, D. Fern and bryophyte endozoochory by slugs. *Oecologia*, v. 172, p. 817–822, 2013.

BOCH, S.; BERLINGER, M.; PRATI, D.; FISCHER, M. Is fern endozoochory widespread among fern-eating herbivores? *Plant Ecology*, v. 217, p. 13–20, 2016.

BODNER, F.; BREHN, G, HOMEIER, J.; STRUTZENBERGER, P.; FIEDLER, K. Caterpillars and host plant records for 59 species of Geometridae (Lepidoptera) from a montane rainforest in southern Ecuador. *Journal of Insect Science*, v. 67, p. 1–22, 2010.

BOEGE, K.; MARQUIS, R. J. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution*, v. 20, p. 441–448, 2005.

BOWERS, M. A.; PORTER, S. D. Effect of foraging distance on water content of substrates harvested by *Atta columbica* (Guerin). *Ecology*, v. 62, p. 273–275, 1981.

BREMNER, J. M.; MULVANEY, C. S. Nitrogen total em Page AL (ed) Methods of soil analysis, 1st edn. *Soil Science Society of America*, Madison, p. 595–624, 1982.

BRUES, C. T. The selection of food-plants by insects, with special reference to lepidopterous larvae. *The American Naturalist*, v. 54, p. 313–332, 1920.

BUKATSCH, F. Bemerkungen zur Doppelfärbung Astrablau-Safranin. *Microkosmos*, v. 61, p. 255, 1972.

CÁRDENAS, R. E.; VALENCIA, R.; KRAFT, N. J. B.; ARGOTI, A.; DANGLES, O. Plant traits predict inter- and intraspecific variation in susceptibility to herbivory in a hyperdiverse Neotropical rain forest tree community. *Journal of Ecology*, v. 102, p. 939–952, 2014.

CARLISLE, D. B.; ELLIS, P. E. Bracken and locust ecdysones: their effects on molting in the desert locust. *Science*, v. 159, p. 1472–1474, 1968.

CARMONA, D.; LAJEUNESSE, M. J.; JOHNSON, M. T. J. Plant traits that predict resistance to herbivores. *Functional Ecology*, v. 25, p. 358–367, 2011.

CHERRETT, J. M. Leaf-cutting ants, biogeographical and ecological studies, em Lieth H, Werger MJ (eds) *Ecosystems of the world 14B, tropical rain forest ecosystems*, 1st edn. Elsevier, New York, p. 473–488, 1989.

CHERRETT, J. M. Some factors involved in the selection of vegetable substrate by *Atta cephalotes* (L.) (Hymenoptera: Formicidae) in Tropical Rain Forest. *Journal of Animal Ecology*, v. 41, p. 647–660, 1972.

COLEY, P. D. Herbivory and defensive characteristics of tree species in lowland tropical forest. *Ecological Monoghaphs*, v. 53, p. 209–233, 1983.

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

COLEY, P.D.; BRYANT, J. P.; CHAPIN, F. S. Resource availability and plant antiherbivore defense. *Science*, v. 230, p. 895–899, 1985.

COOPER-DRIVER, G. A.; HAUFLER, C. The changing role of chemistry in fern classification. *Fern Gazette* 12: 283–294, 1983.

COOPER-DRIVER, G. A. Insect-fern associations. *Entomologia Experimentalis et Applicata*, v. 24, p. 310–316, 1978.

COOPER-DRIVER, G. A. Anti-predation strategies in pteridophytes: a biochemical approach. *Proceedings of the Royal Society of Edinburgh*, v. 86B, p. 397–402, 1985.

COOPER-DRIVER, G. A.; FINCH, S.; SWAIN, T.; BERNAYS, E. Seasonal variation in secondary plant compounds in relation to palatability of *Pteridium aquilinum*. *Biochemical Systematics and Ecology*, v. 5, p. 177–183, 1977.

CORNELISSEN, J. H. C.; LAVOREL, S.; GARNIER, E.; DÍAZ, S.; BUCHMANN, N.; GURVICH, D. E.; REICH, P. B.; TER STEEGE, H.; MORGAN, H. D.; VAN DER HEIJDEN, M. G. A.; PAUSAS, J. G.; POORTER, H. A handbook of protocols for standarised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, v. 51, p. 335–380, 2003.

CORNELISSEN, T. G.; FERNANDES, G. W. Patterns of attack by herbivores on the tropical shrub *Bauhinia brevipes* (Leguminosae): vigour or chance? *European Journal of Entomology*, v. 98, p. 37–40, 2001.

CORNELISSEN, T.; FERNANDES, G. W.; VASCONCELLOS-NETO, J. Size does matter: variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos*, v. 117, p. 1121–1130, 2008.

CORRÊA, P. 2007. *Defesas foliares em resposta à herbivoria em espécies lenhosas de restinga, Ipojuca-PE*. Dissertação em Biologia Vegetal. Universidade Federal de Pernambuco.

COSTA, A. N.; VASCONCELOS, H. L.; VIEIRA-NETO, E. H. M.; BRUNA, E. M. Do herbivores exert top-down effects in neotropical savannas? Estimates of biomass consumption by leafcutter ants. *Journal of Vegetation Science*, v. 19, p. 849–854, 2008.

COSTA, F. V.; FAGUNDES, M. F.; NEVES, F. S. Arquitetura da planta e diversidade de galhas associadas à *Copaifera langsdorffi* i (Fabaceae). *Ecología Austral*, v. 20, p. 9–17, 2010.

COSTA, F. V.; NEVES, F. S.; SILVA, J.O.; FAGUNDES, M. Relationship between plant development, tannin concentration and insects associated with *Copaifera langsdorffii* (Fabaceae). *Arthopod-Plant Interactions*, v. 5, p. 9–18, 2011.

CRAWLEY, M. J. Reduction of oak fecundity by low-density herbivore populations. *Nature*, v. 314, p. 163–164, 1985.

DALIN, P.; AGREN, J.; BJÖRKMAN, C.; KÄRKKÄINEN, K. Leaf trichome formation and plant resistance to herbivory. Pp. 89–105 em Schaller, A (ed.) *Induced Plant Resistance to Herbivory*. Springer, Berlin, 2008.

DAMMAN, H. Patterns of interaction among herbivore species. Pp. 132–169 em Stamp N.E. &. Casey T.M (eds.) *Caterpillars: Ecological and Evolutionary Constraints on Foraging*, Chapman & Hall, New York, 1993.

DOCTERS VAN LEEUWEN, W. M. Uber die von Eriophyes pauropus Nal. an verschiedenen Arten von Nephrolepis gebildeten Blattgallen. *Annales Du Jardin Botanique Buitenzorg*, v. 31, p. 85, 1921.

DOCTERS VAN LEEUWEN, W. M. 1938. *Manual of Pteridology*. The Hague: Martinus Nijhoff. Zoocecidia.

DYER, L. A.; LETOURNEAU, D. K.; DODSON, C. D.; TOBLER, M. A.; STIREMAN, J. O.; HSU, A. Ecological causes and consequences of variation in defensive chemistry of a neotropical shrub. *Ecology*, v. 85, p. 2795–2803, 2004.

EHRLICH, P. R.; RAVEN, P. H. Butterflies and plants: a study in coevolution. *Evolution*, v. 18, p. 586–608, 1964.

EIGENBRODE, S. D.; ESPELIE, K. E. Effects of plant epicuticular lipids on insect herbivores. *Annual Reviews of Entomology*, v. 40, p. 171–194, 1995.

EISNER, T.; EISNER, M.; HOEBEKE, R. E. When defense backfires: detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. *PNAS*, v. 95, p. 4410–4414, 1998.

ELLWOOD, M. D. F.; FOSTER, W. A. Doubling the estimate of invertebrate biomass in a rainforest canopy. *Nature*, v. 429, p. 549–551, 2004.

FAETH, S. H. Indirect interactions between temporally separated herbivores mediated by the host plant. *Ecology*, v. 67, p. 479–494, 1986.

FAGUNDES, M.; FERNANDES, G. W. Insect herbivores associated with *Baccharis dracunculifolia* (Asteraceae): responses of gall-forming and free-feeding insects to latitudinal variation. *Revista de Biología Tropical*, v. 59, p. 1419–1432, 2011.

FALCÃO, P. F. 2004. *Efeito da fragmentação florestal na diversidade de plantas cortadas pela formiga cortadeira Atta laevigata*. Dissertação em Biologia Vegetal, Universidade Federal de Pernambuco, Recife.

FARIA, M. L.; FERNANDES, G. W. Vigor of a dioecious shrub and attack by a galling herbivore. *Ecological Entomology*, v. 26, p.37–45, 2001.

FARIAS, R. P, ARRUDA, E. C. P.; SANTIAGO, A. C. P.; ALMEIDA-CORTEZ, J. S.; CARVALHO-FERNANDES, S. P.; COSTA, L. E. N.; BARROS, I. C. L.; MEHLTRETER, K. First record of galls in the tree fern *Cyathea phalerata* (Cyatheaceae) from Tropical Rainforest, Brazil. *Brazilian Journal of Biology*, v. 78, p. 1–3, 2018.

FARIAS, R. P.; COSTA, L. E. N.; SILVA, I. A. A.; BARROS, I. C. L. Phenological studies of selected leaf and plant traits of *Didymochlaena truncatula* (Dryopteridaceae) in a Brazilian submontane tropical rainforest. *Nordic Journal of Botany*, v. 33, p. 249–255, 2015.

FARJI-BRENER, A. G.; ILLES, A. E. Do leaf-cutting ant nests make "bottom-up" gaps in neotropical rain forest? A critical review of the evidence. *Ecology Letters*, v. 3, p. 219–227, 2000.

FEENY, P. P. Plant apparency and chemical defense. Pp. 1-40 em Wallace, J.W. & Mansell, R.L. (eds.) Recent Advances in Phytochemistry. Plenum Press, New York, 1976.

FERNANDES, G. W.; PRICE, P. W. The adaptive significance of insect gall distribution: survivorship of species in xeric and mesic habitats. *Oecologia*, v. 90, p. 14–20, 1992.

FERNANDES, G. W. PRICE, P. W. Biogeographical gradients in galling species richness: tests of hypotheses. *Oecologia*, v. 76, p. 161–167, 1988.

FERNANDES, G. W.; MARTINS, R. P. As galhas: tumores de plantas. *Ciência Hoje*, v. 4, p. 58–64, 1985.

FERNANDES, G. W.; CARNEIRO, M. A. A.; ISAIAS R. M. S. Gall-inducing insects: from anatomy to biodiversity em A. R. Panizzi, J. R. P. PARRA (eds). *Insect bioecology and nutrition for integrated pest management*. Boca Raton: CRC Press, p. 369-395, 2011.

FERRAZ, F. F. F.; MONTEIRO, R.F. Complex interactions evolving a gall midge *Myrcia myiamaricaensis* Maia (Diptera, Cecidomyiidae), phytophagous modifiers and parasitoids. *Revista Brasileira de Zoologia*, v. 20, p. 433–437, 2003.

FOWLER, H. G.; PAGANI, M. I.; SILVA, O. A.; FORTI, L. C.; PEREIRA-DA-SILVA, V.; VASCONCELOS, H. L. A pest is a pest is a pest? The dilemma of neotropical leaf-cutting ants: keystone taxa of natural ecosystems. *Environmental Management*, v. 13, p. 671−675, 1989.

FRAENKEL, G. S. The raison d'être of secondary plant substances. *Science*, v. 129, p. 1466–1470, 1959.

FUTUYMA, D. J.; AGRAWAL, A. A. Macroevolution and the biological diversity of plants and herbivores. *PNAS*, v. 106, p. 18054–18061, 2009.

GARRETTSON, M.; STETZEL, J.; HALPERN, B.; HEARN, J.; LUCEY, B.; MCKONE, M. Diversity and abundance of understory plants on active and abandoned nest of leaf-cutting ants (*Atta cephalotes*) in Costa Rica rain forest. *Journal of Tropical Ecology*, v. 14, p. 17–26, 1998.

GAY, H.; HENSEN, R.1992. Ant specificity and behaviour in mutualisms with epiphytes: the case of *Lecanopteris* (Polypodiaceae). *Biological Journal of the Linnean Society*, v. 47, p. 261–284, 1992.

GAY, H. 1990. The ant association and structural rhizome modifications of the far-eastern epiphytic fern genus *Lecanopteris* Reinw. (Polypodiaceae). Thesis, University of Oxford.

GAY, H. Ant-houses in the fern genus *Lecanopteris* Reinw. (Polypodiaceae) – the rhizome morphology and architecture of *L. sarcopus* Teijsm and Binnend. and *L. darnaedii* Hennipman. *Botanical Journal of the Linnean Society*, v. 106, p. 199–208, 1991.

GAY, H. Animal-fed plants – an investigation into the uptake of ant-derived nutrients by the Far-Eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). *Biological Journal of the Linnean Society*, v. 50, p. 221–233, 1993a.

GAY, H. Rhizome structure and evolution in the ant-associated epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). *Botanical Journal of the Linnean Society*, v. 113, p. 135–160, 1993b.

GIESENHAGEN, K. Uber zwei Tiergallen an Farnen. Berichte der Deutschen Botanischen Gesellschaft, v. 22, p. 327, 1909.

HANLEY, M. E.; LAMONT, B. B.; FAIRBANKS, M. M.; RAFFERTY, C. M. Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, v. 8, p. 157–178, 2007.

HANSON, P. E.; GÓMEZ-LAURITO, J. Diversity of gall-inducing arthropods of Costa Rica. Pp. 673–692 em Raman, A., Schaefer, C.W. & Withers, T.M. (eds.) *Biology, ecology and evolution of gall-inducing arthropods*. Science Publishers, Enfield, 2005.

HAPER, N. L.; COOPER-DRIVER, G. A.; SWAIN, T. A. survey for cyanogenesis in ferns and gymnosperms. *Phytochemistry*, v. 15, p. 1764–1767, 1976.

HARMS, K. E.; POWERS, J. S.; MONTGOMERY, R. A. Variation in small sapling density, understory cover, and resource availability in four neotropical forests. *Biotropica*, v. 36, p. 40–51, 2004.

HAUKIOJA, E.; RUOHOMÄKI, K.; SUOMELA, J.; VUORISALO, T. Nutritional quality as a defense against herbivores. *Forest Ecology and Management*, v. 39, p. 237–24, 1991.

HAWKES, C. V.; SULLIVAN, J. J. The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology*, v. 82, p. 2045–2058, 2001.

HEADS, P. A.; LAWTON, J. H. Bracken, ants and extrafloral nectaries. III. How insect herbivores avoid ant predation. *Ecological Entomology*, v. 10, p. 29–42, 1985.

HEADS, P. A. Bracken, ants and extrafloral nectaries. IV. Do wood ants (*Formica lugubris*) protect the plant against insect herbivores? *Journal of Animal Ecology*, v. 55, p. 795–809, 1986.

HENDRIX, S. D.; MARQUIS, R. J. Herbivore damage to three tropical ferns. *Biotropica*, v. 15, p. 108–111, 1983.

HENDRIX, S. D. The resistance of *Pteridium aquilinum* (L.) Kuhn to insect attack by *Trichoplusia* ni (Hübn.). *Oecologia*, v. 26, p. 347–361, 1977.

HENDRIX, S. D. An evolutionary and ecological perspective of the insect fauna of ferns. *American Naturalist*, v. 115, p. 171–196, 1980.

HERZ, H.; BEYSCHLAG, W.; HÖLLDOBLER, B. Herbivory rate of leaf-cutting ants in a tropical moist forest in Panama at the population and ecosystem scales. *Biotropica*, v. 39, p. 482–488, 2007.

HIKINO, H.; OKUYAMA, T.; JIN, H.; TAKEMOTO, T. Screening of Japanese ferns for phytoecdysones. I. *Chemical and Pharmaceutical Bulletin*, v. 21, p. 2292–2302, 1973.

HOUARD, C. 1933. *Les Zoocécidies des Plantes de L'Amérique du Sud et de L'Amérique Centrale*. Hermann et Cie, Paris, 549 p.

HOWARD, J. J. Leafcutting ant diet selection: the role of nutrients, water, and secondary chemistry. *Ecology*, v. 68, p. 503–515, 1987.

HOWARD, J. J. Leafcutting ant diet selection: relative influence of leaf chemistry and physical features. *Ecology*, v. 69, p. 250−260, 1988.

HUBBELL, S. P.; WIEMER, D. F. Host plant selection by an attine ant. Pp. 135-154 em Jaisson, P. (ed.) *Social Insects in the tropics*. Université Paris Nord 2, Paris, 1983.

HUBERTY, A. F.; DENNO, R. F. Consequences of nitrogen and phosphorus limitation for the performance of two plant hoppers with divergent life-history strategies. *Oecologia*, v. 149, p. 444–455, 2006.

INUI, Y.; TANAKA, H. O.; HYODO, F.; ITIOKA, T. Within-nest abundance of a tropical cockroach *Pseudoanaplectinia yumotoi* associated with *Crematogaster* ants inhabiting epiphytic fern domatia in a Bornean dipterocarp forest. *Journal of Natural History*, v. 43, p. 1139–1145, 2009.

ISAIAS, R. M. S.; OLIVEIRA, D. C.; CARNEIRO, R. G. S.; KRAUS, J. E. Developmental anatomy of galls in the Neotropics: arthropods stimuli versus host plant constraints. Pp, 15–34 em Fernandes, G. W.; Santos, J. C. (eds) *Neotropical insect galls*, 1st edn. Springer, Netherlands, 2014.

ISAIAS, R. M. S.; CARNEIRO, R. G. S.; SANTOS, J. C.; OLIVEIRA, D. C. Gall morphotypes in the neotropics and the need to standardize them. Pp. 51–68 em Fernandes, G.W. & Santos, J.C. (eds.) Neotropical Insect Galls. Springer, Dordrecht, 2014.

JOHANSEN, D. A. *Plant microtechnique.* McGraw-Hill Book Co., Inc, New York, 1940.

JOHNSON, M. T. J.; IVES, A. R.; AHERN, J.; SALMINEN, J.P. Macroevolution of plant defenses against herbivores in the evening primroses. *New Phytologist*, v. 203, p. 267–279, 2014.

JOLLIFFE, I. T. *Principal component analysis*. Springer-Verlag, New York, 2002.

JUENGER, T.; MORTON, T. C.; MILLER, R. E.; BERGELSON, J. *Scarlet gilia* resistance to insect herbivory: the effects of early season browsing, plant apparency, and phytochemistry on patterns of seed fly attack. *Evolutionary Ecology*, v. 19, p. 79–101, 2005.

KAPLANIS, J. N.; THOMPSON, M. J.; ROBBINS, W. E.; BRYCE, B. M. Insect hormones: alfa ecdysone and 20-hydroxyecdysone in bracken fern. *Science*, v. 157, p. 1436–1438, 1967.

KARBAN, R.; BALDWIN, I. T. *Induced Responses to Herbivory*. University of Chicago Press, Chicago, 1997.

KARBAN, R.; MYERS, J.H. Induced plant responses to herbivory. *Annual Review of Ecology, Evolution and Systematics*, v. 20, p. 331–348, 1989.

KEELER, K.H. A model of selection for facultative, non-symbiotic mutualism. *The American Naturalist*, v. 118, p. 488–98, 1981.

KOPTUR, S.; PALACIOS-RIOS, M.; DÍAZ-CASTELAZO, C.; MACKAY, W.P.; RICO-GRAY, V. Nectar secretion on fern fronds associated with lower levels of herbivore damage: field experiments with a widespread epiphyte of Mexican cloud forest remnants. *Annals of Botany*, v. 111, p. 1277–1283, 2013.

KOPTUR, S.; RICO-GRAY, V.; PALACIOS-RIOS, M. Ant protection of the nectaried fern *Polypodium plebeium* in central Mexico. *American Journal of Botany*, v. 85, p. 736–739, 1998

KOPTUR, S.; SMITH, A. R.; BAKER, I. Nectaries in some neotropical species of *Polypodium* (Polypodiaceae): preliminary observations and analyses. *Biotropica*, v. 14, p. 108–113, 1982.

KRAUS, J. E.; MONTENEGRO, G.; KIM, A. J. Morphological studies on entomogenous stem galls of *Microgramma squamulosa* (Kauf.) Sota (Polypodiaceae). *American Fern Journal*, v. 83, p. 120–128, 1993.

KRAUS, J. E., MONTENEGRO, G.; KIM, A. J. Morphological studies on entomogenous stem galls of *Microgramma squamulosa* (Kauf.) Sota (Polypodiaceae). *American Fern Journal*, v. 83, p. 120-128, 1993.

KULANDAIRAJ, D.; BRITTO, J. D. Antibacterial and antifungal activity of secondary metabolities from some medicinal and other common plant species. *Journal of economic and taxonomic botany*, v. 24, p. 21–24, 2000.

KURSAR, T. A.; COLEY, P. D. Convergence in defense syndromes of young leaves in tropical rainforests. *Biochemical Systematics and Ecology*, v. 31, p. 929–949, 2003.

KURSAR, T. A.; DEXTER, K. G.; LOKVAM, J.; PENNINGTON, R. T.; RICHARDSON, J. E.; WEBER, M. G.; MURAKAMI, E. T.; DRAKE, C.; MCGREGOR, R.; COLEY, P. D. The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proceedings of the National Academy of Sciences*, v. 106, p. 18073–18078, 2009.

LAMARRE, G. P. A.; MENDONZA, I.; FINE, P. V. A.; BARALOTO, C. Leaf synchrony and insect herbivory among tropical tree habitat specialists. *Plant Ecology*, v. 215, p. 209–220, 2014.

LAWTON, J. The structure of the arthropod community on bracken. *Biological Journal of the Linnean Society*, v. 73, p.187–216, 1976.

LAWTON, J. The structure of the arthropod community on bracken. B*iological Journal of the Linnean Society*, v. 73, p. 187–216, 1976.

LAWTON, J. H.; HEADS, P. A. Bracken, ants and extrafloral nectaries. 1. The components of the system. *Journal of Animal Ecology*, v. 53, p.995–1014, 1984.

LAWTON, J. H.; MACGARVIN, M. Interaction between bracken and its insect herbivores. *Proceedings of the Royal Society of Edinburgh*, v. 86, p. 125–131, 1985.

LEGENDRE, P.; LEGENDRE, L. F. *Numerical ecology*. Elsevier, Amsterdam, The Netherlands, 2012.

LEMOINE, N. P.; GIERY, S. T.; BURKEPILE, D. E. Differing nutritional constraints of consumers across ecosystems. *Oecologia*, v. 174, p. 1367–1376, 2014.

LEÓN, B.; YOUNG, K. R. A fortuitous ant-fern association in the Amazon lowlands of Peru. *Revista Peruana de Biología*, v. 17, p. 245–247, 2010.

LITTLEDYKE, M.; CHERRETT, J. M. Direct ingestion of plant sap from cut leaves by leaf-cuttings ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich) (Formicidae, *Attini*). *Bulletin of Entomological Research*, v. 66, p. 205–217, 1976.

LUEDERWALDT, VON H. *Die Cyathaceen aus der Umgebung der Stadat S. Pau*lo. São Paulo, Zeitschriff Deutscher Verein für Wissenschaft und Kunst, 1923

MAIA, V. C.; SANTOS, M. G. A new genus and species of gall midge (Diptera, Cecidomyiidae) associated with *Microgramma vacciniifolia* (Langsd. & Fisch.) Copel. (Polypodiaceae) from Brazil. *Revista Brasileira de Entomologia*, v. 55, p. 40–44, 2011

MAIA, V. C.; SANTOS, M. G. Record of insects in two fern species of the genus *Microgramma* (Polypodiaceae) in the Atlantic Rain Forest, Rio de Janeiro state, Brazil. *Brazilian Journal Biology*, v. 75, p. 253–254, 2015.

MAIA, V. C. A new genus and six new species of gall midges (Diptera, Cecidomyiidae) from Serra de São José (Minas Gerais State, Brazil). *Arquivos do Museu Nacional*, v. 62, p. 69–82, 2004.

MAIA, V. C.; ARAÚJO, L. *Clinodiplosis agerati* (Diptera, Cecidomyiidae), a new galling species associated with *Ageratum conyzoides* (Asteraceae) from Brazil. *Brazilian Journal of Biology*, v. 76, p. 782-786, 2016.

MAIA, V. C.; SILVA, L. O. Insect galls of Restinga de Marambaia (Barra de Guaratiba, Rio de Janeiro, RJ). *Brazilian Journal of Biology*, v. 76, p. 787-795, 2016;

MAIA, V. C.; CONSTANTINO, P. A. L.; Monteiro, R. F. New gall midges (Diptera, Cecidomyiidae) associated with two species of *Eugenia* (Myrtaceae). *Revista Brasileira de Entomologia*, v. 49, p. 347–352, 2005.

MAIA, V. C.; MAGENTA, M. A. G.; MARTINS, S. E. Ocorrência e caracterização de galhas de insetos em áreas de restinga de Bertioga (São Paulo, Brasil). *Biota Neotropica*, v. 8, p. 167–197, 2008.

MANI, M. S. *Ecology of plant gall*s. The Hague, Dr. W. Junk. Publishers. 1964.

MANI, M. S. Introduction to Cecidology. Pp. 1-7 em J.D. Shorthouse, O. Rohfritsch (eds.) *Biology of insect-induced galls*. Oxford: Oxford University Press, 1992.

MARQUIS, R. J. Leaf herbivores decrease fitness of a tropical plant. *Science*, v. 226, p. 537–539. 1984.

MATOS, F. J. A. *Introdução à Fitoquímica Experiment*al. Edições UFC, Fortaleza, 1997.

MATTSON, W. J. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology, Evolution and Systematics*, v. 11, p. 119–161, 1980.

MAURICIO, R. Cost of resistance to natural enemies in field populations of the annual plant, *Arabidopsis thaliana*. *American Naturalist*, v. 151, p. 20–28, 1998.

MEHLTRETER, K.; GARCÍA-FRANCO, G. Leaf phenology and trunk growth of the deciduous tree fern *Alsophila firma* (Baker) D. S. Conant in Lower Montane Mexican Forest. *American Fern Journal*, v. 98, p. 1–13, 2008.

MEHLTRETER, K.; HÜLBER, K.; HIETZ, P. Herbivory on epiphytic ferns of a Mexican cloud forest. *Fern Gazette*, v. 17, p. 303–309, 2006.

MEHLTRETER, K.; SHARPE, J. M. Causes and consequences of the variability of leaf lifespan of ferns. *Fern Gazette*, v. 9, p. 193–202, 2013.

MEHLTRETER, K.; VALENZUELA, J. Leafcutter ants as test organisms for leaf quality of ferns. *Indian Fern Journ*al, v. 29, p. 262–268, 2012.

MEHLTRETER, K.; TOLOME, J. Herbivory on three tropical fern species of a Mexican cloud forest. Pp. 375-381 em Chandra, S. & Srivastava, M. (eds.) *Pteridology in the New Millenium*. Kluwer Academic Publishers, Alphen aan den Rijn, 2003

MEHLTRETER, K.; VALENZUELA, J. Leafcutter ants as test organisms for leaf quality of ferns. *Indian Fern Journal*, v. 29, p. 262–268, 2012.

MEHLTRETER, K. Interactions of ferns with fungi and animals. Pp. 220–254 em Mehltreter, K., Walker, L. R. & Sharpe, J. M. (eds) *Fern ecology*. Cambridge University Press, Cambridge, 2010.

MEHLTRETER, K.; ROJAS, P.; PALACIOS-RIOS, M. Moth larvae-damaged giant leather-fern *Acrostichum danaeifolium* as host for secondary colonization by ants. *American Fern Journal*, v. 93, p. 49–55, 2003.

Mendonça-Jr, M. S. *Ecologia de Eugeniamyia dispar (Diptera: Cecidomyiidae), galhador sobre Eugenia uniflora (Myrtaceae)*. Dissertação em Biologia Animal, Universidade Federal do Rio Grande do Sul. 1996.

MENDONÇA-JR, M. S.; ROMANOWSKI, H. P. Inimigos naturais do galhador *Eugeniamyia dispar* (Diptera, Cecidomyiidae): formigas predadoras e parasitóides. *Brazilian Journal of Biology*, v. 62, p. 269–275, 2002.

MESIPUU M.; SHEFFERSON, R. P.; KULL, T. Weather and herbivores influence fertility in the endangered fern *Botrychium multifidum* (S.G. Gmel.) Rupr. Plant Ecology, v. 203 p. 23–31, 2009.

MICKEL, J. T.; SMITH, A. R. The Pteridophytes of Mexico. M*emoirs of the New York Botanical Garden*, v. 88, p. 1–1055, 2004.

MOLE, S.; ROSS, J. A.; WATERMAN, P. G. Light-induced variation in phenolic levels in foliage of rain-forest plants: I. Chemical changes. *Journal of Chemical Ecology*, v. 14, p. 1–21, 1988.

MOLINA-MONTENEGRO, M. A.; ÁVILA, P.; HURTADO, R.; VALDIVIA, A. I.; GIANOLI E. Leaf trichome density may explain herbivory patterns of *Actinote* sp. (Lepidoptera: Acraeidae) on *Liabum mandonii* (Asteraceae) in a montane humid forest (Nor Yungas, Bolivia). *Acta Oecologia*, v. 30, p. 147–150, 2006.

MORAN, R. C. *A natural history of ferns*. Timber Press, Portland Oregon, 2004.

MOREIRA, X.; PEARSE, I. S. Leaf habit does not determine the investment in both physical and chemical defences and pair-wise correlations between these defensive traits. *Plant Biology*, v. 19, p. 354–359, 2017.

MOREIRA, X.; SAMPEDRO, L.; ZAS, R.; PEARSE, I.S. Defensive traits in young Pine trees cluster into two divergent syndromes related to early growth rate. *PLoS One*, v. 11, p. 1–12, 2016.

MUMM, R.; HILKER, M. Direct and indirect chemical defence of pine against folivorous insects. T*rends in Plant Science*, v. 11, p. 351–358, 2006.

MUMM, R.; SCHRANK, K.; WEGENER, R.; SCHULZ, S.; HILKER, M. Chemical analysis of volatiles emitted by *Pinus sylvestris* after induction by insect oviposition. *Journal of Chemical Ecology* 29: 1235-1251, 2003.

NAKAMURA, M.; MIYAMOTO, Y.; OHGUSHI, T. Gall initiation enhances the availability of food resources for herbivorous insects. *Functional Ecology*, v. 17, v. 851–857, 2003.

NORTH, R. D.; JACKSON, C. W.; HOWSE, P. E. Evolutionary aspects of ant-fungus interactions in leaf-cutting ants. *Trends in Ecology & Evolution*, v. 12, p. 386–389, 1997.

NÚÑEZ-FARFÁN, F. J.; VALVERDE, P. L. The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics*, v. 38, p. 541–566, 2007.

PAGE, C. N. Ecological strategies in fern evolution: a neopteridological overview. *Review Palaeobotany and Palynology*, v. 119, p. 1–33, 2002.

PASCUAL-ALVARADO, E.; CUEVAS-REYES, P.; QUESADA, M.; OYAMA, K. Interactions between galling insects and leaf-feeding insects: the role of plant phenolic compounds and their possible interference with herbivores. *Journal of Tropical Ecology*, v. 24, p. 329–336, 2008.

PATRA, B.; BERA, S.; MEHLTRETER, K. Structure, biochemistry and ecology of entomogenous galls in *Selaginella* Pal. Beauv. (Selaginellaceae) from India. *Journal Plant of Interactions*, v. 5, p. 29–36, 2010.

PEARSE, I. S.; HIPP, A. L. Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences*, v. 106, p. 18097–18102, 2009.

PEETERS, P. J. Correlations between leaf constituent levels and the densities of herbivorous insect guilds in an Australian forest. *Austral Ecology*, v. 27, p. 658–671, 2002.

PELLOTO, J. P., MARTÍNEZ, M. D. P. Chemical defenses in the tree *Ziziphus mistol* against the leaf-cutting ant *Acromyrmex striatu*s. Ecología Austral, v. 12, p. 11–18, 2002.

PENNINGS, S. C.; SIMPSON, J. C. Like herbivores, parasitic plants are limited by host nitrogen content. *Plant Ecology*, v. 196, p. 245–250, 2008.

PÉREZ-HARGUINDEGUY N et al. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, v. 61, p. 167–234, 2013.

PERKINS, M. C.; WOODS, H. A.; HARRISON, J. F.; ELSER, J. J. Dietary phosphorus affects the growth of larval *Manduca sexta*. *Archives of Insect Biochemistry and Physiology*, v. 55, p. 153–168, 2004.

POTES, A. Comparative anatomy of the nectaries of *Aglaomorpha* and *Drynaria* (Polypodiaceae). *American Fern Journal*, v. 100, p. 80– 92, 2010.

PPG I. A community-derived classification for extant lycophytes and ferns. J*ournal of Systematics Evolution*, v. 54, p. 563–603, 2016.

PRADO, A.; SIERRA, A.; WINDSOR, D.; BEDE, J. C. Leaf traits and herbivory levels in a tropical gymnosperm, *Zamia stevensonii* (Zamiaceae). *American Journal of Botany*, v. 101, p. 437–447, 2014.

PRICE, P. W.; LEWINSHON, T. M.; FERNANDES, G. W.; BENSON, W. W. P*lant animal interactions: evolutionary ecology in Tropical and Temperate Regions*. Wiley, New York, 1991.

PRICE, P. W.; FERNANDES, G. W.; WARING, G. L. Adaptive nature of insect galls. *Environmental Entomology*, v. 16, p. 15–24, 1987.

PRICE, P. W.; FERNANDES, G. W.; LARA, A. C. F.; BRAWN, J.; BARRIOS, H.; WRIGHT, M. G.; RIBEIRO, S. P.; ROTHCLIFF, N. Global patterns in local number of insect galling species. *Journal of Biogeography*, v. 25, p. 581–591, 1998.

PRICE, P. W.; LEWINSHON, T. M.; FERNANDES, G. W.; BENSON, W. W. *Plant Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions*. Wiley, New York. 1991.

PRINGLE, E. G.; ADAMS, R. I.; BROADBENT, E.; BUSBY, P. E.; DONATTI, C. I.; KURTEN, E. L.; RENTON, K.; DIRZO, R. Distinct leaf-trait syndromes of evergreen and deciduous trees in a seasonally dry tropical forest. *Biotropica*, v. 43, p. 299–308, 2011.

PURVIS, M.; COLLIER, D.; WALLS, D. *Laboratory techniques in botany*. Butterworths, London, 1964.

QUINLAN, R. J.; CHERRETT, J. M. The role of fungus in the diet of the leaf-cutting ant *Atta cephalotes* (L.). *Ecological Entomology*, v. 4, p. 151–160, 1979.

R Core Team. *R: A Language and Environment for Statistical Computing*. R Core Team, 2016.

RAMAN, A. Insect-induced plant galls of India: unresolved questions. *Current Science*, v. 92, p. 748–757, 2007.

RASMANN, S.; AGRAWAL, A. A. Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. *Current Opinion in Plant Biology*, v. 12, p. 473–478, 2009.

RASMANN, S.; AGRAWAL, A. A. Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. *Ecology Letters*, v. 14, p. 476–483, 2011.

RATHINASABAPATHI, B.; RANGASAMY, M.; FROEBA, J.; CHERRY, R. H.; MCAUSLANE, H. J.; CAPINERA, J. L.; SRIVASTAVA, M.; MA, LQ. Arsenic hyperaccumulation in the Chinese brake fern (*Pteris vittat*a) deters grasshopper (*Schistocerca americana*) herbivory. *New phytologist*, v. 175, p. 363–369, 2007.

RAW, A. An annotated catalogue of the leafcutter and mason bees (Genus *Megachile*) of the Neotropics. *Zootax*a, v. 1601, p. 1–127, 2007.

READ, J.; SANSON, G. D.; CALDWELL, E.; FIONA, J. C.; CHATAIN, A.; PEETERS, P.; LAMONT, B. B.; GARINE-WICHATITSKY, JAFFRÉ, T.; KERR, S. Correlations between leaf toughness and phenolics among species in contrasting environments of Australia and New Caledonia. *Annals of Botany*, v. 103, p. 757–767, 2009.

RIBEIRO, S. P.; BASSET, I. Gall-forming and free-feeding herbivory along vertical gradients in a lowland tropical rainforest: the importance of leaf sclerophylly. *Ecography*, v. 30, p. 663–672, 2007.

ROCKWOOD, L. L. Plant selection and foraging patterns in two species of leaf-cutting ants (*Atta*). *Ecology*, v. 57, p. 48–61, 1976.

ROWELL, C. H. F.; ROWELL-RAHIER, M.; BRAKER, H. E.; COOPER-DRIVER, G.; GÓMEZ, P. L. D. The palatability of ferns and the ecology of two tropical forest grasshoppers. *Biotropica*, v. 15, p. 207–16, 1983.

RUIZ, N. R.; WARD, D.; SALTZ, D. Leaf compensatory growth as a tolerance strategy to resist herbivory in *Pancratium sickenbergeri*. *Plant Ecolog*y, v. 198, p. 19–26, 2008.

RUSSELL, G. B.; FENEMORE, P. G. Insect moulting hormone activity in some New Zealand ferns. *New Zealand Journal of Science*, v. 14, p. 31–35, 1971.

SANKARAN, M.; MCNAUGHTON, S. J. Terrestrial plant-herbivore interactions: integrating across multiple determinants and trophic levels. Pp 265–285 em van der Maarel E (ed) *Vegetation Ecology*, 1st edn. United Kingdom, Blackwell, 2005.

SANTOS, J. C.; SILVEIRA, F. A. O.; FERNANDES, G. W. Long term oviposition preference and larval performance of *Schizomyia macrocapillata* (Diptera: Cecidomyiidae) on larger shoots of its host plant *Bauhinia brevipes* (Fabaceae). *Evolutionary Ecology*, v. 22, p. 123–137, 2008.

SANTOS, J. C.; TAVARES, C. B.; ALMEIDA-CORTEZ, J. S. Plant Vigor Hypothesis refuted: preference-performance linkage of a gall-inducing weevil on small-sized host plant resources. *Brazilian Journal of Biology*, v. 71, p. 65–69, 2011.

SANTOS, J. C.; ALMEIDA-CORTEZ, J.S.; FERNANDES, G. W. Diversity of gall-inducing insects in the high altitude wetland forests in Pernambuco, Northeastern Brazil. *Brazilian Journal of Biology*, v. 71, p. 47-56, 2011.

SANTOS, J. C.; CARNEIRO, M. A. A.; FERNANDES, G. W. Insetos galhadores neotropicais: diversidade e ecologia evolutiva dos herbívoros mais sofisticados da natureza. Pp. 183-199 em K. Del Claro; H.M. Torezan-Silingardi (eds.) E*cologia das Interações Plantas-Animais: Uma Abordagem Ecológico-Evolutiva*. Rio de Janeiro: Technical Books, 2012.

SANTOS, M. G.; MAYHÉ-NUNES, A. J. Contribuição ao estudo das interações entre pteridófitas e formigas. *Revista Brasileira de Biociências*, v. 5, p. 381−383, 2007.

SANTOS, M. G.; CARVALHO, C. E. M.; KELECOM, A.; RIBEIRO, M. L. R. C.; FREITAS, C. V. C.; COSTA, L. M.; FERNANDES, L. V. G. Cianogênese em esporófitos de pteridófitas avaliada pelo teste do ácido pícrico. *Acta Botanica Brasilica*, v. 19, p. 783–788, 2005.

SCHMITT, J. L.; WINDISCH, P. G. Aspectos ecológicos de *Alsophila setosa* Kaulf. (Cyatheaceae, Pteridophyta) no Rio Grande do Sul, Brasil. *Acta Botanica Brasilica*, v. 19, p. 859−865, 2005.

SCHNEIDER, H.; SCHUETTPELZ, E.; PRYER, K. M.; CRANFILL, R.; MAGALLÓN, S.; LUPIA, R. Ferns diversified in the shadow of angiosperms. *Nature*, v. 428, p. 553–557, 2004.

SCHNEIDER, G. *The book of choice fern*s. Gill, London, 1892.

SCHREINER, I.; NAFUS, D.; PIMENTEL, D. Frequency of cyanogenesis in bracken in relation to shading and winter severity. *American Fern Journal*, v. 74, p. 51–55, 1984b.

SCHREINER, I.H. Cyanogenesis and the herbivorous insects of bracken fern (*Pteridium aquilinum*). Ph.D. Thesis, Cornell University, Ithaca. 1980.

SCHREINER, I. H.; NAFUS, D.; PIMENTEL, D. Effects of cyanogenesis in bracken fern (*Pteridium aquilinum* (L.) Kuhn) on associated insects. E*cological Entomology*, v. 9, p. 69−79, 1984a.

SCHREMMER, F. Extranuptiale Nectarien. Beobachtungen an *Salix eteagnos* Scop. und *Pteridium aquilinum* (L.) Kuhn. Österreichische botanische Zeitschrift, v. 11, p. 205−22, 1969.

SCHULTZ, J. C.; BALDWIN, I. T. Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science*, v. 217, p. 149–151, 1982.

SCHVARTSMAN, S. *Plantas venenosas e animais peçonhentos*. Sarvier, São Paulo, 1992.

SCRIBER, J. M. Plant-Insect Interactions. Pp 806–810 em Resh, V.; Carde, R (eds) T*he Encyclopedia of Insects*, 1st edn. Academic Press, New York, 2009.

SHARPE, J. M. Leaf growth and demography of the rheophytic fern *Thelypteris angustifolia* (Willdenow) Proctor in a Puerto Rican rainforest. *Plant Ecolog*y, v. 130, p. 203–212, 1997.

SHARPE, J. M.; MEHLTRETER, K.; WALKER, L. R. Ecological importance of ferns. Pp. 1–21 em Mehltreter, K., Walker, L. R & Sharpe, J. M. (eds) *Fern ecology*. Cambridge University Press, Cambridge, 2010.

SHORTHOUSE, J. D.; ROHFRITSCH, O. *Biology of insect-induced galls*. Oxford University Press, Princeton, 1992.

SILVA, D. M; BATALHA, M. A. Defense syndromes against herbivory in a cerrado plant community. *Plant Ecology*, v. 212, p. 181–193, 2011.

SILVA, F. C. L. *Ecofisiologia de Cyatheaceae (Monilophyta): Fenologia, banco de esporos, anatomia e germinação*. Tese em Biologia Vegetal, Universidade Federal de Pernambuco. 2008.

SIMMS, E. L.; RAUSCHER, M. D. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. *Evolution*, v. 43, p. 573–585, 1989.

SIMMS, E. L.; TRIPLETT, J. Costs and benefits of plant responses to disease. *Evolution*, v. 48, p. 1973–1985, 1994.

SOEDER, R. W. Fern constituents: including occurrence, chemotaxonomy and physiological activity. *The Botanical Review*, v. 51, p. 442–536, 1985.

SOMVANSHI, R.; LAUREN, D. R.; SMITH, B. L.; DAWRA, R. K.; SHARMA, O. P.; SHARMA, V. K.; SINGH, A. K.; GANGWAR, N. K. Estimation of the fern toxin, ptaquiloside, in certain Indian ferns other than bracken. *Current Science*, v. 91, p. 1547–1552, 2006.

SOO HOO, C. F.; FRAENKEL, G. The resistance of ferns to the feeding of *Prodenia eridania* larvae. *Annals of the Entomological Society of America*, v. 57, p. 788–90, 1964.

STAMP, N. E.; CASEY, T. M. *Caterpillars: ecological and evolutionary constraints on foraging.* Chapman and Hall, New York, 1993.

STAMP, N. Out of the quagmire of plant defense hypotheses. *The Quarterly Review of Biology*, v. 78, p. 23–55, 2003.

STEWARD, J. L.; KEELER, K. H. Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae)? *Oikos*, v. 53, p. 79–8, 1988.

STRAUSS, S. Y.; AGRAWAL, A. A. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution*, v. 14, p. 179–185, 1999.

SUDD, J. H.; SUDD, M. E. Seasonal changes in the response of wood-ants (*Formica lugubris*) to sucrose baits. *Ecological Entomology*, v. 10, p. 89–97, 1985.

SWAIN, T.; COOPER-DRIVER, G. Biochemical systematics in the Filicopsida em Jermy, A.C., Crabbe, J.A. & Thomas, B.A. (eds.) The phylogeny and classification of the ferns. *Botanical Journal of the Linnean Society*, v. 67, p. 111–134, 1973.

SWAIN, T.; COOPER-DRIVER, G. A. Biochemical evolution in early land plants. Pp. 103-134 em Niklas K. J. (ed.) *Paleobotany, Paleoecology and Evolution*, Praeger, New York. 1981.

SWEZEY, O. H. Insects attacking ferns in the Hawaiian Islands. Hawaiian Entomological Society, v. 5, p. 57–65, 1922.

TANAKA, H. O.; ITIOKA, T. Ants inhabiting myrmecophytic ferns regulate the distribution of lianas on emergent trees in a Bornean tropical rainforest. *Biology Letters*, v. 7, p. 706–709, 2011.

TANAKA, H. O.; INUI, Y.; ITIOKA, T. Anti-herbivore effects of an ant species, Crematogaster difformis, inhabiting myrmecophytic epiphytes in the canopy of a tropical lowland rainforest in Borneo. *Ecological Research*, v. 24, p. 1393–1397, 2009.

TANNER, E. V. J.; KAPOS, V. Leaf structure of jamaican montane rain-forest trees. *Biotropica*, v. 14, p. 16–24, 1982.

TEMPEL, A. S. Field studies of the relationship between herbivore damage and tannin concentration in bracken (*Pteridium aquilinum* (L.) Kuhn). *Oecologia*, v. 51, p. 97–106, 1981.

TOMA, T. S. P.; SOUZA MENDONÇA, J. R. M. Population ecology of galling arthropods in the Neotropics. Pp 69–98 em Fernandes GW, Santos JC (eds) *Neotropical insect ga*lls, 1st edn. Springer, Netherlands, 2014.

TRAVERS-MARTIN, N.; MÜLLER, C. Matching plant defence syndromes with performance and preference of a specialist herbivore. *Functional Ecology*, v. 22, p. 1033–1043, 2008.

TRYON, R. M. Revision of the genus *Pteridium*. *Rhodora*, v. 43, p. 1-31, 37-67, 1941.

TUOMI, J.; NIEMALA, P. S.; CHAPIN, F. S.; BRYANT, J. P.; SIREN, S. Defensive responses of trees in relation to their carbon/nutrient balance. Pp 57–72 em Mattson WJ, Levieux J, Bernard-Dagan C (eds) *Mechanisms of woody plant defenses against insects: search for pattern*. Springer-Verlag, New York, 1987.

TURCOTTE, M. M.; DAVIES, T. J.; THOMSEN, C. J. M.; JOHNSON, M. T. J. Macroecological and macroevolutionary patterns of leaf herbivory across vascular plants. *Proceedings of the Royal Society* B, v. 281, p. 1–7, 2014.

TURLEY, N. E.; ODELL, W. C.; SCHAEFER, H.; EVERWAND, G.; CRAWLEY, M. J.; JOHNSON, M. T. J Contemporary evolution of plant growth rate following experimental removal of herbivores. *The American Naturalist*, v. 181, p. 21–34, 2013.

VAN DER MEIJEDEN E.; WIJN, E.H.; VERKAAR, J. Defence and regrowth: alternative plant strategies in the struggle against herbivores. *Oikos*, v. 51, p. 355–363, 1988.

VAN GILS, H. A. J. A.; VANDERWOUDE, C. Leafcutter ant (*Atta sexdens*) (Hymenoptera: Formicidae) nest distribution responds to canopy removal and changes in micro-climate in the Southern Colombian Amazon. *Florida Entomologist*, v. 95, p. 914–921, 2012.

WAGNER, H.; BLADT, S. *Plant drug analysis a thin layer chromatography atlas*. Springer-Verlag, Berlin, 1996.

WATKINS JR., J. E.; CARDELÚS, C. L.; MACK, M. C. Ants mediate nitrogen relations of an epiphytic fern. *New phytologist*, v. 180, p. 5–8, 2008.

WEIGAND, A.; LEHNERT, M. The scaly tree ferns (Cyatheaceae-Polypodiopsida) of Brazil. *Acta Botanica Brasilica*, v. 30, p. 336-350, 2016.

WETTERER, J. K. Ontogenetic changes in forager polymorphism and foraging ecology in the leaf-cutting ant *Atta cephalotes*. *Oecologia*, v. 98, p. 235–238, 1994.

WETTERER, J. K. Forager size and ecology of *Acromyrmex coronatus* and other leaf-cutting ants in Costa Rica. Oecologia, v. 104, p. 409–415, 1995.

WIECZOREK, V.H. Zur Kenntnis der Adlerfarninsekten. Ein Beitrag zum Problem der biologischen Bekämpfung von *Pteridium aquilinum* (L) Kuhn in Mitteleuropa. *Zeitschrift für Angewandte Entomologie*, v. 72, p. 337–358, 1973.

WILLIAMS, M. A.; CRONIN, J. T. Gall former guild response to stressed and vigorous roses. *Environmental Entomology*, v. 33, p. 1052–1061, 2004.

WINTERBOURN, M. J. The arthropod fauna of bracken (*Pteridium aquilinum*) on the Port Hills, South Island, New Zealand. *New Zealand Entomologist*, v. 10, p. 99–104, 1987.

WIRTH, R.; MEYER, S. T.; ALMEIDA, W. R.; ARAÚJO, M. V.; BARBOSA, V. S.; LEAL, I. R. Increasing densities of leaf-cutting ants (*Atta* spp.) with proximity to the edge in a Brazilian Atlantic forest. *Journal of Tropical Ecology*, v. 23, p. 501–505, 2007.

WIRTH, R.; HERZ, H.; RYEL, R. J.; BEYSCHLAG, W.; HÖLLDOBLER, B. Herbivory of leaf-cutting ants: a case study on *Atta colombica* in the Tropical Rainforest of Panama. *Ecological Studies* 164, Springer Verlag, Berlin. 2003.

WRIGHT, I. J et al. The world-wide leaf economics spectrum. *Nature*, v. 428, p. 821–827, 2004.

WRITE, T. C. R. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, v. 64, p. 90–105, 1984.

YA-IDE, J. Inter- and intra-shoot distributions of the ramie moth caterpillar, *Arctecoerulea* (Lepidoptera: Noctuidae), in ramie shrubs. *The journal Applied Entomology and Zoology*, v. 41, 49–55, 2006.