BIOTROPICA *(*): ***–*** ****

10.1111/j.1744-7429.2007.00326.x

Host Specificity of Pathogenic *Pythium* Species: Implications for Tree Species Diversity

Carol K. Augspurger[1]

Department of Plant Biology, University of Illinois, 505 S. Goodwin Avenue, Urbana, Illinois 61801, U.S.A.

and

Henry T. Wilkinson

Department of Natural Resources and Environmental Sciences, University of Illinois, 1102 S. Goodwin Avenue, Urbana, Illinois 61801, U.S.A.

ABSTRACT

In the Janzen–Connell hypothesis, host-specific natural enemies enhance species diversity and influence the structure of plant communities. This study tests the explicit assumption of host specificity for soil pathogens of the genus *Pythium* that cause damping-off disease of germinating seeds and seedlings. We isolated *Pythium* spp. from soil of a tropical forest in Panama. Then, in an inoculation experiment, we determined the pathogenicity of 75 tropical isolates of unknown pathogenicity and seven pathogenic temperate isolates of *Pythium* on seeds and/or seedlings of eight tropical tree species. Only three tropical isolates, one identified as *P. ultimum* and two as *P. aphanidermatum*, were pathogenic. Tropical pathogenic isolates were pathogenic on 4–6 of eight tree species. Temperate isolates were pathogenic on 0–4 of eight species, indicating that some tropical tree species are susceptible to novel isolates of *Pythium*. No tree species was susceptible to all isolates and two species were not susceptible to any isolate. Collectively, these results indicate that these *Pythium* isolates vary widely in their pathogenicity, causing differential mortality of potential host species; likewise, the tree species vary in their susceptibility to a given *Pythium* isolate. These differences in pathogenicity and susceptibility indicate some support for the Janzen–Connell assumption of host specificity. While they are not restricted to a single species, their intermediate level of specificity suggests that *Pythium* spp. have the potential to have some effect on forest community structure and diversity.

Abstract in Spanish is available at http://www.blackwell-synergy.com/loi/btp.

Key words: damping-off disease; inoculation; isolation; Janzen–Connell hypothesis; Panama; pathogenicity; seeds and seedlings; soil pathogens; susceptibility; tropical semi-deciduous forest.

THE JANZEN–CONNELL HYPOTHESIS STATES THAT HOST-SPECIFIC NATURAL ENEMIES cause a greater incidence of mortality of seeds and seedlings near the parent tree than farther away because they respond to high density of seeds and seedlings and/or are restricted in their dispersal (Janzen 1970, Connell 1971). Consequently, recruitment by the susceptible tree species occurs at some distance from the parent tree, and other nonsusceptible species may occupy the vacant space near the susceptible parent tree. Hence, species coexistence is enhanced and spatial distributions become less clumped over time. This mechanism is especially important if the natural enemy responds to the high density of common species, thereby favoring rare species (Gillett 1962, Wright 2002).

This hypothesis hinges directly on the explicit assumption of host specificity (Browder & Eversmeyer 1986, Kohmoto *et al.* 1995, Zhou & Hyde 2001). It depends on a given natural enemy killing only its host species, and not other species, resulting in the mechanism operating at the species level. While this strict specificity would bring the most opportunity for species coexistence and maintenance of diversity, in fact, any differential mortality among species by a natural enemy would have some potential to affect the diversity and distribution of species. Despite the centrality of host specificity to the hypothesis and the dominant role this hypothesis has played in tropical forest ecology, this assumption is seldom tested (but see Packer & Clay 2000 for a temperate example). Indeed, Gilbert (2005) points out our nearly total lack of knowledge of pathogen specificity in tropical forests. He concludes that the most important current need to assess how important pathogens are for tropical tree diversity is to determine the extent of their host specificity.

Soil pathogens, dominated by species of *Pythium*, *Phytothophora*, *Fusarium*, and *Rhizoctonia*, are significant natural enemies of tropical tree species. Their host specificity has not been tested in the context of the Janzen–Connell hypothesis. They cause widespread density- and distance-dependent mortality of seeds and seedlings by damping-off disease (Augspurger 1984, Dalling *et al.* 1998, Hood *et al.* 2004). A greater incidence of damping-off occurs in high density because diseased seeds and seedlings facilitate pathogen dispersal to nearby seeds and seedlings via mycelium (Burdon & Chilvers 1975; Neher *et al.* 1987, 1992). In addition, being restricted by the soil, their dispersal is limited except by soil run-off, swimming zoospores, and mycelium growth between plants. Some of the genera, however, disperse through time and do not necessarily require a living host. They form persistent spore-like structures and are saprophytic between periods of infecting living organisms (Hendrix & Campbell 1973).

The extent of host specificity of plant pathogens varies (Agrios 1997, Zhou & Hyde 2001). There are many examples of fungal plant pathogens being recorded on one host (Shivas & Hyde 1997). However, degrees of pathogenesis and aggressiveness, that is, the rate of pathogenesis, on different species are likely for facultative pathogens, such as *Pythium*. Thus, the pathogen would use a diversity of hosts, both in space and time. Indeed, relative to other

Received 3 April 2006; revision accepted 31 January 2007.

[1]Corresponding author; e-mail: carolaug@uiuc.edu

pathogens, soil microbes are considered at the extreme end of the continuum of host specificity, being relative generalists (Hendrix & Campbell 1983, Martin 1992, Carlisle & Watkinson 1994) and have been called the 'Jack of all trades' (Harper 1977).

The host specificity of soil pathogens causing damping-off disease in tropical forests is currently unknown. They have rarely been isolated (Davidson *et al.* 2000), and often only disease symptoms have been used to indicate their presence. Damping-off was first observed to cause heavy morality of seedlings of *Platypodium elegans* J. Vogel, a tree on Barro Colorado Island (BCI), Panama (Augspurger 1983a). Mortality due to damping-off ranged from 35 to 81 percent among four parent trees and was particularly high near the parent in the highest density of seedlings. Subsequent experiments and observations demonstrated damping-off mortality to be both density- and distance-dependent in a range of host species (Augspurger 1983b, 1984; Augspurger & Kelly 1984; but see Davidson *et al.* 2000). In addition, seedling populations incurred a greater incidence of damping-off mortality underneath their own parent than when transplanted under other conspecific parents at the same density (Augspurger & Kelly 1984). These studies raised the possibility that such pathogens could be not only species-specific, but even genotype-specific; alternatively, different pathogen species or strains of the same species, but with different pathogenicity, could occur at the different locations. In a greenhouse study in which seedlings of 18 tree species from BCI were grown in soil from under a 19th species, 13 species showed damping-off disease (Augspurger & Kelly 1984). Either the soil under one parent contained multiple pathogens, each with some degree of host specificity, or one or a few pathogens were capable of infecting many host species. Without isolating specific pathogens and directly testing their pathogenicity on multiple hosts, it is impossible to know the extent of their host specificity or to fully interpret the above studies in the context of the Janzen–Connell hypothesis.

This study's objectives were to determine the extent of: (1) host specificity for *Pythium*, a genus of Oomycetes, isolated from soil on BCI, Panama; and, conversely, (2) susceptibility of tropical tree species to *Pythium* isolates, including novel temperate isolates. Specificity of tropical and temperate isolates was explored on seedlings of three parents of *P. elegans* and of one parent of seven other co-occurring tree species. Simultaneously, temperate isolates of *Pythium* species were tested for their pathogenicity on the same set of tree species to determine host specificity. This inoculation study allowed an interpretation of these plant–pathogen interactions from two perspectives: first, how specific a given isolate is to potential host species; and second, how many isolates a given tree species is susceptible to. Furthermore, inclusion of the temperate *Pythium* isolates determined the susceptibility of tropical tree species to novel pathogens.

The study had two phases. First, from soils collected on BCI, tropical microbes were isolated on media selective for common Oomycetes, principally species of *Pythium* and *Phytothphora*. Special emphasis was placed on *Pythium* species because preliminary attempts to isolate the pathogen from diseased stem tissue of seedlings of *Platypodium* had shown it to be aerial, white, and fast-growing, traits typical of some *Pythium* species. In addition, *Pythium* species are well known to be a dominant group causing damping-off of seeds and seedlings (Hendrix & Campbell 1973, Martin 1992), and are often the primary colonist. Second, an inoculation experiment was conducted in which tropical isolates and the previously isolated temperate *Pythium* spp. were tested for their pathogenicity on tropical tree species.

METHODS

ISOLATION OF SOIL MICROBES.—Tropical isolates of potential soil pathogens were obtained from soil collected on BCI, Panama (9° 10′ N, 79°50′ W), a semi-deciduous forest with a dry season from December to April (Leigh *et al.* 1996, Leigh 1999). In March, three sets of soil samples were collected with a soil corer inserted to a depth of 10 cm. The dry season sampling captured the soil microbes in their survival mode. Sampling occurred on two spatial scales. First, soil was collected at 26 sites on BCI (hereafter 'island' sites) randomly selected over an area of *ca* 3 km^2 centered on the island's plateau. Second, at a smaller scale, soil was collected near two canopy trees of *P. elegans*. At *Platypodium* 1 sites, soil was collected at 60 sites within its seed dispersal range (*ca* 100 m; C. Augspurger, per. obs.) within the Lutz Creek watershed; no other *Platypodium* tree was within this dispersal range. At *Platypodium* 2 sites, soil was sampled at 10 sites within 10 m of the trunk located off the trail at Miller 12. The 96 soil samples were air-dried in Panama, then stored in open bottles in a dark, dry location for 15–33 d until pulverized by a dry roller and sieved (2-mm screen) at the University of Illinois, where the remainder of the study was carried out. The sieved soil samples contained no visible root structures. The soil was brought into the United States using with a USDA APHIS soil permit. All soils and plant material were disposed of according to USDA APHIS specifications.

Soil *Pythium* were isolated using a selective medium consisting of water agar (1.7%), benomyl (10 ppm), nystatin (25 ppm), pentachloronitrobenzene (PCNB; 25 ppm), and rifampicin (20 ppm). This medium was designed to eliminate the growth of *Rhizoctonia*, most *Fusarium*, and gram-negative bacteria. The medium allowed growth of Oomycetes, including *Pythium* and *Phytophthora*. No *Phytophthora* were observed or isolated in this study.

To facilitate isolation of propagules in the soil, autoclaved (121°C, 110 KPa) sterilized soil (Drummer: [mixed mesic Typic Haplaquall]) from Illinois was used to dilute the raw Panama soil to three concentrations: 1:0, 1:9, and 1:99. Prior to plating onto the selective medium, 10-ml soil from each of the three concentrations was wetted with 20 ml of sterile deionized water and incubated for 24 h in the dark at 27°C. Nineteen aliquots (each 5 μl) of soil solution were uniformly applied to each of the three replicate plates per dilution (total nine plates per sample). Plates with soil were incubated at 27°C in the dark for 10 d during which unique isolates were obtained.

MICROBIAL AND TREE SPECIES.—To obtain pure cultures of tropical isolates for use for pathogenicity studies (see below), one representative colony of each unique morphology and growth rate from each soil sample were transferred from the initial dilution plates first to water agar, and then to corn meal agar (DIFCO Voigt Global

Distribution, Lawrence, KS, 17 g/L). A total of *ca* 700 isolates were transferred. They were categorized into 16 unique groups. Traits used in distinguishing the groups included colony color, extent of aerial growth, coarseness and sheen of hyphae, and density, symmetry, zonation, dendritic pattern, and speed of hyphal growth. It is unknown how their morphological differences related to genetic variation within or among species. Chen *et al.* (1992) note that asexual isolates of *Pythium* are difficult to distinguish by their morphology; molecular tests are required. Furthermore, the species concept in *Pythium* remains fluid. The 16 unique groups therefore may be artificial, and the number of unique genetic isolates is unknown.

One typical isolate from each of the 16 groups was designated as a 'core isolate'; four were from *Platypodium* 1 sites, three from *Platypodium* 2 sites, and nine from island sites. Isolates with similar, but not identical, morphologies to these 16 core isolates occurred in multiple samples in a variety of sites; 59 (hereafter 'other isolates') were arbitrarily selected for tests of pathogenicity. It is unknown whether these slight variations in morphology reflected underlying genetic variation and they might be linked to differences in pathogenicity.

Temperate isolates of seven *Pythium* spp. were selected from a reference collection based on their history of demonstrated pathogenicity of temperate graminoid and legume hosts from a variety of soils in the United States (P. Sanders, pers. comm.; Table 1).

In the pathogenicity tests, ≤ 7 temperate *Pythium* species, the 16 tropical core isolates, and ≤ 59 of the tropical other isolates were screened on each tree species. All core isolates were tested on each tree species, but the number of other isolates tested varied among tree species depending on seed and seedling availability (see Table 1).

Eight tropical tree species from BCI were tested as potential host species: *P. elegans* (Leguminosae), *Luehea seemanii* (Tiliaceae), *Cochlospermum vitifolium* (Cochlospermaceae), *Ochroma pyramidale* (Bombaceae), *Cordia alliodora* (Boraginaceae), *Apeiba membranaceae* (Tiliaceae), *Lafonsia punicifolia* (Lythraceae), and *Tabebuia guayacan* (Bignoniaceae). Nomenclature follows Croat (1978). These species include pioneer species (*Cochlospermum* and *Ochroma*) and species from secondary (*Luehea, Cordia, Apeiba*) and primary (*Platypodium, Lafonsia, Tabebuia*) forest. Their seed masses range from 1.9 to 444 mg. Each species is common in the 50-ha forest dynamics plot on BCI, except *Ochroma* (rare), *Lafoensia* (rare), and *Cochlospermum* (absent; Condit *et al.* 1996). In a prior greenhouse study, using soil from beneath one tree of *Dalbergia retusa*, a high incidence of post-emergence damping-off was observed among seedlings of *Platypodium*, *Luehea*, *Cochlospermum*, *Ochroma*, *Cordia*, and *Lafoensia*, especially in high-density stands in the shade (Augspurger & Kelly 1984); *Apeiba* and *Tabebuia* were not included in that study.

Seeds were collected beneath the crown of one parent for all species, except *Platypodium*, for which seeds of three parents (hereafter *Platypodium* 1, 2, and 3) were collected separately. Seeds were not collected near any of the island soil sites. *Platypodium* 1 and 2 were at the beginning of the transects used to collect soils referred to

TABLE 1. *Pathogenicity of temperate and tropical isolates of* Pythium *spp. on eight tropical tree species, including three parents of* Platypodium elegans.[a]

	Potential host species									
	Platypodium parent									
	1	2	3	*Luehea*	*Coch*	*Och*	*Cor*	*Ape*	*Laf*	*Tab*
Isolates										
Temperate										
P. torulosum 1	+[b]	+	+	+/+	+	+	−	−	−	−
P. periplocum				−/−	+	+	−		−	−
P. sp. unknown				−/−	+	−	−		−	−
P. mamillatum				−/−	−	−	−		−	−
P. torulosum 2				−/−	−	−	−		−	−
P. ultimum				−/−	−	−	−		−	−
P. aphanidermatum				−/−	−	−	−		−	−
Tropical										
P. aphanidermatum 1	−	−	−	+/+	+	+	−	+	−	−
P. aphanidermatum 2	+	+	+	+/+	+	+	+	+	−	−
P. ultimum	−	−	−	+/+	+	+	+	−	−	−
Other core isolates	13−	13−	13−	13−/−	13−	13−	13−	13−	13−	13−
Other isolates				59−/−	20−	20−		31−	20−	20−

[a] See methods for tree species identity. Blanks indicate not tested. Numbers indicate number of additional core isolates (13) and number of other isolates (maximum = 59) tested.

[b] +/+pre- and post-germination damping-off; +post-germination damping-off; −/−no pre- or post-germination damping-off; −no post-germination damping-off.

above as *Platypodium* 1 and *Platypodium* 2 sites. *Platypodium* 1 and 2 were separated by 0.8 km; *Platypodium* 3 was 1 km east of *Platypodium* 1. *Platypodium* 1 and 2 were selected because in a prior field study, 81 percent and 63 percent of their seedlings, respectively, died from damping-off disease (Augspurger 1983a). The percent killed by damping-off was negatively correlated with distance from the parent tree.

PATHOGENICITY TESTS.—The overall inoculation design was to use both tropical and temperate *Pythium* isolates to inoculate vermiculite containing germinating seeds and/or young seedlings of the tree species. A complete design was not possible because of insufficient seed and the large number of tropical isolates that emerged from plating soil samples. Pathogenicity was tested at the seedling stage for all species. In addition, *Luehea* was tested at the seed stage.

All seeds were surface-sterilized in 0.6 percent sodium hypochlorite for 1 min and rinsed three times in sterilized water. Seeds of *Ochroma* and *Cochlospermum* were scarified with a razor, and fruits of *Platypodium* were cracked open to enhance immediate and synchronous germination. An experimental unit consisted of seeds placed on top of sterilized vermiculite in a plastic container (4-cm diam × 13.5-cm deep). The number of seeds per experimental unit varied among species from 1 to 15, depending on seed size and availability.

A standardized unit of inoculum consisted of three colonized oat kernels. Inoculum was prepared by placing autoclaved sterilized, premoistened oat kernels onto isolates growing on corn meal agar for 2 d at 25°C. Three colonized oat kernels per experimental unit were removed from the plates and placed on top of the vermiculite. Control experimental units received only seeds and sterile oat kernels. A 1-cm layer of fine vermiculite was added on top of the oat kernels to ensure moist conditions. To test for pre-emergence damping-off in *Luehea*, inoculum was added 2 d after seeding and initial watering. To test for post-emergence damping-off, inoculum was added the day that hypocotyls emerged above the vermiculite (at 4–14 d, depending on species). Three replicate experimental units were used for each isolate in both pre- and post-emergence tests. Tree species were placed randomly in racks of 98 experimental units that were kept in closed clear plastic bags at 30°C in 16 h light and at 100 percent humidity. The vermiculite was maintained moist by daily watering.

All experimental units were inspected daily for seedling emergence (hypocotyls emerging above vermiculite) and symptoms of damping-off were noted for up to 11 d after inoculation. An isolate was deemed pathogenic at the pre-emergence stage if no seedlings emerged in any replicate experimental unit for that isolate, but seedlings emerged from each control experimental unit. Pathogenicity at the post-emergence stage was declared if at least one seedling/experimental unit in each of the three replicated units for a given isolate died after showing symptoms of damping-off (*i.e.*, lesions at or near surface of vermiculite, watery stem, fallen over, and seedling death), while no control seedlings showed these symptoms. In almost all cases, all replicate experimental units showed identical responses and most seedlings per experimental unit died as well.

RESULTS

PATHOGENICITY TESTS.—The majority of both tropical and temperate isolates were nonpathogenic at the seed and young seedling stage of host development (Table 1). Only three of the 75 tropical isolates tested were pathogenic on any tree species. These three isolates were identified as *Pythium* spp. (P. Sanders, pers. comm.). The three pathogenic tropical isolates, all from the core isolate group, showed different pathogenic responses across the host range tested. The isolate of *Pythium aphanidermatum* 1 was nonpathogenic on all three *Platypodium* parents, but was pathogenic on four of seven other species. The second isolate of *P. aphanidermatum* 2 was pathogenic on all three parents of *Platypodium* and five of seven other species. The suite of host species on which the two *P. aphanidermatum* were pathogenic differed. Thus, a given species included multiple strains that differed in pathogenicity. Finally, the isolate of *P. ultimum* was pathogenic on four of eight tree species. None of the other 13 core isolates or the 59 other isolates were pathogenic on any species tested (Table 1).

Only three of seven temperate *Pythium* species were pathogenic on any tree species (Table 1). Only one of two temperate isolates of *Pythium torulosum* was pathogenic on four of eight species, including all three parents of *Platypodium*. The temperate isolate of *Pythium periplocum* was pathogenic on two of six tree species, while the temperate isolate of the unknown *Pythium* species was pathogenic on one of six species (Table 1).

For all isolates tested on both seeds and seedlings of *Luehea*, the results for pre- and post-emergence damping-off were identical. No isolate caused damping-off at only one developmental stage (Table 1).

From the perspective of the tree species, a mixed pattern of susceptibility was also evident (Table 1). Some species were susceptible to both tropical and temperate *Pythium* species. They varied in their susceptibility to tropical isolates. *Luehea*, *Cochlospermum*, and *Ochroma* were susceptible to all three of the pathogenic tropical isolates; *Platypodium*, *Cordia*, and *Apeiba* showed mixed susceptibility, while *Lafoensia* and *Tabebuia* showed no susceptibility to any tropical or temperate isolate. There was no association between susceptibility and species abundance. Both common and rare species were susceptible and nonsusceptible to the various isolates.

DISCUSSION

Pathogens that increase the variation in environments for successful seedling establishment have the potential to enhance species diversity and affect relative species abundance (Bever 1994, Bever *et al.* 1997, Klironomos 2002). In order for soil pathogens to provide this heterogeneity, they cannot be complete generalists, capable of infecting any species. The effect of the pathogens on the spatial pattern of the seedling community would be greatest if each has a single host species. However, heterogeneity in seedling establishment arises whenever a pathogen is not effective against all species.

This study demonstrated that none of the three pathogen isolates are strictly host-specific; conversely, none is a complete

generalist capable of infecting all species. Each isolate has a unique host range. This intermediate level of specificity can be viewed from two perspectives. Their lack of total specificity and fairly broad host range argues for a diminished role in affecting spatial patterns of seedling recruitment. Alternatively, some specificity creates heterogeneity in the environment for successful seedling establishment, suggesting that tropical soil pathogens have some potential to affect seedling recruitment, thereby influencing local and community-wide patterns of tree densities and distributions.

Previous studies of the comparative pathogenicity of *Pythium* species have been limited to temperate crop species (*e.g.*, McCarter & Littrell 1970, Abad *et al.* 1994, Larkin *et al.* 1995), and in natural systems to herbs (Mills & Bever 1998) and a tree (Packer & Clay 2000, Reinhart *et al.* 2005). Mills and Bever (1998) demonstrated that four *Pythium* species each infected all four host species tested, but each species caused a different extent of reduction in plant mass and root:shoot ratios of the different species. Packer and Clay (2000) found that in soil with *Pythium* spp. collected from under *Prunus serotina*, all seedlings of *P. serotina* were killed, but mortality of two other tree species was low. They did not do direct comparative pathogenicity studies of a given isolate of *Pythium* on multiple tree species. The current study is the first to survey the extent of host specificity of soil pathogens in a tropical forest and to demonstrate via pathogenicity trials that *Pythium* species have different host ranges on tropical tree species.

Very few pathogenic isolates were found among the 96 soil samples. This rarity arose, in part, because the initial isolation of soil-borne organisms was done by culturing vegetative propagules and not by using baiting techniques to isolate only pathogens. In addition, more pathogenic isolates may have been detected if more tree species or multiple parent trees of each species had been tested, or if different environmental conditions had been used. Therefore, some isolates classified as nonpathogens may be facultative pathogens. In addition, despite using selective media for *Pythium*, it is possible that not all isolates were *Pythium*.

Each tropical isolate was not family-specific in its pathogenicity, as the host ranges included multiple families. It is possible that an additional pattern of pathogenicity would arise if tested in a more phylogentically oriented design. Some *Pythium* species, for example, are pathogenic on graminoids but not nongraminoids (Mitchell & Deacon 1986). Host specificity of insect herbivores in the study forest is phylogenetically related (Barone 1998).

Only some of the temperate *Pythium* species were pathogenic on novel hosts. Some, but not all, novel isolates of *Pythium* species have been shown to be pathogenic on crop species (Zhang & Yang 2000). These results suggest that not all local dispersal events or invasions to a new habitat by a pathogen will lead to finding a suitable host. Despite many *Pythium* species being cosmopolitan in their distribution with a wide variety of host species, each species nevertheless has somewhat limited pathogenicity. Likewise, each species has intraspecific variation (Garzon *et al.* 2005), suggesting that only some invading pathogen strains of a species will be pathogenic. Indeed, only some temperate isolates, including only one of two strains of one *Pythium* species, were pathogenic on tropical tree species.

From the tree's perspective, the species showed varying degrees of susceptibility to these isolates. Furthermore, they were susceptible to both local and foreign pathogens. This differential susceptibility corroborates the evidence above that pathogens have the potential to enhance tree species diversity. Seedlings were nonsusceptible to some pathogens, as no species was killed by all pathogenic isolates. In fact, two species were susceptible to no isolates. These species cannot be declared nonsusceptible in general, however, but only to those specific isolates tested under those specific environmental conditions.

The tree species were not susceptible to the majority of isolates. Therefore, although the high density of isolates indicated a high probability of a seedling, wherever it establishes, being exposed to many different soil microbes, the probability that it encounters a pathogenic one is low. Escape from pathogens via seed dispersal, however, does not always occur. Seedlings of several species were susceptible to isolates from soil at some distance away from any parent tree of that species, and showed susceptibility to novel isolates as well. Thus, while local dispersal or invasion of a new habitat may not lead to a pathogen-free environment, it likely leads to a site with lower seedling density with less chance of the pathogen spreading to adjacent seedlings. At the same time, because a seedling may be vulnerable to a variety of pathogens, local adaptation to specific pathogens is less likely.

These results indicate that plant–pathogen interactions in a tropical forest are likely to be highly complex. Given both differential pathogenicity and differential susceptibility, the population dynamics of both pathogen and host are more complex than if the pathogen were strictly host-specific or the host uniformly susceptible. Similar complexity due to differential plant host choice in the same tropical forest has been demonstrated for mycorrhizae (Kiers *et al.* 2000, Husband *et al.* 2002), fungal endophytes of leaves (Arnold *et al.* 2000), polypore fungi (Gilbert *et al.* 2002, Ferrer & Gilbert 2003), and insect herbivores (Barone 1998).

Assuming that similar patterns of differential pathogenicity and susceptibility would occur with a greater number of isolates of pathogenic *Pythium* and under field conditions, soil pathogens have the potential to enhance species diversity in the manner envisioned by the pest pressure hypothesis (Gillett 1962), made spatially explicit by the Janzen–Connell hypothesis. The results provide support for the assumption of some degree of host specificity for this particular group of natural enemies. The small number of pathogenic *Pythium* isolates tested limits the strength of this conclusion. Future studies should test more pathogenic isolates, for example, those obtained directly from diseased plant tissues. In addition, to test the predictions of the Janzen–Connell hypothesis in this tropical system, it must be demonstrated that this relative host specificity leads to the greater survival of heterospecific, nonsusceptible hosts, especially rare species, thus leading to greater local plant diversity (Gilbert 2005). While this study examined multiple tree species, it did not do so in the field in a community context. The complexity found in this study indicates that field studies must be expanded from a focus on a single host species to include neighboring species in a community context (Kwit *et al.* 2004), and to examine whether pathogen mortality falls more heavily on common than rare species.

ACKNOWLEDGMENTS

We thank Richard Wagner, Theresa Wilkinson, Roland Willowby, and Diane Pederson for assistance with laboratory techniques to obtain the *Pythium* isolates; Patricia Sanders, Pennsylvania State University, for providing the temperate *Pythium* spp. and identifying the tropical pathogenic isolates; and the Smithsonian Tropical Research Institute and the Republic of Panama for logistic support. Gregory Gilbert, Sabrina Russo, and Peter Thrall provided constructive criticism of an earlier draft of the manuscript. C.K.A. was supported as a College of Liberal Arts and Science Fellow for Study in a Second Discipline, University of Illinois.

LITERATURE CITED

ABAD, Z. G., H. D. SHEW, AND L. T. LUCAS. 1994. Characterization and pathogenicity of *Pythium* species isolated from turfgrass with symptoms of root and crown rot in North Carolina. Phytopathology 84: 913–921.

AGRIOS, G. 1997. Plant pathology. Academic Press, London, UK.

ARNOLD, A. E., Z. MAYNARD, G. S. GILBERT, P. D. COLEY, AND T. A. KURSAR. 2000. Are tropical endophytes hyperdiverse? Ecol. Lett. 3: 267–274.

AUGSPURGER, C. K. 1983a. Seed dispersal by the tropical tree, *Platypodium elegans,* and the escape of its seedlings from fungal pathogens. J. Ecol. 71: 759–771.

AUGSPURGER, C. K. 1983b. Offspring recruitment around tropical trees: Changes in cohort distance with time. Oikos 40: 189–196.

AUGSPURGER, C. K. 1984. Seedling survival of tropical tree species: Interactions of dispersal distance, light-gaps, and pathogens. Ecology 65: 1705–1712.

AUGSPURGER, C. K., AND C. K. KELLY. 1984. Pathogen mortality of tropical tree seedlings: Experimental studies of the effects of dispersal distance, seedling density, and light conditions. Oecologia 61: 211–217.

BARONE, J. A. 1998. Host-specificity of folivorous insects in a moist tropical forest. J. Anim. Ecol. 67: 400–409.

BEVER, J. D. 1994. Feedback between plants and their soil communities in an old-field community. Ecology 75: 1965–1977.

BEVER, J. D., K. M. WESTOVER, AND J. ANTONOVICS. 1997. Incorporating the soil community into plant population dynamics: The utility of the feedback approach. J. Ecol. 85: 561–573.

BROWDER, L. E., AND M. G. EVERSMEYER. 1986. Parasite: Host specificity and resistance/susceptibility, two concepts, two perspectives. Phytopathology 76: 379–381.

BURDON, J. J., AND G. A. CHILVERS. 1975. Epidemiology of damping-off disease (*Pythium irregulare*) in relation to density of *Lepidium sativum* seedlings. Ann. Appl. Biol. 81: 135–143.

CARLISLE, M. J., AND S. C. WATKINSON. 1994. The fungi. Academic Press, New York, New York.

CHEN, W., R. W. SCHNEIDER, AND J. W. HOY. 1992. Taxonomic and phylogenetic analyses of ten *Pythium* species using isozyme polymorphisms. Phytopathology 82: 1234–1244.

CONDIT, R., S. P. HUBBELL, AND R. B. FOSTER. 1996. Changes in tree species abundance in a Neotropical forest: Impact of climate change. J. Trop. Ecol. 12: 231–256.

CONNELL, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. *In* P. J. den Boer and G. R. Gradwell (Eds.). Dynamics of populations, pp. 298–312. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.

CROAT, T. B. 1978. Flora of Barro Colorado Island. Stanford University Press, Stanford, California.

DALLING, J. W., M. D. SWAINE, AND N. C. GARWOOD. 1998. Dispersal patterns and seed bank dynamics of pioneer trees in moist tropical forest. Ecology 79: 564–578.

DAVIDSON, J. M., S. A. REHNER, M. SANTANA, E. LASSO, AND E. A. HERRE. 2000. First report of *Phytophthora hevae* and *Pythium* spp. on native tropical tree seedlings in Panama. Plant Dis. 84: 706.

FERRER, A., AND G. S. GILBERT. 2003. Effect of tree host species on fungal community composition in a tropical rain forest in Panama. Divers. Distrib. 9: 455–468.

GARZON, C. D., D. M. GEISER, AND G. W. MOORMAN. 2005. Diagnosis and population analysis of *Pythium* species using AFPL fingerprinting. Plant Dis. 89: 81–89.

GILBERT, G. S. 2005. Dimensions of plant disease in tropical forests. *In* D. F. R. P. Burslem, M. A. Pinard, and S. Hartley (Eds.). Biotic interactions in the tropics, pp. 141–164. Cambridge University Press, Cambridge, UK.

GILBERT, G. S., A. FERRER, AND J. CARRANZA. 2002. Polypore fungal diversity and host density in a moist tropical forest. Biodivers. Conserv. 11: 947–957.

GILLETT, J. B. 1962. Pest pressure, an underestimated factor in evolution. Syst. Assoc. Pub. 4: 37–46.

HARPER, J. L. 1977. Population biology of plants. Academic Press, New York, New York.

HENDRIX, F. F. JR.,, AND W. A. CAMPBELL. 1973. Pythiums as plant pathogens. Ann. Rev. Phytopathol. 11: 77–98.

HENDRIX, F. F. JR.,, AND W. A. CAMPBELL. 1983. Some Pythiaceous fungi. *In* S.T. Buczacki (Ed.). Zoosporic plant pathogens, pp. 123–160. Academic Press, New York, New York.

HOOD, L. A., M. D. SWAINE, AND P. A. MASON. 2004. The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil. J. Ecol. 92: 816–823.

HUSBAND, R., E. A. HERRE, S. L. TURNER, R. GALLERY, AND J. P. W. YOUNG. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol. Ecol. 11: 2669–2678.

JANZEN, D. H. 1970. Herbivores and the number of tree species in tropical forests. Am. Nat. 104: 501–528.

KIERS, E. T., C. E. LOVELOCK, E. L KRUEGER, AND E. A. HERRE. 2000. Differential effects of tropical arbuscular mycorrhizal fungi inocula on root colonization and tree seedling growth: Implications for tropical forest diversity. Ecol. Lett. 3: 106–113.

KLIRONOMOS, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67–70.

KOHMOTO, K., U. S. SINGH, AND R. P. SINGH. 1995. Pathogenesis and host specificity in plant pathogenic fungi and nematodes. *In* K. Kohnoto (Ed.). Pathogenesis and host specificity in plant diseases VII. Eukaryotes, pp. 21–27. Pergamon Press, Oxford, UK.

KWIT, C., D. J. LEVEY, AND C. H. GREENBERG. 2004. Contagious seed dispersal beneath heterospecific fruiting trees and its consequences. Oikos 107: 303–308.

LARKIN, R. P., J. T. ENGLISH, AND J. D. MIHAIL. 1995. Identification, distribution, and comparative pathogenicity of *Pythium* spp. associated with alfalfa seedlings. Soil Biol. Biochem. 27: 357–364.

LEIGH, E. G. JR. 1999. Tropical forest ecology. Oxford University Press, New York, New York.

LEIGH, E. G. JR., A. S. RAND, AND D. M. WINDSOR. 1996. The ecology of a tropical rain forest (2nd edition). Smithsonian Institution Press, Washington, DC.

MARTIN, F. N. 1992. *Pythium. In* L. L. Singleton, J. D. Mihail, and C. M. Rush (Eds.). Methods for research on soilborne phytopathogenic fungi, pp. 39–49. APS Press, St. Paul, Minnesota.

MCCARTER, S. M., AND R. H. LITTRELL. 1970. Comparative pathogenicity of *Pythium aphanidermatum* and *Pythium myriotylum* to twelve plant species and intraspecific variation in virulence. Phytopathology 60: 264–268.

MILLS, K. E., AND J. D. BEVER. 1998. Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. Ecology 79: 1595–1601.

MITCHELL, R. T., AND J. W. DEACON. 1986. Differential (host-specific) accumulation of zoospores of *Pythium* on roots of graminaceous and non-graminaceous plants. New Phytol. 102: 113–122.

NEHER, D. A., C. K. AUGSPURGER, AND H. T. WILKINSON. 1987. Influence of age structure of plant populations on damping-off epidemics. Oecologia 74: 419–424.

NEHER, D. A., H. T. WILKINSON, AND C. K. AUGSPURGER. 1992. Progression of damping-off epidemics in *Glycine* populations of even-age and mixed-age structure. Can. J. Bot. 70: 1032–1038.

PACKER, A., AND K. CLAY. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404: 278–281.

REINHART, K. O., A. A. ROYO, W. H. VAN DER PUTTEN, AND K. CLAY. 2005. Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. J. Ecol. 93: 890–898.

SHIVAS, R. G., AND K. D. HYDE. 1997. Biodiversity of plant pathogenic fungi in the tropics. *In* K. D. Hyde (Ed.). Biodiversity of tropical microfungi, pp. 47–56. Hong Kong University Press, Hong Kong, China.

WRIGHT, S. J. 2002. Plant diversity in tropical forests: A review of mechanisms of species coexistence. Oecologia 130: 1–14.

ZHANG, B. Q., AND X. B. YANG. 2000. Pathogenicity of *Pythium* populations from corn-soybean rotation fields. Plant Dis. 84: 94–99.

ZHOU, D. O., AND K. D. HYDE. 2001. Host-specificity, host-exclusivity and host-recurrence in saprobic fungi. Mycol. Res. 105: 1449–1457.