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The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil

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Summary

1 *Milicia regia* (Moraceae) is a dioecious light-demanding (Pioneer) timber tree of West Africa. Experiments in shadehouses were used to examine the influence of light and soil source (beneath and away from conspecific adults) on mortality and growth of its seedlings in relation to fungal pathogens and arbuscular mycorrhizal (AM) colonization.
2 The experiment in Ghana included treatments for light (2% and 20% of unshaded irradiance), three soil sources (under female, under male and 200 m distant from adults), and two different fungicide treatments.
3 The results showed significant effects of irradiance, soil source and fungicide on both seedling mortality and accumulated biomass. Biomass was greater and mortality reduced at higher irradiance. Fungicide application reduced mortality in 2% irradiance, but had no significant effects at 20%, although AM infection of seedlings grown in soils from beneath adults was severely reduced. Mortality was greatest in soil from beneath female trees and least in soil distant from adult conspecifics. However, biomass in 20% irradiance was greater in soil from beneath females, possibly because of enhanced AM colonization: seedlings had a significantly higher percentage root length colonized by AM fungi beneath adult conspecifics, and biomass accumulation was significantly correlated with root length colonized by AM fungi.
4 There were significant interactions amongst light, soil source and fungicide treatments that attest to the complexity of spatial effects on seedling establishment in tropical forest. Whilst there was clear evidence of pathogen-mediated effects that provides support for Janzen-Connell spacing mechanisms, and escape from the parent tree increases the chances of arriving in gaps, spatial differences in AM fungal communities may act in the opposite direction and enhance seedling growth close to adult conspecifics.
5 The complexity of interactions between abiotic and biotic factors and the way in which these factors can vary spatially to affect seedling recruitment may be an important factor contributing to the maintenance of tree species diversity in tropical forests.

Key-words: arbuscular mycorrhizal fungi, irradiance, *Milicia regia*, soil fungal pathogens, spatial effects, tropical forest, West Africa

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Introduction

Evidence from neo-tropical forests suggests that pathogens contribute to the maintenance of high species diversity by causing higher mortality of juveniles around parent trees (Augspurger 1983; Augspurger & Kelly 1984; Augspurger 1984a; Gilbert 1994). However, the mechanisms for this remain unclear. The Janzen–Connell hypothesis (Janzen 1970; Connell 1971) predicts that host-specific pathogens present in the soil around parent trees lead to higher disease-induced seedling mortality. Negative feedback mechanisms may also operate whereby repeated annual input of offspring enables individual plants to culture their soil microbial communities (Bever 1994). In addition, adult trees may serve as disease 'incubators' as diseases in the canopy are often shared by juveniles in the understorey (Gilbert 1995).

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Susceptibility to disease amongst tropical tree seedlings seems to vary in relation to individual species' light requirements, with shade-tolerant species more likely than light-demanding species to display an advantage to dispersal away from adult conspecifics in shaded conditions (Augspurger 1984a). Moreover, seedlings dispersed to light gaps are less likely to suffer from damping-off disease, a seedling disease caused by fungal pathogens, than those present in the understorey (Augspurger 1983; Augspurger & Kelly 1984; Augspurger 1984a). Interactions between light and fungal pathogens are therefore likely to be relevant with regard to the degree to which disease may influence seedling establishment. Likewise, as well as abiotic/biotic interactions, arbuscular mycorrhizal (AM) fungi have the potential to influence the diversity and distribution of host species (Grime *et al*. 1987; Alexander *et al*. 1992; Bever *et al*. 1997; Kiers *et al*. 2000), and interaction between AM fungi and root pathogens is believed to be of considerable ecological significance (Newsham *et al*. 1994). Despite their predicted importance, we have only a scant understanding of these types of interactions in tropical forest.

Milicia regia (A.Chev.) C.C. Berg is a dioecious small-seeded light-demanding tree species of the Moraceae family. Along with *Milicia excelsa* (Welw.) C.C. Berg (the two species are rarely distinguished by foresters and are collectively known as Odum or Iroko) it is one of the most valuable timber trees in West Africa. *Milicia* species regenerate poorly in natural forest, even at light intensities that are not limiting, the reasons for which are unclear (Nichols *et al*. 1999).

The dioecious nature of *M. regia* enables investigation of disease incidence around both female and male trees, and therefore provides an opportunity to disentangle the effects of the presence of adult conspecifics from the effects of the input of conspecific seeds and seedlings. The aim of this study was to examine the interactions between light (understorey and small gaps), soil source (beneath and away from conspecific adults) and fungal pathogens (using fungicide application) on the survival, growth and mycorrhizal infection of *Milicia regia* seedlings. This approach allowed us to test the following hypotheses: (i) soil beneath female adults causes higher seedling mortality than soil from beneath males or soil distant from adults; (ii) fungicide application reduces these effects and enhances growth and survival; and (iii) higher irradiance reduces the effects of pathogen infection, resulting in better growth and survival. Furthermore, the potential influence of arbuscular mycorrhizas on seedling-pathogen processes was considered by comparing root colonization in different treatments.

Materials and methods

SOIL COLLECTION

At the end of May 2000, soil samples were collected from Neung South Forest Reserve (5°10′ N, 2°01′ W, 60 m a.s.l.), in the South-west of Ghana. The reserve is typical of the wet evergreen forest found in this region (Hall & Swaine 1981). *Milicia regia* trees exist at low density in the forest, enabling the selection of mature individuals separated by more than 200 m. Six *M. regia* trees that met this criterion (three female and three male) were chosen. Soil was collected from under the crown of these trees, and also at three points within the understorey of the same forest area but at a distance of at least 100 m away from individuals of either *Milicia* species. Soil samples were collected by first removing the litter layer; soil was then collected from the top 10 cm before immediate transport to the shadehouses at the Forestry Research Institute of Ghana (FORIG). Soil samples were not pooled.

EXPERIMENTAL TREATMENTS

The experiment was set up in three shadehouses at FORIG, near Kumasi. The station has a climate representative of moist semi-deciduous forest, and environmental conditions therefore differ somewhat from those at the field site in the wet evergreen forest zone. Temperature is similar in both regions. Relative humidity at the station varies mostly between 80% and 100% during the wet season (Veenendaal *et al*. 1996a). However, annual rainfall is approximately 1500 mm in Kumasi, lower than the 1750 mm minimum experienced in wet evergreen forest (Hall & Swaine 1976).

The experiment consisted of two light treatments, three fungicide treatments and three soil sources collected from Neung South Forest Reserve (under female, under male and distant, as described above). Daily irradiance totals at the station range from ≤ 14 mol m^{-2} day^{-1} in overcast weather to 36 mol m^{-2} day^{-1} in sunny weather (Veenendaal *et al*. 1996a). Light availability within the shadehouses, which ran east–west, was manipulated using bamboo slatting and wooden strips (which provided neutral shade and ensured adequate ventilation, therefore preventing air temperatures and relative humidity conditions from climbing above ambient). Neutral shade was used as tests showed that differences in light quality were not major influences on the species light responses (Swaine *et al*. 1997). Mosquito netting was used to cover the shadehouses and provide protection from herbivores. These shading treatments resulted in one half of each shadehouse having low light (2% of unshaded irradiance, hereafter referred to as 2% irradiance), mimicking conditions under forest canopy in Neung South Forest Reserve, and the other half having higher light conditions (20% of unshaded irradiance, hereafter referred to as 20% irradiance), mimicking light gap conditions in Neung South Forest Reserve. PAR measurements were made using a Decagon sunfleck septometer (PPFD quantum sensor) (Delta T. Devices, Cambridge, UK).

The fungicide treatments were as follows: (i) control, water only; (ii) Octave (Levington, active ingredient Prochloraz), applied as a soil drench at a rate of

5.6 g m^{-2}; and (iii) Octave applied at the same rate, in combination with Fubol Gold (Novartis, active ingredients Metalaxyl-M and Mancozeb), applied at 1.95 g m^{-2}. One hundred millilitres of each treatment were applied to each pot every 10 days as a soil drench. Octave fungicide is generally used to control Fusaria (Whitehead 1995; Motta & Balmas 1996), and would thus target *Fusarium* species, the fungal genus most frequently isolated from *M. regia* seedlings (Mancini *et al*. 2001). Fubol Gold is generally used to control Oomycetes (Leroux *et al*. 1991; Whitehead 1995), such as species of *Phytophthora* and *Pythium*, which had been previously isolated from Neung South forest soil and were suspected to be potentially important due to their nature as aggressive damping-off pathogens (Jarosz & Davelos 1995).

Freshly fallen fruit was collected from a single parent tree in Neung South Forest Reserve and seeds were removed by soaking fruit in water. Seedlings were raised from surface sterilized seeds (exposed to 1% sodium hypochlorite for 5 min) that were germinated on moist filter paper in Petri dishes. Approximately 1-week-old emerging seedlings free of fungal infection were transferred into polypots (10 × 15 cm) filled with forest soil collected from the various sources. Ten *M. regia* seedlings were transferred into each pot and each pot was replicated three times within each of the three shadehouses, giving a total of nine replications of each soil × light × fungicide combination.

SEEDLING MEASUREMENTS

Seedling mortality was measured weekly, and at the end of the experiment (8 weeks after planting) seedlings were harvested and biomass measurements were made. Ten seedlings per treatment per shadehouse (where possible, in some treatments fewer than 10 seedlings survived) were randomly chosen and harvested for the biomass measurements. The remaining seedlings were used to assess mycorrhizal colonization in roots. The roots of *M. regia* seedlings growing in the shade, being fragile and often diseased, were difficult to harvest and so were omitted from biomass and mycorrhizal colonization assessments. Otherwise, roots were preserved in 2% gluteraldehyde and transported back to the UK where the whole root system of each seedling was stained using trypan blue with 2.5% KOH (Koske & Gemma 1989) using a modified syringe method (Claassen & Zasoski 1992). Percentage root length colonized (% RLC) was assessed using the modified root intersect method (Tennant 1975; Giovannetti & Mosse 1979) and root length was measured using the grid-intersect method (Marsh 1971).

STATISTICAL ANALYSIS

To test for differences in *M. regia* seedling survival between treatments a split-plot ANOVA (split for light) was applied to arcsine transformed percentage survival per pot data collected at the end-point of the trial. There were many missing biomass values due to high seedling mortality in the 2% irradiance treatment and the lack of root biomass values in this treatment. A regression analysis was therefore carried out to test for differences in shoot biomass between all treatments using available individual seedlings. Thereafter, shoot biomass results of individual seedlings growing in 2% irradiance were subjected to regression analysis to test for effects of soil source and fungicide treatment. Root, shoot and total biomass and root to shoot ratio of seedlings growing in the light were examined using two-way ANOVA (in randomized blocks) to test for soil source and fungicide treatment effects. Percentage mycorrhizal colonization results required no transformation; analysis was performed using two-way ANOVA (in randomized blocks, where each shadehouse represented a block). Percentage mycorrhizal colonization results were correlated with seedling biomass results. Root length results were logarithmically transformed prior to analysis using appropriate ANOVAs as for percentage colonization. All analyses were carried out using GENSTAT version 5, release 4.1.

Results

SEEDLING SURVIVAL

At the end of the experiment, ANOVA showed that there were significant effects of light, soil source and fungicide treatment on *M. regia* survival (Table 1). In addition, irradiance treatment differentially influenced seedling survival depending on fungicide treatment (there was a light–fungicide interaction).

An illustration of the light–fungicide interaction can be seen in Fig. 1. Overall, *M. regia* seedlings suffered heavier mortality at 2% irradiance than at 20% irradiance. The great majority of seedlings that died showed symptoms of damping-off disease, namely discoloration at the base of the stem and wilting. Similar symptoms had previously been observed amongst diseased *M. regia* seedlings in Neung South Forest Reserve. The

Table 1 ANOVA *F*-ratios for *Milicia regia* seedling survival at the end-point of the trial (8 weeks after planting). Light = light treatment; source = soil source; fungicide = fungicide treatment. Significance shown as follows: NS, not significant, $P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

	d.f.	Survival *F*-ratio
Light	1	411.08**
Source	2	11.08***
Fungicide	2	32.59***
Light × source	2	0.93 NS
Light × fungicide	2	14.14***
Source × fungicide	4	0.92 NS
Light × source × fungicide	4	0.84 NS

Light effects were tested over the light × block mean square (d.f. 2). Other effects were tested over the error mean square (d.f. 113).

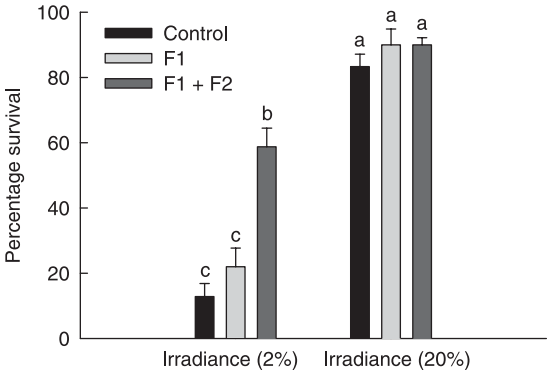

Fig. 1 Percentage of *Milicia regia* seedlings (+ 1 SE) surviving under different fungicide treatments in 2% irradiance and 20% irradiance conditions at the end of the trial, 8 weeks after planting. Control = no fungicide, F1 = Octave, F2 = Fubol Gold. Different letters show significant ($P < 0.05$) differences between treatments.

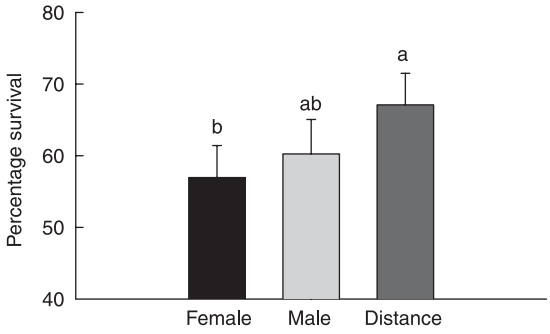

Fig. 2 Percentage of *Milicia regia* seedlings (+ 1 SE) surviving in soil from different sources (soil collected from under mature female and male *Milicia regia* trees, and at a distance of over 100 m from *Milicia regia* trees) at the end of the trial, 8 weeks after planting. Different letters show significant ($P < 0.05$) differences between treatments.

Table 2 Regression analysis *F*-ratios for individual *Milicia regia* seedling shoot biomass. Light = light treatment; source = soil source; fungicide = fungicide treatment. Significance shown as follows: NS, not significant, $P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

	d.f.	Shoot biomass *F*-ratio
Light	1	85.39*
Soil source	2	10.94***
Fungicide	2	20.68***
Light × source	2	3.96**
Light × fungicide	2	0.39 NS
Source × fungicide	4	6.76**
Light × source × fungicide	4	0.49 NS

Light effects were tested over the light × block mean square (d.f. 2). Other effects were tested over the error mean square (d.f. 370).

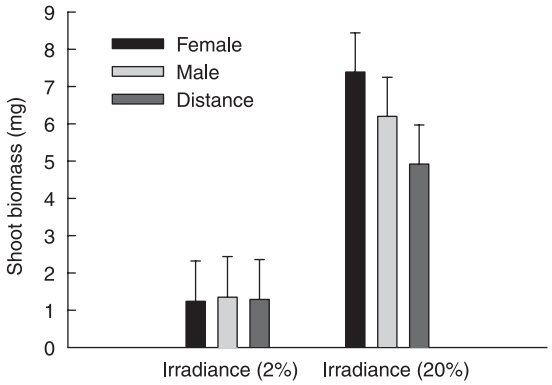

Fig. 3 *Milicia regia* mean shoot biomass per seedling as predicted from the regression model (+ 1 SE) in response to soil source (soil collected from under mature female and male *Milicia regia* trees, and at a distance of over 100 m from *Milicia regia* trees) in 2% irradiance and 20% irradiance conditions at the end of the trial, 8 weeks after planting.

application of fungicides had no significant influence in the 20% irradiance treatment, where seedling survival was 83% even in the control (no fungicide) treatment. In the 2% irradiance treatment, however, the application of Octave and, to a greater degree, Octave and Fubol Gold in combination, markedly improved seedling survival: at the end of the experiment survival among seedlings treated with both fungicides was improved by on average 46%, and seedlings treated with Octave alone experienced a non-significant 10% improvement in survival.

The influence of soil source on seedling survival was less pronounced than the influence of light or fungicide application, but nevertheless there was significantly higher survival when seedlings were grown in soil collected at a distance from parent trees (67.1% survival) compared with survival in soil collected from under female trees (56.9% survival), as seen in Fig. 2. In contrast to the varying effect of fungicides in the different light conditions, the pattern of seedling survival in response to soil source was similar in both 20% irradiance and 2% irradiance treatments.

SEEDLING GROWTH

There were significant effects of light, soil source and fungicide treatment on *M. regia* shoot biomass (Table 2). In general, seedlings had higher biomass in soil from under female trees than in soil from under male trees or at a distance of 100 m from parent trees, and fungicides exhibited a positive influence on seedling growth. However, there was a significant light–soil source interaction: seedlings displayed greater shoot biomass in soil from under female trees at 20% irradiance but not at 2% irradiance (Fig. 3). There was also a source–fungicide interaction for *M. regia* shoot biomass, which is best illustrated by considering other growth parameters, as described below.

Light and shade biomass results were analysed separately as previously explained. Within the 2% irradiance treatment, there were no significant differences in *M. regia* shoot biomass between soil sources ($F = 0.03$, $P > 0.05$) or fungicide treatments ($F = 2.94$, $P > 0.05$). However, in the 20% irradiance treatment a different pattern of biomass results emerged: amongst seedlings

Table 3 ANOVA *F*-ratios for *Milicia regia* seedling growth parameters (root biomass, root length, shoot biomass, root : shoot ratio and total biomass) and percentage root length colonized (%RLC) by AM fungi in 20% irradiance conditions. Source = soil source; fungicide = fungicide treatment. Significance shown as follows: NS, not significant, $P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

		F-ratios					
	d.f.	Root biomass	Root length	Shoot biomass	Root : shoot ratio	Total biomass	%RLC
Source	2	1.03 NS	0.94 NS	18.22***	22.38***	8.40***	15.60***
Fungicide	2	2.92 NS	0.29 NS	10.77***	19.66***	8.71***	60.91***
Source × fungicide	4	2.51*	1.53 NS	2.00 NS	0.62 NS	4.15**	4.39*

Effects were tested over the error mean square (d.f. 253) for all variables except root length and percentage RLC (d.f. 16).

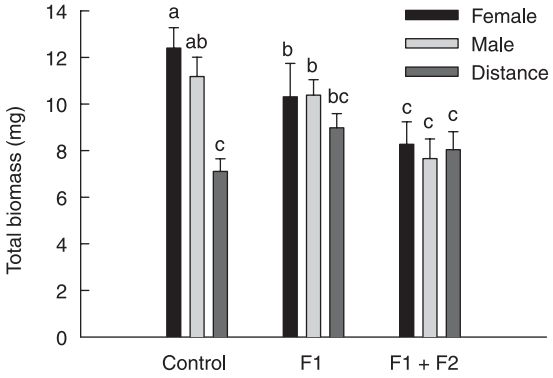

Fig. 4 *Milicia regia* mean total biomass per seedling (+ 1 SE) in 20% irradiance conditions in response soil source treatments (soil collected from under mature female and male *Milicia regia* trees, and at a distance of over 100 m from *Milicia regia* trees) and fungicide treatment at the end of the trial, 8 weeks after planting. Control = no fungicide, F1 = Octave, F2 = Fubol Gold. Different letters show significant ($P < 0.05$) differences between treatments.

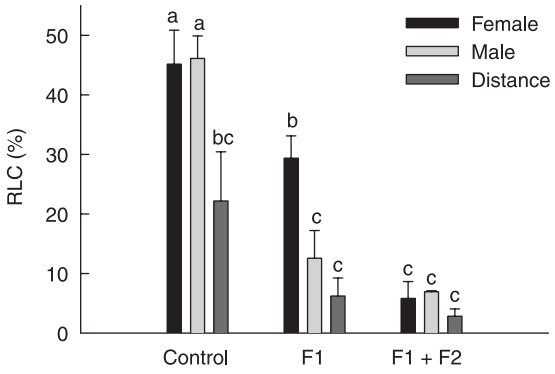

Fig. 5 Mean percentage RLC (percentage root length colonization) by AM fungi (+ 1 SE) in *Milicia regia* seedlings in 20% irradiance conditions subject to different soil source treatments (soil collected from under mature female and male *Milicia regia* trees and at a distance of over 100 m from *Milicia regia* trees) and fungicide treatments at the end of the trial, 8 weeks after planting. Control = no fungicide, F1 = Octave, F2 = Fubol Gold. Different letters show significant ($P < 0.05$) differences between treatments.

growing in 20% irradiance, there was a significant interaction between soil source and fungicide treatment for a number of growth parameters (Table 3). Total biomass of control seedlings in soil collected from under female trees was almost double the total biomass in soil collected at a distance from parent trees, but this effect was non-significant with the application of fungicide (Fig. 4). The individual responses of root and shoot biomass showed a similar pattern.

MYCORRHIZAL COLONIZATION

M. regia roots were colonized by arbuscular mycorrhizal (AM) fungi, with clumps of intense infection observed. There was significantly different percentage root length colonization (%RLC) depending on soil source and fungicide treatments within 20% irradiance conditions (Table 3). This soil source–fungicide interaction is shown in Fig. 5. Fungicide application dramatically reduced AM colonization from an average of 37.8% in control treatments with no fungicide to an average of 5.2% in treatments consisting of a combination of the two fungicides. In addition, when no fungicide was applied, percentage AM colonization was significantly higher in soils collected from under female and male *M. regia* than in soil collected at a distance from parent trees. Application of fungicide not only reduced percentage RLC but also reduced the differences in percentage RLC between the different soil sources.

MYCORRHIZAL COLONIZATION AND SEEDLING GROWTH

There was a significant positive correlation between shoot biomass and percentage AM colonization averaged across treatments (Fig. 6), with an R^2 value of 0.64. *M. regia* root biomass and percentage colonization were not, however, significantly correlated.

Discussion

Milicia regia seedlings had a higher probability of dying due to damping-off disease in low-light conditions characteristic of tropical forest understorey as opposed to higher light conditions that may be found in light gaps. Growth in soil from under adult female trees also

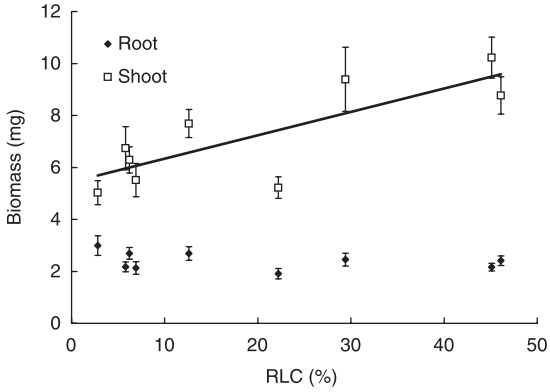

Fig. 6 Correlation between *Milicia regia* mean root and shoot biomass per seedling and percentage RLC (percentage root length colonized) for seedlings growing in 20% irradiance. Data shown are mean biomass per seedling ± 1 SE. Shoot biomass: $r = 0.8$, 7 d.f., $P < 0.01$. Root biomass: $r = 0.33$, 7 d.f., not significant ($P > 0.05$).

increased the likelihood of seedling mortality. However, patterns in seedling growth indicate that there are more complex interactions occurring between *M. regia* seedlings and the soil community that involve small-scale patterns in arbuscular mycorrhizal (AM) infection as well as fungal pathogenic infection.

Reduced incidence of damping-off disease in forest gaps has been reported for a range of tropical tree species (Augspurger & Kelly 1984; Augspurger 1984b). The reasons for this are thought to be a combination of lower humidity and more rapid drying of soil in gaps, which is expected to depress pathogen activity (and lead to smaller pathogen populations if repeated years of unfavourable conditions occur), and also increased light intensity, which is expected to promote growth of the host and thereby limit the period of host vulnerability to these pathogens (Augspurger 1990). The fact that the soil used in this trial was originally collected in forest understorey and then transferred to light conditions simulating gaps meant that pathogen populations were initially the same. Moreover, during the experiment, soil in the 20% irradiance and 2% irradiance treatments was kept more or less equally moist by daily watering. Results here would therefore indicate that the increased growth of the host as observed in gap conditions, rather than differences in the soil community, was the most probable reason why *M. regia* suffered less disease-induced mortality at 20% irradiance.

Mortality of *M. regia* seedlings was higher in soil from under adult female trees than at a distance from adult conspecifics, endorsing similar findings from tropical rain forest (Augspurger 1990; Gilbert 1994). It has been suggested that adult trees serve as reservoirs for disease and various mechanisms for this have been suggested: there may be host-specific pathogens present in the soil around parent trees (Janzen 1970; Connell 1971); there may be a build up of more generalist pathogens in the soil around parent trees through negative feedback mechanisms where repeated annual input of offspring enables individual plants to 'culture' their soil microbial communities (Bever 1994); or adult trees may serve as disease 'incubators' as diseases in the canopy are often shared by juveniles in the understorey (Gilbert 1995). In this study, treatment with fungicide generally thought to be Oomycete specific increased seedling survival to a greater extent than fungicide used to target Fusaria. Previous isolation and identification of pathogens from soil and seedlings showed that these two pathogenic groups were the most probable cause of disease in *Milicia* (Mancini *et al*. 2001). Results found here would therefore suggest that Oomycetes were the main pathogens causing damping-off disease. Oomycetes are soil-borne and are regarded as generalist pathogens (Agrios 1997). However, studies in natural forests have indicated that host-specific Oomycete pathogens can build up higher inoculum levels in soil around parent trees through the input of susceptible seeds and seedlings, whereas at a distance from conspecifics they exist at low density or heterogeneously in the soil (Davidson 1998; Packer & Clay 2000). It therefore seems probable that this is the mechanism responsible for the elevated incidence of disease under female *M. regia* trees. The level of disease-induced mortality in soil from under male trees lay somewhere between the levels of mortality in soils from under female trees and at a distance from conspecifics. This would suggest that canopy inputs of pathogens such as *Fusarium solani*, which is commonly isolated from *Milicia* seedlings and is found in the crowns of *Milicia* trees (Mancini *et al*. 2001), may indeed result in some background disease, but that the majority of disease is caused by the build up of soil-borne Oomycete pathogens rather than the source being aerial inoculum from an infected parent.

Although mortality was higher under parent trees, results indicate that the growth of seedlings at 20% irradiance was actually improved under parent trees. This apparent anomaly may be best explained by the increased AM colonization of roots growing in soil from under parent trees and the positive correlation observed between AM infection and shoot growth. AM fungi tend to be generalists but the results here lend support to the argument that there may be high functional diversity of AM fungi within and across habitats, with AM populations varying both seasonally and spatially (Musoko *et al*. 1994; Allen *et al*. 1995; Merryweather & Fitter 1998; Husband *et al*. 2002; Lovelock *et al*. 2003), and the identity of AM fungi, as well as their presence or absence, is likely to be important. AM fungi associated with host trees have been found to have varying effects on growth of conspecific seedlings, with both negative effects (Kiers *et al*. 2000) and improved growth responses observed (Onguene 2000). In addition, spore numbers in soil are not always correlated with the proportion root length colonized by AM fungi (Merryweather & Fitter 1998). Although spore numbers were not investigated in this study, results here indicate that host tree-specific AM fungi not only colonize seedling roots to a greater extent close to adult

conspecifics, but that this colonization can be beneficial in terms of seedling growth.

At 2% irradiance, however, positive growth effects were not observed close to parent trees. As AM colonization was not measured in this situation it is not known whether this was due to lower colonization, as may be predicted in low light where there is lack of carbon benefit to the mycorrhizal fungus (Smith & Smith 1996), or because the increased virulence of seedling pathogens growing at low light outweighed the potentially beneficial effects of AM colonization. Whichever is the case, the importance of soil pathogens and AM fungi to *M. regia* seedling establishment seems to vary greatly depending on whether a seedling germinates in light conditions typical of gaps or in the understorey. The specific effects of each fungal group in these situations are, however, difficult to elucidate due to the confounding effects of the fungicides used. Problems with experimental use of fungicides have previously been noted, with a lack of plant response to mycorrhizas in some studies suggested to be due to the fungicides used to exclude mycorrhizas also eliminating pathogenic fungi (Newsham *et al*. 1994). A similar effect has occurred here except that the fungicides that were used to remove pathogenic fungi also reduced AM fungi colonization.

The results presented here demonstrate that it is not sufficient simply to look at variation in responses to a single factor when examining spatial effects on seedling mortality and growth around parent trees, but that there is the potential for spatial variation in a number of factors, both biotic and abiotic. This is demonstrated by the fact that pathogens, AM fungi and light environment interact to affect *M. regia* seedlings establishment in tropical forest soil. *M. regia* displays a twofold advantage to dispersal, both to escape from pathogen pressure around parent trees and to increase its chances of arriving in a gap, where improved seedling performance lessens the effects of pathogens. However, *M. regia* seedlings seem preferentially to associate with host-specific AM fungi, suggesting that if a seedling could survive pathogenic attack then there would be a growth advantage incurred by existing close to the parent tree, acting in the opposite direction to spacing mechanisms. This would imply that there is perhaps even more complexity than expected in interactions between abiotic and biotic factors, the way in which these factors can vary spatially in tropical forest, and ultimately, the way in which these factors may contribute to tropical forest diversity.

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References

Agrios, G. (1997) *Plant Pathology*. Academic Press, London.

Alexander, I., Ahmad, N. & See, L. (1992) The role of mycorrhizas in the regeneration of some Malaysian forest trees. *Philisophical Transactions of the Royal Society of London B*, **355**, 379–388.

Allen, E., Allen, M., Helm, D., Trappe, J., Molina, R. & Rincon, E. (1995) Patterns of regulation of mycorrhizal plant and fungal diversity. *Plant and Soil*, **170** (1), 47–62.

Augspurger, C. (1983) Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of its seedlings from fungal pathogens. *Journal of Ecology*, **71**, 759–771.

Augspurger, C. (1984a) Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps and pathogens. *Ecology*, **65** (6), 1705–1712.

Augspurger, C. (1984b) Light requirements of neotropical tree seedlings: a comparative study of growth and survival. *Journal of Ecology*, **72**, 777–795.

Augspurger, C. (1990) Spatial patterns of damping-off disease during seedling recruitment in tropical forests. *Pests, Pathogens and Plant Communities* (eds J. Burdon & S. Leather), pp. 131–143. Blackwell Scientific, Oxford.

Augspurger, C. & Kelly, C. (1984) Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. *Oecologia*, **61**, 211–217.

Bever, J. (1994) Feedback between plants and their soil communities in an old field community. *Ecology*, **75**, 1965–1977.

Bever, J., Westover, K. & Antonovics, J. (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*, **85**, 561–573.

Claassen, V.P. & Zasoski, R.J. (1992) A containerized staining system for mycorrhizal roots. *New Phytologist*, **121**, 49–51.

Connell, J. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. *Dynamics in Populations* (eds P. Den Boer & G. Gradwell), pp. 298–310. Center for Agricultural Publishing and Documentation, Wageningen.

Davidson, J.M. (1998) *Pathogen Mediated Mortality of Tropical Tree Seedlings: Implications for Density-Dependent Regulation of Tropical Tree Diversity*. INTECOL VII International Congress of Ecology. Backhuys Publishers, Neiden.

Gilbert, G. (1994) Density- and distance-to-adults effects of a canker disease of stress in a moist tropical forest. *Oecologia*, **98**, 100–108.

Gilbert, G. (1995) Rain forest plant diseases: the canopy-understory connection. *Selbyana*, **16** (1), 75–77.

Giovannetti, M. & Mosse, B. (1979) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, **84**, 489–500.

Grime, J., Mackey, M., Hillier, S. & Read, D. (1987) Floristic diversity in a model system using experimental microcosms. *Nature*, **328**, 420–422.

Hall, J. & Swaine, M. (1976) Classification and ecology of closed-canopy forest in Ghana. *Journal of Tropical Ecology*, **64**, 913–951.

Hall, J. & Swaine, M. (1981) *Distribution and Ecology of Vascular Plants in a Tropical Rain Forest: Forest Vegetation in Ghana*. W Junk, The Hague.

Husband, R., Herre, E.A., Turner, S.L., Gallery, R. & Young, J.P.W. (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology*, **11**, 2669–2678.

Janzen, D. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist*, **104**, 501–528.

Jarosz, A. & Davelos, A. (1995) Tansley Review Number 81. Effects of disease in wild plant populations and the evolution of pathogen aggressiveness. *New Phytologist*, **129**, 371–387.

Kiers, E., Lovelock, C., Krueger, E. & Herre, E.A. (2000) Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecology Letters*, **3**, 106–113.

Koske, R.E. & Gemma, J.N. (1989) A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*, **92** (4), 486–505.

Leroux, H., Whner, F., Kotze, J. & Grech, N. (1991) Combining Fosetyl-Al trunk injection or Metalaxyl soil drenching with soil application of Aldicarb for control of citrus decline. *Plant Disease*, **75** (12), 1233–1236.

Lovelock, C.E., Andersen, K. & Morton, J.B. (2003) Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. *Oecologia*, **135**, 268–279.

Mancini, F., Apetorgbor, M., Cobbinah, J. & Ragazzi, A. (2001) Potential fungal pathogens on seeds and seedlings of *Milicia excelsa* of three ecological zones in Ghana. *Journal of Plant Disease and Protection*, **108** (1), 31–38.

Marsh, B.B. (1971) Measurement of length in random arrangements of lines. *Journal of Applied Ecology*, **8**, 265–267.

Merryweather, J. & Fitter, A. (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. II. Seasonal and spatial patterns of fungal populations. *New Phytologist*, **138**, 131–142.

Motta, E.T., A. & Balmas, V. (1996) Seedborne fungi in Norway spruce: testing methods and pathogen control by seed dressing. *European Journal of Forest Pathology*, **26**, 307–314.

Musoko, M., Last, F. & Mason, P. (1994) Populations of spores of vesicular-arbuscular mycorrhizal fungi in undisturbed soils of secondary semideciduous moist tropical forest in Cameroon. *Forest Ecology and Management*, **63**, 359–377.

Newsham, K., Fitter, A. & Watkinson, A. (1994) Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. *Journal of Ecology*, **82** (4), 805–814.

Nichols, J., Agyeman, V., Agurgo, F., Wagner, M. & Cobbinah, J. (1999) Patterns of seedling survival in the tropical African tree *Milicia excelsa*. *Journal of Tropical Ecology*, **15**, 451–461.

Onguene, N.A. (2000) *Diversity and dynamics of mycorrhizal associations in tropical rain forests with different disturbance regimes in south Cameroon*. PhD thesis. Wageningen University, Wageningen.

Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, **404** (6775), 278–281.

Smith, F. & Smith, S. (1996) Mutualism and parasitism: diversity and function in structure in the 'arbuscular' (VA) mycorrhizal symbiosis. *Advances in Botanical Research*, **22**, 1–43.

Swaine, M., Agyeman, V., Kyereh, B., Orgle, T., Thompson, J. & Veenendaal, E. (1997) *Ecology of Forest Trees in Ghana*. ODA Forestry Series No. 7, London.

Tennant, D. (1975) A test of a modified line intersect method of estimation of root length. *Journal of Ecology*, **63**, 995–1001.

Veenendaal, E., Swaine, M., Lecha, R., Walsh, M., Abebrese, I. & Owusu-Afriyie, K. (1996a) Responses of West African forest tree seedlings to irradiance and soil fertility. *Functional Ecology*, **10**, 501–511.

Whitehead, R. (1995) *The UK Pesticide Guide*. CAB International, Wallingford.

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