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Chaetomium elatum (Kunze: Chaetomiaceae) as a root-colonizing fungus in avocado: is it a mutualist, cheater, commensalistic associate, or pathogen?[1]

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Plants support numerous root colonists that may share morphological characteristics with mycorrhizal fungi but may play different roles in the rhizosphere. To determine the function of one such root-colonizing fungus, *Chaetomium elatum*, the infectivity and composition of inoculum containing *C. elatum* were varied independently of and in association with the known mutualist *Glomus intraradices* under two light intensities. Maximum plant benefit occurred with mixtures of both *G. intraradices* and *C. elatum* and under high light intensity. Under low light intensity and in monoculture, *C. elatum* functioned as a weak pathogen that was able to kill host plants. Here, maximum plant mortality was associated with the highest levels of *C. elatum* infectivity. When *G. intraradices* was present, no negative impact of *C. elatum* was detected. Intraspecific interactions were important in predicting sporulation rates for both fungi, whereas no interspecific fungal interactions were detected. In the presence of *G. intraradices*, *C. elatum* appears to function as a "commensalistic associate," neither impacting plant growth nor sporulation by *G. intraradices*. Overall, *C. elatum* appears to be multifunctional, serving as both a rhizoplane and rhizophere fungus, opportunistically colonizing plant roots and only becoming pathogenic when resources are severely limited and intraspecific competition is high. This multifunctional strategy may be shared with other fungi that form similar structures in roots.

Key words: *Chaetomium*; cheater; dark-septate endophyte; density dependence; *Glomus*; light; mycorrhizae.

Associations between root-colonizing fungi and plants range from pathogenic to mutualistic (Johnson et al., 1997; Smith and Read, 1997; Jumpponen, 2001). Mutualistic associations are beneficial to both partners, whereas parasitic relationships are defined by the gain of one partner resulting in a loss to the other partner (Addicott, 1984). In some cases, a "third party" may take advantage of a preexisting mutualistic association or mimic a mutualist without providing a host benefit (Bidartondo et al., 2000). An organism with this capability can be referred to as a "cheater" if this results in a relationship where the benefit to one of the participants is reduced (Addicott and Tyre, 1994) or as a "commensalistic associate" if no cost is imposed on the other participants [also referred to as "casual associates" by Read (1991)]. Although mycorrhizal associations are the most widely observed, plant root systems support numerous fungal colonizers for which functions have yet to be determined (Newsham et al., 1995).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil organisms that entirely depend on host plants for carbohydrates and often form a mutually beneficial symbiosis with most plants (Graham and Miller, 2005). However, outcomes of interactions between closely associated organisms can depend upon environmental conditions (Bronstein, 1994). This means that a beneficial relationship can become parasitic if the very resources upon which the association depends are severely limiting or available in excess (Johnson, 1993; Graham and Eissenstat, 1998; Jones and Smith, 2004). Further, the presence of other organisms with which AMF share space and resources may alter the plant growth response to AMF (Hetrick, 1986). Thus interspecific interactions between root colonists may impact plants, and this may change under different environmental conditions.

We found a fungal colonist of unknown function sporulating prolifically in the root system of mycorrhizal and nonmycorrhizal avocado [*Persea americana* Mill. var. *drymifolia* (Schltdl. & Cham.)] and used culturing and molecular techniques, to identify this root colonist as the ubiquitous soil fungus, *Chaetomium elatum* (Kunze). Although *Chaetomium* spp. have been frequently isolated from surface-sanitized healthy roots (Bartoli et al., 1978; Ishiba et al., 1979; Skipp and Christensen, 1981), it is not widely recognized as a rhizosphere organism. Hyphae and chlamydospores of the anamorph of *C. elatum* were observed in association with avocado roots. In our isolate, these masses of chlamydospores can remain as resting spores (as observed in pot cultures and on media) for months or progress to become perithecia of *C. elatum*. Root colonization has been observed to start as an infection cushion with what appears to be a simultaneous formation of a loose "mantle" and dense partial to complete intracellular infection of epidermal cells. No symptoms of disease, poor nutrient status, or root tissue disorganization were detected to imply that *C. elatum* is a pathogen or parasite on avocado.

This morphology closely resembles a large number of fungal species that are simply called rhizoplane fungi or lumped into a number of large, loosely defined groups of root colonists (including E-strain and dark-septate endophytes). Many fungi in Ascomycota, and even in Sordariales (the taxon to which *Chaetomium* belongs), form very similar structures in roots

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(Jumpponen et al., 2003). Although these root colonists share some common morphological characteristics with mycorrhizal associations, these characteristics are not specifically indicative of a mutualistic association (as would be arbuscules). Numerous examples of organisms that "mimic" mutualists but provide no benefit to their hosts have been documented in natural systems (Morris et al., 2003).

The study of these root colonists is also important because, not only are there a large number of fungal colonists that form these structures in roots, but they are also frequently observed in a wide range of plant species. For instance, ascomycetous anamorphs forming similar structures have been documented in association with roots of 135 angiosperm species (Peyronel, 1924; Haselwandter and Read, 1980). Jumpponen and Trappe (1998) stated hosts for root colonists forming similar structures can include ecto-, nonmycorrhizal, arbuscular, ericoid, or orchid mycorrhizal plant species. Girlanda et al. (2002) noted that although such structures have been observed in some 600 plant species (Jumpponen and Trappe, 1998), and the first observations coincided with foundational mycorrhizal research, a limited number of studies have sought to determine their function or clarify group membership. Few of these fungi have been associated with teleomorphs or even identified as anamorphs. As a result, these frequently observed fungal root colonists provide few useful morphological characteristics and consequently often fit the descriptions of multiple groups. An aim of this study was to determine the role of the ascomycetous anamorph, *C. elatum*, as a root colonist in association with and independent of the arbuscular mycorrhizal fungus *Glomus intraradices*.

The relationships among *G. intraradices*, *C. elatum*, and plant growth were examined under two light intensities and under low P conditions (ca. 10 ppm). We expected that carbon-limiting conditions would improve the probability of detecting either a parasite or a strong mutualist because where carbon is limiting plants may be more sensitive to the carbon cost of supporting a root colonizer (Fredeen and Terry, 1988; Thomson et al., 1992). By contrast, noncarbon-limiting but P-limiting conditions were expected to improve the probability of detecting mutualistic associations.

To determine if a mutualistic association between the fungi and avocado was established, two hallmarks of a mycorrhizal mutualistic relationship were used: (1) a growth benefit provided to the host by the symbiont and (2) an increased investment in the symbiosis. The greatest investment in the symbiosis was presumed to be detectable under low light for which we predicted plants would allocate the greatest proportion of their carbon to leaves. This prediction is based on the hypothesis of balanced growth, which states that plants will allocate more biomass to the organ that is acquiring the most limiting resource (Shipley and Meziane, 2002), and if the plant P status was improved due to the symbiosis, the most limiting resource under low light would then be carbon. Therefore, the maximum growth benefit to the host should be acquired from the mycorrhizal fungus under high light conditions where carbon fixation was presumably least limiting, whereas the greater investment in leaf area should be evident under carbon-limiting conditions. If the presence of *C. elatum* alters this relationship, this should be detectable when comparing plants inoculated with *G. intraradices* monocultures with those inoculated with both fungi.

Alternatively, the interaction of these fungi may not directly impact plants but instead may influence one another's sporulation. This could have indirect effects on plant growth because changes in the infectivity, or inoculum potential, of both pathogenic and mutualistic soil fungi can influence plant growth (Abbott and Robson, 1981; Newton et al., 1997). The potential effect of changes in fungal inoculum potential is not easily predicted. Whereas increases in AMF inoculum potential can improve host benefit, in some instances, interspecific fungal interactions can favor assemblages of less beneficial fungi than those initially present.

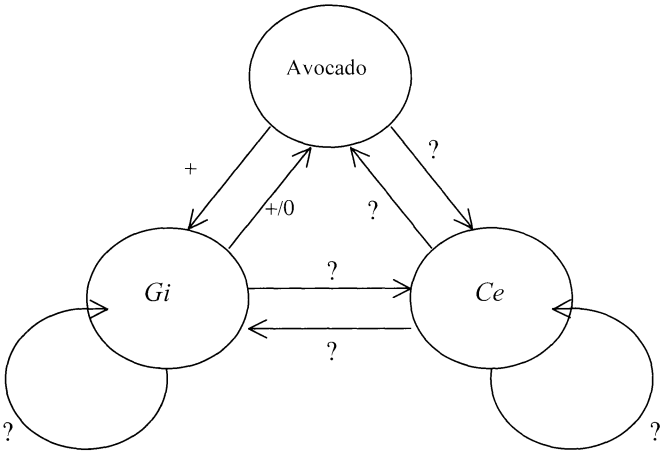


Fig. 1. A conceptual diagram illustrating the possible interaction effects among avocado, *Glomus intraradices* (*Gi*), and *Chaetomium elatum* (*Ce*) prior to the experiment. These relationships were defined for fungal inoculum that varied in composition and infectivity and for two light intensities. Question marks indicate which effects were tested in this experiment. The effect of *G. intraradices* on avocado is well studied and has been observed as positive to neutral (indicated in diagram as "+/0"). The function of *C. elatum* was unknown. Thus, the influence of inter- and intraspecific interactions between and within these fungi and their effects on avocado were also tested.

Our aim was to determine the function of *C. elatum* as a root colonizer in mycorrhizal and nonmycorrhizal avocado under two light intensities. We asked (1) how *C. elatum* affected plant growth and C allocation by comparing it to a known mutualistic arbuscular mycorrhizal fungus (AMF), *Glomus intraradices*; (2) if intra- and interspecific interactions (individual fungal species density or competition between fungi) influenced fungal effects on plant growth and C allocation; and/or (3) if intra- and interspecific interactions affect sporulation of either fungal species (Fig. 1). Comparisons of C allocation, plant biomass, and fungal fecundity were made by manipulating the propagule density (inoculum potential) of *G. intraradices* and *C. elatum* both in monoculture and mixture under two light regimens.

MATERIALS AND METHODS

Inocula production and estimation of inoculum potential—*Glomus intraradices* (INVAM DN989) whole soil inoculum was obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (Morgantown, West Virginia, USA), and *Chaetomium elatum* inoculum was produced via soil-extracted chlamydospores. To produce enough inocula for the experiment, we applied approximately 20 mL of the *G. intraradices* inoculum or 20 surface-sanitized chlamydospores of *Chaetomium elatum* on 10-d-old *Sorghum vulgare* roots, which were then potted in silica sand in 0.4-L pots. Chlamydospores of *C. elatum* were extracted from soil in which avocado was

grown using sucrose-density-gradient centrifugation (Furlan et al., 1980), isolated on filter paper, and surface sanitized with 70% ethanol. After 2 mo, plants and soil were transferred with minimum disturbance to 1-L pots containing silica sand, and additional seed was sown around the plants. Plants were fertilized once per wk with a modified half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1938) without P and were otherwise watered up to several times per day. Cultures were grown in growth chambers for 4.5–5 mo before being dried and harvested. Growth chambers and the shortest period possible for producing adequate inocula were used to reduce the probability of contamination by non-target organisms. All cultures were checked for contaminants before use in experiments. Inocula, consisting of soil and *S. vulgare* roots with either *G. intraradices* or *C. elatum*, were stored in paper bags in a cold room (4°C) for 1 mo before use in the experiment.

Before the inoculum was used in the experiment, the propagule density or infectivity was measured for both fungi. To do this, we performed a most probable number (MPN) test using inocula, avocado seedlings, and silica sand with a solid-phase buffer (Haas et al., 1983). The MPN test included three 10-fold dilutions of each inoculum type and five replicates (plants) per dilution. Avocado seedlings inoculated with either *G. intraradices* or *C. elatum* were grown in separate pots for 25 d. To determine if infection occurred in plants inoculated with *C. elatum*, we examined 40 roots from each pot for infection and checked the sand for chlamydospores. Any detectable infection or an abundance of chlamydospores (that is, there were more chlamydospores than were used to inoculate the plants) resulted in a positive score for that plant.

To determine if *G. intraradices* successfully infected avocado roots, we performed a PCR using primers based on small-subunit (SSU) rRNA gene sequences. The genus-specific primers used were GlomusF and NS2R (Schussler et al., 2001). Roots from avocado inoculated with *G. intraradices* were immediately frozen at −80°C until they were processed. Approximately 30 roots (1–2 cm long) were ground in liquid nitrogen from which 1 mg ground material was used for DNA extraction using the QIAGEN DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The PCR program was as follows: an initial denaturing period of 3 min at 95°C; then 40 cycles of denaturing at 94°C for 1 min, annealing of primers to single-stranded template DNA at 55°C for 1 min, and an extension period at 72°C for 1 min; followed by a final extension of 7 min at 72°C. PCR products were run on a 1% agarose gel in Tris-borate buffer (TBE) buffer and visualized under UV light after staining with ethidium bromide.

The MPN test was applied to the data for each fungus separately. Specifically, the number of positive scores per dilution factor per fungus were summed and used to calculate the number of infective propagules per gram sand per fungus with the MPN equation described in Alexander (1982). The resulting infectivity values are the basis for the inoculum treatments used in the experiments. Results gave a 2 : 1 ratio of *C. elatum* to *G. intraradices* inoculum to achieve a matched inoculum potential or infectivity. These infectivity values were needed to standardize the amount of inoculum per species used for the different inoculum treatments such that (1) at the 50 : 50 level each inoculum type had equal potential to infect plant roots and (2) all treatments where the infective number of propagules for both fungi summed to 100 were of equal total infectivity (i.e., the *G. intraradices* to *C. elatum* inoculum ratios of 100 : 0, 75 : 25, 50 : 50, 25 : 75, and 0 : 100 all had the same total number of infective fungal propagules).

Plant cultivation and inoculation—*Persea americana* var. *drymifolia* seeds were cleaned and then surface sterilized in 1% bleach solution. Approximately 300 seeds were planted in sterile 0.5-L pots with vermiculite. To induce germination, the top quarter of each seed was cut off. After 1 mo, 128 similar seedlings, each with a radical and shoot of similar length but no fully formed leaves, were selected. Seedlings were weighed immediately before inoculation and planting. Seedlings were inoculated with variable amounts of either or both types of inocula consisting of sand, root material, and spores of *C. elatum* or *G. intraradices*. Inocula consisting of soil and roots from pot cultures were used for each fungus. Controls were inoculated with soil filtrate produced by soaking inocula in distilled water for 45 min and then twice passing the soil solution through a 40-μm filter. This insured that bacterial soil microbes were consistent throughout the experiment.

Experimental conditions—The system was an open flood and drain sand culture system containing 128 2-L clay pots modified after Lynch et al. (1990). The experiment was repeated on separate greenhouse benches (64 pots per bench). An automated irrigation system supplied water to pots that then drained from trays under pots. In order to attain more realistic P concentrations in the soil solution and spatial distributions in the sand culture system, an Al_2O_3 solid-phase buffer was used in a silica sand substrate. Sand was ~99% pure mixed grade silica sand (SiO_2) (75 : 25 grade #12 : #16 to achieve an average grain size of 1 mm). Alumina (Al_2O_3) was mixed with silica sand to 2% mass to volume ratio after silica sand was autoclaved twice for 45 min within a 24-h period. Olsen's P concentrations were estimated before introducing other nutrients to the substrate. Methods for acid washing sand and establishing P regimes followed Lynch et al. (1990). This involved loading P onto the buffer to create 10 μmol P concentrations. The pH of the sand solution was maintained at six by the buffer. Inoculated and control plants were fertilized as needed with a -P half-strength Hoagland's nutrient solution. Temperatures were maintained between 24 and 27°C. For the high and low light treatment, photon flux densities were 1250 and 125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ mid-day, respectively. Shade cloth was used on all five sides of each of the two bench areas to create low light conditions. Reflective shade cloth was used over the low light intensity treatments, and strong air circulation across greenhouse benches was maintained to reduce any temperature differences between the treatments.

Experimental design—We used a response surface design including two light treatments and *G. intraradices* to *C. elatum* inoculum ratios of 0 : 0, 100 : 0, 50 : 0, 75 : 25, 50 : 50, 0 : 50, 25 : 75, and 0 : 100. One hundred represents the highest number of propagules per gram sand per fungus used in the experiment, and 0 indicates no fungal propagules were added to the inoculum. For instance, the 0 : 0 ratio represents the filtrate control (no fungal propagules), and the 50 : 0 ratio represents inoculum that contains half the maximum amount of *G. intraradices* propagules used in the experiment and no *C. elatum* propagules. The amount of each inoculum type used to create these *C. elatum* to *G. intraradices* ratios was based on the number of propagules per gram estimated using the MPN tests as previously described. Controls were inoculated with a filtrate. There were 16 treatments total and eight replicates per treatment. The 128 pots were randomly relocated to new locations within a given light treatment twice during the course of the experiment. Inoculum containing chlamydospores of only one of the two study fungi is referred to as a monoculture despite the presumed presence of other nontarget microorganisms. Mixtures refer to inoculum containing both fungi.

Plant variables—Plant fresh mass was determined at the beginning of the experiment. Seedlings were destructively harvested after 6 mo. Leaf area was measured photometrically with a leaf area meter (Model Li-3100, LI-COR, Lincoln, Nebraska, USA). Leaves, stems, cotyledons, fine roots, and coarse roots were dried for 2 d at 65°C and weighed.

Fungal variables (sporulation)—To estimate sporulation rates for both fungi, 15 mL of sand was collected from two sides of each pot using a ~1 cm wide soil core three times during the course of the study. Chlamydospores were extracted from sand using a sucrose floatation method (Furlan et al., 1980). Propagule numbers per fungus were estimated for time 0, month 1, month 2, and month 4, and progagule numbers were used to estimate sporulation rates as described in the "Statistical analysis" section. Chlamydospores for *G. intraradices* and *C. elatum* were estimated on a per volume sand basis. For verifying the identity of putative *C. elatum* chlamydospores, a subsample was surface-sanitized with 70% ethanol or 1% bleach solutions and mounted on potato dextrose agar (PDA) media. *Chaetomium elatum* perithecia were produced for over 100 separate chlamydospores after 2–5 wk on PDA. No other fungi producing similar chlamydospores were detected during the experiment.

Statistical analyses—All analyses were performed in JMP version 5.1.1 (SAS Institute, Cary, North Carolina, USA). To determine the effect of inocula and light on plant performance, both relative growth rate (RGR) and total plant biomass were included in analyses. Plant response data were analyzed using a factorial least squares fit with all fixed factors including light and inocula infectivity. First, the effects of light and inocula potential on RGR were analyzed. Relative growth rate was calculated using the equation $RGR = (\log_e (B_f) - \log_e (B_0))/t$, where B_f is final total plant dry mass, B_0 is initial seed mass, and t is time. The effects of light and inocula infectivity on total plant biomass were also analyzed after means were adjusted for initial seed mass [adj mean = $Y_i - \beta(X_i - X...)$] in a standard least fit model. In the formula, Y_i is the unadjusted score, β the regression coefficient, and $(X_i - X...)$ represents the deviation due to initial seed mass.

To test for differences in C allocation patterns across treatments, fine root biomass, leaf area, leaf mass, and cotyledon mass were then analyzed independently with the same fixed factors using a least squares fit with total

TABLE 1. Results of standard least squares fit showing degrees of freedom and F ratios for the effects of inocula potential and light (L) on relative growth rate [RGR = ($\log_e B_f - \log_e B_0$)/t], where B_f = final total plant dry mass, B_0 = initial seed mass, t = time).

Source of variation	RGR df	RGR F
Model	6	5.13***
Light (L)	1	8.45**
Inoculum potential *G*	1	9.02**
G × L	1	0.05
Inoculum potential *C*	1	0.37
C × L	1	5.52*
G × *C*	1	6.26*
Error (MS)	121	0.01

Notes: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and $r^2 = 0.20$. *G* = *Glomus intraradices*, *C* = *Chaetomium elatum*, MS = mean square.

biomass as a covariate. Total biomass was used as a covariate in the model as opposed to the more common use of ratios of these ecophysiological traits to total biomass because it allowed for the data to be analyzed in raw form. To determine if model fit was adequate, a lack of fit test was performed. Specifically, this involved a chi-square test, which determined if the pure error log-likelihood is significantly better than the fitted model (SAS Institute, 2002). Normality of residuals was determined by inspecting histograms of residuals for each test.

Student's t tests were used for all post-hoc comparisons at the level deemed appropriate in the least squares fit model. For post-hoc tests, adjusted means [adj mean = $Y_i - \beta(X_i - X...)$] were again used as opposed to raw means because the effects of the covariates were significant. In the equation, Y_i is the unadjusted score, β the regression coefficient, and $(X_i - X...)$ represents the deviation due to total plant biomass (for leaf and fine root mass) or initial seed mass (for plant biomass).

Fungal response variables included sporulation rates for both fungi. *Glomus intraradices* grew exponentially, thus the following formula was used to calculate sporulation rate: $r = (\log_e N_t - \log_e N_0)/t$, where N_t and N_0 represent the number of spores per gram of sand at the end of the experiment and within the initial inoculum, respectively, t is time, and r is the rate of spore production. *Chaetomium elatum* sporulation occurred in shorter cycles and was more erratic. Curve fitting techniques failed to identify a function that described sporulation adequately for all treatments containing this fungus over the course of the experiment. For this reason, we estimated sporulation rates for each of the three sampling periods using the exponential growth equation already described for *G. intraradices*.

Response surface analyses were performed to test the strength of fungal interspecific relative to intraspecific density-dependent interactions (Inouye, 2001) on fungal sporulation rates. Specifically, to determine the strength of intraspecific density-dependent factors on sporulation, quadratic terms (*C. elatum* × *C. elatum* and *G. intraradices* × *G. intraradices*) for inoculum potential were included in the model. Interspecific fungal interactions were simply tested by including a two-way interaction term for fungal inoculum potential (*C. elatum* × *G. intraradices*). The high number of terms for fungal inoculum potential could have led to the inclusion of superfluous terms in the model and multicolinearity among terms could reduce the value of the model (Myers, 1990). For this reason, prior to data analysis, a stepwise linear regression was used to determine which model terms best explained the variation in the response variables. Terms included in a forward combined stepwise regression were then used as predictor variables in a least squares fit model.

RESULTS

Plant growth—Light and *G. intraradices* inoculum potential significantly affected the relative growth rate of avocado (Table 1). Both factors positively affected plant growth. The effect of *C. elatum* alone on plant growth was not significant, but the interaction between light and *C. elatum* and the interaction *G. intraradices* × *C. elatum* were significant (Table 1). There was a significant negative effect of *C. elatum*, when inoculated at the highest density, on plant relative growth rate under low light conditions and in the absence of *G. intraradices* (Table 1, Fig. 2). This interpretation of the interactions is supported by several facts. First, the highest plant growth rates were associated with *G. intraradices* inoculum under high light (although there was no statistical difference between these growth rates and those of the controls under either light treatment). Second, plant relative growth rates for the 100 : 0, 75 : 25, 50 : 50, and 25 : 75 inoculum treatments (ratios represent *G. intraradices* to *C. elatum* infectivity) under high light were significantly higher than those associated with plants grown under low light conditions and inoculated with *C. elatum* monocultures (Fig. 2). Third, the slowest growing plants were associated with low light and the highest *C. elatum* inoculum potential (*C. elatum* monoculture 0 : 100) treatment. The values for plants subjected to such a treatment were significantly lower than all other treatment combinations except for intermediate inoculum potential *C. elatum* monocultures (0 : 50) grown under low light conditions (Fig. 2). Last, plant growth rates for low light, *C. elatum* monocultures (0 : 100) had significantly lower means than uninoculated control plants grown under either the high or the low light levels (Fig. 2). Negative values associated with plants inoculated with high inoculum potential *C. elatum* monocul-

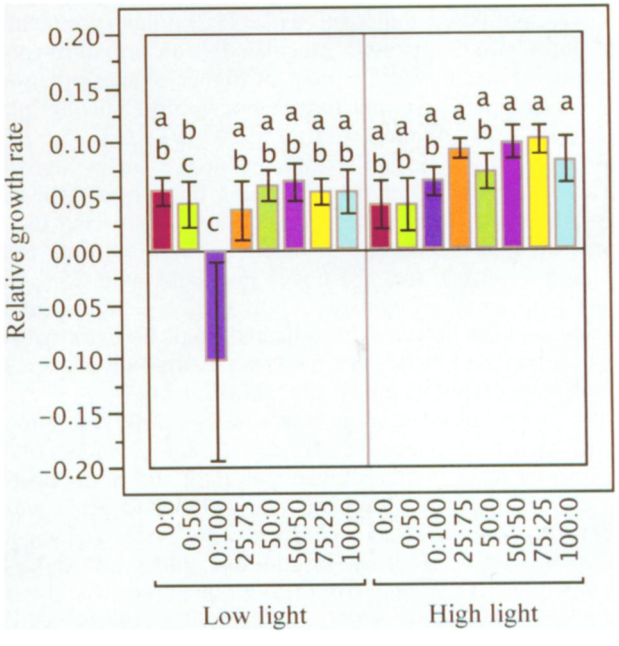


Fig. 2. Results for relative growth rate [RGR = ($\log_e B_f - \log_e B_0$)/t] for eight fungal inoculum treatments and two light treatments. B_f is final total plant dry mass, B_0 is initial seed mass, and t is time. Inoculum treatments are defined by the initial inocula potential of *Glomus intraradices* and *Chaetomium elatum* added to the substrate. Each color corresponds to a different inoculum treatment. $N = 8$ per treatment, and bars represent the adjusted means for the covariate initial seed mass with ±1 SE. Means (compared with t tests) not followed by the same letter are significantly different at $P \leq 0.05$.

TABLE 2. Results of standard least squares fit showing degrees of freedom and F ratios for the effects of inocula potential and light on plant biomass (dry mass in grams). Plant biomass was adjusted for initial seed mass (X) using adj. mean $= Y_i - \beta(X_i - X...)$, where Y_i is the unadjusted score, β the regression coefficient, and $(X_i - X...)$ represents the deviation due to initial seed mass.

	Adj. biomass (g)	
Source of variation	df	F
Model	6	5.68***
Light (L)	1	12.12***
Inoculum potential G	1	14.14***
$G \times$ L	1	3.77*
Inoculum potential C	1	3.09
$C \times$ L	1	5.38*
$G \times C$	1	5.89*
Error (MS)	121	314.22

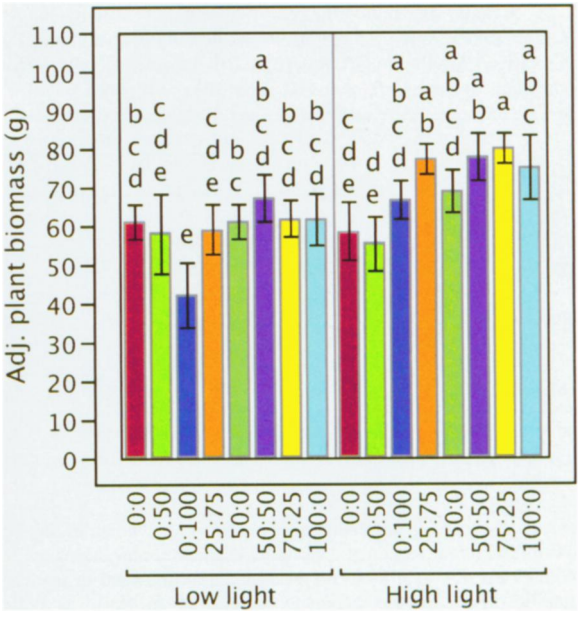
Notes: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and $r^2 = 0.22$. G = *Glomus intraradices*, C = *Chaetomium elatum*, MS = mean square.

tures were due to plant death. Plant growth rates did not differ between *G. intraradices* monocultures or mixtures (Fig. 2).

Light and *G. intraradices* both also significantly and positively affected total plant biomass when controlling for initial seed mass (Table 2). Similar to plant growth rate, *C. elatum* inocula alone did not affect plant biomass, but the interactions between *C. elatum* and light and between *G. intraradices* and *C. elatum* were significant (Table 2). In contrast to plant growth rate, the interaction *G. intraradices* × light was significant and positive (Table 2). Plants grown under high light conditions and inoculated with mixtures of *G. intraradices* and *C. elatum* were of higher biomass than high light control plants without fungal inocula. Specifically, plants inoculated with mixtures (25 : 75, 50 : 50, and 75 : 25 *G. intraradices* to *C. elatum* ratios) and grown under high light had significantly higher plant biomass than those grown (1) with *C. elatum* 0 : 50 under either light intensity and 0 : 100 under low light inoculum; (2) under high light with no fungal inoculum (control); and (3) under low light with 25 : 75 *G. intraradices* to *C. elatum* inoculum (Fig. 3). No significant differences were detected for adjusted plant biomass between plants inoculated with *G. intraradices* monocultures and control plants without fungal inocula (Fig. 3).

The significant interaction between *C. elatum* and light can be attributed to the negative effect of *C. elatum* monocultures (0 : 100) on plant biomass under low light and in the absence of *G. intraradices* (all treatments that included *G. intraradices*, except the low light 25 : 75 treatment, were greater than 0 : 100 treatment) (Fig. 3). In addition, under low light, the high infectivity *C. elatum* (0 : 100) monoculture was the only treatment to be significantly less than the control with no fungal inoculum (Fig. 3). In summary, differences among treatments with equal inoculum potential strongly imply that inoculum composition affected plant biomass. Most importantly, differences in inoculum composition accounted for the low plant biomass of *C. elatum* monocultures under low light and the high plant biomass associated with mixtures of both fungi grown under high light.

Plant carbon allocation—Differences could also be detected in patterns of C allocation among light and inoculum treatments, although no significant differences among the



G. intraradices: *C. elatum* inoculum potential

Fig. 3. Pairwise comparisons (t tests) for the effects of inocula potential and light on plant biomass (mean dry mass in grams) [adjusted for initial seed mass (X) calculated adj. mean $= Y_i - \beta(X_i - X...)$]. Y_i is the unadjusted score, β the regression coefficient, and $(X_i - X...)$ represents the deviation due to initial seed mass. Inoculum treatments are defined by the initial inocula potential of *Glomus intraradices* and *Chaetomium elatum* added to the substrate. Each color corresponds to a different inoculum treatment. $N = 8$ per treatment, and bars represent the adjusted means for the covariate initial seed mass with ±1 SE. Means (compared with t tests) not followed by the same letter are significantly different at $P \leq 0.05$. Means were compared at levels deemed appropriate in the main model (Table 2).

treatments could be detected in the proportion of cotyledon mass relative to total biomass at the end of the study ($F_{2,125} = 1.91$, $P = 0.153$). This indicates that plants used similar amounts of reserves during the course of the experiment. Plants grown under low light had the highest leaf mass (Model, $F_{5,122} = 3.19$, $P = 0.004$, $r^2 = 0.16$; Light, $F = 9.99$, $P = 0.002$). Light and inocula potential had significant effects on adjusted leaf area (Model, $F_{5,122} = 25.09$, $P \leq 0.0001$, $r^2 = 0.51$). Significantly higher values were associated with plants grown under the low light intensity (Light, $F = 102.88$, $P \leq 0.0001$). *Glomus intraradices* inoculum potential positively affected biomass allocation to leaf area (*G. intraradices* inoculum potential, $F = 10.31$, $P \leq 0.002$), whereas *C. elatum* inoculum potential did not significantly affect C allocation to leaf area. Under both light treatments, control plants (without fungal inocula) allocated significantly less carbon to leaf area than did plants inoculated with *G. intraradices* at most levels of inoculum potential (Table 3).

Light and inocula potential had significant effects on adjusted fine root biomass (Model, $F_{5,122} = 4.14$, $P = 0.002$, $r^2 = 0.15$). For plants grown under low light, the highest inoculum potential for *G. intraradices* (100 : 0) positively affected C allocation to fine root mass (Light intensity, $F = 11.25$, $P = 0.001$; *G. intraradices* inoculum potential, $F = 6.75$, $P = 0.010$). Whereas no significant contribution of *C. elatum*

TABLE 3. Pairwise comparisons (t tests) for the effects of *Glomus intraradices* inoculum potential on adjusted leaf area (dm^2) for each light treatment. Leaf area was adjusted for total plant biomass (X) using adj mean $= Y_i - \beta(X_i - X...)$, where Y_i is the unadjusted score, β the regression coefficient, and $(X_i - X...)$ represents the deviation due to total plant biomass. Values for *G. intraradices* inoculum potential are at time 0. Means were compared at levels deemed appropriate in the main model. Post-hoc tests of *G. intraradices* inoculum potential effects were therefore done for each light treatment separately.

	Glomus intraradices inoculum potential	Adj. leaf area (cm^2)
A. High light	0	145.955[b]
	25	189.489[a]
	50	185.734[a]
	75	184.029[ab]
	100	194.876[a]
B. Low light	0	243.041[b]
	25	275.315[ab]
	50	304.109[a]
	75	321.957[a]
	100	304.842[a]

Notes: Means not followed by the same letter are significantly different at $P \leq 0.05$.

inoculum alone was detected, the interaction between *G. intraradices* and *C. elatum* was again significant (*C. elatum* × *G. intraradices* inoculum potential, $F = 3.89$, $P = 0.048$). Under high light intensity, plants inoculated with the *C. elatum* 0 : 50 monoculture allocated proportionately less biomass to fine roots than did plants inoculated with the 25 : 75 and 50 : 50 mixtures of the two fungi and control plants (Fig. 4).

Fungal responses and interactions—Response surface analyses showed *G. intraradices* inoculum potential and light to be significant factors positively affecting *G. intraradices* sporulation rate (Table 4). *Glomus intraradices* inoculum potential positively affected *G. intraradices* sporulation rate although intraspecific density-dependent factors limited sporulation such that maximum sporulation did not occur with maximum inoculum potential for *G. intraradices* (Table 4). This is indicated by the negative and significant quadratic *G. intraradices* × *G. intraradices* effect (Table 4). Neither the effect of *C. elatum* inoculum potential nor the *G. intraradices* × *C. elatum* interaction were significant to sporulation of *G. intraradices* (Table 4). The maximum sporulation rate [mean = (0.70) $\log_e$ chlamydospores·mL^{-1} sand·$month^{-1}$] occurred at 50 : 50 ratio of *G. intraradices* to *C. elatum* inoculum. The lowest sporulation rates [means both equal (0.56) $\log_e$ chlamydospores·mL^{-1} sand·$month^{-1}$] were estimated for the 75 : 25 and the 25 : 75 ratios of *G. intraradices* : *C. elatum* inocula under low light conditions. Within the first 3 mo of the study, no significant differences in sporulation rates were detected among treatments.

As shown in Table 5, *C. elatum* sporulation was significantly affected by the linear inoculum term in the first month of the experiment (time 1), by both linear and quadratic (*C. elatum* × *C. elatum* inocula potential) terms during the final months (time 3) (Fig. 5A–C and see Materials and Methods for details on calculating sporulation rates). High levels of inoculum potential in *C. elatum* inocula reflected high levels of sporulation during the first month, but this effect was not significant during the second month of the experiment (Table 5 and Fig. 5C). For the first month of the experiment, the highest

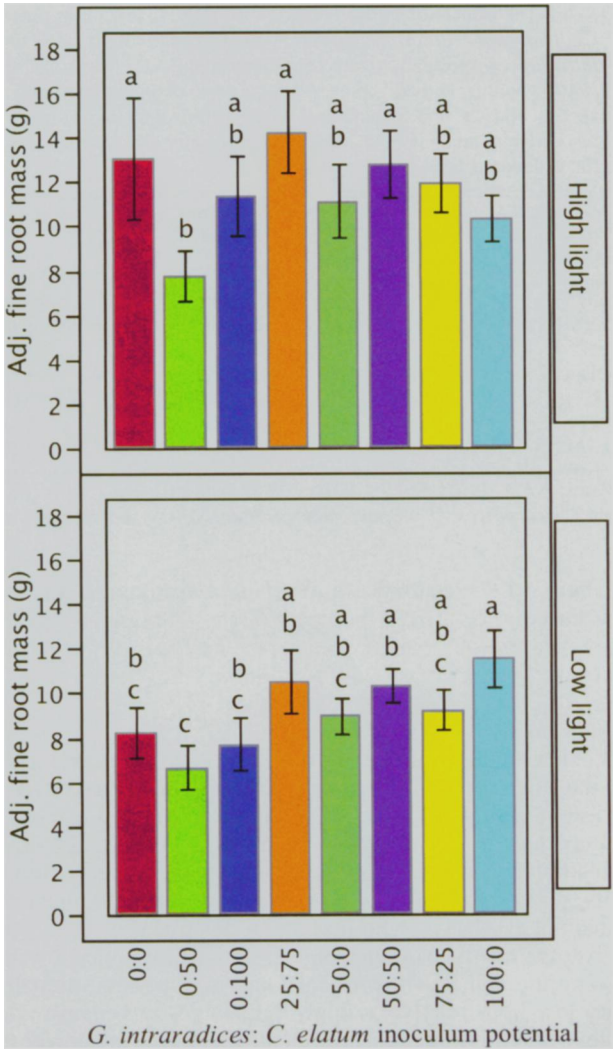


Fig. 4. Pairwise comparisons (t tests) for the effects of inocula potential on adjusted fine root dry mass (g). Fine root dry mass was adjusted for total plant biomass (X) using adj mean $= Y_i - \beta(X_i - X...)$, where Y_i is the unadjusted score, β the regression coefficient, and $(X_i - X...)$ represents the deviation due to total plant biomass. Inoculum treatments are defined by the initial inocula potential of *Glomus intraradices* and *Chaetomium elatum* added to the substrate. Each color corresponds to a different inoculum treatment. $N = 8$ per treatment, and bars represent the adjusted means for the covariate initial seed mass with ±1 SE. Means (compared with t tests) not followed by the same letter are significantly different at $P \leq 0.05$. Means were compared at levels deemed appropriate in a standard least squares fit model.

C. elatum sporulation rate [mean = (1.55) $\log_e$ chlamydospores·mL^{-1} sand·$month^{-1}$] was detected under low light conditions for the highest *C. elatum* inoculum potential (0 : 100), whereas the lowest rate [mean = (0.12) $\log_e$ chlamydospores·mL^{-1} sand·$month^{-1}$] was also obtained under low light but for plants inoculated with ratios of 75 : 25 *G. intraradices* to *C. elatum*. Neither light nor *G. intraradices* inocula potential were important for predicting *C. elatum* sporulation during the experiment (Table 5). The highest rate of *C. elatum* sporulation [mean = (2.33) $\log_e$ chlamydospores·mL^{-1} sand·$month^{-1}$] was detected during the final months of the experiment under high light for plants inoculated

TABLE 4. The effects of initial *Glomus intraradices* inoculum potential, *Chaetomium elatum* inoculum potential and light (L) on *G. intraradices* sporulation rate. Sporulation rate: $r = (\log_e N_t - \log_e N_0)/t$, where N_t and N_0 represent the number of spores per gram sand at the end of the experiment and within the initial inoculum, respectively, and t is time. ANOVA, parameter estimates and t ratios for a standard least squares fit model.

Source of variation	df	Estimate	t Ratio
Model (F ratio)	7	—	39.39***
Light (L)	1	0.0749	2.13*
G	1	0.0095	12.75***
C	1	0.0011	1.16
L × *G*	1	0.0010	0.91
G × *G*	1	−0.0001	−5.50***
L × *C*	1	−0.0000	0.00
G × *C*	1	0.0000	0.43
Error (MSE)	120	—	1917.50

Notes: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and $r^2 = 0.70$. *G* = *Glomus intraradices*, *C* = *Chaetomium elatum*, MSE = mean square error.

with 50 : 50 *G. intraradices* to *C. elatum* mixtures (Fig. 5A–C).

DISCUSSION

We determined that patterns of plant C allocation and growth for the light and *G. intraradices* combinations tested are consistent with what could be predicted from well-established relationships in the literature (Ferguson and Menge, 1982; Bethlenfalvay and Pacovsky, 1983; Tester et al., 1986; Son and Smith, 1988). Our results also mirror a study conducted by Graham et al. (1982), who detected a positive growth response in mycorrhizal plants under high light but not under low light and limiting soil P. Mycorrhizal fungi may be less mutualistic under low light relative to high light intensity because where photosynthate production is limited, there is a greater cost of supporting mycorrhiza formation to plants (Fredeen and Terry, 1988; Thompson et al., 1992). Because *G. intraradices* interacted with light and avocado as predicted, using this AMF in determining the function of *C. elatum* was deemed appropriate.

TABLE 5. ANOVA followed by standard least squares fit parameter estimates for the effects of initial *Glomus intraradices* inoculum potential, *Chaetomium elatum* inoculum potential, and light (L) on *C. elatum* sporulation rates for two of the three periods analyzed (the model for the second time interval was not significant).

Source of variation	df	Estimate (time 1)	t Ratio	Estimate (time 3)	t Ratio
Light (L)	1	−0.072	−0.93	0.3123	1.28
G	1	−0.001	−0.42	0.0024	0.37
C	1	0.009	5.51***	0.0148	2.86**
L × *G*	1	−0.001	−0.23	0.0098	1.23
L × *C*	1	−0.004	−1.71	−0.0005	−0.07
G × *C*	1	0.000	−0.54	0.0001	0.28
C × *C*	1	0.000	0.96	−0.0004	−2.53**

Notes: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; time 1, $r^2 = 0.46$; and time 3, $r^2 = 0.16$. *G* = *G. intraradices*, *C* = *C. elatum*.

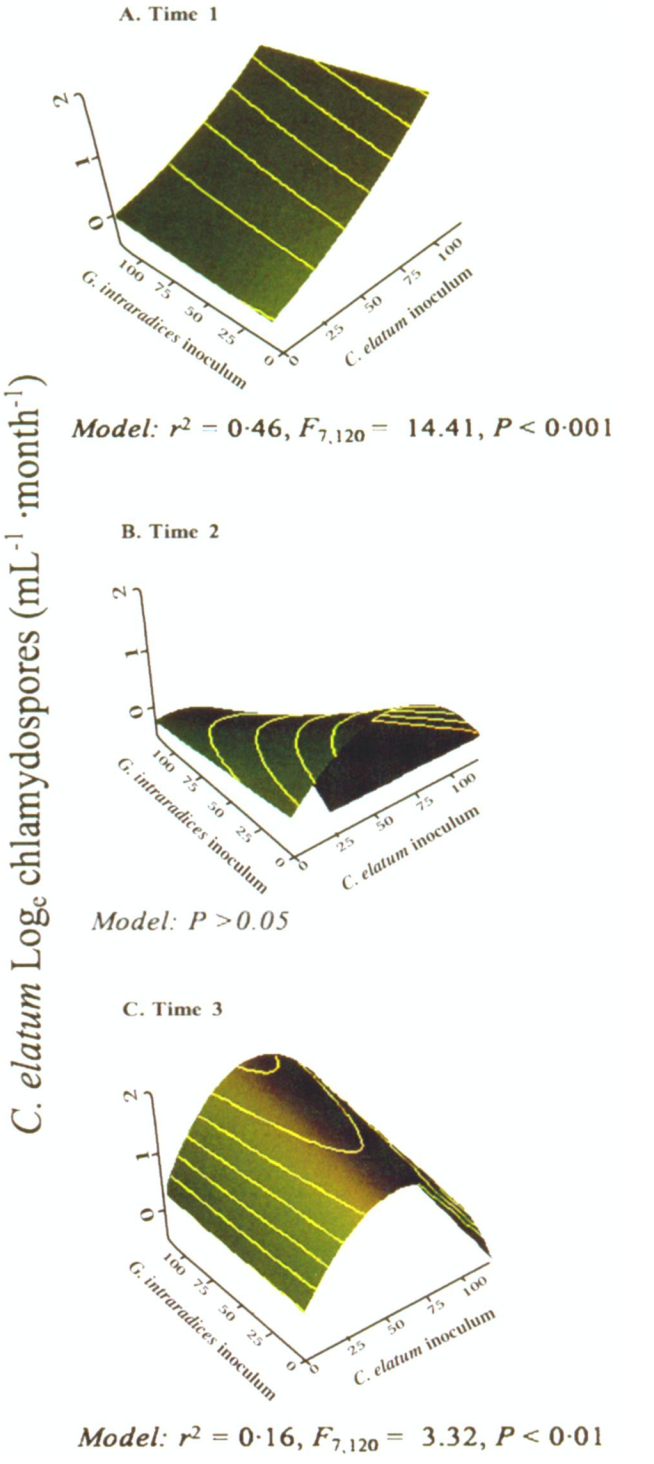


Fig. 5. Sporulation rates for *Chaetomium elatum* [$r = (\log_e N_t - \log_e N_0)/t$] predicted as a function of *Glomus intraradices* and *C. elatum* inocula potential at time 0. *Chaetomium elatum* sporulation rates are fitted for three periods: (A) time 1 (month 0–1); (B) time 2 (month 1–2) and (C) time 3 (month 2–5) of the study (no significant effects of inoculum infectivity were detected for time 2). $N = 16$ for all graphs. The effect of light was not significant.

Plant–fungal interactions—Within the neutral to mutualistic benchmarks established with *G. intraradices,* no independent mutualistic role for *C. elatum* as a root colonist on avocado was detected. In the absence of the known mutualist *G. intraradices* and under low light where C fixation was relatively limiting, there was a negative effect of *C. elatum* on plant biomass and on relative growth rate. In addition, plants grown under these conditions had a decrease in the proportion of biomass allocated to fine roots and no concurrent increase in the proportion of biomass allocated to leaves.

The negative effect of *C. elatum* on plants under C-limiting conditions is consistent with the findings of Mikola (1965) for fungi forming similar structures in pine roots under low light. However, the results obtained in this experiment also suggest that *C. elatum* and *G. intraradices* interact to improve plant growth under high light. This statement is based on the fact that only with inocula containing mixtures of the two fungi and under high light intensity did plants achieve a biomass greater than did the controls. Thus interspecific fungal interactions "protected" the plant from the negative impact of *C. elatum* under low light. Similar differences in interspecific fungal effects on plant growth between differing light intensities are not well documented. The apparent improved host benefit associated with the addition of a weak pathogen to mycorrhizal inoculum is particularly unusual.

Fungal interactions—Where fungi coexisted in the root system, any additional costs of supporting *C. elatum* in the root system were not reflected in changes to *G. intraradices* sporulation rates. Further, response surface design and repeated measurements of fungal sporulation revealed some similarities between the two fungi. For example, our data provided no evidence that sporulation was limited by interspecific density-dependent factors but instead, strongly suggested intraspecific density-dependent factors were limiting sporulation rates at high initial fungal infectivity for both fungi. This was most evident when comparing sporulation rates of monocultures of different infectivity.

Interestingly, under low light and as *C. elatum* inoculum potential increased, the negative impact of *C. elatum* on plant biomass was greatest. High *C. elatum* sporulation within the first month of the study was associated with the lowest plant relative growth rates under low light conditions. This indicates that 1 mo after seed germination under low light, plants were most susceptible to *C. elatum* pathogenesis. Also, as intraspecific density-dependent factors became stronger, the cost to the plant was greater and in some cases even killed the host. Alternatively, as *G. intraradices* intraspecific density-dependent factors presumably became stronger (at the highest level *G. intraradices* inoculum potential), the benefit to the host benefit was lower relative to some levels of intermediate inoculum potential. Moreover, at intermediate levels of inoculum potential, *G. intraradices* sporulation rates peaked.

Although intraspecific competition was detectable in both cases, differences in sporulation rates cannot be interpreted the same way for both fungi. Unlike *G. intraradices*, *C. elatum* is not an obligate symbiont and it has a sexual stage. *Chaetomium elatum* chlamydospore production rates may be indicative of a fungal stress response to plant-related, resource-limiting conditions. *Chaetomium elatum* may shift to pathogenesis only under strong intraspecific competition for plant resources, whereas maximum benefit to *G. intraradices* in terms of fecundity is achieved under conditions that make *G. intraradices* a stronger mutualist. For *G. intraradices,* sporulation can be considered a component of fungal fitness. However for *C. elatum,* sporulation may be a sign of stress, perhaps in response to host defense mechanisms.

Presumably both fungi used in this study primarily rely on plant-produced resources, but *G. intraradices* may increase availability of some plant resources, such as fine roots, for *C. elatum*. This may explain why there was no detectable interaction between the two fungi in terms of spore production or host benefit. There is no apparent cost of supporting *C. elatum* in the presence of *G. intraradices* for the host or for *G. intraradices* and no large differences in *C. elatum* sporulation compared to *C. elatum* monocultures of equal inoculum potential. Further, if *C. elatum* elicits a systemic plant defense response, the lack of a negative impact of *C. elatum* on *G. intraradices* sporulation also implies that the ability of *G. intraradices* to colonize the root system is unimpeded by any defense response associated with *C. elatum*. This study also raises the question of "resource use complementarity" because these root colonizers occupy the same physical location on the root without impacting one another's fecundity (Petchey, 2003).

Does C. elatum function similarly to fungi that form analogous structures in roots?—There is wide taxonomic diversity among loosely categorized ascomycetous anamorphs that form structures similar to that of *C. elatum* in avocado. These ascomycetous anamorphs are called E-strain, dark-septate endophytes, or ectendomycorrhizal fungi and, like *C. elatum*, are characterized by some or all of the following: chlamydospores, a thin mantle, a Hartig net (O'Dell et al., 1993; Ahlich and Sieber, 1996) and/or to varying degrees of epidermal and cortical cell penetration (Yu et al., 2001). They also closely resemble a large number of fungi that colonize roots and that are simply called rhizoplane fungi.

According to Egger et al. (1991), E-strain fungi only include species within the genus *Wilcoxina*. Dark-septate endophytes are a polyphyletic group that contains, but is not limited to, members from *Pleosporales, Diaporthales,* and *Pezizales*. Jumpponen (2001, p. 207) defines dark-septate endophytes "as conidial or sterile ascomycetous fungi colonizing living plant roots without apparent negative effects such as tissue disorganization." Examples of genera within these taxa documented to form structural features characteristic of ectendomycorrhizae include *Wilcoxina, Sphaerosporella, Phialophora,* and *Chloridium* (Wilcox and Wang, 1987). Mikola (1988) proposes that the term ectendomycorrhizal fungi be reserved for fungi that simultaneously form a Hartig net while penetrating host cell walls in order to distinguish this group from fungi associated with senescent ectomycorrhizas. To compound the confusion, many ectendomycorrhizal fungi and dark-septate endophytes are suspected to be Basidiomycetes that are capable of forming ectomycorrhizae but are not physiologically adapted for their particular host (see discussion in Jumpponen, 2001).

Chaetomium elatum could justifiably be categorized as a dark-septate endophyte because, like dark-septate endophytes, it does not produce a complete mantle and older mycelium is often dematiaceous. Further, no sign of tissue disorganization was detected in colonized roots. However, to simply categorize *C. elatum* as a dark-septate endophyte does little to further our understanding of the functional roles of these understudied fungal root colonists.

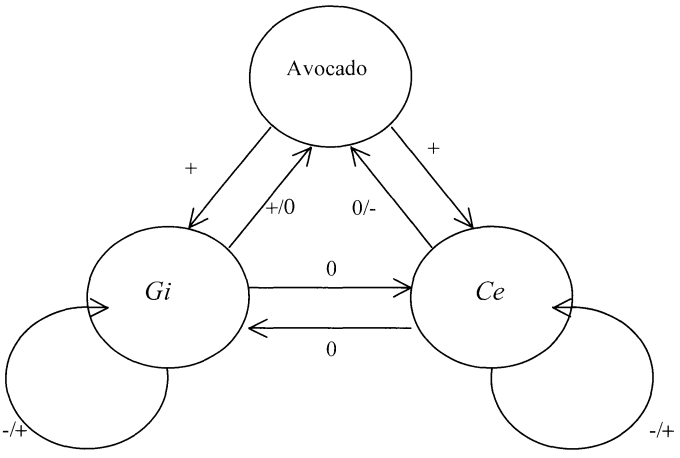


Fig. 6. Diagram in Fig. 1 recast with evidence from experimental data. Interaction effects among avocado, *Glomus intraradices* (*Gi*) and *Chaetomium elatum* (*Ce*) were defined for fungal inoculum that varied in composition and infectivity and for two light intensities. Data on fungal fecundity provide no evidence of interspecific interactions (indicated as "0") but do imply intraspecific interactions reduced or limited fungal sporulation rates over time (indicated as "–"). The influence of *Chaetomium elatum* on plants was neutral under high light intensity but parasitic under low light intensity.

Categorizing *C. elatum* as a dark-septate endophyte does little to further our understanding of their ecology largely because functional roles for dark-septate endophytes (and/or other similar groups discussed) have been classified as positive, negative, and neutral as measured in the cost of a given fungus to host plant biomass (Smith and Read, 1997; Jumpponen, 2001). Proposed functions include a protective role against root pathogens (Narisawa, 2003) and that of latent pathogens, which become active following root senescence (Yu et al., 2001). To further complicate this matter, an individual fungus that fits the morphological definition of a dark-septate endophyte can be multifunctional.

Chaetomium elatum is in fact a multifunctional fungus and may be able to shift among types of organic substrates when a given substrate becomes limiting. In this study, the negative effect on avocado may be related to a shift in resource use with the development of intraspecific density-dependent competition by *C. elatum* over time and under low light. Root-colonizing fungi in the genus *Chaetomium* (Sordariales) may serve as both opportunistic rhizoplane and rhizosphere species as well as soil cellulose degraders. This hypothesis is consistent with that of Jumpponen and Trappe (1998) for many fungi forming similar structures in plant roots. They suggest "considerable functional and ecological overlap" (p. 295) may exist between rhizosphere and rhizoplane fungi categorized as saprophytic, strict pathogens, mutualists, and endophytes due to the dynamic nature of host–fungi associations.

Because pathogenesis was only detected in the absence of *G. intraradices* and because there was no observed benefit of pathogenesis to *C. elatum*, this fungus seems to be a saprophyte that becomes parasitic under severely C-limiting conditions. Because neither *G. intraradices* nor plants in the presence of *G. intraradices* were negatively affected by *C. elatum, C. elatum* does not appear to be a cheater as hypothesized. The fact that *Chaetomium* has both cellulolytic abilities (Alexopoulos et al., 1996) and can act as a biocontrol agent (Soytong et al., 2001) may help to explain how plants were parasitized when hosting *C. elatum* alone but may have benefited from hosting both fungi (possibly benefiting from antibiotic properties of *C. elatum*). For instance, colonization of barley by the congener *C. globosum* can reduce disease caused by powdery mildew (Vilich et al., 1998), but under different conditions (in the absence of other microbes), this same species caused disease in barley (Reissinger et al., 2003).

Our work also reinforces the idea that interactions among hosts, "mutualistic" and "pathogenic" organisms often show "conditionality" (Bronstein, 1994). Meaning that the relationships between these root colonists and their hosts probably change depending upon the type of organic resources available, light intensity, fungal density, time, and the presence of other microorganisms, such as mycorrhizal fungi.

Implications for plant ecology—Our data imply that the effect of *C. elatum* on plants will be greater when *C. elatum* infectivity is high and in the absence of AMF. The presence of *C. elatum* in greenhouse experiments should be noted because these fungi often survive or recolonize fumigated or heat-sterilized soil and, hence, are a common fungus on the roots of plants grown in these treated substrates (Warcup, 1976). Similarly, in the field, stochastic events can lead to fungi within the spore bank, namely, those that have superior dispersal capabilities (Jumpponen et al., 2003), being in greater relative abundance in soils due to the disturbance-related reduction of common mycorrhizal associates (Jumpponen et al., 2002). Consistent with this hypothesis, fungi that form similar structures in plant roots (mainly dark-septate endophytes) have been noted among the first and most abundant root associates found in colonizing seedlings (Mikola, 1965, 1988; Danielson, 1991; Cazares et al., 1992; Horton et al., 1998). Early colonization dominated by dark-septate endophytes has been observed for bishop pine in chaparral and forest communities following fire (Horton et al., 1998), spruce in mine tailings (Danielson, 1991), and seedlings colonizing areas in the wake of glacial retreat (Helm et al., 1996).

Some ecologists postulate that primary successional processes may sometimes commence with a heterotrophic community that relies on deposited organic material (Hodkinson et al., 2002; Jumpponen et al., 2003), and multifunctional fungi, such as *C. elatum*, have the capability of using this substrate. With time, there may be a reduction in organic matter or other substrates that commonly support these fungi, causing a shift to more severe competition for alternative substrates such as plant roots.

Dark-septate endophytes been observed not only as early root colonists but also as persistent and common root colonizers (Danielson, 1991; Horton et al., 1998; Girlanda et al., 2002). Consistent with these field observations, our data demonstrated that *C. elatum* is a persistent root colonizer occupying the avocado rhizosphere for nearly a year.

Conclusions—This study provides good evidence that *C. elatum* is an opportunistic fungus functioning within the range of a "commensalistic associate" to a weak pathogen in avocado (Fig. 6). In the presence of the aggressive colonizer *G. intraradices*, no significant biomass loss was detectable for avocado hosting *C. elatum,* but in the absence of *G. intraradices*, under low light and when occurring in high density, *C. elatum* appeared to function as a pathogen. *Chaetomium elatum*, and other ascomycetous anamorphs that

do not have independent mutualistic capabilities, may become plant pathogens when disturbance lowers the inoculum potential of mycorrhizal fungi. Intraspecific density dependent interactions, light, and the presence of a strongly mutualistic mycorrhizal fungus are all likely factors in determining the function of opportunistic root colonizers such as *C. elatum*. Coexistence between AMF, opportunistic fungi and plants may be the norm and maintained by a balance between the AMF host benefit and the plant's ability to provide resources to AMF. Where mutualists and opportunists coexist under favorable conditions, these variables may function to increase net C gain to plants. Remarkably, these root colonists appeared to share space and resources without negatively impacting one another's fecundity.

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