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Summary

• Plant symbiotic fungi are generally thought to express a single lifestyle that might increase (mutualism), decrease (parasitism), or have no influence (commensalism) on host fitness. However, data are presented here demonstrating that plant pathogenic *Colletotrichum* species are able to asymptomatically colonize plants and express nonpathogenic lifestyles.

• Experiments were conducted in growth chambers and plant colonization was assessed by emergence of fungi from surface sterilized plant tissues. Expression of symbiotic lifestyles was assessed by monitoring the ability of fungi to confer disease resistance, drought tolerance and growth enhancement.

• Several pathogenic *Colletotrichum* species expressed either mutualistic or commensal lifestyles in plants not known to be hosts. Mutualists conferred disease resistance, drought tolerance, and/or growth enhancement to host plants. Lifestyle-altered mutants expressing nonpathogenic lifestyles had greater host ranges than the parental wildtype isolate. Successive colonization studies indicated that the ability of a symbiont to colonize a plant was dependent on previous colonization events and the lifestyles expressed by the initial colonizing fungus.

• The results indicate that the outcome of symbiosis is controlled by the plant's physiology.

Key words: symbiosis, fungal lifestyle, plant fitness, drought tolerance, disease resistance, *Colletotrichum*.

Introduction

Few, if any, plants exist in natural ecosystems independent of symbiotic associations with endophytic and/or mycorrhizal fungi (Petrini, 1986). In the last several decades it has become apparent that symbiotic fungi play a critical role in the structure, function, and health of plant communities (Read, 1999). In fact, it is conceivable that plant communities may not survive many environmental stresses without these symbiotic associations.

Fungal symbionts express a variety of symbiotic lifestyles including mutualism, commensalism and parasitism (Lewis, 1985). Mutualistic fungi may confer several benefits to plants such as drought tolerance (Read & Camp, 1986; Bacon, 1993), metal tolerance (Read, 1999), growth enhancement (Belesky *et al.*, 1987; Marks & Clay, 1990; Varma *et al.*, 1999), disease resistance (Carroll, 1986; Freeman & Rodriguez, 1993; Redman *et al.*, 1999a), herbivore resistance (Latch, 1993), and enhanced nutrient acquisition (Read, 1999). Commensal symbioses benefit the symbiont but have no apparent beneficial or detrimental affects on the host. The impacts of parasitic fungi on plants range in severity from decreased growth rates and/or fecundity, to lethality.

Although the importance of symbiotic fungi in the health and dynamics of plant communities is well established (Read & Camp, 1986; Bacon & Hill, 1996; Rodriguez & Redman, 1997; Clay & Holah, 1999), the genetic and biochemical processes responsible for host colonization and the expression of different symbiotic lifestyles remain an enigma. It is unclear if the outcome of symbiotic interactions (mutualism, commensalism, parasitism) is genetically

predetermined, a physiological response to biotic or abiotoic environmental factors, or both.

Successful plant-fungal symbioses involve at least three events: penetration by the fungus into plant tissues; colonization of plant tissues by the invading fungus; expression of a fungal symbiotic lifestyle. It is not known what type of communication occurs between symbionts and hosts that results in the expression of different symbiotic lifestyles, or if symbionts are recognized by hosts before lifestyle expression. However, it appears that some form of biochemical and/or genetic communication occurs that allows mutualists to confer physiological benefits to hosts.

Some fungi express different symbiotic lifestyles depending on the plant host and/or environmental conditions. For example, both pathogenic and nonpathogenic fungi may be isolated from the same asymptomatic plant tissues (Schulz *et al.*, 1999). This suggests that pathogens either express nonpathogenic lifestyles, or colonize and then remain quiescent in different hosts. Some mycorrhizas may be mutualistic or parasitic depending on the plants physiology and environmental conditions (Francis & Read, 1995; Johnson *et al.*, 1997; Graham & Eissenstat, 1998). These studies indicate that individual fungal symbionts may either positively or negatively influence the fitness of a single host. Therefore, to understand plant community dynamics, characterization of the factors controlling the outcome of symbiosis is required.

To begin characterizing the biochemical and genetic bases of fungal symbiotic lifestyle expression, mutation studies were performed on the *Colletotrichum magna* wildtype isolate L2.5 (pathogenic and 100% lethal to cucurbit plants). UV irradiation and gene disruption of *C. magna* has resulted in the conversion of a virulent pathogen to a series of nonpathogenic, symbiotic mutants that express either mutualistic, commensal, or intermediate mutualistic lifestyles (Freeman *et al.*, 1993; Redman *et al.*, 1999b). In this system, mutualism is based on benefits to both the host (disease resistance against pathogenic isolates of *C. magna*, *C. orbiculare*, and *Fusarium oxysporum*) and the symbiont (acquisition of nutrients from hosts). Designation of the mutants as mutualists, intermediate mutualists, or commensals is based on the ability of the mutants to confer 80–100%, 30–70%, and 0% disease resistance to cucurbit plants, respectively (Redman *et al.*, 1999b).

Here, five questions concerning fundamental aspects of plant/fungal symbioses are addressed: does the symbiotic lifestyle expressed by fungi influence host ranges; are pathogens capable of expressing nonpathogenic symbiotic lifestyles; do mutualists differ in the number and extent of benefits conferred to hosts; does the host, invader, or both control the outcome of symbiosis; and does the lifestyle expressed by initial colonizing fungi influence successive colonization events? Addressing these questions will increase our knowledge of the ecological and evolutionary roles that fungal symbionts have in plant communities.

Materials and Methods

Fungal strains, media, and plant cultivars

Pathogenic wildtype isolates of *Colletotrichum* spp. (*C. magna*, *C. coccodes*, *C. orbiculare*, *C. musae*, *C. lindemuthianum*, *C. graminicola*, *C. gloeosporioides*, and *C. acutatum*) were obtained from the *Colletotrichum* repository (http://www.uark.edu/Departments/plant). The plant origin, tissue of isolation and infection characteristics of the *Colletotrichum* species are described in Table 1. All fungal species were taxonomically verified by microscopy and/or PCR analysis (Freeman *et al.*, 1993). Mutants of *C. magna* expressing nonpathogenic symbiotic lifestyles were obtained through UV irradiation (designated path-1 (mutualist)) and restriction enzyme-mediated integration (designated R1-A (mutualist) and R21-C (commensal)) (Freeman & Rodriguez, 1993;

Table 1 Fungal isolates, infection characteristics and the plants of origin

		Plant of origin			
Fungal species (isolate)	Infection characteristics[1]	Common name	Genus	Species	Tissue of isolation
Colletotrichum magna (L2.5)	Sinp + Ihp	watermelon	*Citrillus*	*lanatus*	fruit
Colletotrichum coccodes (155)	Sinp	tomato	*Lycopersicon*	*esculentum*	fruit
Colletotrichum orbiculare (683)	Ihp	squash	*Cucurbita*	*pepo*	stem
Colletotrichum musae (927)	Sinp	banana	*Musa*	*acuminata*	fruit
Colletotrichum gloeosporioides (95–41A)	–	strawberry	*Fragaria*	*ananassa*	crown
Colletotrichum gloeosporioides (95–63B)	–	strawberry	*Fragaria*	*ananassa*	crown
Colletotrichum acutatum (216)	–	strawberry	*Fragaria*	*ananassa*	fruit
Colletotrichum graminicola (003NY85)	Ihp	corn	*Zea*	*mays*	stalk
Colletotrichum lindemuthianum (BA10)	Ihp	bean	*Phaseolus*	*vulgaris*	stem

[1]The abbreviations Sinp (Subcuticular, intramural necrotrophic pathogen) and Ihp (Intracellular hemibiotrophic pathogen) were described by Bailey *et al*. (1992). –, not tested.

Redman *et al.*, 1999b). Fungi were cultured on either liquid or solid modified Mathur's (MS) (Tu, 1985), 0.1X potato dextrose agar (PDA) (pH 5.0), V8C (1 : 4 dilution of V8 Juice, 14.49 g l^{-1} $CaCO_3$), and/or hygromycin (HM) media containing 100 μg/ml of hygromycin as previously described (Redman & Rodriguez, 1994). Plant seeds were purchased from Petoseed Company (Woodland, CA, USA), Territorial Seed Company (Cottage Grove, OR, USA), MBS Seed LTD. Company (Denton, TX, USA), W. Altee Burpee Company (Warminster, PA, USA), and Nakamura Seed Company (Auburn, WA, USA).

Plant colonization and symbiotic lifestyle expression

Seeds were surface sterilized for 15–20 min in 1% sodium hypochlorite solution, rinsed five times in 100 volumes of sterile water, planted in sterile vermiculite, and grown for 7–10 d in growth chambers (95% humidity, 12 h daily light exposure at an irradiance of 180 μE m^{-2} s^{-1}, 22°C). The light source in the growth chambers consisted of eight 60 W coolwhite fluorescent bulbs and four 60 W incandescent bulbs. To inoculate plants, a minimum of 30 seedlings were placed into sterile glass beakers containing fungal spore suspensions ($1.0–3.0 \times 10^6$ conidia/ml) as previsously described (Redman *et al.*, 1999b). Fluid in the beakers was maintained so the roots and lower stems were fully immersed over the course of each experiment. These plants were used to determine if fungi expressed pathogenic or nonpathogenic lifestyles, and if nonpathogenic fungi colonized plant tissues as described below.

To determine if fungi expressed pathogenic lifestyles, inoculated and uninoculated control plants were incubated in growth chambers and plant mortality assessed after 5–14 d. Fungi were designated pathogenic if they induced 95–100% plant mortality and nonpathogenic if there was 0% plant mortality. All assays were repeated a minimum of three times with standard deviations of less than 10%.

Colonization of plants by fungi expressing nonpathogenic lifestyles was assessed 3–14 d after inoculation. Colonization was assessed by surface sterilization of 10 plants (Redman *et al.*, 1999b), cutting the plants into sections (roots; lower, middle and upper stem sections; and cotyledons or leaves (corn and strawberry only)), and plating the sections on fungal growth medium. The effectiveness of surface sterilization was verified by the imprint technique (Schulz *et al.*, 1999). Species of *Colletotrichum* that emerged from surface sterilized tissues were identified by both microscopic and PCR analysis (Freeman *et al.*, 1993, data not shown). *C. magna* isolates were identified microscopically (conidial differences between path-1 and L2.5) or by hygromycin resistance (for REMI mutants R1-A and R21-C).

The symbiotic lifestyles expressed by fungi that asymptomatically colonized plants were assessed as previously described (Redman *et al.*, 1999b). Seedlings were colonized with primary inocula (conidia of fungi expressing nonpathogenic lifestyles) for 36–48 h as described above. Colonized plants were then exposed to lethal conidial concentrations ($1.0–3.0 \times 10^6$ conidia/ml) of a wildtype *Colletotrichum* sp. that would induce 100% mortality in the respective plant species. Plant mortality was assessed 5–14 d after exposure to the pathogens. Symbiotic lifestyles of the nonpathogenic primary inocula were designated mutualistic, intermediate mutualistic, or commensal if the fungi conferred 80–100%, 30–70% and 0% disease protection, respectively. In these experiments, surviving plants were indistinguishable from uninoculated control plants (data not shown). All assays were repeated a minimum of three times with standard deviations of < 10%.

Plant physiological responses to fungal symbionts

The influence of path-1 and the *C. magna* wildtype L2.5 on plant growth was quantified by measuring the height and/or biomass of plants of colonized and uncolonized tomato (Seattle Best and Big Beef cultivars) and pepper (California Wonder) grown under glasshouse conditions. The initial experiment involved height measurements of 16 path-1-colonized and 16 uninoculated Seattle Best tomato plants. All other experiments involved measuring the size and f. wt of six path-1 colonized, six L2.5 colonized, and six uninoculated control tomato or pepper plants.

The ability of path-1 to confer drought tolerance was measured in 100 colonized and 100 uncolonized 5-d-old watermelon seedlings (Sugar Baby cultivar). Ten plants were placed in separate 100 ml beakers containing 30 ml water. Every 24 h, one of the beakers was emptied and the plants left dry. This process was continued for 9 d (the water in one of the beakers was maintained as a nondrought stress control) and all the beakers were re-filled with 30 ml water, the plants left to recover for 48 h and assessed for mortality. All assays were repeated a minimum of three times with standard deviations of < 10%.

The ability of *Colletotrichum* spp. to confer drought tolerance in Seattle Best and Big Beef tomato cultivars was measured in six plants colonized with either *C. magna* (path-1 and L2.5), *C. orbiculare* (683), *C. musae* (927), *C. gloeosporioides* (95–51 A), and six uncolonized 3-wk-old plants. Two plants were placed in separate 2 l beakers containing 500 ml water. Every 24 h, one of the beakers was emptied and the plants left dry. This process was continued for 3 d and 500 ml water were replaced in all beakers, the plants left to recover for 24 h and assessed for mortality. All plants were then surface sterilized and stems plated on to fungal growth media to verify colonization.

The ability of path-1 to protect squash (multipik vericolor cultivar) plants against *Phytophthora capsici* was tested by planting 50 colonized and 50 uncolonized seedlings into *P. capsici*-infested soils (provided from Dr G. Holmes, North Carolina State University, NC, USA). Plant mortality was

assessed after 4 wk in growth chambers (95% humidity, 12 h daily light exposure at an irradiance of 180 μE m^{-2} s^{-1}, 22°C). Controls consisted of the same soils autoclaved for 1 h and planted with 20 uncolonized squash seedlings. Plants exhibiting mortality or disease symptoms were sectioned and internal plant fragments plated on V8C growth medium to culture *P. capsici* from diseased tissues.

The interaction between fungi expressing different symbiotic lifestyles *in planta* was addressed by successive colonization studies. Ten watermelon plants (Sugar Baby cultivar) were colonized with either path-1, R1-A, or R21-C (primary inoculum) for 48 h and then exposed to a secondary inoculum of path-1, R1-A, R21-C, or wild type L2.5 and assessed for secondary colonization by plant mortality and/or re-isolation of fungi from surface sterilized tissue (as described above). All assays were repeated a minimum of three times with standard deviations of < 10%.

Results

Host range and lifestyle expression of *C. magna*

The host range and lifestyles expressed by *C. magna* wild-type isolate L2.5 and the lifestyle-altered mutants path-1 (mutualist), R1-A (mutualist), and R21-C (commensal) were determined on several cucurbit species (Tables 2a and 3). L2.5 was pathogenic resulting in 100% mortality on all cultivars with the exception of two resistant watermelon cultivars and one resistant cucumber cultivar. Re-isolation studies indicated that L2.5 did not colonize the resistant cultivars (data not shown). The two mutualists, path-1 and R1-A, colonized all eight of the cucurbit cultivars, including the resistant ones, without eliciting disease symptoms and conferred 100% disease protection against challenge by L2.5. By contrast, the commensal R21-C colonized all of the cucurbit cultivars without eliciting disease symptoms but did not confer disease resistance against L2.5. These results indicated that the expression of symbiotic lifestyles by the mutants was consistent among cucurbit species. More importantly, the expression of nonpathogenic lifestyles expanded host ranges to include cucurbit cultivars resistant to pathogenic isolates of *C. magna*.

L2.5 and path-1 were also assessed for the ability to asymptomatically colonize noncucurbit plant species not known to be hosts (Tables 2b,c and 3). Both isolates asymptomatically colonized several plant species indicating that the host range of *C. magna* encompasses at least four dicotyledenous plant families and is much greater than previously thought (Jenkins, 1963). Additionally, path-1 and L2.5 expressed a variety of nonpathogenic lifestyles including mutualism in noncucurbit plants. In general, the noncucurbit host ranges of L2.5 and path-1 were similar. However, L2.5 did not colonize Roma tomato while path-1 was an intermediate mutualist in this host. Although path-1 and L2.5 differed in the nonpathogenic lifestyles expressed on several plants, both isolates were able to express mutualistic, commensal and intermediate mutualistic lifestyles in noncucurbit hosts. These data indicated that the subtle genetic differences between path-1 and L2.5 (Freeman & Rodriguez, 1993) affected both host range and expression of symbiotic lifestyle. More importantly, these data demonstrate that a fungal plant pathogen is able to express a pathogenic lifestyle in certain plant species and several nonpathogenic lifestyles in other plant species.

Host ranges and lifestyles expressed by other *Colletotrichum* spp.

Seven additional *Colletotrichum* species were analysed for colonization and expression of symbiotic lifestyle on plant cultivars of known hosts and several plant species not known to be hosts (Tables 4a–c and 5). Three patterns emerged from this study and fungi either had: a narrow host range and expressed a single lifestyle; a wide host range and expressed a single lifestyle; or a wide host range and expressed multiple lifestyles. The first pattern was observed with *C. gloeosporioides* (95–63B), *C. graminicola* (NY00385), and *C. lindemuthianum* (BA10) which only colonized known hosts (strawberry, corn, and bean, respectively) and expressed pathogenic lifestyles. By contrast, *C. coccodes*, described as a 'cosmopolitan pathogen' with a large host range (Dillard, 1992), colonized both known hosts (tomato, pepper, cucurbits, and eggplant) and plants not known to be hosts (strawberry), and expressed only a pathogenic lifestyle (the second pattern).

The third pattern was observed with the remaining *Colletotrichum* species (Table 4). For example, *C. orbiculare* (683), a foliar pathogen of cucurbits (Table 4a), exhibited a pathogenic lifestyle on one cultivar of tomato (Seattle Best) and eggplant (Bambino), a commensal lifestyle on eggplant cultivar Kurumi, and a mutualistic lifestyle on tomato cultivar Big Beef (Table 4b). *C. musae*, a pathogen of banana fruit, exhibited both commensal (tomato, squash, eggplant), and intermediate mutualistic (tomato, watermelon, pepper) lifestyles. *C. acutatum*, a strawberry fruit pathogen (Table 4c), was a commensal on several cultivars of watermelon (Table 4a) and a pathogen on eggplant cultivar Kurumi (Table 4b). The two strains of *C. gloeosporioides* (95–63B and 95–41 A) isolated from diseased strawberry tissues differed in host ranges and lifestyle expression. Isolate 95–63B was pathogenic and specific to strawberry while the isolate 95–41 A was a commensal on a variety of plants (tomato, pepper, watermelon, eggplant) and a pathogen on strawberry (Table 4c).

Additional mutualistic benefits conferred by *Colletotrichum* spp.

The designations of symbiotic lifestyle described above were based on the ability of *Colletotrichum* spp./isolates to confer disease resistance against pathogenic *Colletotrichum* wildtypes.

Table 2 Symbiotic lifestyle expression of *Colletotrichum magna* wildtype and mutants on different plant species

(a)

	Family	Cucurbitaceae							
	Plant Genus Species	Watermelon *Citrullus lanatus*				Cucumber *Cucumis sativus*		Squash *Cucurbita pepo*	
Isolate	Cultivar	J	CG	CS	SB	M76	Pe	GZ	VS
L2.5 (wildtype)		nh	nh	P	P	P	nh	P	P
path-1		M	M	M	M	M	E	M	M
R1-A		E	E	M	M	M	E	M	M
R21-C		E	E	C	C	C	E	C	C

(b)

	Family	Solanaceae							
	Plant Genus Species	Tomato *Lycopersicon esculentum*			Pepper *Capsicum chinense*		*annuum*	Eggplant *Solanum melongena*	
Isolate	Cultivar	S	R	BB	H	LT	CW	B	K
L2.5 (wildtype)		IM	nh	M	M	M	M	C	C
path-1		M	IM	M	IM	IM	IM	C	IM

(c)

	Family	Fabaceae		Poaceae		Rosaceae	
	Plant Genus Species	Bean *Phaseolus vulgaris*		Corn *Zea mays*		Strawberry *Fragaria ananassa*	
Isolate	Cultivar	TC	BT	KK	J	SC	RL
L2.5 (wildtype)		M	nh	nh	nh	E	E
path-1		M	nh	nh	nh	E	E

A minimum of 30 plants of each cultivar were inoculated with conidia of the *C. magna* isolates listed in the left-hand columns and incubated for 5–14 days to determine if pathogenic lifestyles were expressed and if plants were colonized. Interactions denoted by P resulted in 100% plant mortality and all other interactions were asymptomatic. Fungal lifestyles were determined by exposing 30 asymptomatic plants (colonized with the *C. magna* isolates for 36 h) with conidia of compatible virulent pathogens as follows: *Cucurbitaceae* (*C. magna*), *Solanaceae* (*C. coccodes*), *Fabaceae* (*C. lindemutianum*), *Poaceae* (*C. graminicola*). Letters in the tables indicate the lifestyles expressed by the fungi: P, pathogen (induced 100% mortality); M, mutualist (conferred 80–100% disease resistance); IM, intermediate mutualist (conferred 30–70% disease resistance); C, commensal (conferred 0% disease resistance); E, nonpathogenic endophyte not tested for lifestyle; nh, nonhost (no fungal colonization detected). Abbreviations for cultivars: J, Jubilee; CG, Charleston Grey; CS, Crimson Sweet; SB, Sugar Baby; M76, Marketmore 76; Pe, Pepino; GZ, Golden Zucchini; VS, Vegtable Spaghetti; S, Seattle Best; R, Roma; BB, Big Beef; H, Habenero; LT, Long Thin Cayenne; CW, California Wonder; B, Bambino; K, Kurumi; TC, Top Crop; BT, Black Turtle Soup; KK, Kandy Korn; SC, Sweet Charlie; RL, Rosa Linda.

To determine if this was *Colletotrichum*-specific resistance, susceptibility to the aggressive root pathogen *Phytophthora capsici* was assessed with path-1-colonized and uncolonized squash plants (cultivar – Multipik Vericolor) planted in *P. capsici*-infested soils. Multipik Vericolor was used in this study because it was highly susceptible to *P. capsici* in fields containing these same soils (G. Holmers, pers. comm.). Two of the 50 path-1-colonized plants died (4% mortality) while 100% mortality was observed in uninoculated controls (Fig. 1a). *P. capsici* was isolated from dead plants but not from any of the path-1 colonized plants (data not shown). Additional controls consisted of 20 uncolonized plants grown in autoclaved soils resulting in 0% mortality (data not shown). It appears that disease resistance conferred by path-1 applies to a wide range of fungal pathogens encompassing at least two genera of ascomycetes (Freeman & Rodriguez, 1993; Redman *et al.*, 1999b) and the oomycete, *Phytophthora capsici*. This suggests that mutualist-conferred disease resistance to diverse fungal pathogens involves a common mechanism of activation.

Table 3 Asymptomatic colonization of plants by *Colletotrichum magna*

Plant Species	Cultivar	Isolate	Colonization (%)				
			R	LS	MS	US	C
Citrullus lanatus	J	path-1	65	100	100	0	0
	J	R1-A	85	100	100	15	0
	J	R21-C	69	100	100	77	0
	CG	path-1	70	100	95	5	0
	CG	R1-A	69	100	100	77	0
	CG	R21-C	56	100	100	44	0
	CS	path-1	78	96	100	65	0
	CS	R1-A	100	100	82	73	0
	CS	R21-C	82	100	100	23	0
	SB	path-1	79	100	100	79	0
	SB	R1-A	75	100	100	38	0
	SB	R21-C	75	100	100	80	0
Cucumis sativus	M76	path-1	88	100	100	0	0
	M76	R1-A	93	100	93	14	0
	M76	R21-C	79	100	100	7	0
	Pe	path-1	56	100	88	13	0
	Pe	R1-A	50	94	78	22	0
	Pe	R21-C	41	100	96	23	0
Cucurbita pepo	GZ	path-1	58	100	75	0	0
	GZ	R1-A	80	100	80	27	0
	GZ	R21-C	88	100	100	50	0
	VS	path-1	50	100	54	0	0
	VS	R1-A	57	100	100	0	0
	VS	R21-C	94	100	94	0	0
Lycopersicon esculentum	S	L2.5	100	100	100	94	0
	S	path-1	100	100	100	100	0
	R	path-1	100	100	100	100	7
	BB	L2.5	63	93	100	67	0
	BB	path-1	84	96	93	96	25
Capsicum chinense	H	L2.5	100	100	100	100	0
	H	path-1	92	100	85	23	0
	LT	L2.5	100	100	100	21	0
	LT	path-1	100	100	100	100	0
Capsicum annuum	CW	L2.5	100	100	100	54	0
	CW	path-1	100	100	100	60	0
Solanum melongena	B	L2.5	100	100	100	100	0
	B	path-1	100	100	100	23	0
	K	L2.5	70	100	90	20	0
	K	path-1	94	100	69	0	0
Phaseolus vulgaris	TC	L2.5	100	100	40	0	0
	TC	path-1	40	100	20	0	0
Fragaria ananassa	SC	L2.5	100	100	100	100	0
	SC	path-1	92	100	85	23	0
	RL	L2.5	60	100	30	0	0
	RL	path-1	40	100	20	0	0

The cultivars listed above did not develop disease symptoms when inoculated with conidia of the *C. magna* isolates listed next to them. To determine if the inoculted plants were colonized by the fungi, 10 plants/isolate were surface sterilized, cut into sections, and the sections plated on fungal growth medium (MS). The data indicate the % of plant sections of all 10 plants from which *Colletotrichum* emerged. Re-isolated fungi were characterized by microscopy (path-1 and L2.5) and hygromycin resistance (RI-A and R21-C). The plant sections are indicated by R (roots), LS (lower 1/3 of stem), MS (middle 1/3 of stem), US (upper 1/3 of stem), and C (cotyledons or leaves (corn and strawberry only)). Cultivar abbreviations: J, Jubilee; CG, Charleston Grey; CS, Crimson Sweet; SB, Sugar Baby; M76, Marketmore 76; Pe, Pepino; GZ, Golden Zucchini; VS, Vegtable Spaghetti; S, Seattle Best; R, Roma; BB, Big Beef; H, Habenero; LT, Long Thin Cayenne; CW, California Wonder; B, Bambino; K, Kurumi; TC, Top Crop; SC, Sweet Charlie; RL, Rosa Linda.

Table 4 Symbiotic lifestyle expression of wildtype *Colletotrichum* species on various plants

(a)

	Family	Cucurbitaceae							
	Plant Genus Species	Watermelon *Citrullus lanatus*				Cucumber *Cucumis sativus*		Squash *Cucurbita pepo*	
Isolate	Cultivar	J	CG	CS	SB	M76	Pe	GZ	VS
C. magna (L2.5)		nh	nh	P	P	P	nh	P	P
C. coccodes (155)		P	P	P	P	P	P	P	P
C.orbiculare (683)		nh	nh	P	P	P	P	P	P
C. musae (927)		E	E	IM	IM	IM	E	C	C
C. gloeosporioides (95–41A)		E	E	C	C	–	–	–	–
C. gloeosporioides (95–63B)		nh	nh	nh	nh	nh	nh	nh	nh
C. acutatum (216)		E	E	C	C	nh	nh	nh	nh
C. graminicola (003NY85)		nh	nh	nh	nh	nh	nh	nh	nh
C. lindemuthianum (BA10)		nh	nh	nh	nh	nh	nh	nh	nh

(b)

	Family	Solanaceae							
	Plant Genus Species	Tomato *Lycopersicon esculentum*			Pepper *Capsicum chinense*	*annuum*	Eggplant *Solanum melongena*		
Isolate	Cultivar	S	R	BB	H	LT	CW	B	K
C. magna (L2.5)		IM	nh	M	M	M	M	C	C
C. coccodes (155)		P	P	P	P	P	P	P	P
C.orbiculare (683)		P	nh	M	P	P	nh	P	C
C. musae (927)		C	C	IM	nh	M	IM	C	C
C. gloeosporioides (95–41A)		C	–	C	–	–	C	C	C
C. gloeosporioides (95–63B)		nh	nh	nh	nh	nh	nh	nh	nh
C. acutatum (216)		nh	nh	nh	nh	nh	nh	–	P
C. graminicola (003NY85)		nh	nh	nh	nh	nh	nh	nh	nh
C. lindemuthianum (BA10)		nh	nh	nh	nh	nh	nh	nh	nh

(c)

	Family	Fabaceae		Poaceae		Rosaceae	
	Plant Genus Species	Bean *Phaseolus vulgaris*		Corn *Zea mays*		Strawberry *Fragaria ananassa*	
Isolate	Cultivar	TC	BT	KK	J	SC	RL
C. magna (L2.5)		M	nh	nh	nh	E	E
C. coccodes (155)		nh	nh	nh	nh	P	P
C.orbiculare (683)		nh	nh	nh	nh	nh	nh
C. musae (927)		nh	nh	nh	nh	nh	nh
C. gloeosporioides (95–41A)		–	–	–	–	P	P
C. gloeosporioides (95–63B)		nh	nh	nh	nh	P	P
C. acutatum (216)		nh	nh	nh	nh	P	P
C. graminicola (003NY85)		nh	nh	P	P	nh	nh
C. lindemuthianum (BA10)		P	P	nh	nh	nh	nh

A minimum of 30 plants of each cultivar were inoculated with conidia of the *Colletotrichum* species listed in the left-hand columns and incubated for 5–14 days to determine if pathogenic lifestyles were expressed and if plants were colonized (see Table 3). Interactions denoted by P resulted in 100% plant mortality and all other interactions were asymptomatic. Fungal lifestyles were determined by exposing 30 asymptomatic plants (colonized with the *Colletotrichum* species for 36 h) with conidia of compatible virulent pathogens as follows: *Cucurbitaceae* (*C. magna*), *Solanaceae* (*C. coccodes*), *Fabaceae* (*C. lindemutianum*), *Poaceae* (*C. graminicola*). Letters in the tables indicate the lifestyles expressed by the fungi: P, pathogen (induced 100% mortality); M, mutualist (conferred 80–100% disease resistance); IM, intermediate mutualist (conferred 30–70% disease resistance); C, commensal (conferred 0% disease resistance); E, nonpathogenic endophyte not tested for lifestyle; nh, nonhost (no fungal colonization detected); –, not tested. Cultivar abbreviations: J, Jubilee; CG, Charleston Grey; CS, Crimson Sweet; SB, Sugar Baby; M76, Marketmore 76; Pe, Pepino; GZ, Golden Zucchini; VS, Vegtable Spaghetti; S, Seattle Best; R, Roma; BB, Big Beef; H, Habenero; LT, Long Thin Cayenne; CW, California Wonder; B, Bambino; K, Kurumi; TC, Top Crop; BT, Black Turtle Soup; KK, Kandy Korn; SC, Sweet Charlie; RL, Rosa Linda.

Table 5 Asymptomatic colonization of plants by *Colletotrichum* species

Plant species	Cultivar	Fungal Species	Isolate	Colonization (%)				
				R	LS	MS	US	C
Citrullus lanatus	J	*C. musae*	927	76	100	82	41	0
		C. gloeosporioides	95–41A	93	100	93	80	7
		C. acutatum	216	20	100	80	0	0
	CG	*C. musae*	927	63	100	100	69	0
		C. gloeosporioides	95–41A	81	100	94	75	19
		C. acutatum	216	40	93	93	7	0
	CS	*C. musae*	927	79	93	86	36	0
		C. gloeosporioides	95–41A	78	100	100	83	11
		C. acutatum	216	50	100	83	6	0
	SB	*C. musae*	927	79	100	86	21	0
		C. gloeosporioides	95–41A	44	100	100	81	13
		C. acutatum	216	8	100	100	0	0
Cucumis sativus	M76	*C. musae*	927	44	100	94	38	0
	Pe	*C. musae*	927	69	100	100	56	0
Cucurbita pepo	GZ	*C. musae*	927	31	100	31	0	0
	VS	*C. musae*	927	77	94	69	23	0
Lycopersicon esculentum	S	*C. musae*	927	100	100	100	92	8
		C. gloeosporioides	95–41A	100	100	100	100	33
	R	*C. musae*	927	64	100	100	64	21
	BB	*C. orbiculare*	683	15	85	38	15	0
		C. musae	927	100	100	100	94	13
		C. gloeosporioides	95–41A	100	100	100	100	33
Capsicum chinense	LT	*C. musae*	927	100	100	100	100	0
Capsicum annuum	CW	*C. musae*	927	100	100	100	69	0
		C. gloeosporioides	95–41A	93	100	100	93	21
Solanum melongena	B	*C. musae*	927	69	100	100	38	0
		C. gloeosporioides	95–41A	100	100	100	80	0
	K	*C. orbiculare*	683	41	100	15	0	0
		C. musae	927	100	100	100	46	0
		C. gloeosporioides	95–41A	100	100	100	80	0

The cultivars listed above did not develop disease symptoms when inoculated with conidia of the *Colletotrichum* species listed next to them. To determine if the inoculted plants were colonized by the fungi, 10 plants/isolate were surface sterilized, cut into sections, and the sections plated on fungal growth medium (MS). The data indicate the % of plant sections of all 10 plants from which *Colletotrichum* emerged. Re-isolated fungi were characterized by microscopy, colony morphology, and PCR analysis. The plant sections are indicated by R (roots), LS (lower 1/3 of stem), MS (middle 1/3 of stem), US (upper 1/3 of stem), and C (cotyledons or leaves (corn and strawberry only)). Cultivar abbreviations: J, Jubilee; CG, Charleston Grey; CS, Crimson Sweet; SB, Sugar Baby; M76, Marketmore 76; Pe, Pepino; GZ, Golden Zucchini; VS, Vegtable Spaghetti; S, Seattle Best; R, Roma; BB, Big Beef; H, Habenero; LT, Long Thin Cayenne; CW, California Wonder; B, Bambino; K, Kurumi; TC, Top Crop; SC, Sweet Charlie; RL, Rosa Linda.

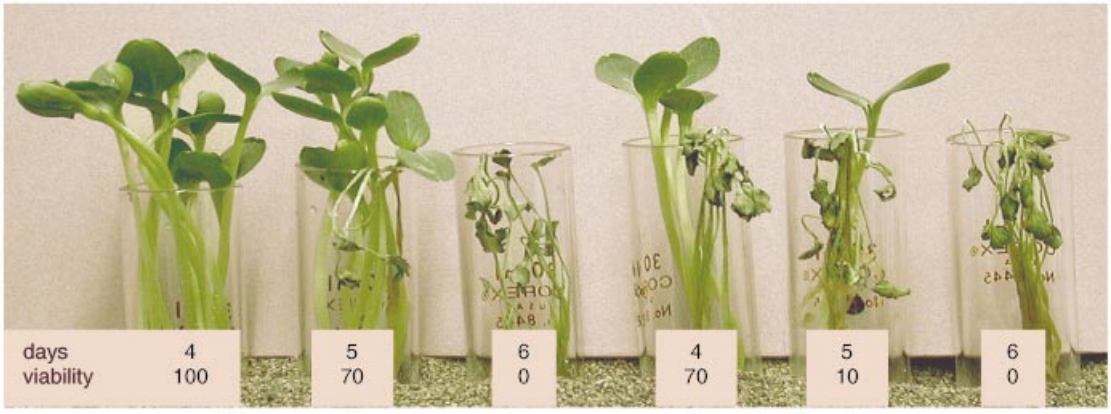
To determine if the benefits conferred to plants by the *C. magna* mutualists were restricted to disease resistance, path-1 and R1-A were tested for the ability to confer drought tolerance and growth enhancement to watermelon and tomato seedlings. In drought studies, uncolonized watermelon seedlings exhibited 50–70% mortality after 4 d without water and 90–100% mortality after 5 d without water (Fig. 1b). Path-1 colonized plants exhibited 20–30% mortality after 5 d without water and 100% mortality after 6 d without water. Although these experiments represent extreme drought stress, it is clear that path-1 colonized plants tolerate this stress better than uncolonized controls. R1-A conferred drought tolerance

Fig. 1 Mutualistic benefits conferred by path-1 colonized by plants vs uncolonized controls. Experimental details are described in the Materials and Methods section. (a) Disease resistance against the root pathogen *Phytophthora capsici*. Seeds were planted in *P. capsici*-infested soil obtained from an agricultural field. Uncolonized plants became stunted and chlorotic after 4 wk of growth (shown) and experienced 100% mortality by week 6. (b) Drought tolerance conferred to watermelon seedlings. The upper and lower numbers refer to days without water and plant viability, respectively. Path-1-colonized plants were significantly more tolerant to drought than uncolonized controls. (c) Growth enhancement of tomato plants grown for 1 month under glasshouse conditions. Path-1 colonized plants were approximately twice the size of uncolonized controls.

(a) Disease resistance

Colonized Uncolonized

(b) Drought tolerance



Colonized Uncolonized

(c) Growth enhancement

Colonized Uncolonized

Table 6 Growth enhancement and drought tolerance conferred by *Colletotrichum* species in tomato and pepper

	Plant Species	*Lycopersicon esculentum* (tomato)									*Capsicum annuum* (pepper)				
	Cultivar	Big Beef				Seattle Best					California Wonder				
Fungal Species (isolate)		fsl-dr[1]	weight[2]	sd[3]	p[4]	dt[5]	fsl-Dr	weight	sd	p	dt	fsl-Dr	weight	sd	p	dt
C. magna (path-1)		M	19.1	2.6	< 0.05	72	M	15.8	3.7	<0.05	48	IM	10.77	2.8	0.93	24
C. magna (L2.5)		M	17.9	3	< 0.05	72	IM	11.2	2.7	0.11	72	M	8.82	1.43	0.05	72
C. orbiculare (683)		M	17.9	10.6	< 0.05	72	P	–	–	–	–	nh	–	–	–	–
C. musae (927)		IM	12	9.3	0.61	48	C	–	–	–	–	IM	11.25	3.01	0.19	72
C. gloeosporioides (95–41A)		C	17.03	10.5	< 0.05	72	C	7.2	0.8	0.18	72	C	14.95	2.38	0.00	72
Uncolonized control			11.2	1.2		48		8	4		48		10.65	1.42		24

[1]Fungal symbiotic lifestyles were determined by exposing 30 asymptomatic plants (colonized with the isolates for 36 hours) with conidia of the virulent pathogen *C. coccodes*. Letters in the table indicate the lifestyles expressed by the fungi; M, mutualist (conferred 80–100% disease resistance); IM, intermediate mutualist (conferred 30–70% disease resistance); C, commensal (conferred 0% disease resistance); nh, nonhost (no fungal colonization detected). [2]Average fresh weight in grams of the entire plant for 6 plants before drought treatment. [3]Standard deviation of plant weights. [4]*P*-value from ANOVA (one-way analysis of variance) for plant weight comparisons. [5]Drought tolerance measured as the number of hours of survival without water over a 72-h period. –, not tested.

Table 7 Successive colonization of watermelon by *Colletotrichum magna* isolates expressing different lifestyles

	Secondary Inocula			
Primary Inocula	Path-1	R1	R21	L2.5
Path-1	+[a]	+	–	–
R1-A	+	nd	+	–
R21-C	+	nd	nd	+

Ten watermelon seedlings (Crimson Sweet) were colonized with primary inoculum for 48 h, then exposed to secondary inoculum and assessed for secondary colonization by monitoring plant mortality and/ or re-isolation of fungi from surface sterilized tissue. + and – indicate 100% and 0% colonization, respectively. nd = not determined. [a]The secondary isolate was path-1 transformed with a hygromycin resistance gene to allow for detection (Redman *et al.*, 1999b).

similar to path-1 and R21-C colonized plants responded the same as uncolonized controls (data not shown). Path-1 also conferred significant growth enhancement to both cucurbit (data not shown) and tomato plants (Fig. 1c & Table 6). After 30 d of glasshouse growth, path-1 colonized plants were approximately twice the size of uninoculated controls. R1-A conferred growth enhancement similar to path-1 and R21-C colonized plants grew at the same rate as uncolonized controls (data not shown).

Pathogenic *Colletotrichum* isolates that were capable of expressing nonpathogenic lifestyles (Tables 2 and 4) were screened for the ability to confer drought tolerance and growth enhancement to tomato and pepper plants (Table 6). Plants were colonized with *C. magna* (path-1 and L2.5), *C. gloeosporioides* (95–41 A), *C. orbiculare* (683), and *C. musae* (927) and grown in a glasshouse for 4 wk. There was a significant increase in the biomass of Big Beef plants colonized with path-1, L2.5, 95–41 A and 683 compared with water controls. However, *C. musae* (927) did not increase the biomass of Big Beef. Similarly, path-1, L2.5, 95–41 A and 683 (but not 927) conferred drought tolerance to Big Beef plants. The results with Seattle Best were very different. Only path-1 conferred a significant growth response and only L2.5 and 95–41 A conferred drought tolerance suggesting that the mechanisms of growth enhancement and drought tolerance are distinct. Results with pepper indicated that 95–41 A conferred growth enhancement and drought tolerance, path-1 had no affect on this host, 927 had no effect on growth but conferred drought tolerance, and L2.5 decreased growth but conferred drought tolerance.

Successive colonization vs lifestyle expression

Previous studies and data presented here demonstrate that path-1 prevents colonization of hosts by pathogenic fungi (Freeman & Rodriguez, 1993; Redman *et al.*, 1999a,b). To determine if fungi expressing nonpathogenic lifestyles

influenced subsequent colonization by other nonpathogenic symbionts, successive inoculation studies were undertaken (Table 7). Watermelon plants precolonized with path-1 allowed mutualists (R1-A, and path-hyg) to colonize (with 0% plant mortality), but did not allow a commensal (R21-C), or a pathogen (L2.5) to do so. In addition, plants precolonized with R1-A were subsequently colonized with path-1 but not L2.5. Interestingly, R1-A did not block subsequent colonization by R21-C. In fact, successive inoculation of R1-A colonized plants with R21-C resulted in 100% plant mortality indicating that these strains have different mutations capable of complementation resulting in the expression of the wildtype pathogenic lifestyle. Therefore, the mutualistic lifestyles expressed by path-1 and R1-A were similar but not identical (Table 7). Plants precolonized with the commensal R21-C were amenable to colonization by mutualists and pathogens resulting in 0% and 100% plant mortality, respectively.

Discussion

The prevailing view of symbiotic fungi is that selection pressures have imposed a directionality on the evolution of symbiotic lifestyles resulting in the generation of mutualistic endophytes from parasitic or pathogenic fungi (Saikkonen *et al.*, 1998). However, some pathogenic *Colletotrichum* spp. have the ability to express different symbiotic lifestyles based on host genotypes. This suggests that either these species have evolved to possess maximum symbiotic flexibility or directional evolution has occurred in a host genotype-specific manner.

Although some *Colletotrichum* spp. were restricted to a single host and expressed one lifestyle, this may reflect the limited number of plant species analysed. It is conceivable that all *Colletotrichum* spp. colonize 'nondisease' hosts which may explain why these species are so commonly isolated from asymptomatic plant tissues during studies on endophytes (Fisher *et al.*, 1994). It is unlikely that the expression of nonpathogenic lifestyles is a phenomenon specific to *Colletotrichum* because so many 'pathogens' can be isolated from asymptomatic plant tissues (Schulz *et al.*, 1998).

The data in Tables 2–5 suggest that the lifestyles expressed by fungi and the outcome of symbiosis is controlled by the plant. This may result from differences in fungal gene expression in response to the plant or differences in the ability of a plant to respond to the fungus. Regardless, subtle genetic differences between cultivars of a single plant species may greatly alter the outcome of fungal/plant symbioses. It is interesting that although we have converted a pathogenic isolate of *C. magna* into gene disruption mutants that express either mutualistic and commensal lifestyles in a single plant species, this phenomenon occurs with wildtype *Colletotrichum* spp. in several plant species not previously known to be hosts.

The host-fitness attributes screened to assess symbiotic lifestyle expression may significantly bias lifestyle designations. For example, *C. gloeosporioides* (95–41 A) was designated a commensal on tomato and pepper in the disease resistance studies (Table 4b) but was a mutualist on both hosts in the growth and drought experiments (Table 6). *C. musae* (927) was an intermediate mutualist in the disease studies on Big Beef and California Wonder (Table 4b), a commensal on both hosts with regard to growth enhancement, and a mutualist on pepper with regard to drought tolerance (Table 6). Therefore, when fungi are assessed for symbiotic lifestyle expression, the screening method (drought tolerance, growth enhancement, disease resistance) may significantly bias the lifestyle designation.

The ability of a pathogen to express nonpathogenic symbiotic lifestyles suggests that the role of fungi in plant community structure and dynamics is not a simple scenario of pathogens causing disease, mutualists conferring benefits, and saprophytes degrading biomass. In specific geographical locations, a single fungal isolate may be pathogenic in some plant species, provide mutualistic benefits such as disease resistance, drought tolerance, and growth enhancement to other plant species, and asymptomatically colonize other plants as a commensal. The ramifications of this phenomenon warrant a re-evaluation of hypotheses that describe the evolution, ecology, and dynamics of fungal and plant communities. In addition, this phenomenon may be used to develop new hypotheses to describe complex aspects of plant community dynamics such as the ability of some nonindigenous plants to outcompete native species. For example, it is possible that nonindigenous plants may transport fungal symbionts that act either as mutualists conferring competitive benefits, or as pathogens to native plant species thereby decreasing plant competition for the invaders. A similar scenario may occur if nonindigenous plants establish mutualistic symbioses with native fungi in new habitats.

If observations with *Colletotrichum* spp. are common in plant/fungal symbioses, then successive colonization of plants by fungi is dependent on the symbiotic lifestyles expressed by primary and secondary colonizing fungi. This conclusion has a significant impact on the practice of restoration of plant communities and biological control of nonindigenous plants. For example, current biological control strategies for nonindigenous plants involve either 'host-specific' fungal pathogens or herbivorous insects. However, depending on their symbiotic potential and history, nonindigenous plants may be colonized with mutualists that confer resistance to pathogenic fungi (Freeman & Rodriguez, 1993; Redman *et al.*, 1999a) and/or insect herbivory (Clay, 1991) making these biological control strategies potentially ineffective.

As our understanding of plant symbiotic interactions increases, it will be possible to design more effective restoration strategies that involve plant communities (plants and microbes) rather than individual plant species. It will also be possible to develop environmentally safe plant protectants as alternatives to chemical fungicides used in agriculture.

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