Field Performance of Cucurbit and Tomato Plants Colonized with a Nonpathogenic, Mutualistic Mutant (path-1) of *Colletotrichum magna* (Teleomorph: *Glomerella magna*; Jenkins & Winstead)

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Abstract

Path-1 is a UV-induced non-pathogenic mutant of a virulent *Colletotrichum magna* isolate that establishes mutualistic symbioses with cucurbit and tomato species. Under laboratory conditions, this mutualism results in plant growth enhancement, drought tolerance, and disease protection against fungal pathogens. This study focuses on the efficacy of this symbiosis and the symbiotic lifestyle expressed by path-1 under field conditions in the absence of disease stress. The effects of colonization by path-1 on fruit yields and growth was measured in field plots with four cucurbit species including four watermelon cultivars, and two tomato cultivars, over four growing seasons. The persistence of the symbiosis, extent of colonization, and path-1 transmission were also assessed. Yields from path-1 infected plants were equivalent to or greater than yields from non-inoculated control plants and path-1 systemically colonized plants throughout each growing season. Path-1 also increased the growth rates of tomato plants and was not transmitted to uncolonized plants. The results indicate that there are no metabolic costs of this symbiosis and the symbiosis is maintained under field conditions.

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1. Introduction

There are numerous reports of controlling fungal diseases with microorganisms and the mechanisms of control involve either biochemical inhibition, enzymatic digestion, nutrient competition, physical displacement, mycoparasitism, or induced resistance (Campbell, 1989; Whipps, 1997; Martin and Loper, 1999; Alabouvette, 2000). All of these mechanisms, with the exception of induced resistance, require the biological control agents to be inoculated, maintained at high cell densities, and compete with resident microbial populations, which often limits their agricultural usefulness (Handelsman and Stabb, 1996; Deacon and Berry, 1993; Becker and Schwinn, 1993; Larkin et al., 1993a and b; Alabouvette et al., 1993; Cook, 1991; Campbell, 1991).

One alternative to standard biological control strategies is to establish mutualistic symbioses between fungi and plants prior to planting. Fungal mutualists are beneficial to plants because they may provide enhanced nutrient acquisition, drought tolerance, and/or disease protection (Read and Camp, 1986; Carroll, 1986; Bacon, 1993; Freeman and Rodriguez, 1993; Redman et al., 1999; Redman et al., 2001; Read, 1999). However, host specificity can limit the usefulness of individual fungal symbionts in biological control efforts.

Colletotrichum spp. are filamentous fungal plant pathogens that have caused devastating diseases in numerous crop plants worldwide (Sutton, 1992). *C. magna* is a pathogen of cultivated cucurbits (Jenkins, 1963). A nonpathogenic mutant of *C. magna*, path-1, that was generated by UV mutagenesis, asymptomatically colonizes cucurbit and tomato plants. Path-1 is defined as a mutualistic symbiont because it confers disease resistance against wildtype *C. magna*, *C. orbiculare*, *C. coccodes*, *Fusarium oxysporum*, and *Phytophthora capsici* (Freeman and Rodriguez, 1993; Redman et al., 1999; Redman et al., 2001). Depending on the plant genotype, path-1 may also confer drought tolerance and/or growth enhancement (Redman et al., 2001). However, all of the previous analyses were conducted under laboratory conditions, and the metabolic cost of path-1 infection, the longevity of the symbiosis during a normal growing season, and its ability to protect against disease under field conditions, were not assessed.

The objectives of this study, were to determine the following: 1 – if path-1 imposed measurable metabolic costs on field grown plants, 2 – the persistence of the symbiosis throughout a growing season, 3 – the phenotypic stability of path-1 in field grown plants, and 4 – if path-1 was transferred from colonized plants to adjacent uncolonized plants. This research was performed in several cucurbit and tomato cultivars over four field seasons.

2. Materials and Methods

Fungal and plant culture conditions and plant inoculation

C. *magna* (Jenkins and Winstead) isolate path-1 was originally obtained by UV mutagenesis of the wildtype strain L2.5 (Freeman and Rodriguez, 1993). The fungus was cultured on liquid or solid modified Mathur's (MS) (Tu, 1985) medium containing 100 μg/ml of ampicillin as previously described (Redman and Rodriguez, 1994). Seeds of anthracnose resistant (R) and susceptible (S) watermelon [*Citrillus lanatus* (Thunb.) Matsumura & Nakai, cvs Sugar Baby (S), Crimson Sweet (S), Allsweet (S), and Jubilee (R)], cucumber [*Cucumis sativus* L., cv Pickler (S)], squash [*Cucurbita pepo* L., cv Early White Bush Scallop (S), Banana Pink (S), Yellow Crook Neck (S), Vegetable Spaghetti (S), and Seneca Zucchini (S)] were purchased from Petoseed Company (Woodland, CA) and MBS Ltd. Co. (Denton, TX). Anthracnose susceptible seeds of tomato [*Lycopersicon esculentum* P. Mill, cvs Big Beef and Seattle's Best] were purchased from Territorial Seed Company (Cottage Grove, OR). Seeds were germinated and grown in vermiculite for 5–14 days. The seedlings were removed from the vermiculite and the roots and lower stems submerged in conidial suspensions (2×10^6/ml) of path-1 for 48 hours as previously described (Redman et al., 1999b). Inoculated seedlings were planted in 5.0 cm peat pots containing Metromix 350 (Scotts Co., Marysville, OH) and maintained in a greenhouse for 2–3 weeks until field planting. Greenhouse plants were watered daily with Hogland's solution. Non-inoculated control seedlings were treated in the same manner as path-1 colonized seedlings but were not exposed to fungal conidia.

To determine the level of path-1 colonization in inoculated plants, fifty inoculated and non-inoculated seedlings of each cultivar used in this study were surface sterilized and plated onto fungal growth medium (described below). Seedlings were analyzed prior to planting and 100% of the inoculated plants were colonized with path-1 and 0% of the non-inoculated plants were colonized (data not shown). Therefore, inoculated and non-inoculated plants are referred to in the text as colonized and non-colonized, respectively.

Field plot design and planting

The first field test of path-1 colonized plants was conducted in Seattle (1995) by comparing fruit production from path-1 colonized plants and non-inoculated controls in small field plots without disease challenge. The field (7.6 m × 24.4 m) was prepared by tilling 21.0 m of plant compost (15.2 cm depth) into a sandy-loam soil and establishing a drip irrigation system. Each plot (1.5 m × 1.5 m) was planted with six seedlings of either cucumber, squash, or zucchini. Each

species was planted into six randomly distributed plots, three of which had path-1 colonized plants and three with non-inoculated controls. As plants matured, fruit was collected on a weekly basis for weight measurements.

The results from the small scale field plots led to the establishment of a large scale field experiment in 1997. Metabolic cost was assessed by determining plant survival, fruit yield and fruit weight at the end of the growing season. A field plot (0.142 km^2) at the Samuel Roberts Noble Foundation's Red River Farm in southern Oklahoma were tilled into 37 bedrows allowing for 2.7 m distances between rows and 64 plants were placed at 1.8 m intervals in each row. Blocks of 8 plants representing 2 treatments (path-1 colonized and non-colonized) and 2 watermelon cultivars (Sugar Baby and Jubilee) were alternately planted throughout the field plot to eliminate bias from possible variation in soil and environmental conditions. The field was fertilized with 23 kg each of nitrogen and potassium prior to planting and plants were watered with overhead irrigation as required. Weeds were controlled by tillage early in the growing season and no insecticides were applied. As a result, a significant number of plants were overcome by weeds and killed by insect infestations. The experiment was initiated with 1280 Sugar Baby and 347 Jubilee watermelon plants but due to plant mortality only 1054 plants were assessed for fruit yields and path-1 colonization.

In 1998 a field trial was performed at the Red River Farm using the same plot design as in 1997. The field was fertilized with 23 kg of nitrogen prior to planting and plants were watered with overhead irrigation as required. The watermelon cultivars Crimson Sweet, Allsweet, and Jubilee were planted. Weeds were controlled by tillage throughout the growing season and insecticides were applied as required. As a result, plant survival was significantly greater compared to the 1997 experiment.

Colonization analyses

Path-1 colonization was assessed at the end of each growing season either by microbiological and/or PCR (Polymerase Chain Reaction; Saiki et al., 1985; Mullis and Faloona, 1987) analysis. In 1997, stem sections (10 cm lengths taken approximately 5.0 cm above the crowns) were collected from 100 path-1 inoculated and 100 non-inoculated Sugar Baby plants for microbiological analyses. In 1998, one leaf was collected from the end of 40 inoculated and 40 non-inoculated Jubilee watermelon vines for PCR analysis. In 2000, two terminal leaves were collected from 18 inoculated and 18 non-inoculated Seattle's Best tomato plants for microbiological and PCR analysis.

Microbiological analysis involved submerging plant tissues into 2.0% (v/v) sodium hypochlorite for 20–30 minutes with moderate agitation followed by a

rinse with 10–20 volumes of sterile distilled water (Redman et al., 1999a). The effectiveness of surface sterilization was verified by the imprint technique (Schultz et al., 1999). Using aseptic technique, plant stems were cut longitudinally and interior sections plated onto MS media medium (containing 100 μg ml^{-1} of ampicillin). Surface sterilized leaves were cut into sections and plated on MS medium. The plates were incubated at room temperature under cool fluorescent lights for 5–7 days to allow for the emergence of the fungi. Identification of fungi was verified after conidiation by microscopic analysis.

Molecular assessment of colonization involved the extraction of DNA from plant leaf samples and PCR amplification using *C. magna* specific primers (Rodriguez et al., 2002). Total nucleic acid was extracted from watermelon leaves using a previously described protocol (Roossinck et al., 1997). Final pellets were resuspended in 30 μl of ddH2O, and 2 μl were used as template for PCR. In the initial pilot experiment, one plant was identified as a positive control for path-1 colonization and another was identified as negative control. Total DNA was isolated from these plants and were used as positive and negative control templates for all future PCR reactions. In addition, template-free reactions were included as a negative control for contamination. To detect *C. magna* in host tissues, species specific primers were designed for dual primer PCR (dpPCR) and nested primer PCR (npPCR) (Rodriguez et al., 2002). Briefly, species-specific PCR products were amplified by apPCR (arbitrarily primed PCR) with a 15 bp primer comprised of five GTC repeats. One of the apPCR products was cloned and sequenced. *C. magna*-specific dpPCR primers [CGAATCTGTAACTCTTCCTGC (p365) and ACAGACAGGATTCTCAATTTC (p366)] were constructed based on the terminal nucleotide sequences of the cloned apPCR product. *C. magna*-specific npPCR primers were AACCGTCTCATGCA AAAGTCA (p413), which was 20 base pairs from the end of p365 and GGTATGTCCCTTCCTGAACAC (p415), which was 10 bp from the end of p366. These primers amplify genomic DNA from all isolates of *C. magna* including the wildtype and path-1.

Dual primer PCR was carried out in 20 μl volumes containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl$_2$, 0.2% Triton X-100, 200 μM each of dATP, dCTP, dGTP, dTTP (Pharmacia), 0.2 units Taq DNA polymerase [Boehringer Mannheim (Indianapolis, IN)], 500 ng each of p365 and p366, and 0.4 to 400 ng of fungal DNA. PCR reactions involved 35 cycles of a temperature regime consisting of denaturation at 93°C for 15 s, primer annealing at 52°C for 2 min, and synthesis at 72°C for 2 min. Prior to the initiation of the cycles, the reactions were incubated at 93°C for 2 min. Nested primer PCR was performed by diluting dpPCR products 5-fold with 10 mM Tris-HCl (pH 9.0). Two μl of diluted dpPCR product was transferred to a 0.5 ml microcentrifuge tube containing 20 μl of fresh reaction buffer and two nested primers (p413 and p415). The thermocyclers were programmed as described for dpPCR reactions.

Electrophoresis of the amplified products was performed for 1.5 h at 12 to 16 V/cm in 2% agarose (Sambrook et al., 1989). PCR products were stained with ethidium bromide, visualized using 305 nm ultraviolet light, and documented with a UVP photodocumentation system.

Plant bioassays

Phenotypic stability of path-1 was assessed by exposing anthracnose susceptible watermelon varieties (Sugar Baby, Allsweet, and Crimson Sweet) to each of the 77 isolates collectively recovered from plants at the end of the growing seasons (described above). Anthracnose susceptible seedlings were placed in spore suspensions (2×10^6 spores/ml) as previously described (Redman et al., 1999). Ten seedlings were inoculated with each recovered path-1 isolate and assessed for disease symptoms 7 days after inoculation. Non-inoculated plants and plants inoculated with the *C. magna* wildtype L2.5 (2×10^6 spores/ml) were used as controls as previously described (Redman et al., 1999).

3. Results

Cucurbitaceae

The effect of path-1 on three cultivars of squash and one cultivar of cucumber was tested in small field plots during the 1995 season. The yields from path-1 colonized plants were greater than non-colonized plants for all four cultivars (Table 1). However, there were no differences in the growth rates, flowering time, and number of flowers set between colonized and non-colonized plants (data not shown). Although the number of plants was low, these experiments provided the impetus to perform larger field trials with greater numbers of plants.

Over two consecutive growing seasons (1997 and 1998) path-1 colonized cultivars of watermelon were compared to non-colonized plants in a 0.142 km^2 plot in southern Oklahoma. In 1997, cultivars Sugar Baby and Jubilee plants were tested. The differences in yields between path-1 colonized and non-colonized Sugar Baby plants were not statistically significant (Table 2). However, path-1 colonized Jubilee plants produced significantly higher yields than non-colonized plants (Table 2). Mortality rates were high in 1997 and there were no significant differences between colonized and non-colonized plants. Mortality rates were ascribed to insect infestations and weed encroachment.

In 1998 three watermelon cultivars were tested in the Oklahoma field plot. The differences in yields between path-1 colonized and non-colonized Crimson

Table 1. The effect of path-1 on fruit yields from cucumber and squash plants

Plant	Cultivar	Treatment	Plant no.	Total yield (g)	Yield/ plant (g)	Control (%)	P-value
Cucumber	Pickler	Control	12	8539	712	100	
		Path-1	12	10113	843	118	0.573
Squash	EWB Scallop*	Control	4	4865	1216	100	
		Path-1	4	5520	1380	113	0.612
Squash	Banana Pink	Control	4	2714	679	100	
		Path-1	4	5051	1263	186	0.369
Squash	Seneca Zucchini	Control	4	5197	1299	100	
		Path-1	4	6578	1645	127	0.212

Planting occured during the first week of June, 1995 and fruit harvest completed in the last week of September. Statistical analysis (ANOVA) of the data revealed that there were no significant differences between colonized and non-colonized plants (P>0.1). * = Early white Bush Scallop.

Sweet, Jubilee, and Allsweet were not statistically significant (Table 2). The mortalities in 1998 were lower than in 1997, and were ascribed to insect and rodent damage.

In the 1997 and 1998 field experiments no differences were observed in the growth rates, flowering time, and number of flowers set between colonized and non-colonized plants (data not shown).

Solanaceae

Recently, several isolates of *C. magna* were screened for the ability to infect and colonize plant species not previously recognized as hosts (Redman et al., 2001). Several plant species were asymptomatically colonized by wildtype isolates of *C. magna* and path-1. In tomato plants, both wildtype *C. magna* isolates and path-1 expressed mutualistic lifestyles and protected the plants against disease from the tomato pathogen *C. coccodes* (Redman et al., 2001). Therefore, we assessed the performance of path-1 colonized and non-colonized tomato plants (cvs Seattle's Best & Big Beef) in small field plots located in Seattle, Washington (Table 3). The results with the tomato trials were similar to those observed with the watermelon trials. Path-1 colonized tomato plants produced slightly greater yields than non-colonized controls. However, the differences in yields between colonized and non-colonized plants were not statistically significant (ANOVA).

Table 2. The effect of path-1 on fruit yields of watermelon vines

Year	Cultivar	Treatment	Initial plant no.	Surviving plant no.[a]	Mortality (%)	Total yield (kg)	Yield/plant (kg)	Control (%)	P-value
1997	Sugar Baby	Control	636	.407	36	3695	9	100	0.282
		Path-1	644	433	33	3848 b	9	104	
1997	Jubilee	Control	120	75	38	1640	12	100	0.027
		Path-1	227	139	39	2176 b	14	120	
1998	Crimson Sweet	Control	634	480	24	16049	33	100	0.784
		Path-1	695	507	27	16264 b	34	101	
1998	Jubilee	Control	537	434	19	11560	27	100	0.700
		Path-1	599	487	19	11206 b	26	97	
1998	Allsweet	Control	201	160	20	4839	30	100	0.122
		Path-1	161	140	13	5289 b	33	109	

Planting occured in the second week of May and yield data represent fruit harvested during the first week of August. Statistical analysis (ANOVA) of the data revealed that there were no significant differences (P>0.1) between colonized and un-colonized plants except in Jubilee (P<0.1) during the 1997 season. a - Due to the weed and insect infestations and rodent herbivory, the number of plants/treatment varied. b - Total yield X (number of surviving control plants/number of surviving colonized plants).

Table 3. The effect of path-1 on fruit yields of tomato plants

Year	Cultivar	Treatment	Plant no.	Total yield (g)	Yield/plant (g)	Control (%)	P-value
2000	Big Beef	Control	18	39853	2214	100	0.517
		Path-1	17	41196	2423	109	
2000	Seattle's Best	Control	16	22128	1383	100	0.357
		Path-1	16	22603	1413	102	

Planting occured in the third week of June and yield data represent fruit harvested during the third week of September. Statistical analysis (ANOVA) of the data revealed that there were no significant differences between colonized and un-colonized plants (P>0.1).
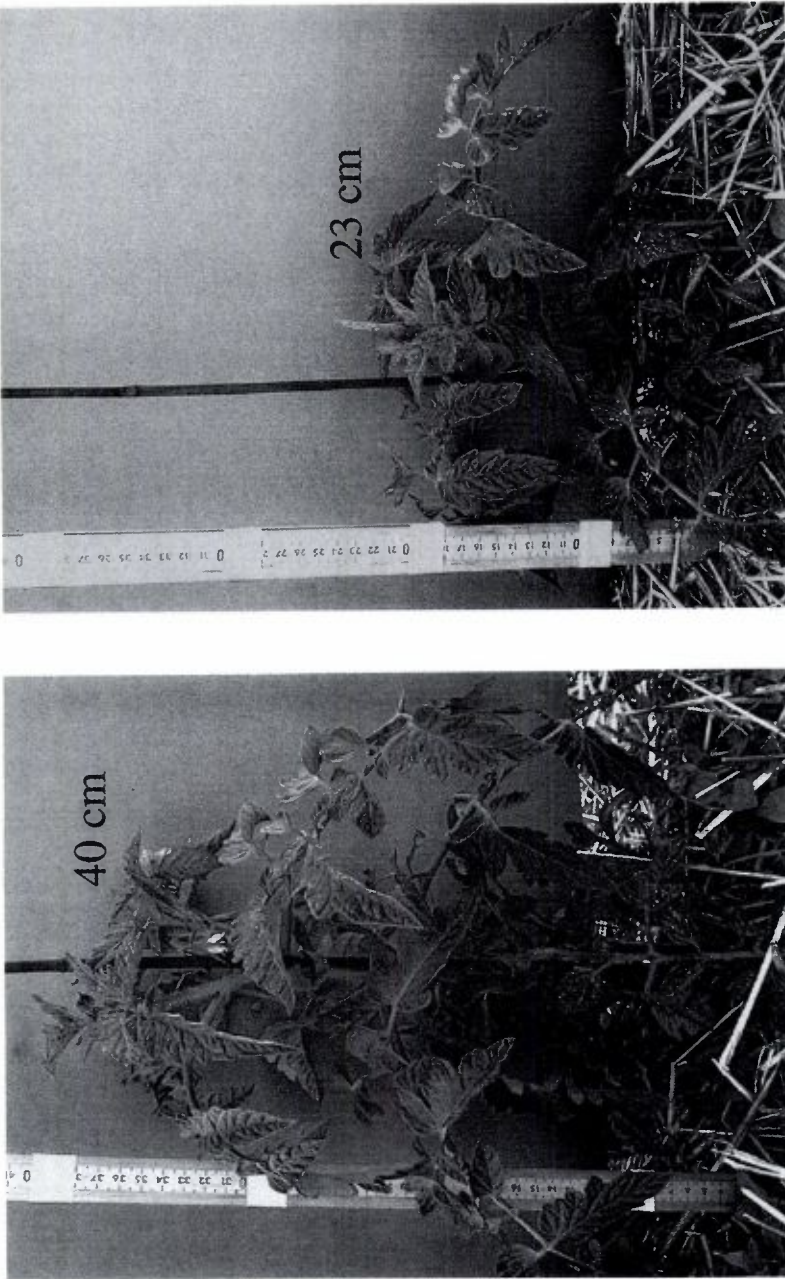
Colonized **Un-colonized**

Figure 1. Path-1 induced growth enhancement of tomato plants (cv Big Beef). Both colonized and uncolonized plants were maintained in a greenhouse for two weeks prior to planting. The plants shown represent the average difference between 20 colonized and 20 uncolonized plants after one month of growth in field plots. Heights of the plants are indicated above the top leaves. Statistical analysis (ANOVA) revealed significant differences in treatments (P<0.05).
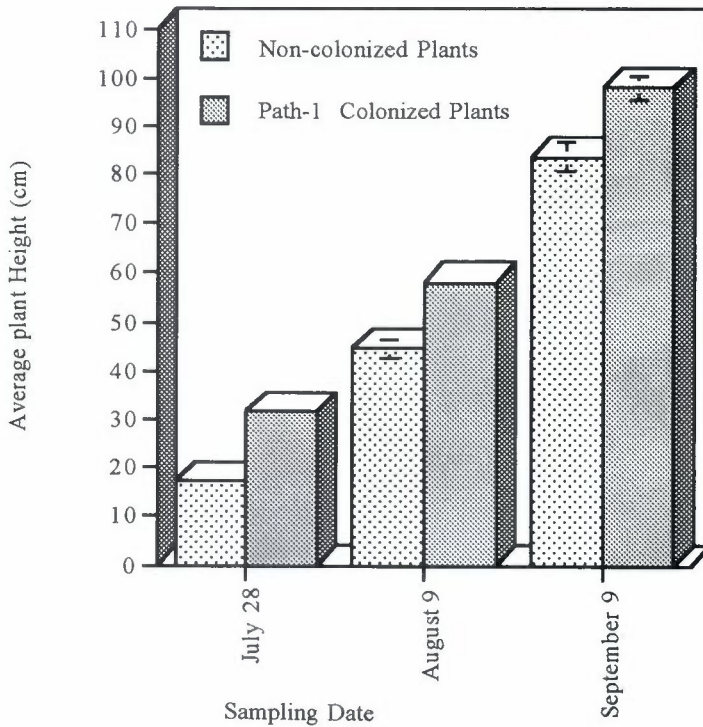
Figure 2. Growth differences between path-1 colonized and non-colonized tomato plants
(cv Big Beef) throughout a growing season. Twenty colonized and 20 non-
colonized plants were maintained in a greenhouse for two weeks prior to
planting on June 20. Columns represent average heights of colonized and non-
colonized plants with standard errors denoted by bars on each column.
Statistical analysis (ANOVA) revealed significant differences in size between
treatments that were maintained throughout the growing season ($P<0.01$).

Path-1 colonized tomato plants were significantly larger than non-colonized
plants under both greenhouse and field conditions. After one month of growth in
field plots, path-1 colonized plants tomato plants (cv Big Beef) were
approximately twice the size of non-colonized controls (Fig. 1). Although this
size difference diminished during the growing season, a statistically
significant (ANOVA) difference between path-1 colonized plants and non-
colonized controls was maintained throughout the growing season (Fig. 2).

Colonization and persistence

Microbiological and molecular methods were used to determine if path-1

Figure 3. Nested primer PCR analysis of DNA from path-1 colonized and non-colonized plant leaf tissues. The *C. magna*-specific npPCR product is approximately 450 bp. Lanes 1 and 2 represent reactions containing pure *C. magna* DNA and devoid of DNA, respectively. Lanes 3–18 represent reactions containing equivalent amounts of DNA from either path-1 colonized or non-colonized control plants as indicated above the lane numbers. The size of amplified products was determined with DNA size markers and one size is denoted in bp on the left.

Table 4. Detection of path-1 in plants at the end of growing seasons

Year	Plant/cultivar	Treatment	Plant no. sampled	Tissue samples	Assay method	Colonization %
1997	Watermelon	Control	100	Stem	Microbiol.	0
	Sugar Baby	Path-1	100			70
1998	Watermelon	Control	40	Terminal	PCR	0
	Jubilee	Path-1	40	leaf		68
2000	Tomato	Control	18	Terminal	Microbiol.	0
	Seattle's Best	Path-1	18	leaf	and PCR	98

Plant tissues were collected for analysis immediately after fruit harvest with adjacent colonized and non-colonized plants sampled. Plants sampled from the watermelon trials were collected randomly throughout the fields and all tomato plants were analyzed. Microbiological and PCR analysis of tomato leaf tissues yielded identical results.

persisted in colonized plants and if the symbiont spread to control plants during the growing season. At the end of the 1997 cucurbit growing seasons, 10.0 cm stem sections were excised 5.0 cm above the crown from 100 colonized and 100 non-colonized Sugar Baby plants, surface sterilized, sectioned and plated on fungal growth medium (MS). Path-1 was recovered from 70% of all colonized

plants and 0% of non-colonized controls (Table 4). In 1998, terminal watermelon vine leaves were collected from 40 colonized and 40 non-colonized Jubilee plants, and DNA extracted for PCR analysis using *C. magna*-specific primers (Fig. 3). Path-1 was detected in 68% of the leaves from colonized plants and 0% of the non-colonized plants (Table 4).

Following the harvest of fruit in 2000, tomato plants were assessed for colonization by microbiological and PCR analyses. Path-1 was isolated from 98% of surface sterilized stem sections from colonized plants and 0% of the non-colonized controls (Table 4). The same results were obtained by PCR analysis.

Phenotypic stability

Path-1 isolates re-isolated from field grown tomato and watermelon plants were screened for the ability to colonize plant hosts, and the symbiotic lifestyle expressed in planta (pathogenic or mutualistic). All of the isolates asymptomatically colonized watermelon plants and expressed a mutualistic lifestyle (protected plants 100% against the *C. magna* wildtype isolate L2.5; Redman et al., 2001).

4. Discussion

The path-1 mutant of *C. magna* is a mutualist of cucurbits and tomato that asymptomatically colonizes plants and confers disease protection under laboratory conditions (Freeman and Rodriguez, 1993; Redman et al., 1999; Redman et al., 2001). In addition, path-1 also confers drought tolerance and growth enhancement to some host species and cultivars (Redman et al., 2001). However, the efficacy of using path-1 under field conditions, where plants grow throughout a season of 90 days or more, and where environmental conditions vary from cool and moist to hot and dry, was unknown. Results from three years of field trials in two locations with cucurbit species indicated that the fruit yields from path-1 colonized plants was equivalent to or better than non-colonized control plants (Tables 1 and 2). A similar scenario was observed in tomato plants with path-1 colonized plants producing equivalent or greater fruit yields than non-colonized controls (Table 3). These results indicate that colonization by path-1 imposed no metabolic cost to the host plants.

Emergence of path-1 from surface sterilized stem sections indicated that once established, the symbiosis was stable and maintained throughout the growing season. More importantly, path-1 was detected by PCR amplification of DNA from terminal cucurbit and tomato leaves at the end of the growing season indicating that path-1 extensively colonized the plants (Fig. 3, Table 4). The growth pattern of path-1 in planta indicated that host defenses were not

activated against the mutant as was previously observed in laboratory studies (Redman et al., 1999). This implies that a mutualistic symbiosis was maintained throughout the growing season. In addition, these studies indicate that although plants were originally colonized in the lower sections of the plant, over time path-1 was able to grow throughout the plant.

Path-1 significantly enhanced the growth of tomato plants (Fig. 1) and the growth difference between path-1 colonized and non-colonized plants diminished throughout the growing season (Fig. 2). There are several reports of endophyte-induced plant growth responses from bacterial and fungal endophytes which may occur due to either protection of plants against pathogens (Nandakumar et al., 2001; Sturz and Nowak, 2000; Redman et al., 2001) and/or symbiosis-induced altered plant biochemistry (see Clay, 1990 and references therein). Several potential biochemical mechanisms may be responsible for symbiont-induced growth enhancement such as increased rates of photosynthesis, altered hormone balance, altered source-sink relationships, and altered nutrient acquisition (Clay, 1990). However, there seems to be little consensus between different studies with regard to the basis of symbiont induced growth enhancement (Clay, 1990).

It is clear that the symbiotic lifestyle expressed by fungal symbionts can change in response to plant genotypes and/or environmental factors (Francis and Read, 1995; Graham and Eissenstat, 1998; Johnson et al., 1997; Redman et al., 2001). Several *Colletotrichum* species are able to express pathogenic, mutualistic, or commensal lifestyles depending on the host genotype (Redman et al., 2001). The field data presented here confirm laboratory observations (Freeman and Rodriguez, 1993; Redman et al., 1999; Redman et al., 2001) that unlike wildtype *C. magna* isolates, path-1 is restricted to expressing non-pathogenic symbiotic lifestyles.

An alternative to standard biological control strategies is to establish mutualistic symbioses prior to planting that will protect plants against diseases. However, fungal mutualists must maintain active symbioses throughout a growing season, and effectively confer disease resistance without imposing significant metabolic costs to host plants. Here, we demonstrate that a mutualistic symbiosis between path-1 and different host species achieved two of these requirements under field conditions in the absence of disease pressure. Future field studies will assess the efficacy of this strategy under fungal disease pressure.

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