Biological and Application-Oriented Factors Influencing Plant Disease Suppression by Biological Control: A Meta-Analytical Review

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ABSTRACT

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Studies to evaluate the effectiveness of biological control in suppressing plant disease often report inconsistent results, highlighting the need to identify general factors that influence the success or failure of biological control in plant pathology. We conducted a quantitative synthesis of previously published research by applying meta-analysis to determine the overall effectiveness of biocontrol in relation to biological and application-oriented factors. For each of 149 entries (antagonist-disease combinations) from 53 reports published in *Biological & Cultural Tests* between 2000 and 2005, an effect size was calculated as the difference in disease intensity expressed in standard deviation units between the biocontrol treatment and its corresponding untreated control. Effect sizes ranged from -1.15 (i.e., disease strongly enhanced by application of the biocontrol agent) to 4.83 (strong disease suppression by the antagonist) with an overall weighted mean of 0.62, indicating moderate effectiveness on average. There were no significant ($P > 0.05$) differences in effect sizes between entries from studies carried out in the greenhouse versus the

field, between those involving soilborne versus aerial diseases, or among those carried out in conditions of low, medium, or high disease pressure (expressed relative to the disease intensity in the untreated control). However, effect sizes were greater on annual than on perennial crops, regardless of whether the analysis was carried out for all entries ($P = 0.0268$) or for those involving only soilborne diseases ($P = 0.0343$). Effect sizes were not significantly different for entries utilizing fungal versus bacterial biocontrol agents or for those targeting fungal versus bacterial pathogens. However, entries that used *r*-selected biological control agents (i.e., those having short generation times and producing large numbers of short-lived offspring) were more effective than those that applied antagonists that were not *r*-selected ($P = 0.0312$). Interestingly, effect sizes for entries that used *Bacillus* spp. as biological control agents were lower than for those that applied other antagonists ($P = 0.0046$ for all entries and $P = 0.0114$ for soilborne diseases). When only aerial diseases were considered, mean effect size was greater for entries that received one or two sprays than for those that received more than eight sprays of the biocontrol agent ($P = 0.0002$). This counterintuitive result may indicate that investigators often attempt unsuccessfully to compensate for anticipated poor performance in antagonist-disease combinations by making more applications.

Several factors have led to an increased interest in the application of biological control for plant disease management (10,30,37,42), including the desire for a more sustainable approach to agriculture in general, concerns about the impact of synthetic agrichemicals on human health and the environment, the high frequency of pathogen populations resistant to commonly used fungicides, the increasing cost of soil fumigation, and the continued search for methyl bromide alternatives. As a result of the increased investment in biological control research and development, numerous biocontrol products have been labeled for commercial use. For example, at least 37 biocontrol agents were available commercially in the United States in 2000 (29). Nonetheless, biological products represent only a minute fraction of the global pesticide market; in fact, the global value of biofungicide sales in 1998 was valued at <0.2% of the global market for synthetic fungicides in the same year (33).

The relatively low adoption of biological control in agricultural practice emphasizes the need to critically and quantitatively examine the factors that determine success or failure of biological control for plant disease management. Although biocontrol has been effective in controlling a range of plant diseases, there are various reports in the literature that question its general effective-

ness (9,18,30,35,42). Vasudevan et al. (44) observed that this inconsistency in performance has plagued researchers and their efforts to exploit biocontrol agents for commercial use. It generally is acknowledged that disease suppression by biological control is the result of complex interactions among the antagonist, the pathogen, the host plant and its associated microbial community, and the physical environment (2,17,26,46). However, we are unaware of quantitative studies to synthesize data on the relative efficacy of a range of biocontrol agents in suppressing plant disease in relation to biological and application-oriented factors. As such, several fundamental questions regarding the level of disease suppression by biocontrol agents still remain unanswered. For example, is biological control generally more successful for soilborne or for aerial diseases? To what extent is its effectiveness determined by overall disease pressure? Is biocontrol really more effective in the greenhouse than in the field, as is assumed generally (35)? Are there certain factors related to the biology of the pathogen or the biocontrol agent (e.g., fungus versus bacterium, *r*- versus *K*-selected life history) that help predict success or failure of biological control? And how significant and consistent are these effects across a wide range of antagonist-disease combinations?

Answering the types of questions posed above requires the quantitative synthesis of research results across a large number of studies. This can be accomplished with meta-analysis, a set of statistical techniques developed to integrate and analyze results obtained from independent studies. Meta-analysis utilizes infor-

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mation on "effect size" (i.e., the relative magnitude of the observed effect—disease suppression in this case—weighted by intrinsic error rate) (15,21,34). Here, we present results of a meta-analysis to synthesize previously published research on the use of biological control for plant disease suppression with the objective of understanding the relative efficacy of biocontrol in relation to selected biological and application-oriented factors. Results of a similar analysis that focused on biological control of arthropod pests and weeds have been reported recently (43).

EFFICACY DATABASE

Data were extracted from reports published in *Biological & Cultural Tests* (B&C Tests) between 2000 (vol. 15) and 2005 (vol. 20). This source was chosen because it publishes a diverse range of disease biocontrol studies irrespective of whether their results are positive (i.e., statistically significant) or negative. In contrast, a tendency toward publishing mostly positive results in more traditional research journals could lead to biased or erroneous conclusions when applying meta-analysis (38). In addition, reports published in B&C Tests were considered most appropriate for our analysis in that they tend to be application-oriented rather than focusing on laboratory or model systems. As such, the results are likely to be more indicative of biocontrol performance in field conditions given the current state of technology.

The following criteria were applied to determine whether or not a particular report could be included in the analysis. First, the report had to provide at least one measure of disease intensity such as disease incidence, disease severity, area under the disease progress curve, or disease intensity expressed as an index or on an arbitrary scale. In cases where both incidence and severity were available, the latter was used in the analysis. Second, an estimate of the pooled sample standard deviation s_{pooled} from the analysis of variance had to be provided for the report to be useful. In a number of cases, s_{pooled} was calculated from Fisher's least significant difference (LSD) values presented in the reports (8). Third, reports that evaluated combinations of multiple biological control agents or of biological agents applied together with cultural practices or chemical products were not included because it would have been impossible to calculate the contribution of each individual antagonist in such cases. Of the 92 disease biocontrol reports published in B&C Tests between 2000 and 2005, 53 reports ("studies") with 149 "entries" (antagonist–disease combinations) met the criteria outlined above; most of the studies that could not be included lacked statistical information sufficient to calculate s_{pooled} , a common problem in scientific data reporting (15). The 53 studies were conducted in either the greenhouse or the field to evaluate the effectiveness of registered or experimental biocontrol agents in suppressing disease for a range of annual and perennial pathosystems (Table 1).

TABLE 1. Antagonist-pathogen combinations included in a meta-analysis to determine the effect size of disease suppression by biological control based on reports published in *Biological & Cultural Tests* between 2000 and 2005

Biocontrol agent species	Number of entries	Strain or product name	Target pathogens	Hosts
<i>Arthrobacter ilicis</i>	1	3026C	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Wheat
<i>Azospirillum brasilense</i>	1	Recharge	<i>Fusarium solani</i>	Cotton
<i>Azotobacter chroococcum</i>	1	Agropro ZX	<i>Erwinia tracheiphila</i>	Cucumber
<i>Bacillus amyloliquefaciens</i>	2	BioYield, IN937a	<i>Alternaria solani</i> , <i>E. tracheiphila</i>	Cucumber, tomato
<i>Bacillus licheniformis</i>	2	EcoGuard	<i>Colletotrichum graminicola</i> , <i>Rhizoctonia solani</i>	Bermudagrass
<i>Bacillus pumilus</i>	7	SE34, Sonata, Yield Shield	<i>A. solani</i> , <i>F. solani</i> , <i>Gibberella zeae</i> , <i>Podosphaera leucotricha</i> , <i>R. solani</i> , <i>Sphaerotheca humuli</i>	Apple, hop, soybean, snap bean, tomato, wheat
<i>Bacillus subtilis</i>	44	Companion, GB03, HiStick, Kodiak, MBI600, OSU140/1, QRD713AS, QRD713/4/5, QST, Serenade, Subtilex, TrigoCor	<i>A. solani</i> , <i>Botryosphaeria dothidea</i> , <i>Fusicladosporium carpophilum</i> , <i>Colletotrichum gloeosporioides</i> , <i>Erwinia amylovora</i> , <i>Fusarium oxysporum</i> , <i>F. solani</i> , <i>Fusicoccum amygdali</i> , <i>Monilinia fructicola</i> , <i>Pythium aphanidermatum</i> , <i>Pythium</i> sp., <i>R. solani</i> , <i>Sclerotinia homoeocarpa</i> , <i>S. minor</i> , <i>Sphaerotheca fusca</i> , <i>Thielaviopsis basicola</i> , <i>Tilletia caries</i> , <i>Uncinula necator</i> , <i>Xanthomonas axonopodis</i> pv. <i>vesicatoria</i>	Apple, cotton, cyclamen, dry bean, bermudagrass, geranium, gladiolus, grape, pansy, peach, peanut, pepper, potato, snap bean, soybean, squash, tomato, wheat
<i>Beauveria bassiana</i>	3	...	<i>R. solani</i>	Cotton, dry bean, soybean
<i>Burkholderia cepacia</i>	13	Deny	<i>F. oxysporum</i> , <i>F. solani</i> , <i>R. solani</i>	Cyclamen, gladiolus, dry bean, potato
<i>Clonostachys rosea</i>	1	63-28	<i>Pythium</i> sp.	Cucumber
<i>Comamonas acidovorans</i>	4	C4712	<i>F. solani</i> , <i>R. solani</i>	Cotton
<i>Coniothyrium minitans</i>	5	Contans WG	<i>Cylindrocladium parasiticum</i> , <i>S. minor</i> , <i>S. sclerotiorum</i> , <i>Sclerotium rolfsii</i>	Peanut, soybean
<i>Fusarium oxysporum</i>	5	CS-20NPF, CWB318, Fo47NPF	<i>F. oxysporum</i> , <i>R. solani</i>	Asparagus, cyclamen, gladiolus
<i>Gliocladium catenulatum</i>	3	Primastop	<i>F. solani</i> , <i>T. basicola</i>	Dry bean, pansy
<i>Gliocladium virens</i>	10	SoilGard	<i>F. oxysporum</i> , <i>R. solani</i>	Gladiolus, potato
<i>Paenibacillus macerans</i>	3	GB49, OSU142/3	<i>R. solani</i> , <i>X. axonopodis</i> pv. <i>vesicatoria</i>	Dry bean, pepper
<i>Pantoea agglomerans</i>	2	C91, E325	<i>E. amylovora</i>	Apple, pear
<i>Pseudomonas flourescens</i>	8	BL915, BlightBan A506, C7, Dagger, 89B61	<i>A. solani</i> , <i>E. amylovora</i> , <i>F. oxysporum</i> , <i>Oidium</i> sp., <i>Pythium</i> sp., <i>R. solani</i> , <i>X. axonopodis</i> pv. <i>vesicatoria</i>	Apple, cucumber, cyclamen, daisy, dry bean, pear, tomato
<i>Pseudomonas mendocina</i>	1	3027C	<i>G. graminis</i> var. <i>tritici</i>	Wheat
<i>Pseudomonas putida</i>	1	SB40	<i>G. graminis</i> var. <i>tritici</i>	Wheat
<i>Streptomyces griseoviridis</i>	5	Mycostop G	<i>F. oxysporum</i> , <i>F. solani</i> , <i>T. basicola</i>	Dry bean, gladiolus, pansy
<i>Streptomyces lydicus</i>	4	Actinovate	<i>F. oxysporum</i> , <i>Pythium</i> sp.	Cyclamen, geranium, gladiolus
<i>Stenotrophomonas maltophilia</i>	4	C3	<i>R. solani</i> , <i>Uromyces appendiculatus</i>	Dry bean, ryegrass, tall fescue
<i>Trichoderma harzianum</i>	19	PlantShield, RootShield, T-22, T-22 Planter Box	<i>Botrytis cinerea</i> , <i>Colletotrichum acutatum</i> , <i>F. oxysporum</i> , <i>F. solani</i> , <i>P. aphanidermatum</i> , <i>Pythium</i> sp., <i>R. solani</i> , <i>T. caries</i>	Cotton, cyclamen, dry bean, geranium, gladiolus, potato, raspberry, soybean, strawberry, wheat

META-ANALYSIS

Effect size estimates. The first step in the analysis was the calculation of a common metric ("effect size") for the effectiveness of disease suppression that could be applied to all 149 entries. We used the standardized group mean difference, commonly referred to as Cohen's d , which is among the most commonly used and intuitively appealing in meta-analysis (25). For each entry, d was calculated as $(\bar{X}_C - \bar{X}_T) / \sigma_{\text{pooled}}$, where $\bar{X}_T$ and $\bar{X}_C$ are the disease intensity means of the treated and control groups, respectively, and σ_{pooled} is the pooled population standard deviation, calculated from s_{pooled} (39). Thus, d is the difference in standard deviation units between the biocontrol treatment and its corresponding untreated control. Values of $d > 0.2, 0.5, 0.8$, and 1.0 are considered to represent small, medium, large, and very large effects, respectively (8,15). The variance of d is given by

$$\sigma^2(d) = \frac{n_T + n_C}{n_T n_C} + \frac{d^2}{2(n_T + n_C)}$$

where n_T and n_C are the numbers of replicates for the treated and control groups, respectively.

In meta-analysis, each effect size d_i (where $i = 1$ to k denotes the number of a given entry) is weighted by its corresponding variance, $\sigma^2(d_i)$, whereby the weight, w_i , assigned to each effect size estimate is inversely proportional to its variance,

$$w_i = \frac{1/\sigma^2(d_i)}{\sum_{i=1}^k [1/\sigma^2(d_i)]}$$

The combined estimate of the effect size across all studies, d_+ , was calculated as

$$d_+ = \sum_{i=1}^k w_i d_i$$

and its variance,

$$\sigma^2(d_+) = \frac{1}{\sum_{i=1}^k [1/\sigma^2(d_i)]}$$

was used to compute a 95% confidence interval around d_+ . The total heterogeneity Q_T across all k entries is given by

$$Q_T = \sum_{i=1}^k w_i (d_i - d_+)^2$$

(21,34); a value of Q_T greater than the theoretical χ^2 value (with $k - 1$ degrees of freedom) indicates that the variance is greater than expected due to sampling error, warranting a test for moderator variables.

Moderator variables. Several dichotomous or ordinal moderator variables describing biological and application-oriented factors were defined and used to group entries into specific classes (Table 2). Entries were grouped broadly based on whether the corresponding study was carried out in the greenhouse or in the field and whether soilborne or aerial diseases were targeted. Depending on the type of production system, crops were classified as either annual or perennial. A disease pressure variable was defined based on the disease intensity of the untreated control; the corresponding classes were low (<35% incidence or severity in the untreated control), moderate (35 to 55%), and high (>55%). In cases where disease intensity was reported as an index or on an arbitrary scale, disease pressure was computed by dividing values for the untreated control by the maximum value of the scale to obtain a percentage.

Entries were further classified based on whether they used fungal or bacterial biocontrol agents and were targeted against fungal or bacterial pathogens. In addition, groups were established based on life history traits of pathogen and biological control agent (i.e., whether these organisms were r -selected, K -selected, or intermediate). In general, r -selected species have shorter generation times and produce larger numbers of shorter-lived offspring than K -selected species (1). For example, in our data set, *Pseudomonas fluorescens* and *Ucinula necator* were considered r -selected, *Fusarium oxysporum* and *Rhizoctonia solani* K -selected, and *Bacillus subtilis* and *Gibberella zeae* were intermediate biocontrol agents and pathogens, respectively. Inasmuch as possible, we further attempted to distinguish classes based on antagonist-pathogen interactions (e.g., whether a patho-

TABLE 2. Statistics for the effects of moderator variables on the effect size of disease suppression by biological control based on a meta-analysis of reports published in *Biological & Cultural Tests* between 2000 and 2005

Variable	Classes	Entire data set		Soilborne diseases ^a	
		<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>
Disease pressure ^b	Low	39	0.3911	26	0.4142
	Moderate	45		35	
	High	48		36	
Crop type	Annual	95	0.0268	73	0.0343
	Perennial	54		34	
Study environment	Field	128	0.1790	89	0.0592
	Greenhouse	21		18	
Disease type	Soilborne	107	0.9671	—	—
	Aerial	42		—	
Pathogen type	Fungus	133	0.5031	—	—
	Bacterium	16		—	
Biocontrol agent type	Fungus	44	0.1604	40	0.2080
	Bacterium	105		67	
Pathogen type × biocontrol agent type	Fungus × fungus	44	0.3472	—	—
	Fungus × bacterium	89		—	
	Bacterium × bacterium	16		—	
Pathogen life history	K -selected	97	0.5138	97	0.2077
	Intermediate or r -selected ^c	52		10	
Biocontrol agent life history	r -selected	89	0.0312	73	0.1002
	Intermediate or K -selected ^c	60		34	
Taxon of biocontrol agent	<i>Bacillus</i> spp.	55	0.0046	29	0.0114
	Other	94		78	

^a The lower number of entries for soilborne diseases resulted in classes in which the numbers of data points were too low or unbalanced for some moderator variables; no analyses were carried out in these cases.

^b For each entry, disease pressure classes were defined as low, medium, or high based on disease incidence or severity values in the untreated control of <35%, 35 to 55%, and >55%, respectively.

^c Classes were grouped together in the analysis to obtain larger and more balanced numbers of entries.

genic fungus was targeted by an antagonist fungus or bacterium or vice versa); however, not all of these groups could be included based on small or unbalanced numbers of entries per group (Table 2).

A total of 55 of the 149 entries in the data set used *Bacillus* spp. as biocontrol agents (Table 1). This allowed us to define another moderator variable based on whether *Bacillus* spp. or other bacterial or fungal species were used as antagonists.

Where possible, the above groupings were defined for the entire data set (i.e., all 149 entries) and separately for entries involving soilborne diseases (107 entries); separate groups were not established when the numbers of entries per group were too low or unbalanced. Because of the relatively low number of entries for aerial diseases ($n = 42$), only one analysis was carried out using this subset of data to determine the effect of number of spray applications of the biocontrol agent (1 or 2, 3 to 8, or >8 sprays) on d .

Analysis of moderator variables. Data were analyzed using meta-analytic methods analogous to one-way analysis of variance to determine whether effect sizes were affected by the moderator variables defined above. For each moderator variable, the SAS macros *within*, *wavgeff*, and *fixconst* (45) were applied to obtain a weighted mean d value for each category (with associated 95% confidence intervals). Two categories of a moderator variable were considered significantly different from one another if the 95% confidence interval around the difference of their d values did not include zero (16).

HOW EFFECTIVE IS BIOLOGICAL DISEASE SUPPRESSION?

The extent of disease suppression by biological control varied widely among entries, with d ranging from -1.15 (i.e., disease strongly enhanced by application of the biocontrol agent) to 4.83 (strong disease suppression by the antagonist) (Fig. 1). Of the 149 entries in the data set, 42% had a large magnitude of disease suppression ($d > 0.8$) while 26% of all entries had d values ≤ 0 . This wide range reflects the contrasting opinions in the literature regarding the usefulness of biological control in controlling plant disease (9,18,27,30,35,37). The combined mean effect size across studies (d_+) was 0.62 , indicating that biocontrol was moderately effective on average. The 95% confidence interval of d_+ ranged

Fig. 1. Frequency distribution of the effect size, d , of disease suppression by biological control based on a meta-analysis of reports published in *Biological & Cultural Tests* between 2000 and 2005 ($n = 149$); d is the difference in standard deviation units between the biocontrol treatment and its corresponding untreated control, with values of >0.2 , 0.5 , 0.8 , and 1.0 representing small, medium, large, and very large effects, respectively.

from 0.51 to 0.73 , showing that the overall effect size was significantly >0 .

The null hypothesis of homogeneity across studies was rejected ($Q_T = 181.7$, $df = 148$, $P < 0.05$), indicating that variability in d was greater than would be expected due to random sampling error. Thus, a fixed-effect meta-analysis was performed to determine the effect of moderator variables on disease suppression.

WHAT ARE THE FACTORS AFFECTING BIOLOGICAL DISEASE SUPPRESSION?

Host and environmental factors. There were no significant ($P > 0.05$) differences in effect sizes among entries from studies carried out in conditions of low, moderate, or high disease pressure (Table 2). However, biocontrol was significantly more effective on annual than on perennial crops, regardless of whether the analysis was carried out for all entries or for those involving only soilborne diseases (Fig. 2). The significantly lower magnitude of disease suppression in perennial crops may be due to the larger number of potential pathogen inoculum sources (such as cankers, leaf scars, or buds harboring pathogen populations) in perennial pathosystems, which give rise to polyetic epidemics that generally are more difficult to control. Furthermore, in the absence of crop rotation in perennial crops, pathogen populations may build up and the resident microbial community may be more resilient, hindering colonization by externally applied biocontrol agents. The fact that d was, on average, $\approx 40\%$ lower on perennial crops than on annual crops highlights the need for further research on the dynamics of pathogen populations, the resident microflora, and the introduced biocontrol agent on such crops and how their interactions affect the success or failure of biological control.

In a review of biological control of plant diseases in greenhouse systems, Paulitz and Bélanger (35) pointed out that the greenhouse environment presents a unique situation that enables disease managers to optimize the effectiveness of biological control agents through manipulation of environmental conditions. For example, biocontrol agents against powdery mildews are more efficient when relative humidity can be maintained $>80\%$, a condition that is easily monitored and adjusted in the greenhouse (4).

Fig. 2. Effect of crop type (annual versus perennial) on the effect size, d , of disease suppression by biological control based on a meta-analysis of reports published in *Biological & Cultural Tests* between 2000 and 2005 ($n = 149$); d is the difference in standard deviation units between the biocontrol treatment and its corresponding untreated control, with values of >0.2 , 0.5 , 0.8 , and 1.0 representing small, medium, large, and very large effects, respectively. Error bars indicate the 95% confidence interval, while the dotted vertical line shows the overall weighted mean across all entries. $CI(\Delta d)$ is the 95% confidence interval around the difference in effect sizes between the two crop types.

Thus, it is reasonable to expect greater disease suppression in greenhouse than in the field applications of biocontrol agents. However, our analysis showed no significant difference in mean effect sizes between entries in which biological agents were applied in these two environments (Table 2). We hypothesize that this may have been due to the fact that the studies carried out in the greenhouse generally included multiple treatments, most of which involved different biocontrol agents (as opposed to different application schedules or rates, for example). Given the different environmental preferences of the different antagonists, it would have been impossible to adjust the greenhouse environment so as to optimize performance of all of the included agents simultaneously. The dilemma faced by greenhouse managers when attempting to adjust environmental conditions to enhance biocontrol without at the same time encouraging disease development has been discussed previously by Jewett and Jarvis (24).

Pathogen and antagonist-related factors. Effect sizes were not significantly different between entries involving fungal versus bacterial biocontrol agents or between those targeting fungal versus bacterial pathogens or soilborne versus aerial diseases (Table 2). Similarly, there were no significant differences when fungal pathogens were targeted with bacterial biocontrol agents, fungal pathogens were targeted with fungal biocontrol agents, or bacterial pathogens were targeted with bacterial biocontrol agents. (There were no entries in the data set for bacterial pathogens targeted with fungal biocontrol agents.) Mean effect sizes also did not differ between entries in which pathogens were *K*-selected versus *r*-selected or intermediate along the *r*-to-*K* continuum. However, entries in which *r*-selected microorganisms were used as biocontrol agents had a greater mean effect size than those in which biological agents were not *r*-selected (Fig. 3), although this effect was not statistically significant when the analysis was restricted to entries involving only soilborne diseases (Table 2).

For effective disease suppression, most biological control agents need to build up large population sizes within their respective niches (30,31,46). This may be the reason why treatments with *r*-selected biological control agents had significantly greater effect sizes than those in which biological control agents that are not *r*-selected were applied. Although *r*-selected microorganisms ex-

hibit larger fluctuations in population size (1), in favorable environmental conditions they reproduce prolifically, reaching larger numbers within a relatively short time. As such, *r*-selected biological agents have been described as the equivalents to protectant fungicides, being in place before infection starts (7). This characteristic is highly compatible with the general strategy of inundative biocontrol used for plant disease control, where the antagonist is applied at an instantly high population size with the intent to preempt the pathogen.

A total of 55 of the 149 entries in the data set used *Bacillus* spp. as biocontrol agents (Table 1). Interestingly, the mean effect size for these entries was significantly lower compared with entries that applied other biocontrol organisms, regardless of whether the analysis was carried out for all studies or separately for those involving soilborne diseases. In both cases, the mean effect size for the entries utilizing *Bacillus* spp. was >40% lower (Fig. 4). This finding is at odds with the fact that the most thoroughly studied biological control bacteria have been species in the genus *Bacillus* (32). Furthermore, considerable efforts have been devoted to integrating *Bacillus* spp.-based biological control agents into practical disease management systems (6,23). *Bacillus* spp. have many advantages as biocontrol agents, including their ability to produce broad-spectrum antibiotics (28) as well as endospores, which facilitate commercial formulation and enhance shelf life (41). However, given the wide availability of commercial *Bacillus*-based biocontrol products (13), it is tempting to include these products in indiscriminate efficacy screens without due consideration of whether the biocontrol agent is a good match for the pathogen or host niche in question. For example, the epiphytic fitness of *Bacillus* spp. such as *B. subtilis* on aboveground plant surfaces generally is low (40), yet close to 20% of the entries in the data set that applied *Bacillus* spp. did so against aerial diseases. Cook (9) suggested that inconsistencies in the efficacy of biological control may be due to the use of a single strain of the agent everywhere, and a similar indiscriminate application of a few widely used *Bacillus* strains may be reflected in our data set. Indeed, 21 of the 55 *Bacillus* entries in the data set utilized the QRD and QST strains of *B. subtilis* commercially

Fig. 3. Effect of biocontrol agent life history (*r*-selected versus *K*-selected or intermediate) on the effect size, *d*, of disease suppression by biological control based on a meta-analysis of reports published in *Biological & Cultural Tests* between 2000 and 2005 ($n = 149$); *d* is the difference in standard deviation units between the biocontrol treatment and its corresponding untreated control, with values of >0.2, 0.5, 0.8, and 1.0 representing small, medium, large, and very large effects, respectively. Error bars indicate the 95% confidence interval, while the dotted vertical line shows the overall weighted mean across all entries. $CI(\Delta d)$ is the 95% confidence interval around the difference in effect sizes between the two life history categories.

Fig. 4. Effect of biocontrol taxon (*Bacillus* spp. versus all other bacterial and fungal species) on the effect size, *d*, of disease suppression by biological control based on a meta-analysis of reports published in *Biological & Cultural Tests* between 2000 and 2005 ($n = 149$); *d* is the difference in standard deviation units between the biocontrol treatment and its corresponding untreated control, with values of >0.2, 0.5, 0.8, and 1.0 representing small, medium, large, and very large effects, respectively. Error bars indicate the 95% confidence interval, while the dotted vertical line shows the overall weighted mean across all entries. $CI(\Delta d)$ is the 95% confidence interval around the difference in effect sizes between the two taxonomic groups.

formulated as Serenade biofungicide. When these 21 entries were omitted from the analysis, the difference in efficacy between *Bacillus*-based biocontrol agents and other antagonist species was no longer statistically significant for the full data set ($P = 0.0724$, $n = 128$); however, when the analysis was restricted to soilborne diseases, the *Bacillus*-based entries still were significantly less effective ($P = 0.0372$, $n = 103$).

Number of applications against aerial diseases. Because of the relatively low number of entries involving aerial diseases ($n = 42$), only one analysis was carried out using this subset of data to determine the effect of number of spray applications of the biocontrol agent. In this analysis, entries with few applications (one or two sprays) of the biological control agents had a significantly ($P = 0.0002$) greater mean effect size than those in which a large number of applications (more than eight sprays) was made (Fig. 5). Theoretically, if a biological agent is inherently effective in suppressing a pathogen on a given host, a limited number of applications should be sufficient to achieve the intended results. Therefore, it is reasonable to conclude that, in instances where the biocontrol agent is deemed less effective (e.g., in cases where season-long control in a niche unfavorable for the antagonist is needed), more applications will be made in an effort to compensate for the anticipated low efficacy of biocontrol agents. As such, our result may indicate that investigators often attempt unsuccessfully to compensate for anticipated poor performance in antagonist–disease combinations by making more applications.

Factors not included in the analysis. We acknowledge that there are additional factors potentially affecting the effectiveness of biological control that were not considered in our analyses. Foremost among these is the mechanism of action of the biocontrol agent (i.e., whether the antagonist operates via competition, antibiosis, hyperparasitism, or induced host resistance). Assigning a single mechanism of action is highly problematic because most antagonists are believed to suppress disease via multiple mechanisms acting simultaneously (11,19). For example, although mycoparasitism and antibiosis (3,49) traditionally have been viewed as the most important mechanisms involved in biological control by *Trichoderma harzianum*, it is now recognized

that induced host resistance plays a major role in some pathosystems (5,12,20,22,47) and that different mechanisms operating at the same time can act additively or synergistically (3,14,18,48). In the absence of more precise data on the relative importance of different mechanisms of action in different host–pathogen–antagonist systems, we considered it imprudent to include this factor in our analysis.

As stated above, studies that evaluated combinations of multiple biological control agents or of biological agents applied jointly with cultural practices or chemical products were not included in our analyses because it would have been impossible to calculate the contribution of each individual antagonist. Thus, we were not able to test whether and to what extent combined applications of antagonists are more effective than single applications, as has been suggested in numerous individual studies (11,14,36). In a recent meta-analysis of factors affecting the success of biological control of arthropod pests (43), multi-species releases of biocontrol agents consistently were more effective than single-species releases. Future research using meta-analytical techniques should determine whether a similar general conclusion applies to plant pathogens as well.

CONCLUSIONS

This is the first meta-analysis to provide quantitative information on selected factors impacting the effectiveness of biological control in suppressing plant disease for a wide range of antagonist–disease systems. The analysis identified several general factors that consistently affected the performance of biological control of plant disease across a wide range of environments and host–pathogen–antagonist combinations. Some of the findings confirmed theoretical expectations (e.g., the greater effect size associated with *r*-selected antagonists), whereas others appear to be at odds with conventionally held wisdom (e.g., similar effect sizes in studies carried out in the greenhouse versus the field). Future research, perhaps involving a larger data base, is needed to test some aspects that were not included in the present analysis. A larger data base also would allow one to test more specific hypotheses related to specific groups of biocontrol agents or even specific disease–antagonist combinations, based on the increased recognition that antagonist strains, formulations, and application methods may need to be optimized for each disease on a particular crop at a particular site (9,42). Nonetheless, we believe there must be some universal principles that apply to a wide range of antagonist–disease–crop combinations, and we hope that this research has provided a step toward their quantitative analysis.

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Fig. 5. Effect of number of applications on the effect size, d , of disease suppression by biological control based on a meta-analysis of reports published in *Biological & Cultural Tests* between 2000 and 2005. Analysis restricted to entries involving aerial diseases ($n = 42$); d is the difference in standard deviation units between the biocontrol treatment and its corresponding untreated control, with values of >0.2, 0.5, 0.8, and 1.0 representing small, medium, large, and very large effects, respectively. Error bars indicate the 95% confidence interval, while the dotted vertical line shows the overall weighted mean across all entries. $CI(\Delta d)$ is the 95% confidence interval around the pairwise differences in effect sizes between the three application frequency categories.

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