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(54) **FUNGAL ENDOPHYTES FOR IMPROVED CROP YIELDS AND PROTECTION FROM PESTS**

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None
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(57) **ABSTRACT**

The invention provides a synthetic combination of a crop and at least one fungal endophyte, wherein the crop is a host plant of the endophyte. Provided are also methods and compositions for producing such synthetic combinations. The endophyte reproduces and enhances the agronomic characteristics of the crop. Methods for inoculating the host plant with the endophyte, for propagating the host-endophyte combination, and for detecting the presence of the endophyte and of its metabolites within a host plant are also described.

21 Claims, 29 Drawing Sheets

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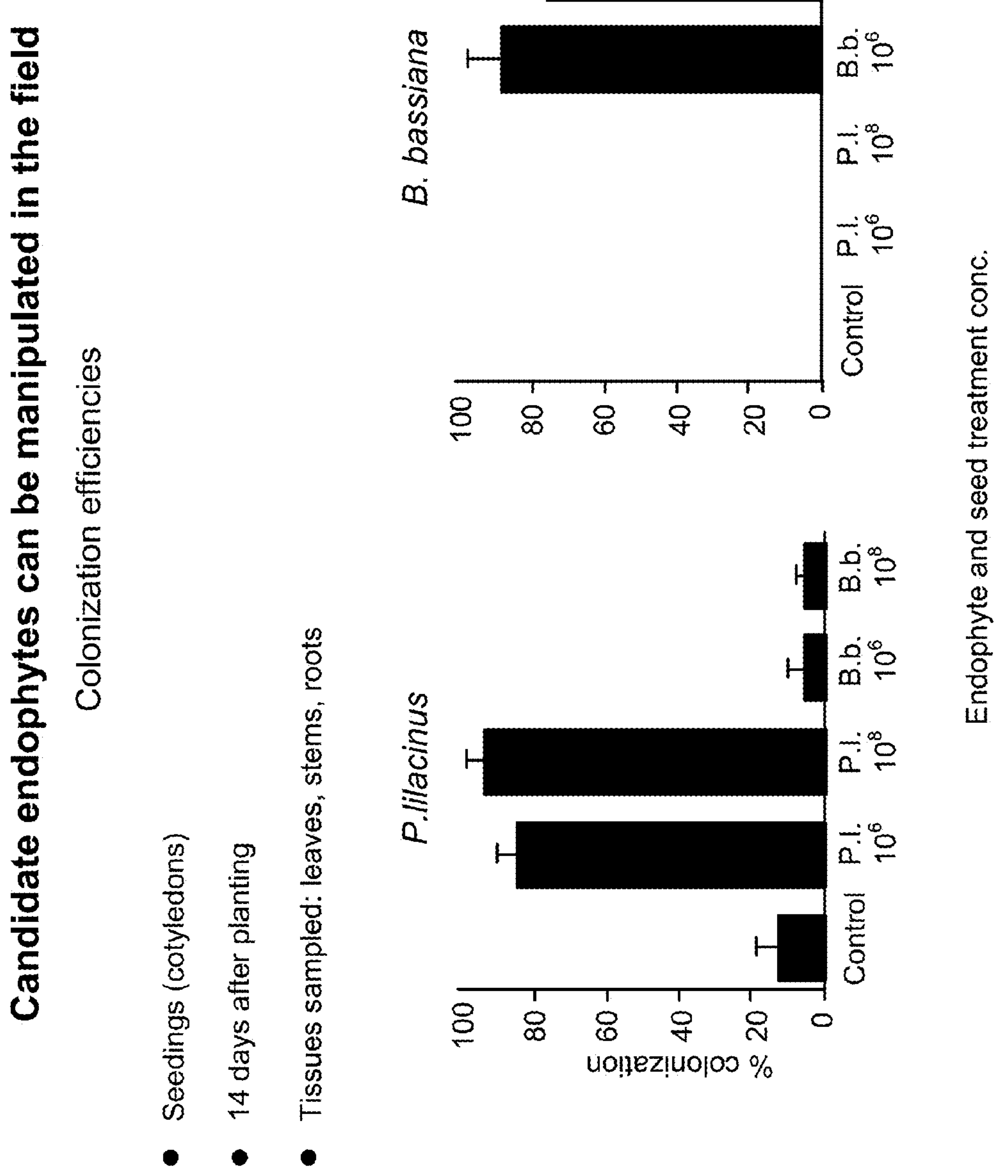
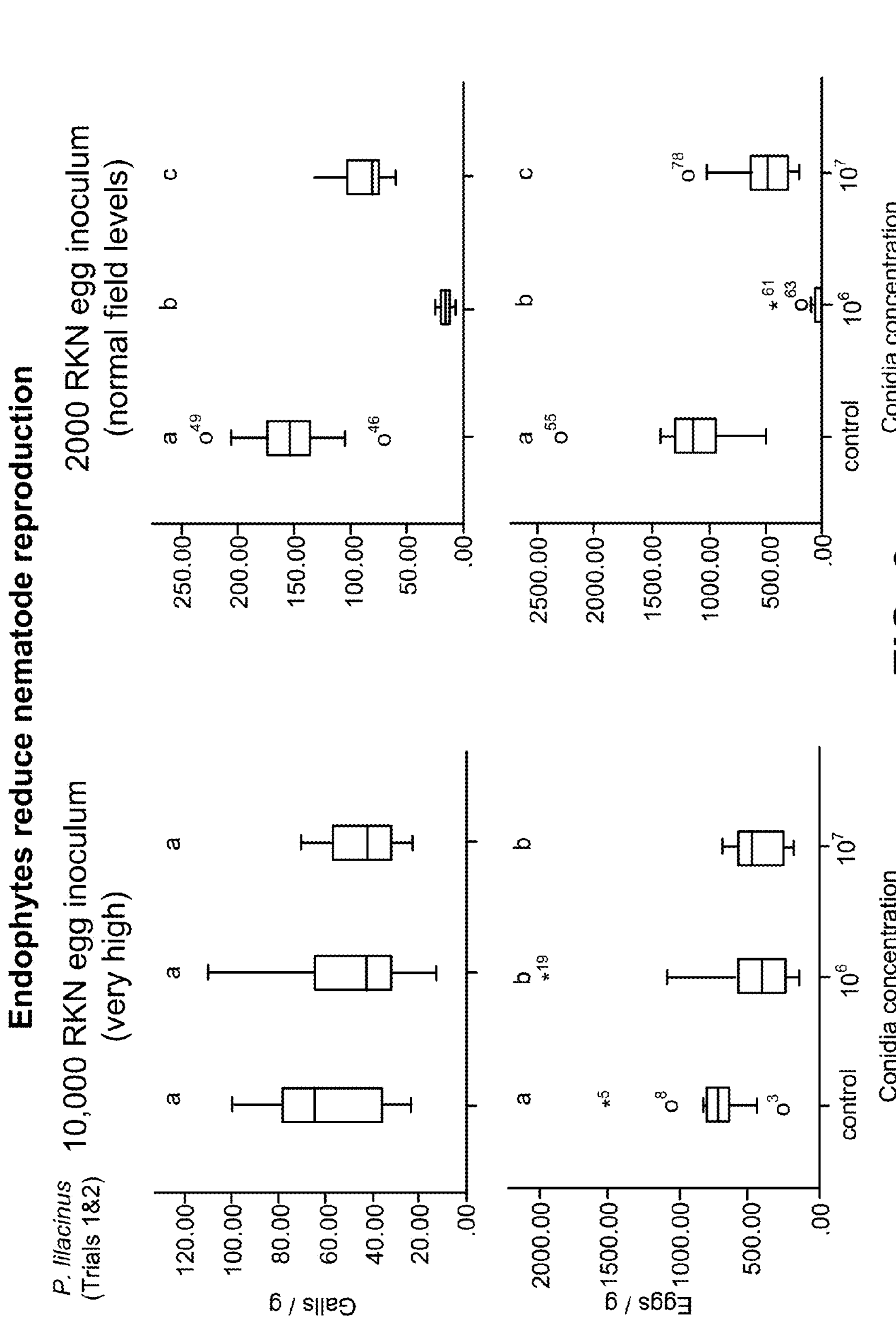

Candidate endophytes can be manipulated in the field
Colonization efficiencies
Seedings (cotyledons)
14 days after planting
Tissues sampled: leaves, stems, roots
P.lilacinus
B. bassiana
% colonization
100
80
60
40
20
0
Control
P.l. 10^6
P.l. 10^8
B.b. 10^6
B.b. 10^8
Endophyte and seed treatment conc.

FIG. 1

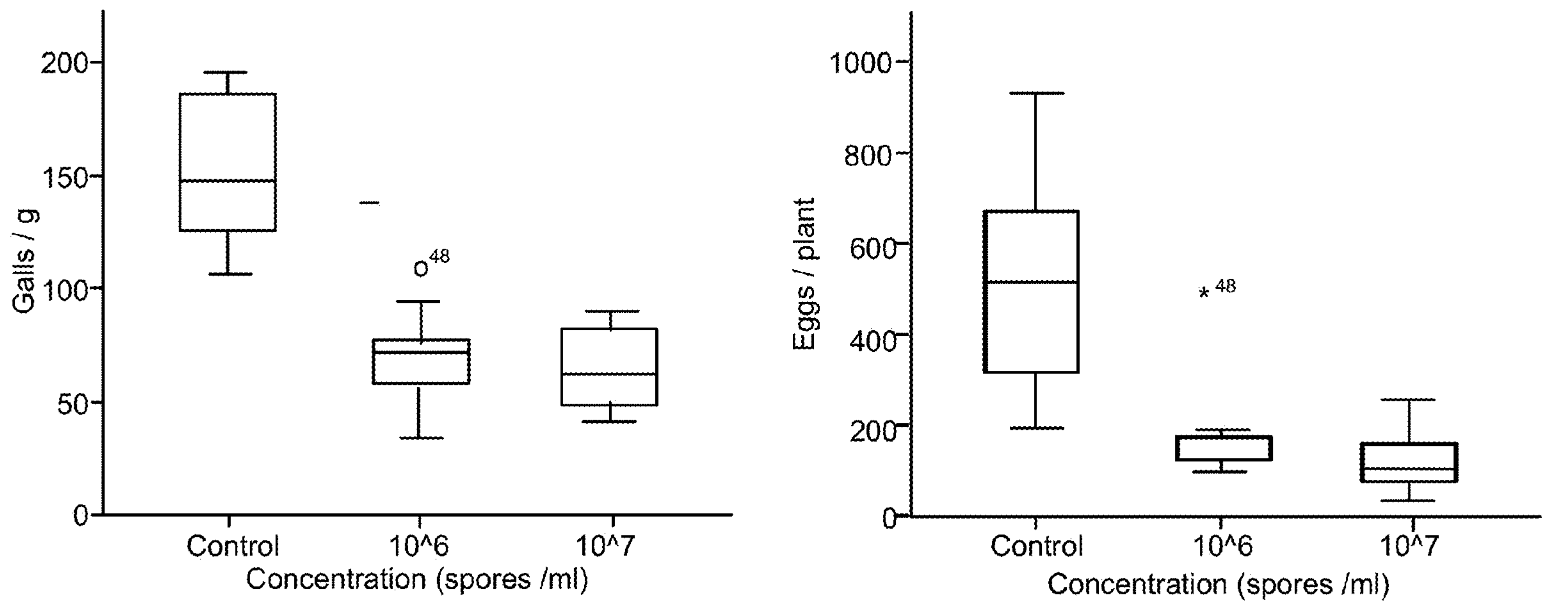


Endophytes reduce nematode reproduction
P. lilacinus (Trials 1&2)
10,000 RKN egg inoculum (very high)
2000 RKN egg inoculum (normal field levels)
Galls / g
Eggs / g
120.00
100.00
80.00
60.00
40.00
20.00
.00
250.00
200.00
150.00
100.00
50.00
2000.00
1500.00
1000.00
500.00
2500.00
a
b
c
control
10^6
10^7
Conidia concentration

FIG. 2

Endophytic *Chaetomium globosum* negatively affects root-knot nematode reproduction

Major reductions in galls and egg production independent of concentration.



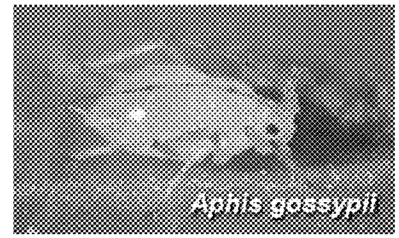
- Two replicate trials with very similar results
- Insect trials ongoing
- Candidate for 2014 field trials
- Six more genetically-distinct *C. globulosum* isolates still to be tested.

FIG. 3

Fungal endophytes negatively affect aphid reproduction

Greenhouse trials – *Beauveria bassiana* (strain GHA)

- Inoculated cotton seeds
- Intact plant assays
- N=10 plants per treatment
- Ten 2nd instar aphids per plant
- Aphids applied at 3rd true leaf stage
- Assayed at 7 & 14 days



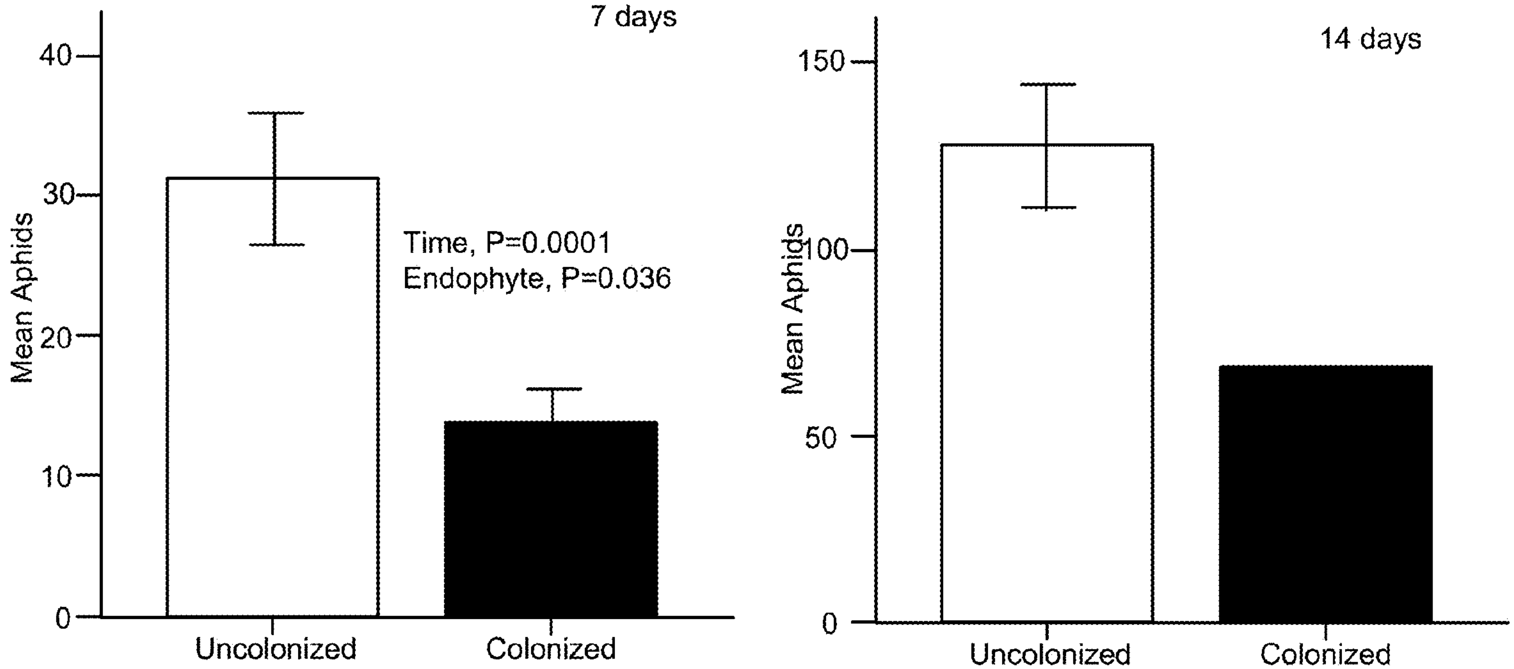


FIG. 4A

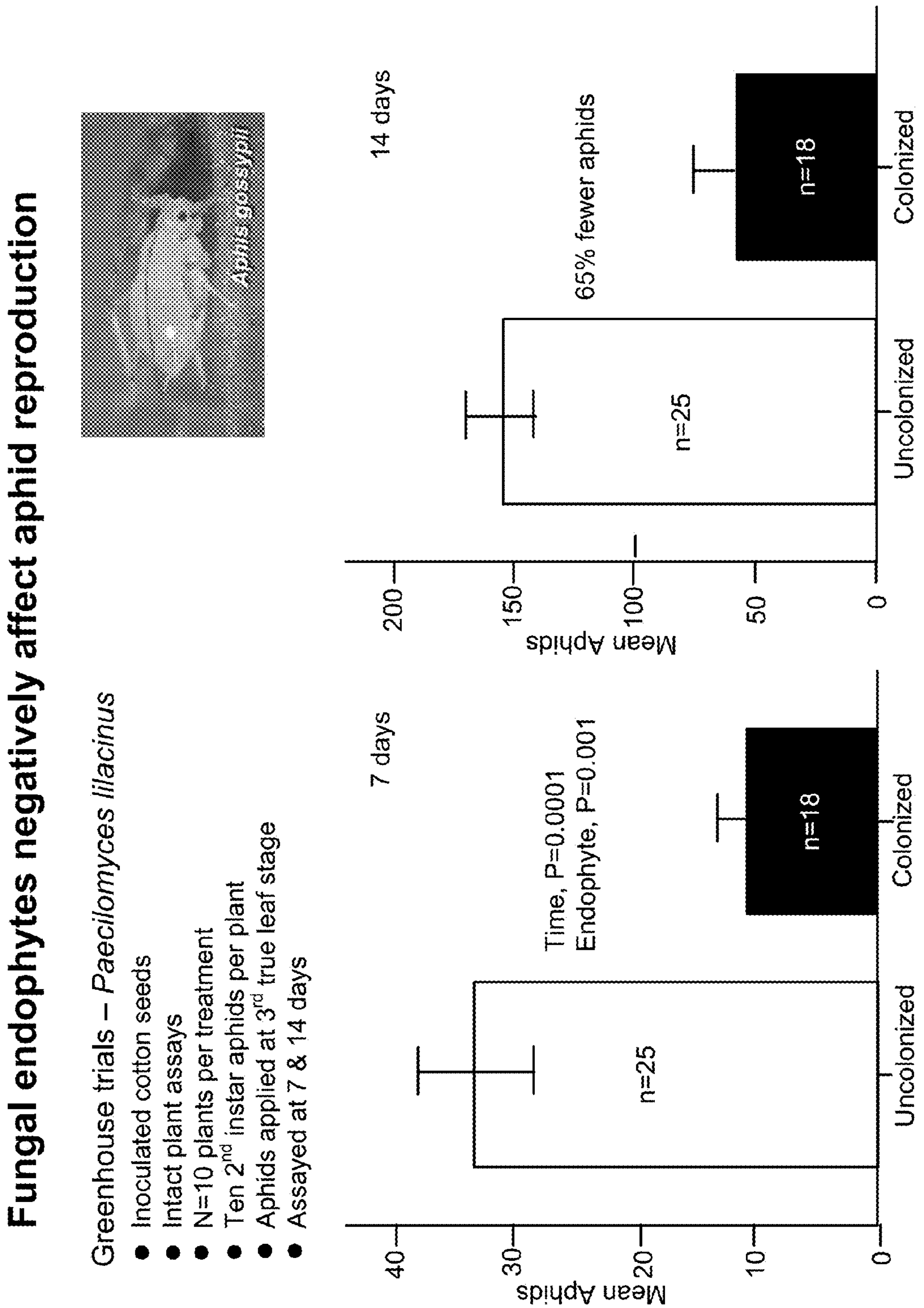

Fungal endophytes negatively affect aphid reproduction
Greenhouse trials – Paecilomyces lilacinus
Inoculated cotton seeds
Intact plant assays
N=10 plants per treatment
Ten 2nd instar aphids per plant
Aphids applied at 3rd true leaf stage
Assayed at 7 & 14 days
Aphis gossypii
7 days
Time, P=0.0001
Endophyte, P=0.001
Mean Aphids
0
10
20
30
40
n=25
n=18
Uncolonized
Colonized
14 days
65% fewer aphids
Mean Aphids
0
50
100
150
200
n=25
n=18
Uncolonized
Colonized

FIG. 4B

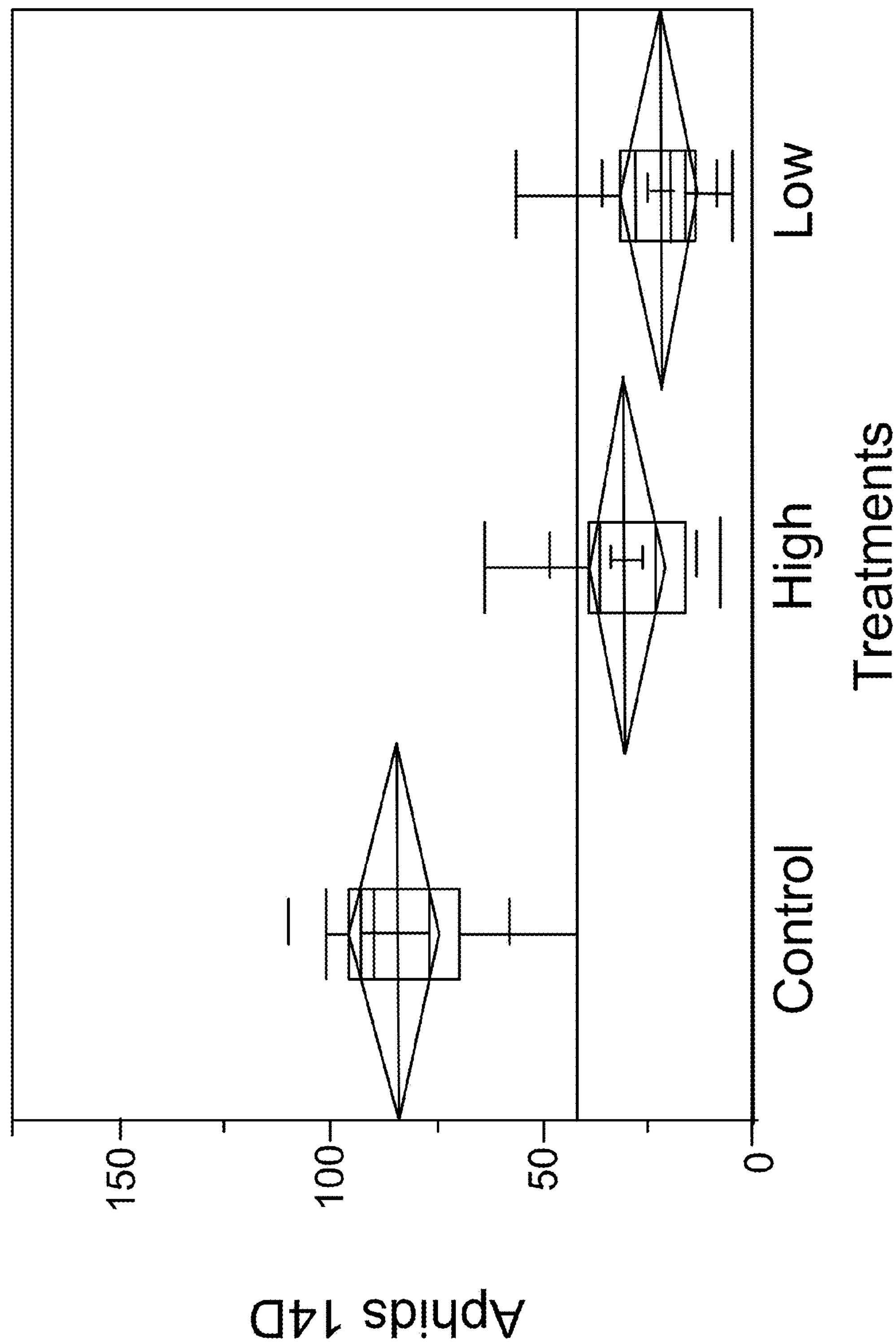


FIG. 5

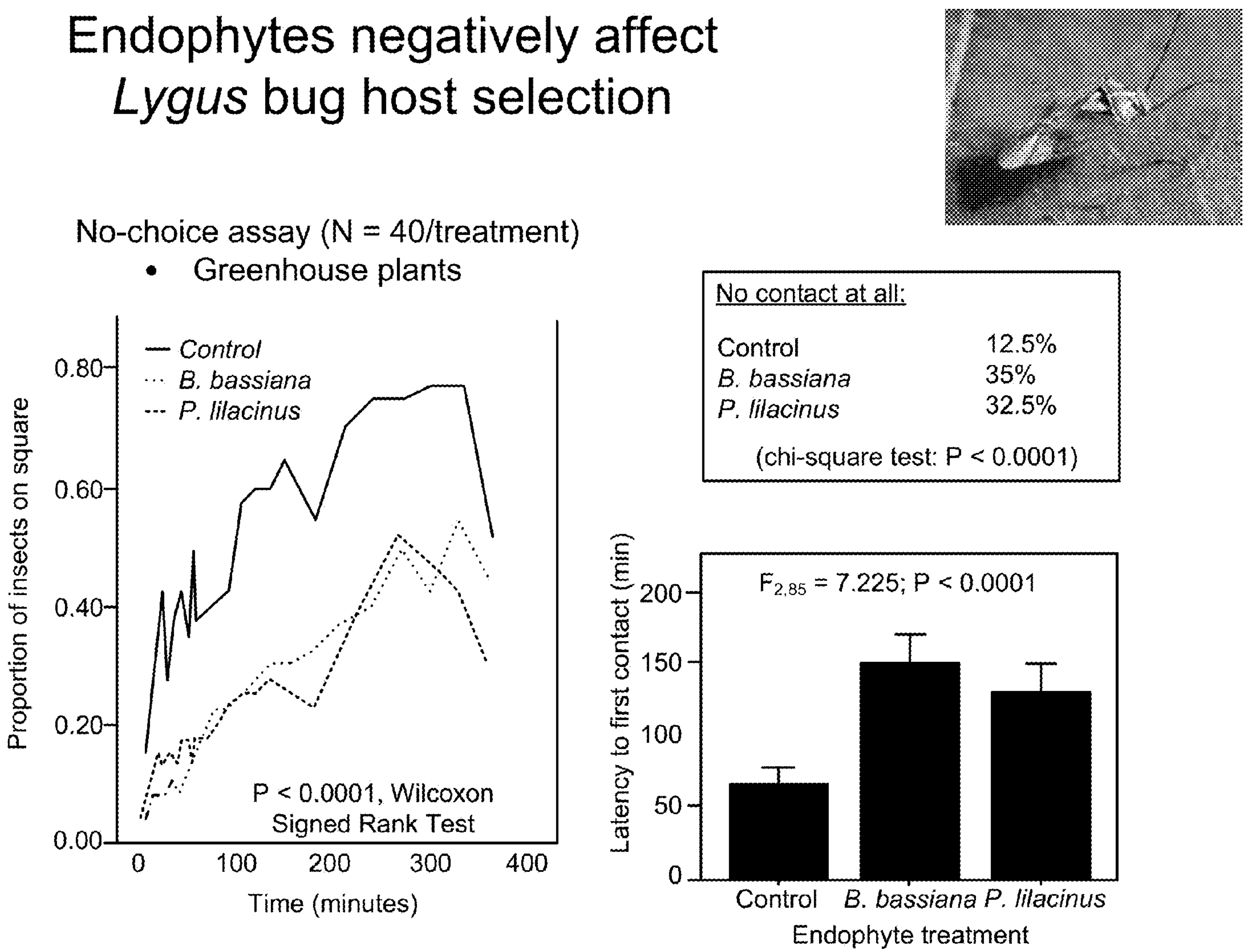

Endophytes negatively affect
Lygus bug host selection
No-choice assay (N = 40/treatment)
• Greenhouse plants
Control
B. bassiana
P. lilacinus
P < 0.0001, Wilcoxon
Signed Rank Test
Proportion of insects on square
0.00
0.20
0.40
0.60
0.80
0
100
200
300
400
Time (minutes)
No contact at all:
Control 12.5%
B. bassiana 35%
P. lilacinus 32.5%
(chi-square test: P < 0.0001)
$F_{2,85}$ = 7.225; P < 0.0001
Latency to first contact (min)
0
50
100
150
200
Control
B. bassiana
P. lilacinus
Endophyte treatment

FIG. 6A

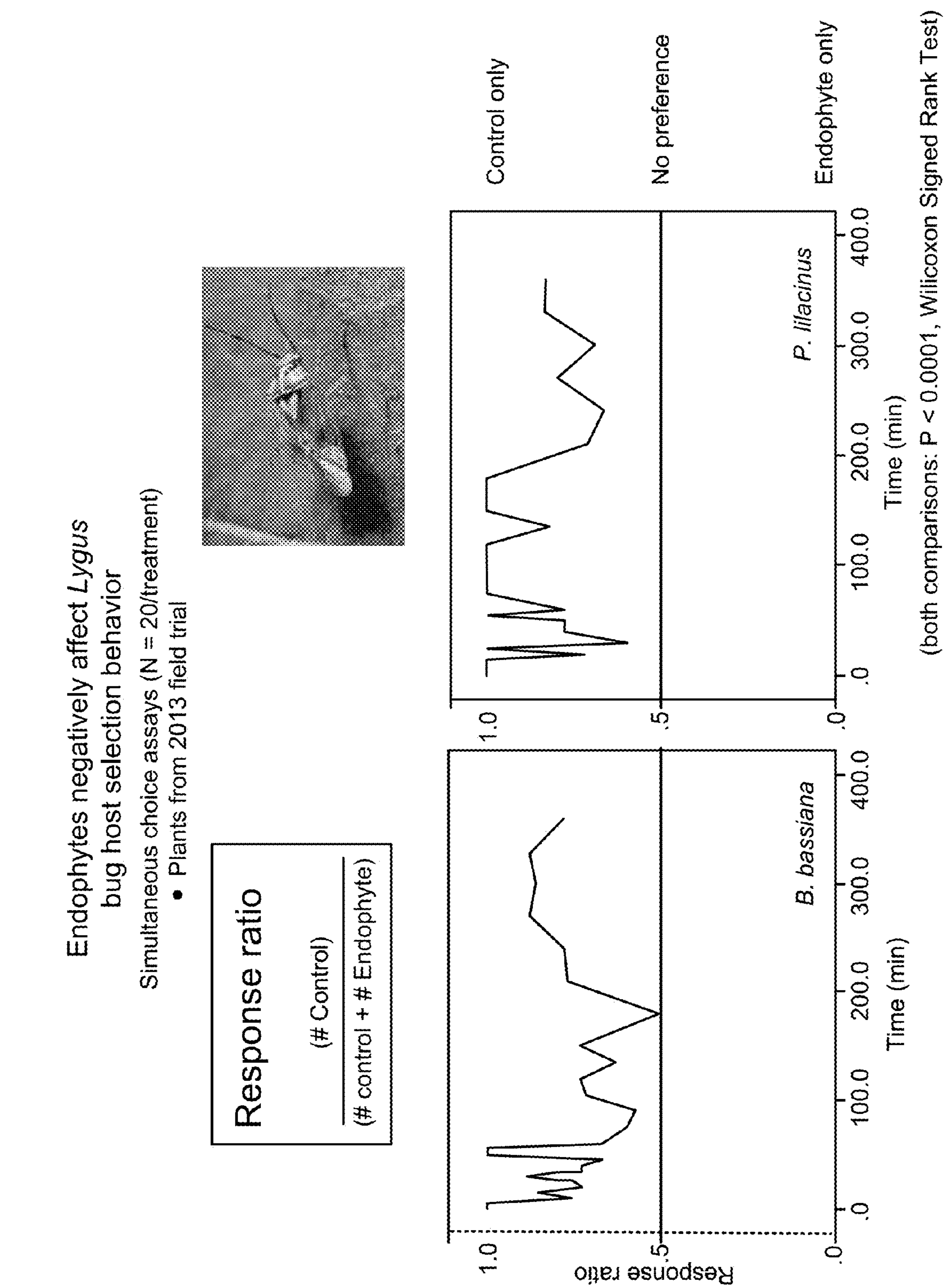

Endophytes negatively affect *Lygus* bug host selection behavior
Simultaneous choice assays (N = 20/treatment)
• Plants from 2013 field trial
Response ratio
(# Control) / (# control + # Endophyte)
Response ratio
1.0
.5
.0
.0
100.0
200.0
300.0
400.0
Time (min)
B. bassiana
P. lilacinus
Control only
No preference
Endophyte only
(both comparisons: P < 0.0001, Wilicoxon Signed Rank Test)

FIG. 6B

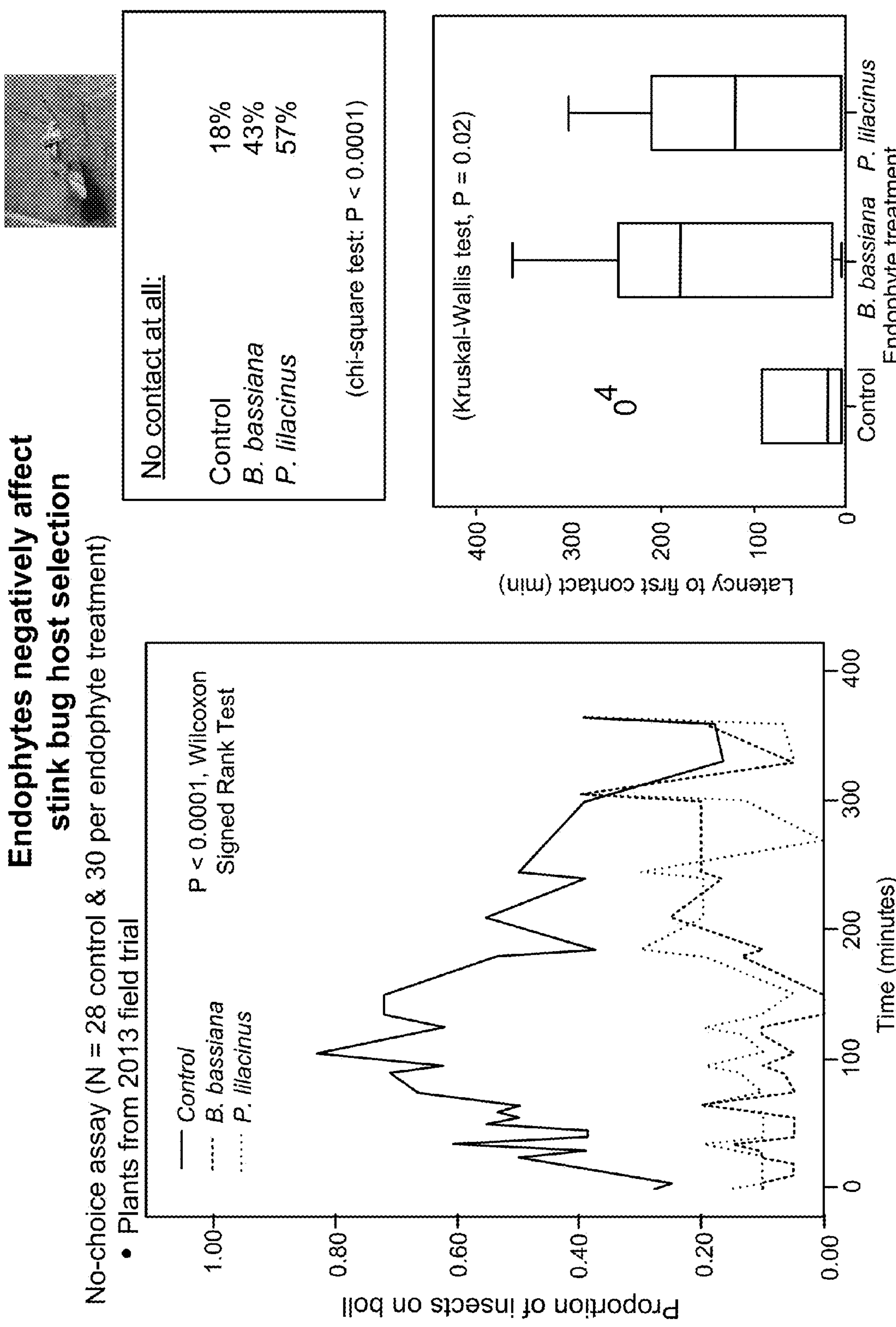

Endophytes negatively affect
stink bug host selection
No-choice assay (N = 28 control & 30 per endophyte treatment)
• Plants from 2013 field trial
Control
B. bassiana
P. lilacinus
P < 0.0001, Wilcoxon
Signed Rank Test
Proportion of insects on boll
Time (minutes)
No contact at all:
Control 18%
B. bassiana 43%
P. lilacinus 57%
(chi-square test: P < 0.0001)
(Kruskal-Wallis test, P = 0.02)
Latency to first contact (min)
Endophyte treatment
Control
B. bassiana
P. lilacinus

FIG. 7A

Endophytes negatively affect stink bug host selection behavior

Simultaneous choice assay (N = 20/treatment)

- Plants from 2013 field trial

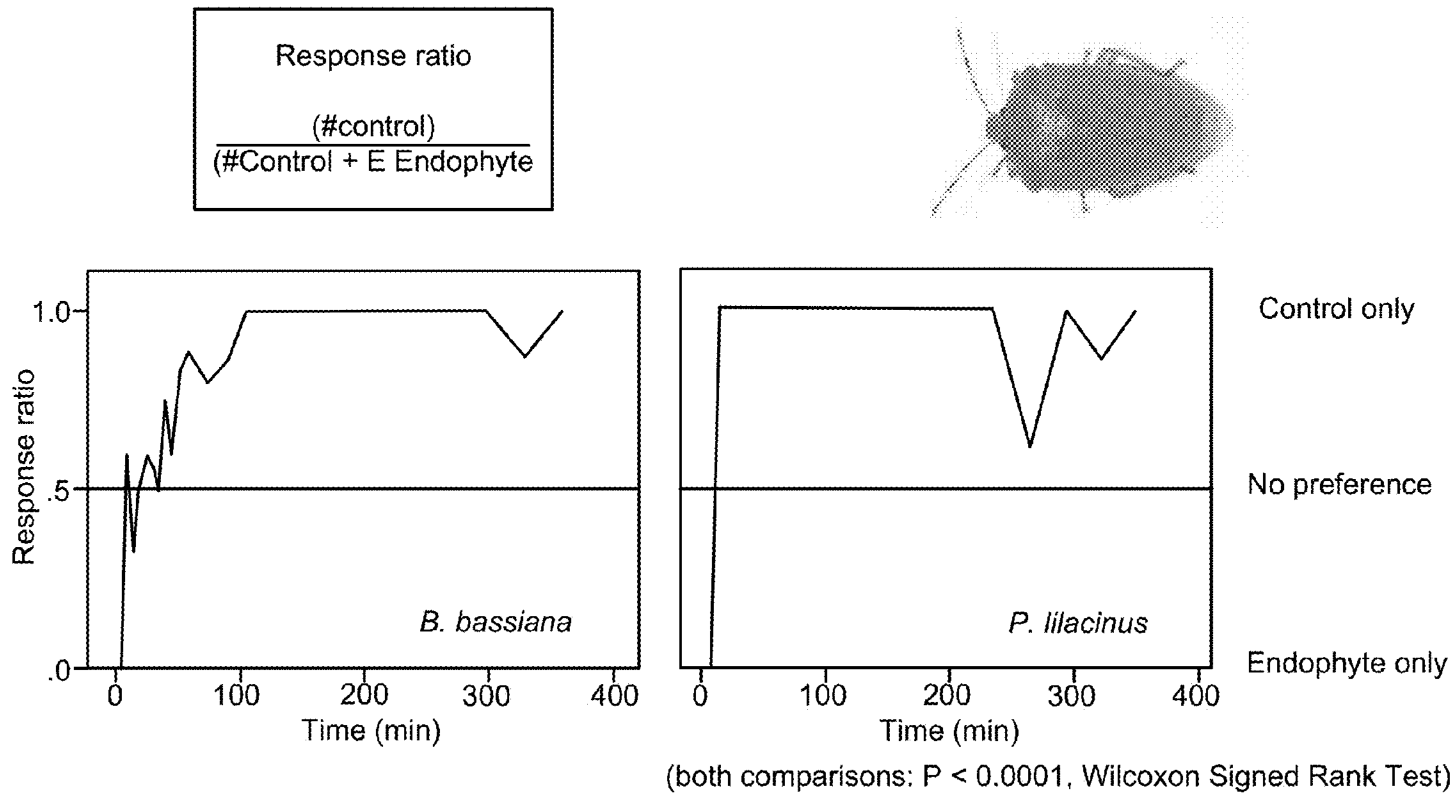
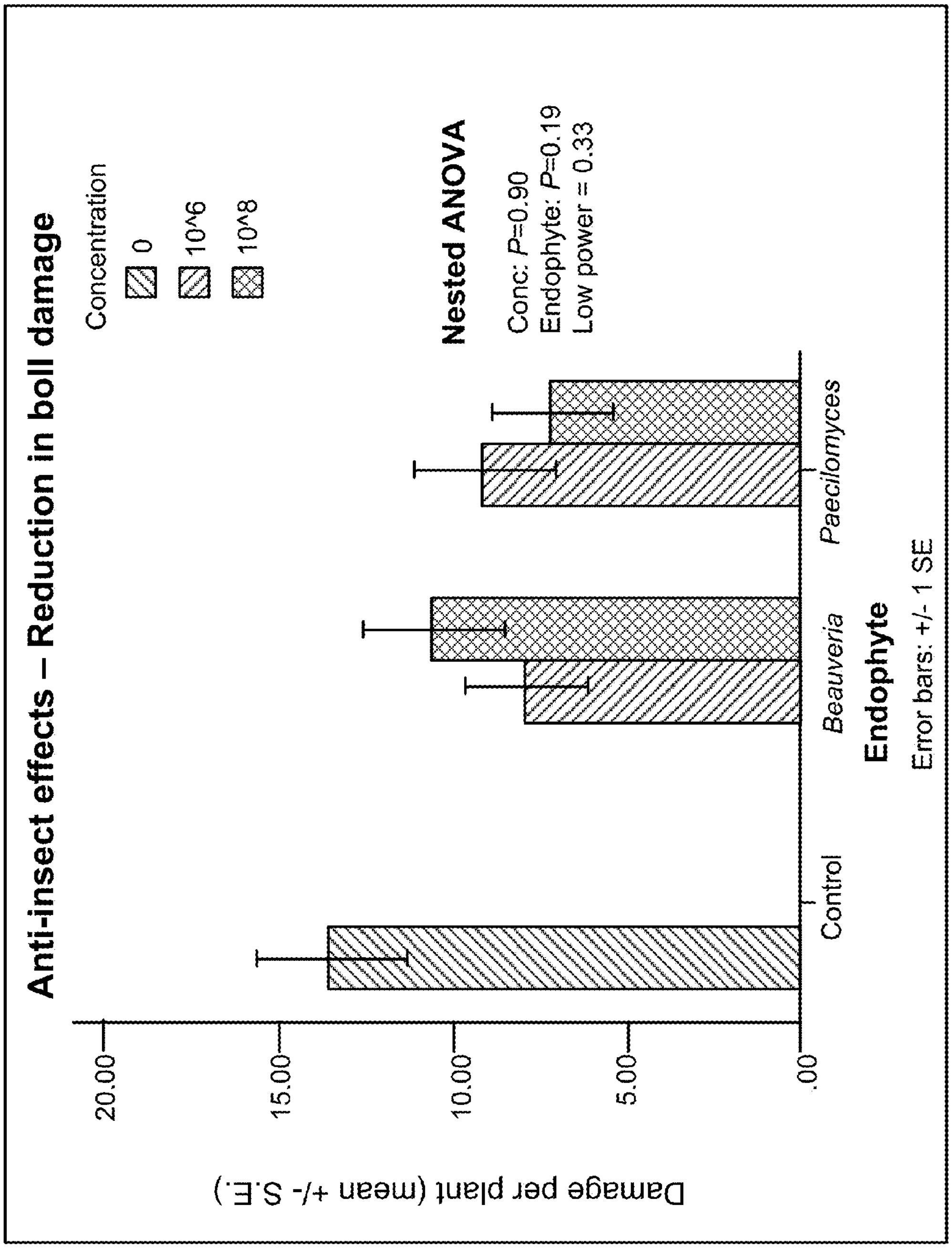


FIG. 7B



Damage = # of open bolls punctured or with locule damage

FIG. 8

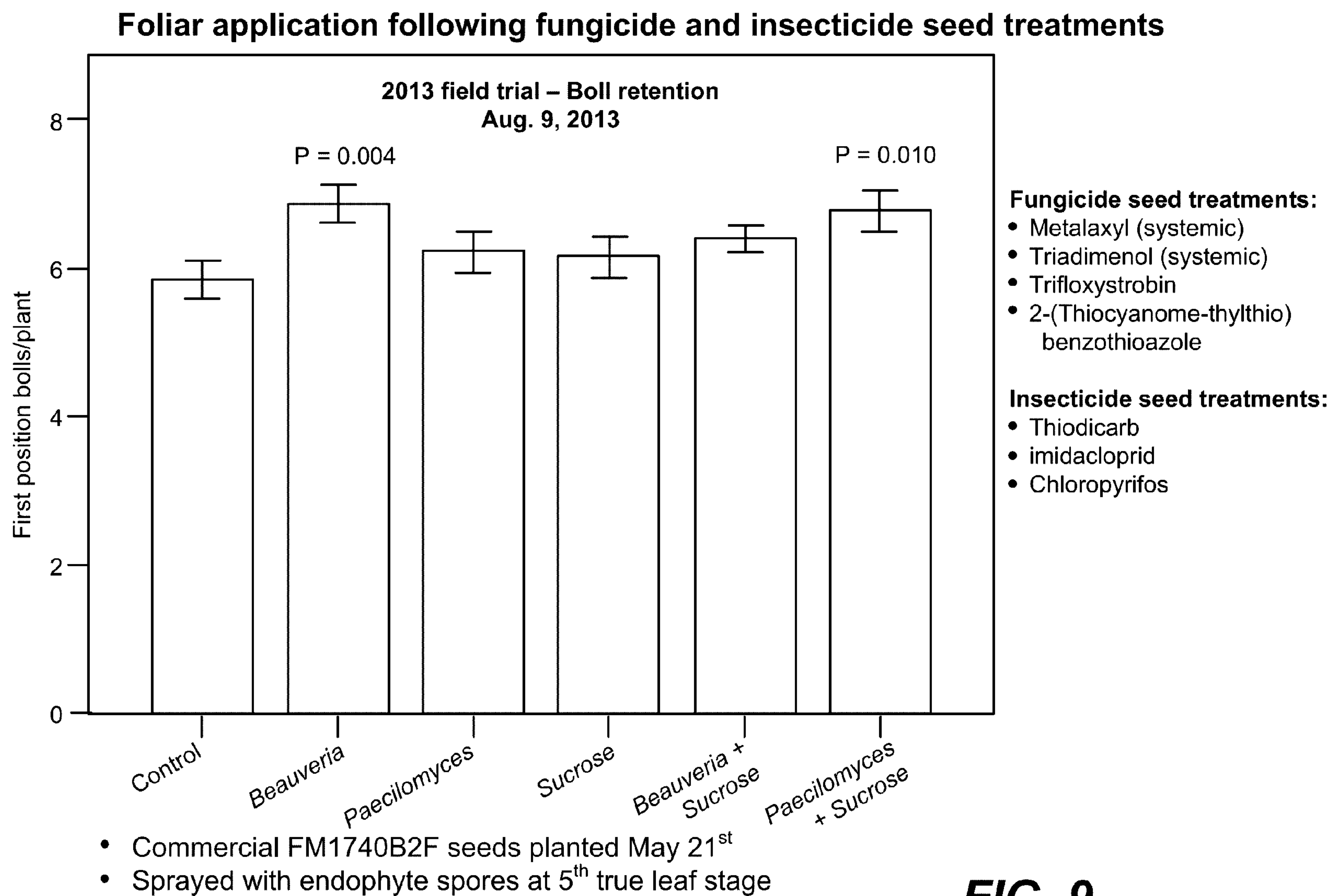

Foliar application following fungicide and insecticide seed treatments
2013 field trial – Boll retention
Aug. 9, 2013
P = 0.004
P = 0.010
First position bolls/plant
0
2
4
6
8
Control
Beauveria
Paecilomyces
Sucrose
Beauveria + Sucrose
Paecilomyces + Sucrose
Fungicide seed treatments:
• Metalaxyl (systemic)
• Triadimenol (systemic)
• Trifloxystrobin
• 2-(Thiocyanome-thylthio) benzothioazole
Insecticide seed treatments:
• Thiodicarb
• imidacloprid
• Chloropyrifos
• Commercial FM1740B2F seeds planted May 21st
• Sprayed with endophyte spores at 5th true leaf stage

FIG. 9

More squares & bolls retained in *all* endophyte treatments

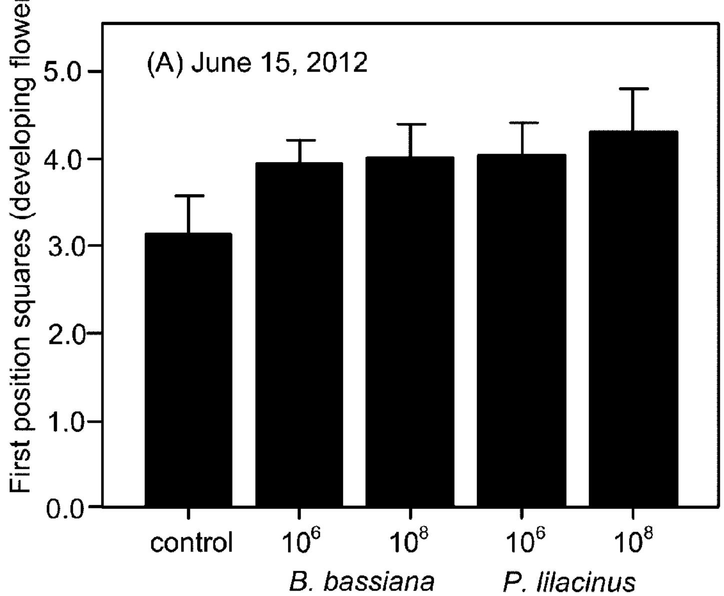


FIG. 10A

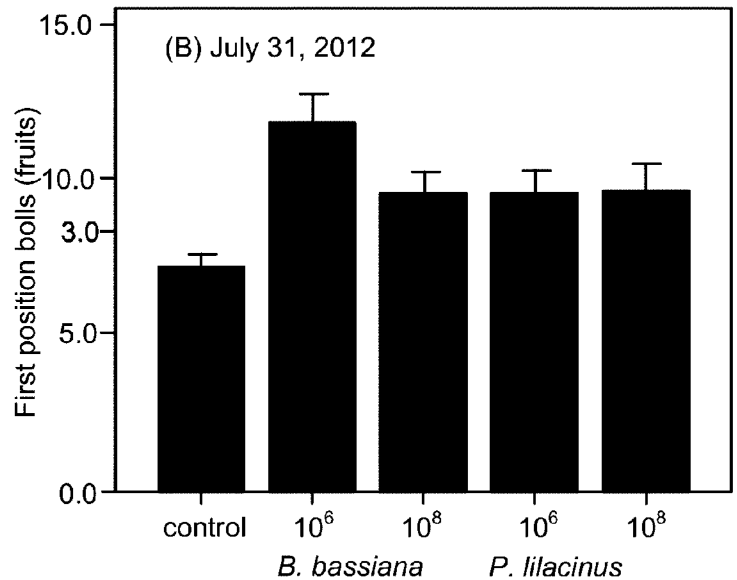
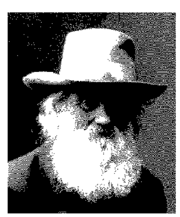
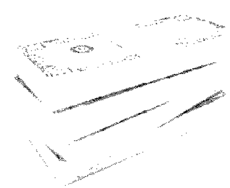


FIG. 10B

Repeated measures ANOVA (Time, P < 0.001; Time*Endophyte, P=0.045, Endophyte, P=0.003)

Positive effects of endophytes on plant reproductive traits

Fitness? 

Yields? 

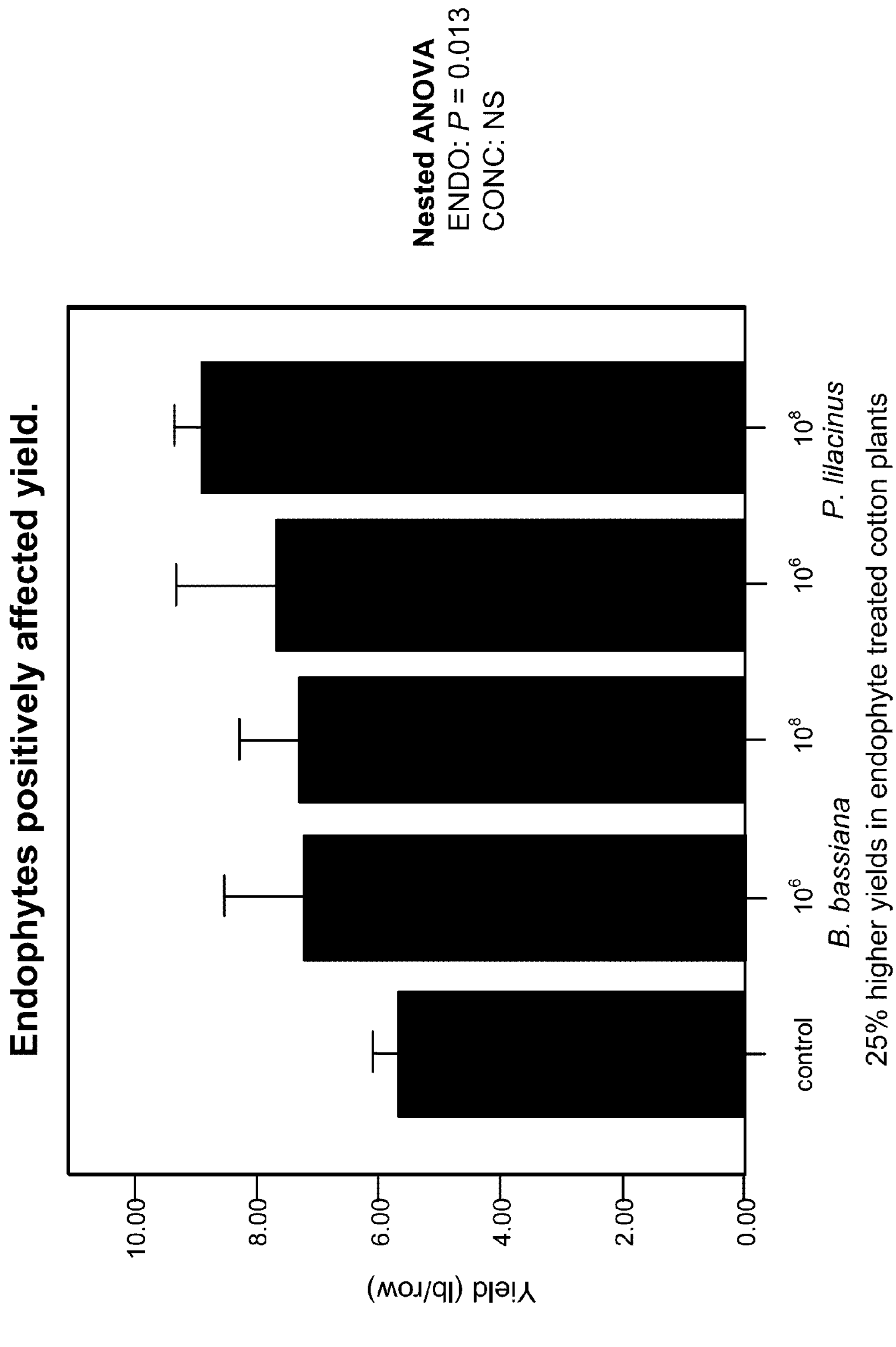

Endophytes positively affected yield.
Nested ANOVA
ENDO: P = 0.013
CONC: NS
Yield (lb/row)
0.00
2.00
4.00
6.00
8.00
10.00
control
10^6
10^8
B. bassiana
10^6
10^8
P. lilacinus
25% higher yields in endophyte treated cotton plants

FIG. 11

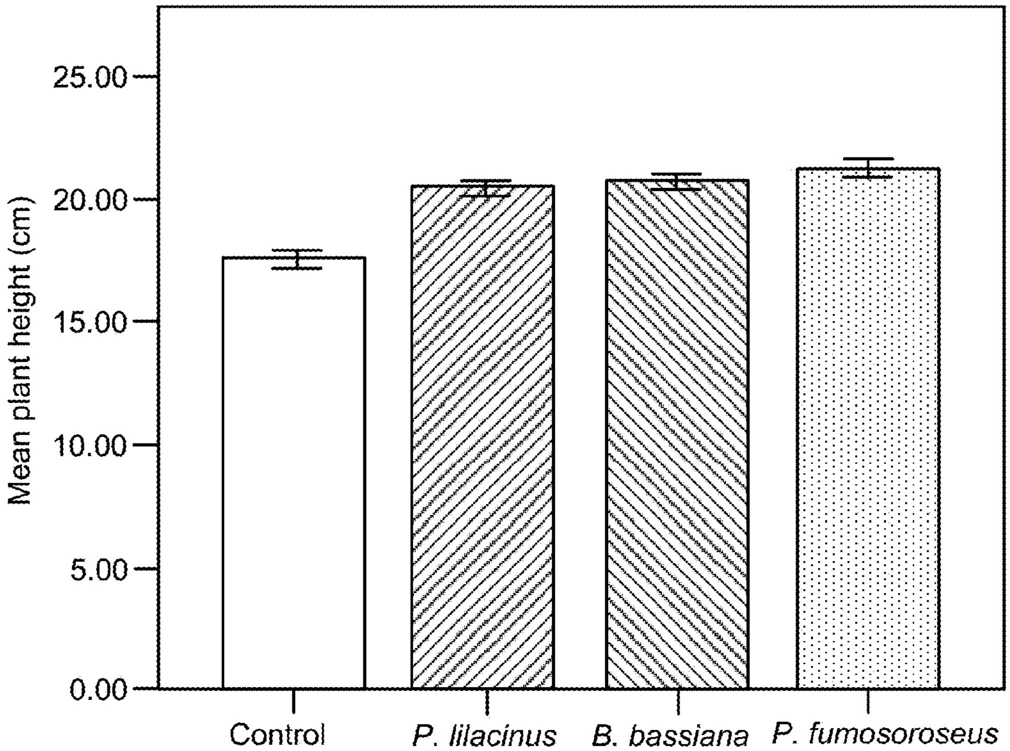


FIG. 12A

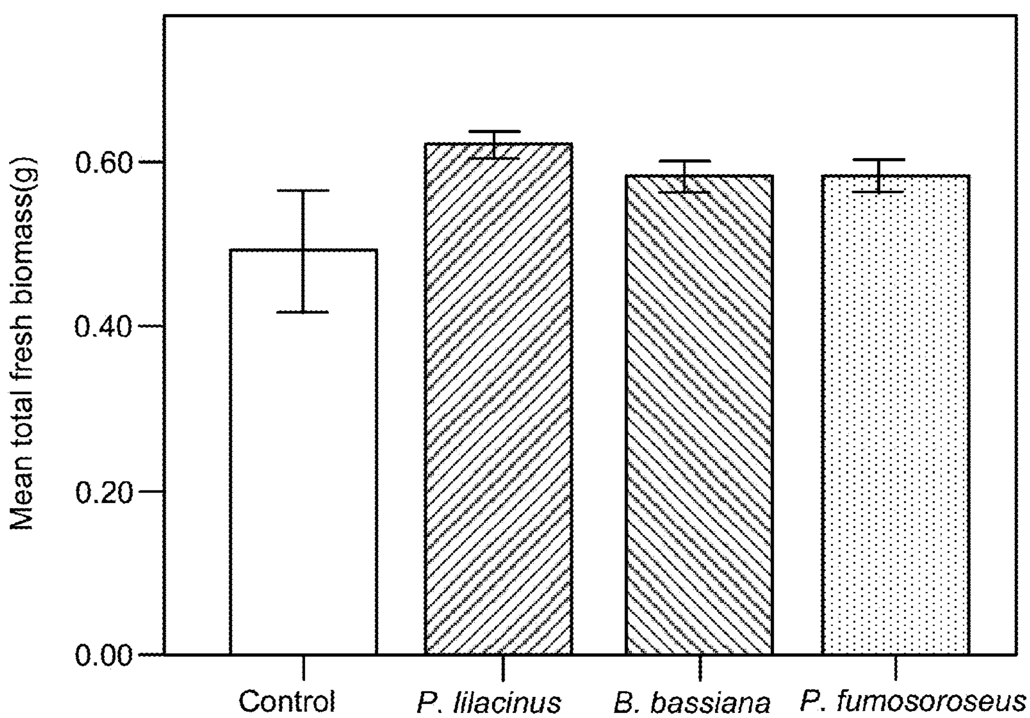


FIG. 12B

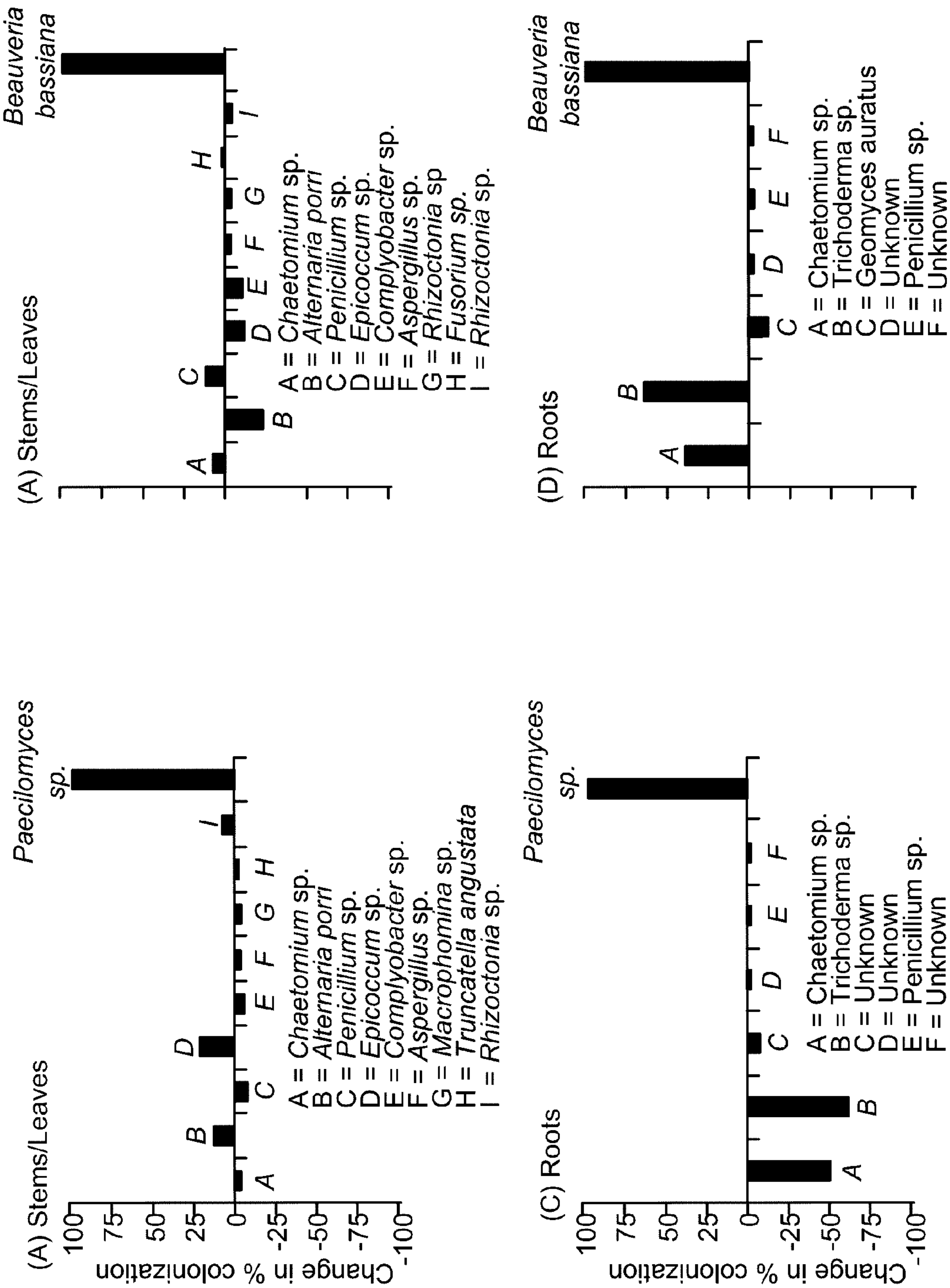

(A) Stems/Leaves
Paecilomyces sp.
Change in % colonization
100
75
50
25
0
-25
-50
-75
-100
A B C D E F G H I
A = Chaetomium sp.
B = Alternaria porri
C = Penicillium sp.
D = Epicoccum sp.
E = Complyobacter sp.
F = Aspergillus sp.
G = Macrophomina sp.
H = Truncatella angustata
I = Rhizoctonia sp.
(A) Stems/Leaves
Beauveria bassiana
A B C D E F G H I
A = Chaetomium sp.
B = Alternaria porri
C = Penicillium sp.
D = Epicoccum sp.
E = Complyobacter sp.
F = Aspergillus sp.
G = Rhizoctonia sp
H = Fusorium sp.
I = Rhizoctonia sp.
(C) Roots
Paecilomyces sp.
Change in % colonization
100
75
50
25
0
-25
-50
-75
-100
A B C D E F
A = Chaetomium sp.
B = Trichoderma sp.
C = Unknown
D = Unknown
E = Penicillium sp.
F = Unknown
(D) Roots
Beauveria bassiana
A B C D E F
A = Chaetomium sp.
B = Trichoderma sp.
C = Geomyces auratus
D = Unknown
E = Penicillium sp.
F = Unknown

FIG. 13

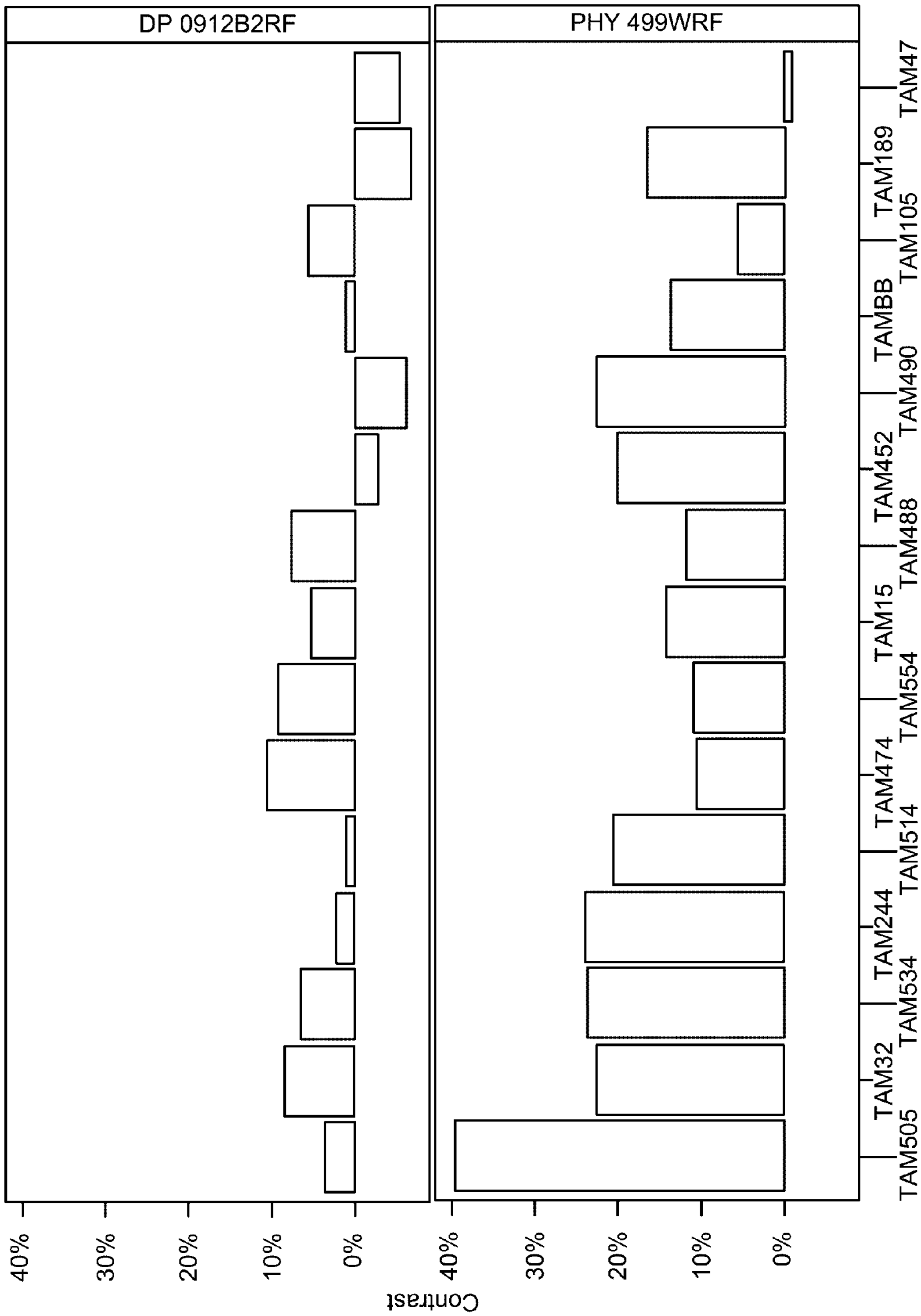

DP 0912B2RF
PHY 499WRF
TAM47
TAM189
TAM105
TAMBB
TAM490
TAM452
TAM488
TAM15
TAM554
TAM474
TAM514
TAM244
TAM534
TAM32
TAM505
40%
30%
20%
10%
0%
40%
30%
20%
10%
0%
Contrast

FIG. 14

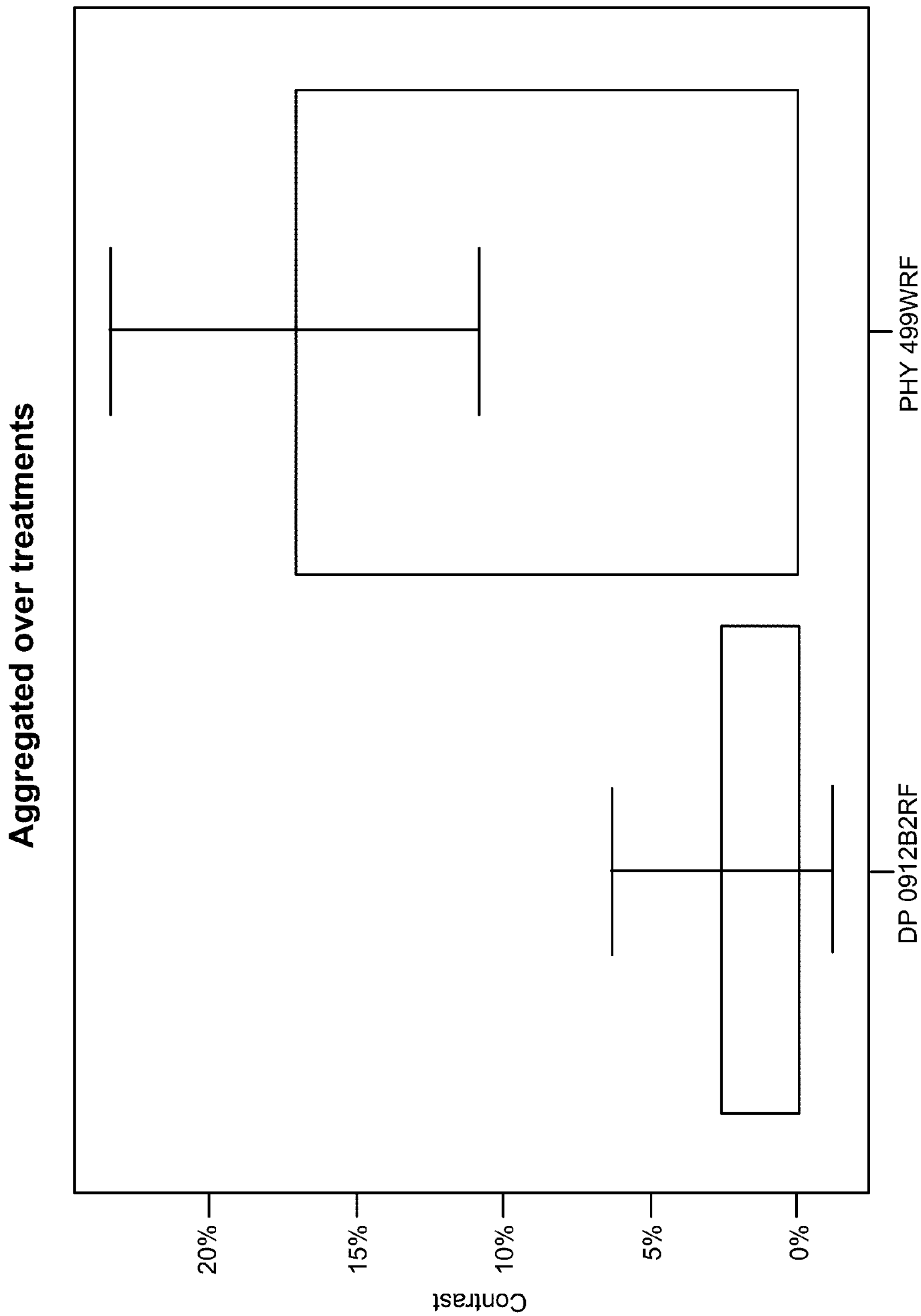

Aggregated over treatments
Contrast
20%
15%
10%
5%
0%
DP 0912B2RF
PHY 499WRF

FIG. 15

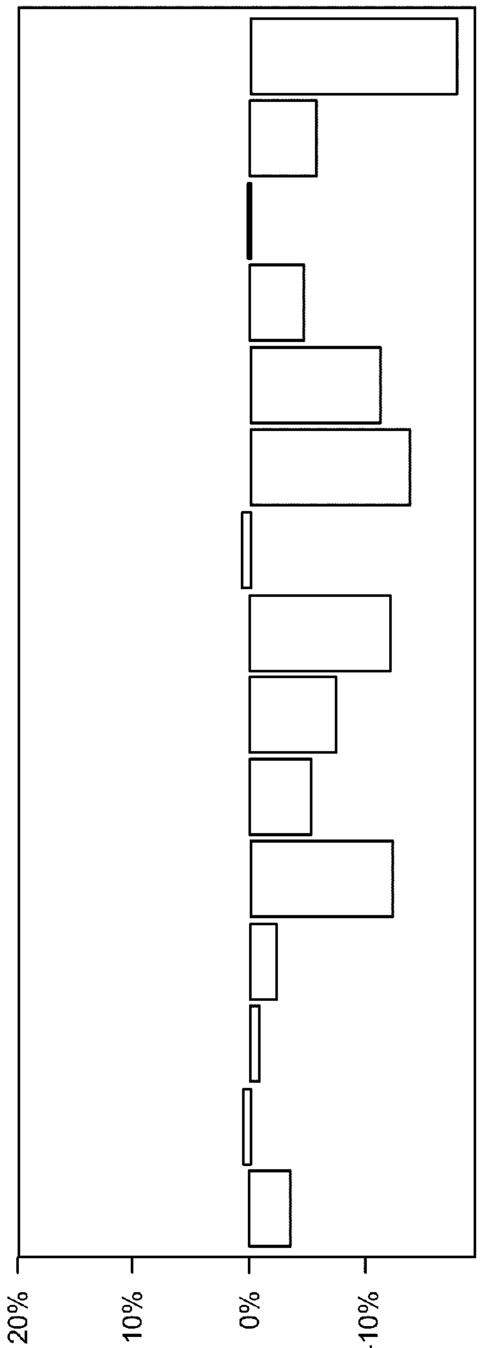


FIG. 16A

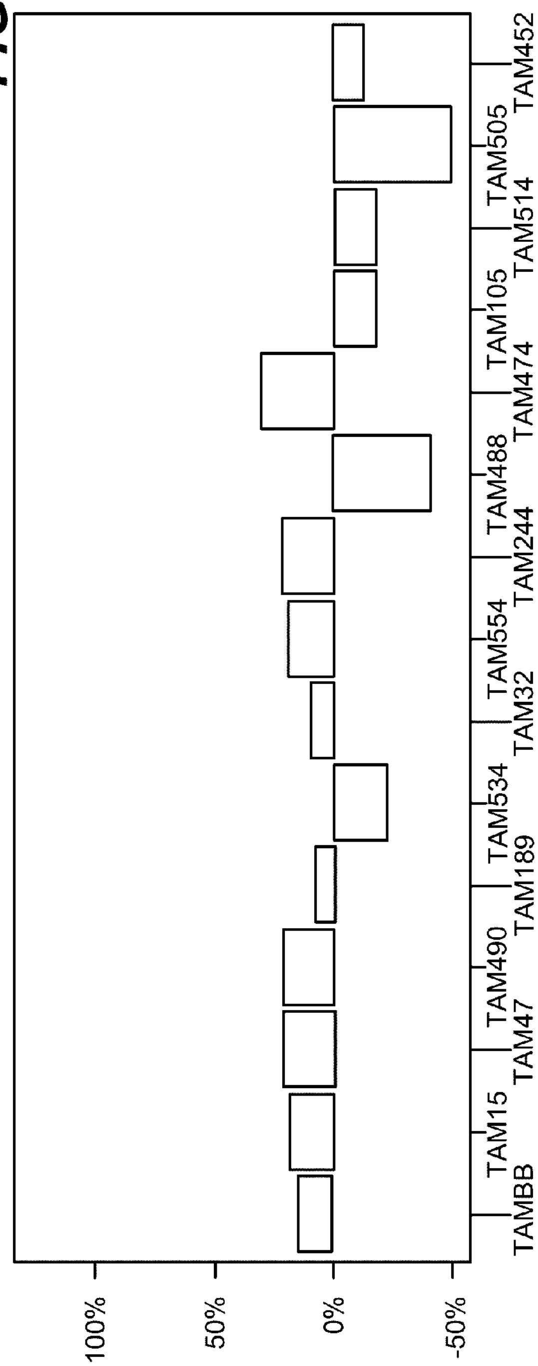


FIG. 16B

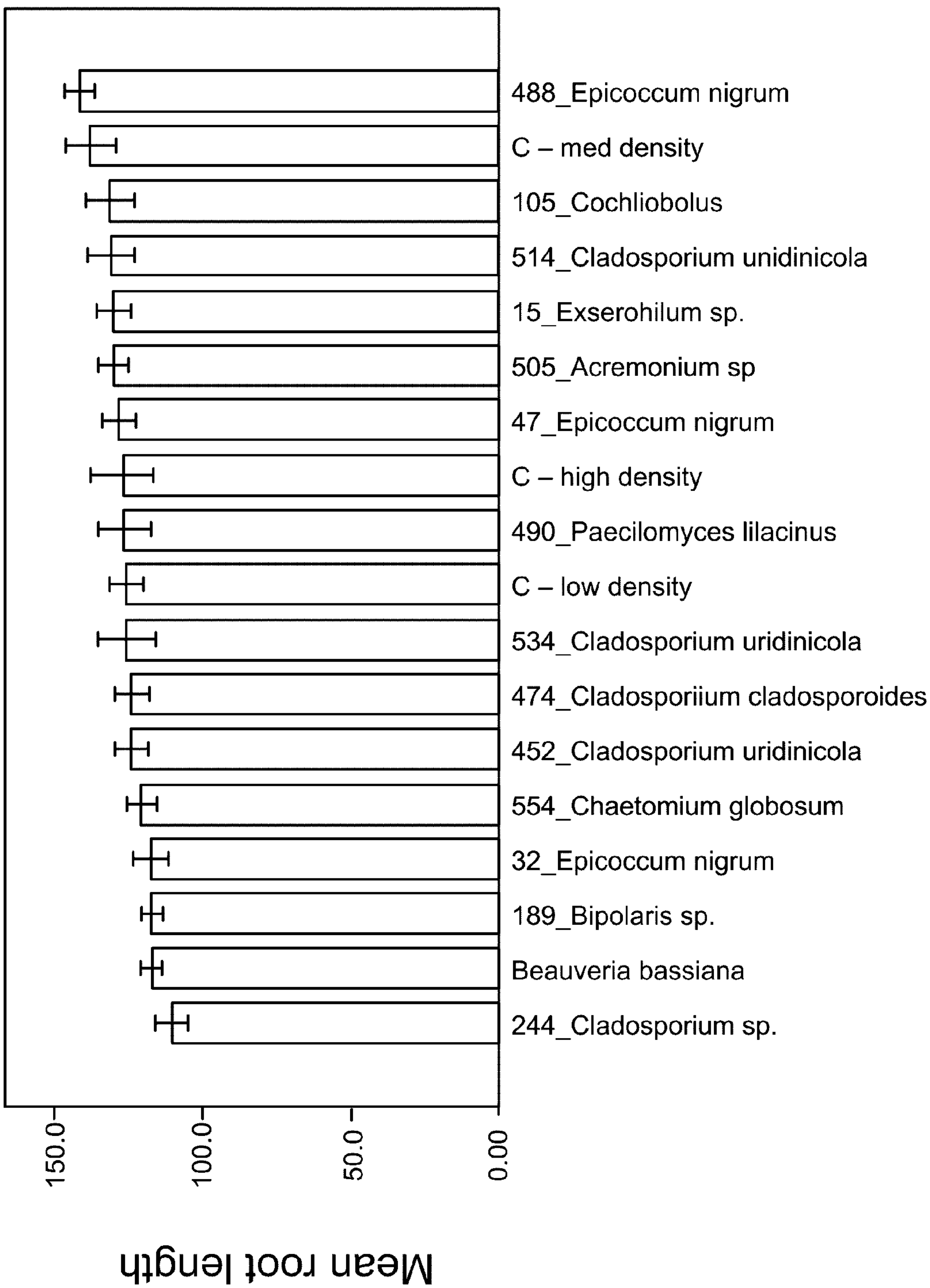

488_Epicoccum nigrum
C – med density
105_Cochliobolus
514_Cladosporium unidinicola
15_Exserohilum sp.
505_Acremonium sp
47_Epicoccum nigrum
C – high density
490_Paecilomyces lilacinus
C – low density
534_Cladosporium uridinicola
474_Cladosporiium cladosporoides
452_Cladosporium uridinicola
554_Chaetomium globosum
32_Epicoccum nigrum
189_Bipolaris sp.
Beauveria bassiana
244_Cladosporium sp.
150.0
100.0
50.0
0.00
Mean root length

FIG. 17A

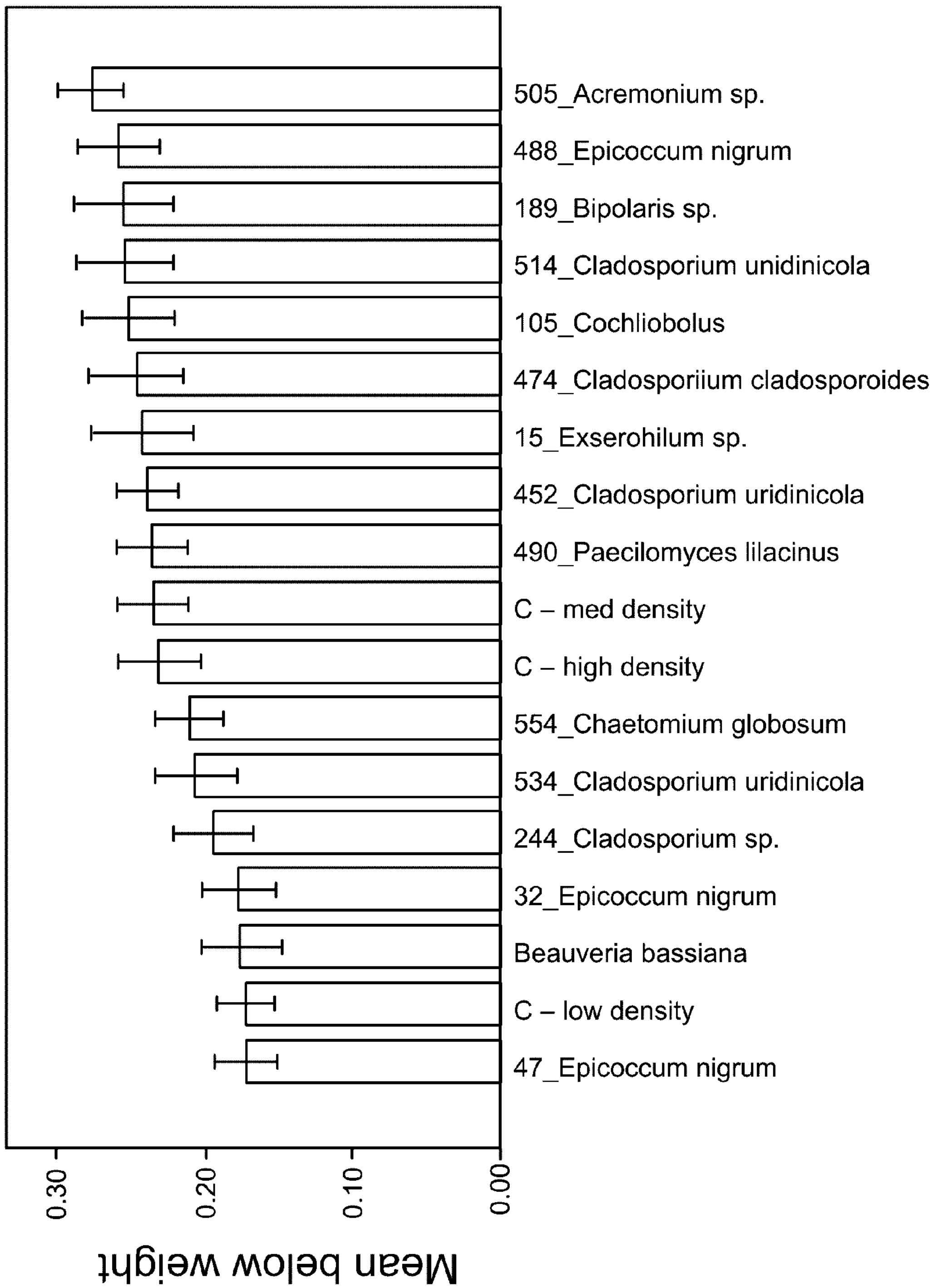
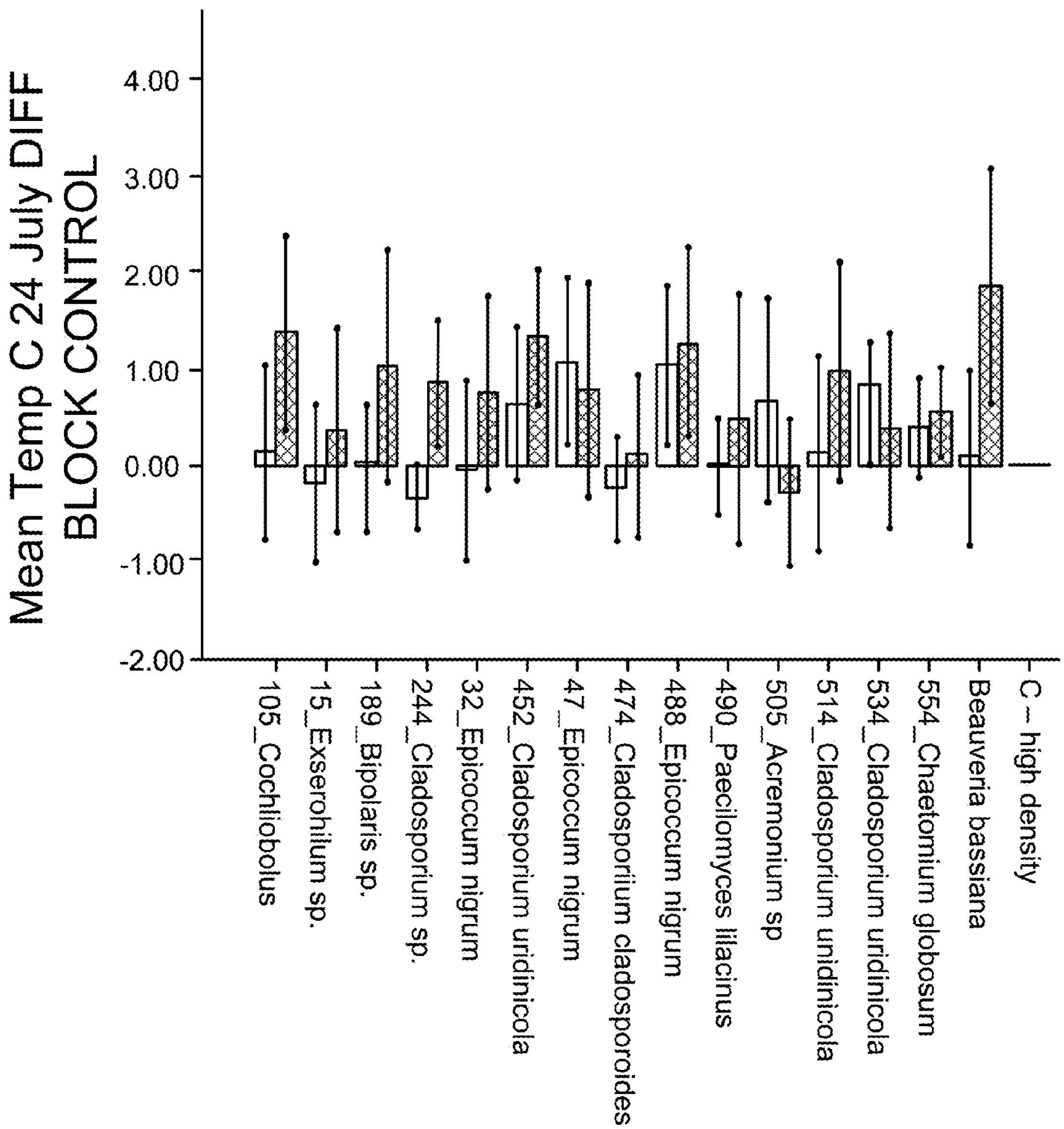

505_Acremonium sp.
488_Epicoccum nigrum
189_Bipolaris sp.
514_Cladosporium unidinicola
105_Cochliobolus
474_Cladosporiium cladosporoides
15_Exserohilum sp.
452_Cladosporium uridinicola
490_Paecilomyces lilacinus
C – med density
C – high density
554_Chaetomium globosum
534_Cladosporium uridinicola
244_Cladosporium sp.
32_Epicoccum nigrum
Beauveria bassiana
C – low density
47_Epicoccum nigrum
0.30
0.20
0.10
0.00
Mean below weight

FIG. 17B



Mean Temp C 24 July DIFF BLOCK CONTROL
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
105_Cochliobolus
15_Exserohilum sp.
189_Bipolaris sp.
244_Cladosporium sp.
32_Epicoccum nigrum
452_Cladosporium uridinicola
47_Epicoccum nigrum
474_Cladosporium cladosporoides
488_Epicoccum nigrum
490_Paecilomyces lilacinus
505_Acremonium sp
514_Cladosporium unidinicola
534_Cladosporium uridinicola
554_Chaetomium globosum
Beauveria bassiana
C -- high density

FIG. 18

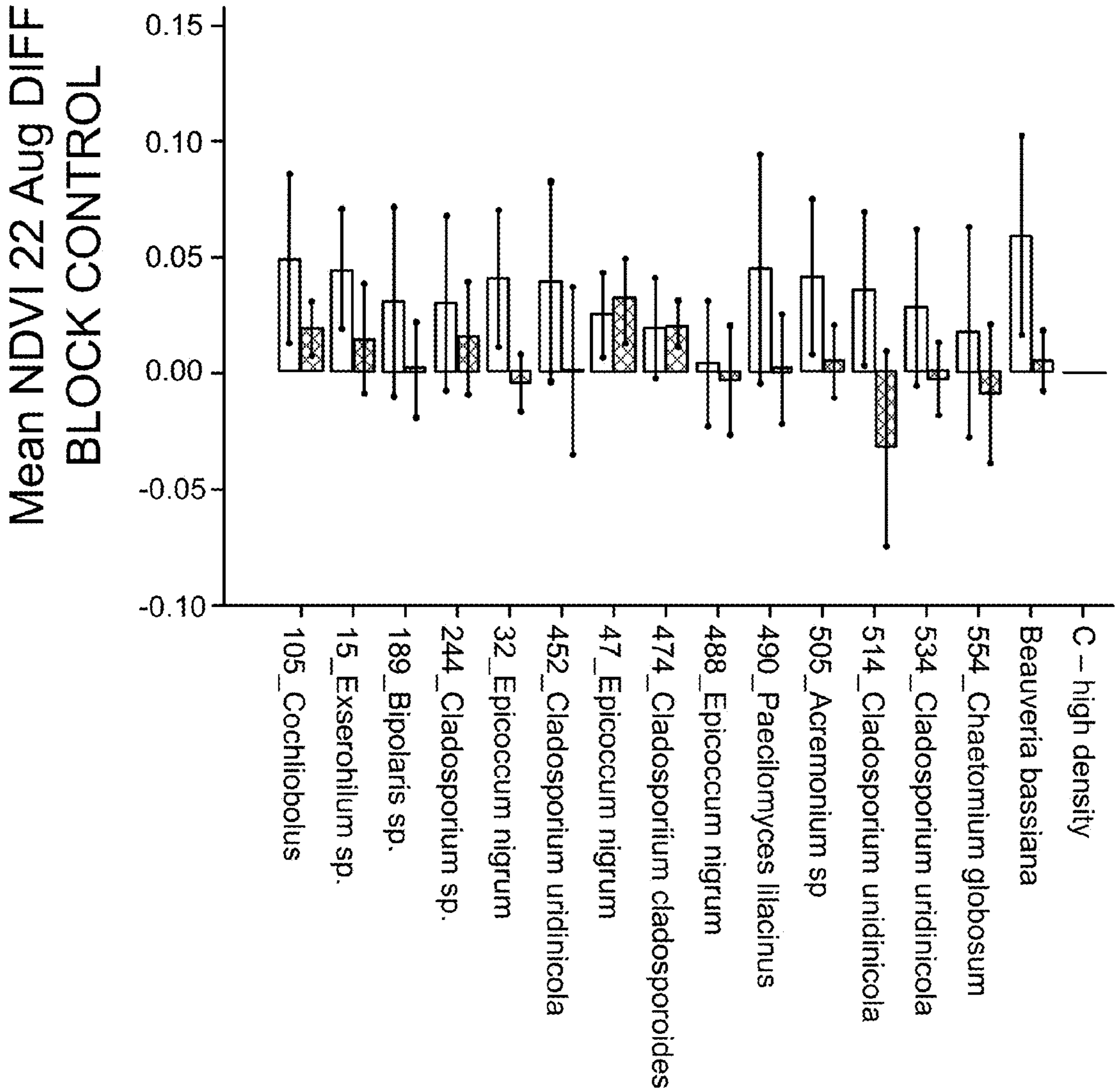
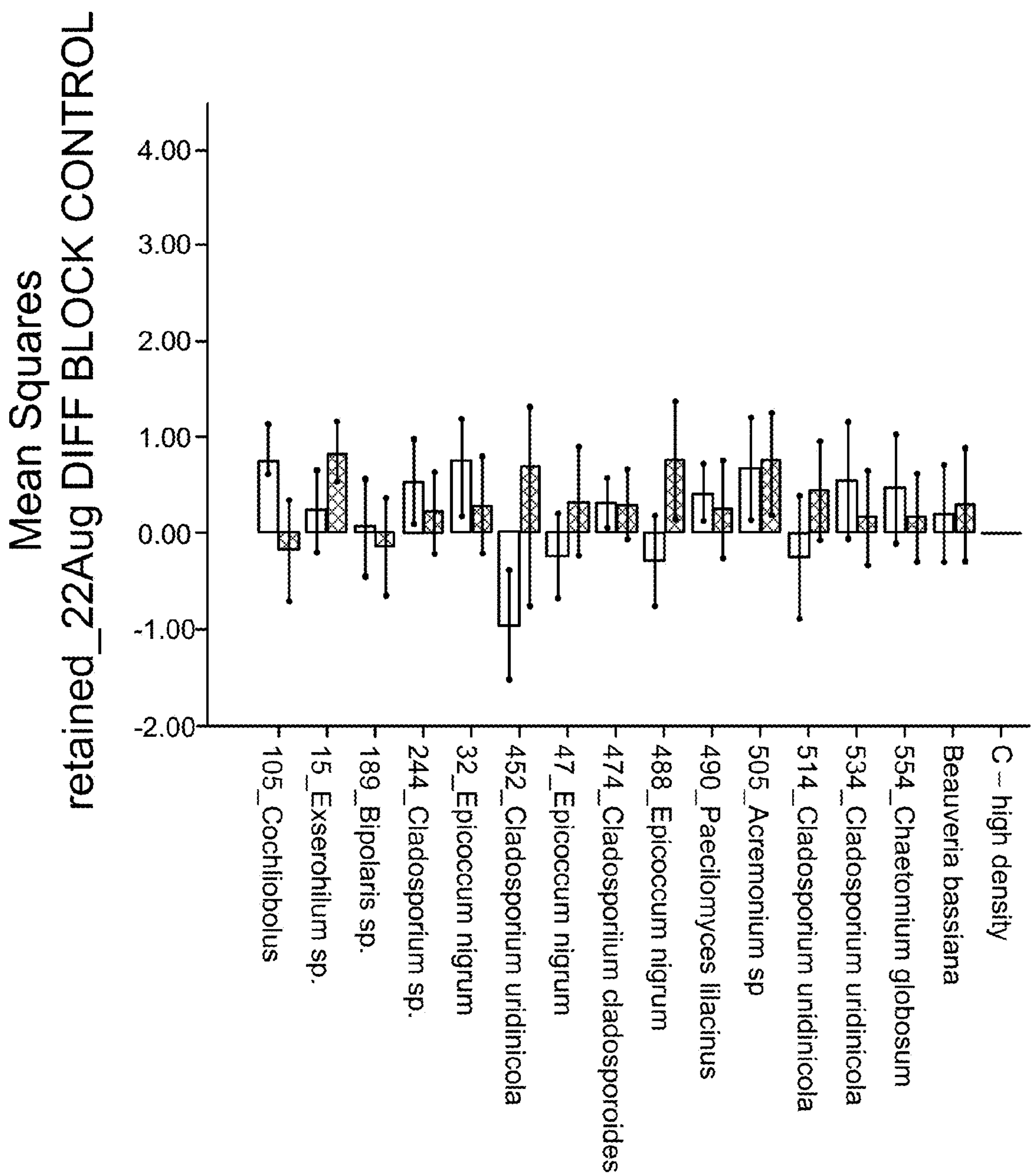

Mean NDVI 22 Aug DIFF
BLOCK CONTROL
0.15
0.10
0.05
0.00
-0.05
-0.10
105_Cochliobolus
15_Exserohilum sp.
189_Bipolaris sp.
244_Cladosporium sp.
32_Epicoccum nigrum
452_Cladosporium uridinicola
47_Epicoccum nigrum
474_Cladosporiium cladosporoides
488_Epicoccum nigrum
490_Paecilomyces lilacinus
505_Acremonium sp
514_Cladosporium unidinicola
534_Cladosporium uridinicola
554_Chaetomium globosum
Beauveria bassiana
C – high density

FIG. 19



Mean Squares
retained_22Aug DIFF BLOCK CONTROL
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
105_Cochliobolus
15_Exserohilum sp.
189_Bipolaris sp.
244_Cladosporium sp.
32_Epicoccum nigrum
452_Cladosporium uridinicola
47_Epicoccum nigrum
474_Cladosporium cladosporoides
488_Epicoccum nigrum
490_Paecilomyces lilacinus
505_Acremonium sp
514_Cladosporium unidinicola
534_Cladosporium uridinicola
554_Chaetomium globosum
Beauveria bassiana
C – high density

FIG. 20

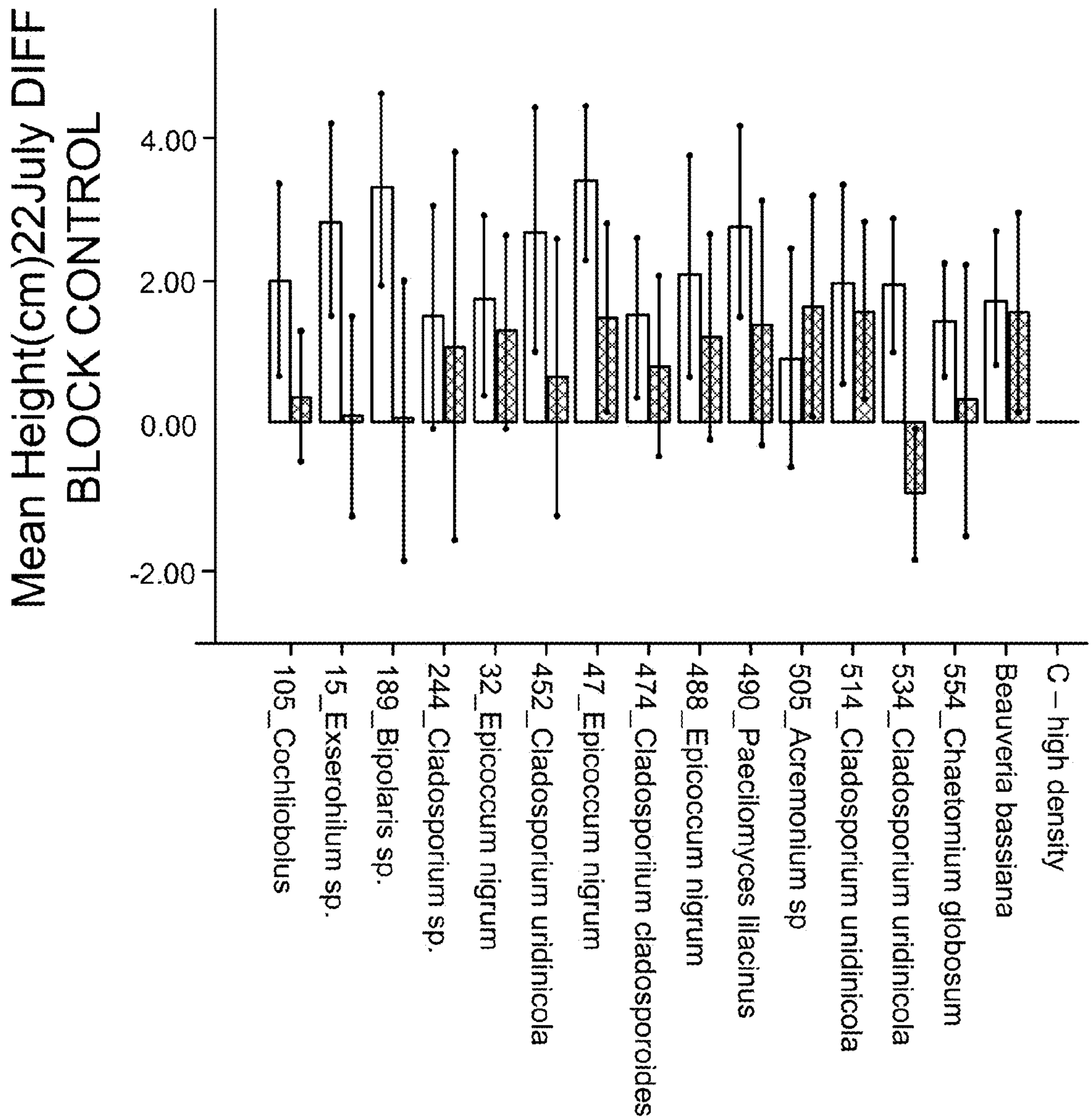

Mean Height(cm)22July DIFF
BLOCK CONTROL
4.00
2.00
0.00
-2.00
105_Cochliobolus
15_Exserohilum sp.
189_Bipolaris sp.
244_Cladosporium sp.
32_Epicoccum nigrum
452_Cladosporium uridinicola
47_Epicoccum nigrum
474_Cladosporium cladosporoides
488_Epicoccum nigrum
490_Paecilomyces lilacinus
505_Acremonium sp
514_Cladosporium uridinicola
534_Cladosporium uridinicola
554_Chaetomium globosum
Beauveria bassiana
C – high density

FIG. 21

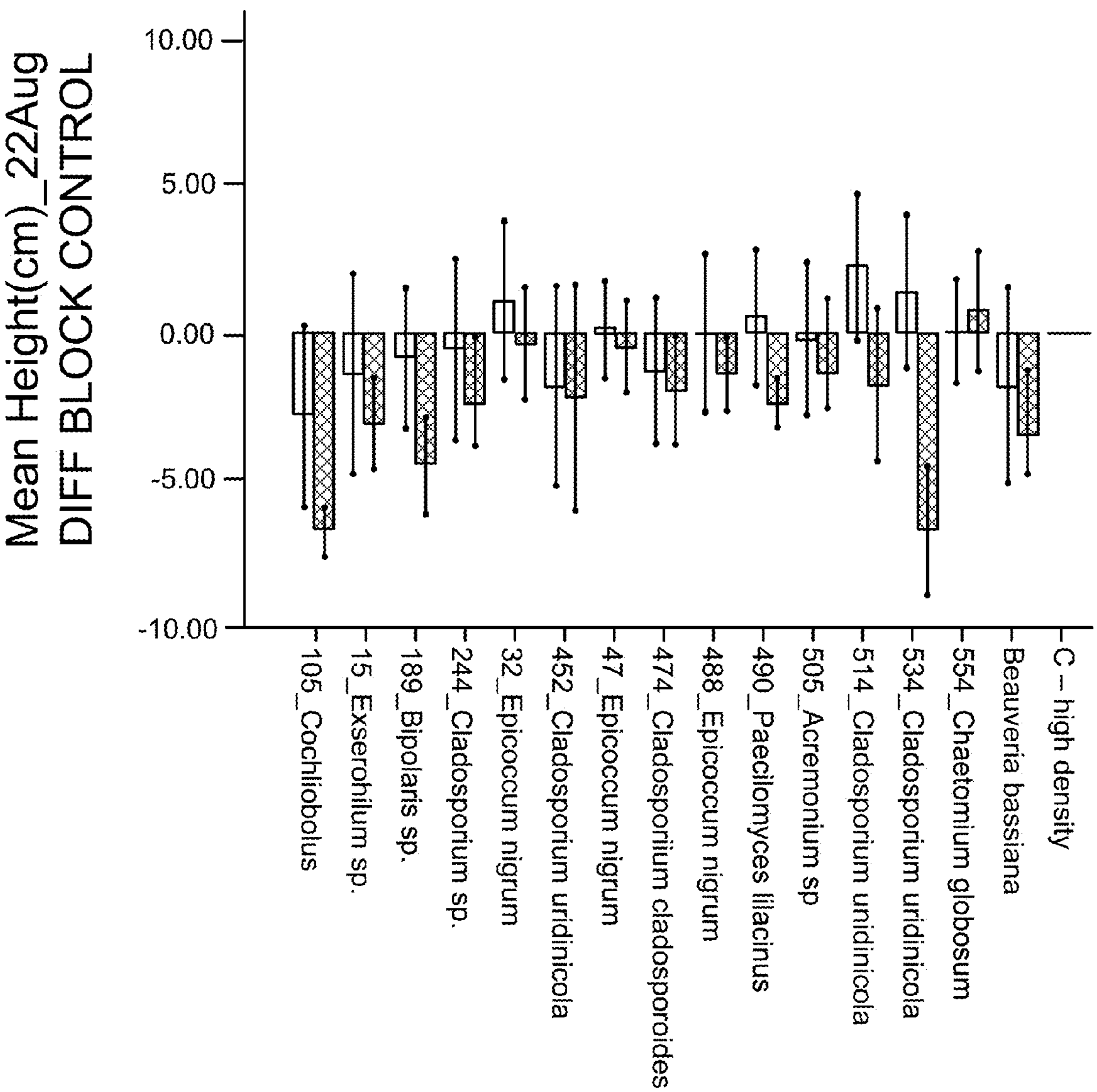

Mean Height(cm)_22Aug
DIFF BLOCK CONTROL
10.00
5.00
0.00
-5.00
-10.00
105_Cochliobolus
15_Exserohilum sp.
189_Bipolaris sp.
244_Cladosporium sp.
32_Epicoccum nigrum
452_Cladosporium uridinicola
47_Epicoccum nigrum
474_Cladosporium cladosporoides
488_Epicoccum nigrum
490_Paecilomyces lilacinus
505_Acremonium sp
514_Cladosporium unidinicola
534_Cladosporium uridinicola
554_Chaetomium globosum
Beauveria bassiana
C – high density

FIG. 22

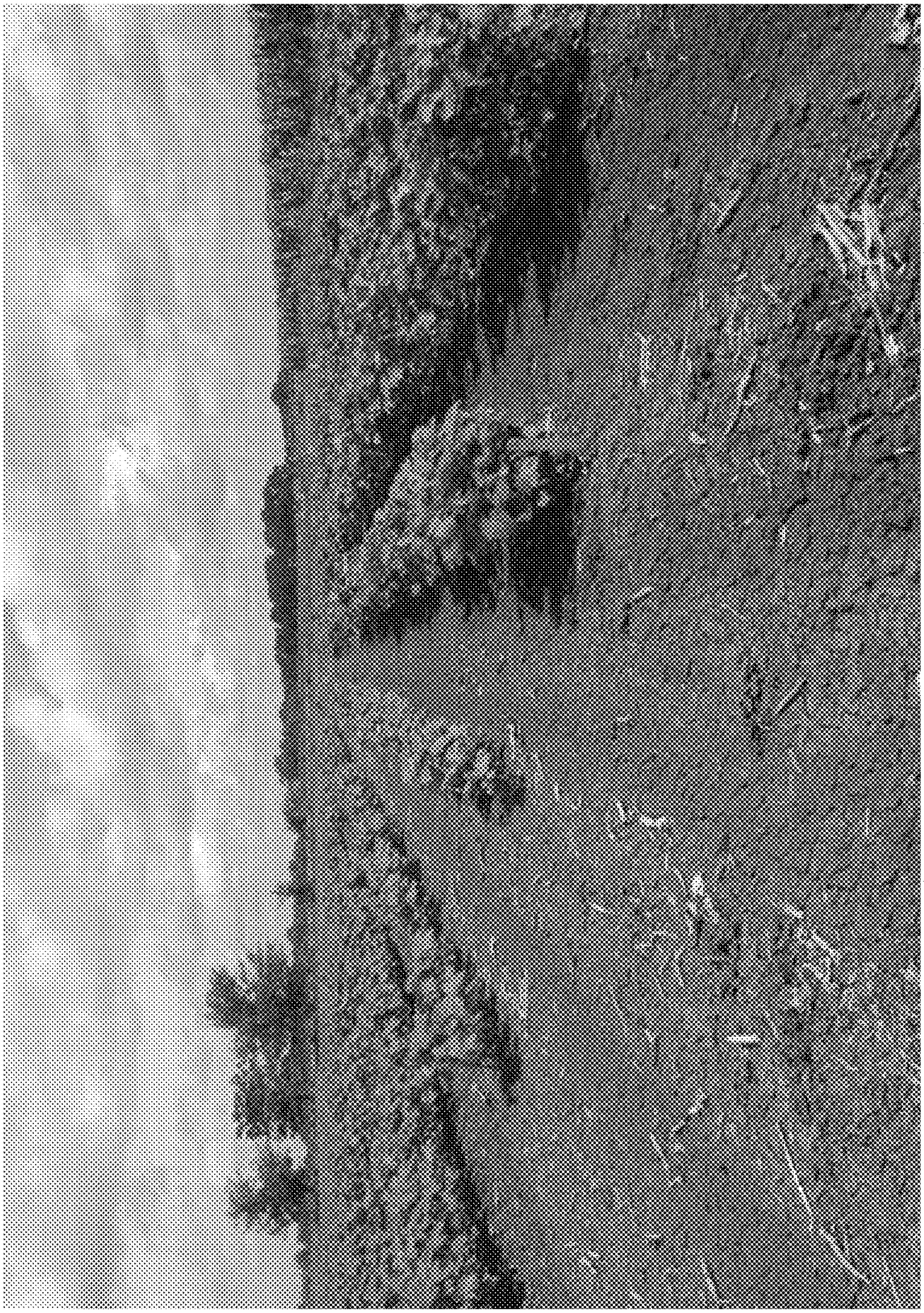


FIG. 23

Genotype	Treatments	Estimate	Std. Error
DTP	194/Epic	15.000	.323
	249/Clad	15.778	.173
	355/Chae	16.500	.345
	46/Epico	17.125	.364
	463/Clad	16.571	.291
	534/Clad	15.722	.289
	554/Chae	15.571	.272
	58/Epico	15.438	.433
	control	16.000	.296
	Overall	15.952	.116
PHY	194/Epic	15.706	.329
	249/Clad	15.000	.331
	355/Chae	14.471	.194
	46/Epico	18.000	.257
	463/Clad	15.438	.288
	534/Clad	14.333	.347
	554/Chae	16.294	.254
	58/Epico	14.824	.376
	control	16.722	.289
	Overall	15.682	.135
Overall	Overall	15.816	.089

FIG. 24

Genotype	Treatments	Estimate	Std. Error
DTP	194	18.899	.332
	249	19.000	.370
	355	19.389	.244
	46	20.188	.248
	463	19.357	.289
	534	19.444	.258
	554	19.429	.374
	58	19.563	.343
	control	20.286	.294
	Overall	19.479	.107
PHY	194	19.176	.246
	249	18.357	.341
	355	17.647	.363
	46	20.353	.171
	463	19.125	.340
	534	18.200	.279
	554	19.529	.244
	58	19.706	.319
	control	19.667	.354
	Overall	19.115	.118
Overall	Overall	19.296	.080

FIG. 25

FUNGAL ENDOPHYTES FOR IMPROVED CROP YIELDS AND PROTECTION FROM PESTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 14/535,292 filed Nov. 6, 2014, allowed, which claims priority to U.S. Provisional Patent Application Nos. 61/900,929 and 61/900,935, both filed Nov. 6, 2013, which are herein incorporated by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

The sequence listing that is contained in the file named 32601_US_Sequence_Listing.txt, includes 77 sequences and is 33 kilobytes as measured in Microsoft Windows operating system and was created on Dec. 5, 2015, is filed electronically herewith and incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to fungal endophytes of agricultural crops for improving yield and/or for protection from pests.

DESCRIPTION OF RELATED ART

Fungal endophytes are fungi that internally colonize plant tissues without causing evident damage or disease. Particular fungal endophytes, such as mycorrhiza, survive within various host plant tissues, often colonizing the intercellular spaces of host leaves, stems, flowers or roots. The symbiotic endophyte-host relationships can provide several fitness benefits to the host plant, such as enhancement of nutrition, and/or increased drought tolerance. Root-colonizing mycorrhizae survive on photosynthetic carbohydrates from the plant, and in return, aid in the solubilization and uptake of water and minerals to the host, which can lead to the promotion of seed germination and plant growth. Additionally, the association of a fungal endophyte with a host plant can provide tolerance to a variety of biotic and abiotic stresses. Host growth, fitness promotion and protection are thought to be achieved through multiple beneficial properties of the endophyte-host association. For instance, the endophytic organisms may produce growth-regulating substances to induce biomass production and alkaloids or other metabolites. Additionally, fungal endophytes may directly suppress or compete with disease-causing microbes, protecting the plant from potential pathogens.

SUMMARY OF THE INVENTION

In one aspect, the invention provides methods for improving a trait in an agricultural plant comprising contacting an agricultural seed of said plant with a formulation comprising a purified facultative fungal endophytes of at least one species, wherein the endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both, and wherein the endophytes are present in the formulation in an amount effective to modulate the colonization frequencies of the endophytes that are native to the agricultural plant grown from the seed compared to a

reference seed that is planted in an agricultural environment, and to provide a benefit to the seeds or the agricultural plants grown from the seeds.

In another aspect, the invention provides methods for providing a benefit to an agricultural plant comprising treating said plant, the seed of said plant, or the rhizosphere of said plant or seed with a composition comprising purified facultative fungal endophytes and an agriculturally-acceptable carrier, wherein the endophyte is capable of at least one of: reducing pest reproduction, killing pests, and deterring pests, and wherein the endophyte is present in the composition in an amount effective to provide a benefit to the seeds or the agricultural plants derived from the seeds.

In yet another aspect, the invention provides methods for providing a benefit to an agricultural plant, comprising obtaining a synthetic combination of an agricultural plant seed and a purified facultative fungal endophyte, wherein the endophyte is capable of at least one of: reducing pest reproduction, killing pests, and deterring pests, and wherein the endophyte is present in the synthetic combination in an amount effective to provide a benefit to the seeds or the agricultural plants derived from the seeds.

In another embodiments, methods of producing a plant with a non-naturally occurring ratio of endophytes is provided, where the methods comprise contacting an agricultural seed of the plant with a formulation comprising facultative fungal endophytes of at least one species, wherein endophytes are present in the formulation in an amount effective to modulate the colonization frequencies of the endophytes that are native to the agricultural plant grown from the seed compared to a reference seed that is planted in an agricultural environment, wherein the plant with the non-naturally occurring ratio of endophytes has an improved trait as compared to a plant with a naturally-occurring ratio. In a further aspect, the facultative fungal endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both.

In another aspect, the invention provides methods for altering the systemic defensive pathway in a plant comprising contacting an agricultural seed of said plant with a formulation comprising a purified facultative fungal endophytes of at least one species, wherein the endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both, and wherein the endophyte is present in the synthetic combination in an amount effective to modulate the level of at least one phytohormone within an agricultural plant grown from the plant seed, and to provide a benefit to the seeds or the agricultural plants grown from the seeds. In a further aspect, the facultative fungal endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both.

In other embodiments, the invention provides methods of modulating the colonization frequencies of endophytes that are native to the agricultural plant grown from the seed compared to a reference seed that is planted in an agricultural environment, comprising contacting the seed of the agricultural plant with a formulation comprising facultative fungal endophytes of at least one species, and wherein endophytes are present in the formulation in an amount effective to modulate the colonization frequencies of native endophytes and to provide a benefit to the seeds or the agricultural plants grown from the seeds. In certain aspects, the native endophytes are of genus *Alternaria*. In a further aspect, the facultative fungal endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both.

In another aspect, the invention provides methods for altering the systemic defensive pathway in a plant comprising contacting an agricultural seed of said plant with a formulation comprising a purified facultative fungal endophytes of at least one species, and wherein the endophyte is present in the synthetic combination in an amount effective to modulate the level of at least one phytohormone within an agricultural plant grown from the plant seed, and to provide a benefit to the seeds or the agricultural plants grown from the seeds. In a further aspect, the facultative fungal endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both.

In yet another aspect, the invention provides methods of producing a plant with a network of fungal endophytes that comprises endophytes of the genus *Alternaria*, comprising (a) contacting the seed of an agricultural plant with a formulation comprising facultative fungal endophytes of at least one non-*Alternaria* species, wherein endophytes are present in the formulation in an amount effective to provide a benefit to the seeds or the agricultural plants grown from the seeds, and wherein the plant grown from the seed comprises endophytes of the genus *Alternaria*. In a further aspect, the facultative fungal endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both.

Also provided herein are synthetic combinations of an agricultural plant seed and a composition comprising purified entomopathogenic fungal endophytes of at least one species, wherein the endophytes are capable of (1) colonizing the agricultural plant grown from the plant seed (2) and at least one of: reducing pest reproduction, killing pests, and deterring pests, from within the agricultural plant; wherein the endophytes are not of species *Beauveria bassiana*, and wherein the endophyte is present in the synthetic combination in an amount effective to provide a benefit other than enhanced resistance to biotic stress to the seeds or the agricultural plants derived from the seeds when the seeds or plants are grown in an agricultural setting.

In yet another aspect, the invention provides synthetic combinations of an agricultural plant seed and a composition comprising purified facultative fungal endophytes of at least one species, wherein the endophyte is present in the synthetic combination in an amount effective to modulate the level of at least one phytohormone within an agricultural plant grown from the plant seed, and to provide a benefit to the seeds or the agricultural plants grown from the seeds. In a further aspect, the facultative fungal endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both.

In another embodiment, the invention provides synthetic combinations of an agricultural plant seed and a composition comprising purified facultative fungal endophytes of at least one species, wherein the facultative fungal endophytes are present in the synthetic combination in an amount effective to modulate the colonization frequencies of endophytes that are native to the agricultural plant grown from the seed compared to a reference seed that is planted in an agricultural environment, and to provide a benefit to the seeds or the agricultural plants grown from the seeds. In a further aspect, the facultative fungal endophytes are capable of producing substances that are beneficial to plants or detrimental to pests or both. In certain aspects, the facultative fungal endophytes are present in the synthetic combination in an amount effective to modulate the colonization frequencies of endophytes of genus *Alternaria* that are native to the agricultural plant grown from the seed compared to a reference seed that is planted in an agricultural environment.

In a further aspect for certain of these methods and synthetic combinations, the composition comprising purified facultative fungal endophytes also comprises an agriculturally acceptable carrier.

In a further aspect for certain of these methods and synthetic combinations, the facultative fungal endophyte may be a filamentous fungal endophyte. In other embodiments, the facultative endophyte may be spore-forming. In yet other embodiments, the facultative fungal endophyte may be a septate fungal endophyte. In yet other embodiments, the facultative fungal endophyte may be a dark septate fungal endophyte. In some embodiments, the facultative endophyte may be an entomopathogen. In some embodiments, the facultative fungal endophyte may belong to the phylum Ascomycota or Basidiomycota. In a further aspect, the facultative fungal endophyte may belong to subphylum Pezizomycotina, Agaricomycotina, or Ustilaginomycotina. In yet another aspect, facultative fungal endophyte may belong to class Sordariomycetes, Dothideomycetes, Agaricomycetes, Ustilaginomycetes, Orbiliomycetes, or Eurotiomycetes. In yet another aspect, the facultative fungal endophyte may belong to order Hypocreales, Pleosporales, Capnodiales, Sordariales, *Polyporales*, Diaporthales, Ustilaginales, Xylariales, Orbiliales, Trichosphaeriales, or Eurotiales.

In a further aspect, the facultative fungal endophyte may be a species from Table 1, namely *Acremonium alternatum, Alternaria alternata, Alternaria brassicae, Alternaria compacta, Alternaria dianthi, Alternaria longipes, Alternaria mali, Alternaria sesami, Alternaria solani, Alternaria* sp., *Alternaria tenuissima, Ascomycota* sp., *Bipolaris spicifera, Cercospora canescens, Cercospora capsici, Cercospora kikuchii, Cercospora zinnia, Chaetomium globosum, Chaetomium piluliferum, Chaetomium* sp., *Cladosporium cladosporioides, Cladosporium* sp., *Cladosporium uredinicola, Cochliobolus* sp, *Phanerochaete crassa, Phoma americana, Phoma subherbarum, Phomopsis liquidambari, Phomopsis* sp., *Pleospora* sp., *Pleosporaceae* sp., *Polyporales* sp., *Preussia africana, Preussia* sp., *Pseudozyma* sp., *Pyrenophora teres, Colletotrichumcapsici, Coniolariella gamsii, Coniothyrium aleuritis, Coniothyrium* sp., *Corynespora cassiicola, Diaporthe* sp., *Diatrype* sp., *Drechslerella dactyloides, Embellisia indefessa, Epicoccum nigrum, Epicoccum* sp., *Exserohilum rostratum, Fusarium chlamydosporum, Fusarium* sp., *Gibellulopsis nigrescens, Gnomoniopsis* sp., *Lewia infectoria, Mycosphaerella coffeicola, Mycosphaerellaceae* sp., *Nigrospora oryzae, Nigrospora* sp., *Nigrospora sphaerica, Paecilomyces* sp., *Penicillium citrinum, Retroconis* sp., *Rhizopycnis* sp., *Schizothecium inaequale, Stagonospora* sp., *Stemphylium lancipes, Thielavia hyrcaniae, Thielavia* sp., *Ulocladium chartarum, Verticillium* sp., *Beauveria bassiana, Aspergillus parasiticus, Lecanicillium lecanii*, and *Paecilomyces lilacinus.*

In a further aspect, the facultative fungal endophyte comprises a nucleic acid that is at least 97% identical, for example, at least 98% identical, at least 99% identical, at least 99.5% identical, or 100% identical to the nucleic acids provided in any of SEQ ID NO:7 through SEQ ID NO:77, for example those listed in Example 16.

In another aspect for certain of these methods is an additional step of packaging the contacted seeds in a container may be included. In certain aspects, the packaging material may be selected from a bag, box, bin, envelope, carton, or container, and may comprise a dessicant.

In a further aspect for certain of these methods and synthetic combinations, the benefit to the treated seed or plant grown from the treated seed is measured at the level of the population, as compared to a reference population of plants. In certain aspects, the facultative fungal endophyte may be providing a benefit to a crop comprising a plurality of agricultural plants produced from the seeds treated with the endophyte. In certain aspects, the present invention discloses a substantially uniform population of plants produced by growing the population of seeds described above. In one embodiment, at least 75%, at least 80%, at least 90%, at least 95% or more of the plants comprise in one or more tissues an effective amount of the endophyte or endophytes. In another embodiment, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, at least 80%, at least 90%, at least 95% or more of the plants comprise a microbe population that is substantially similar.

In a further aspect for certain of these methods and synthetic combinations, the plant is grown in an agricultural setting or environment, including a greenhouse. In one embodiment, the agricultural setting or environment comprises at least 100 plants. In another embodiment, the population occupies at least about 100 square feet of space, wherein at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% of the population comprises an effective amount of the microbe. In another embodiment, the population occupies at least about 100 square feet of space, wherein at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% of the population comprises the microbe in reproductive tissue. In still another embodiment, the population occupies at least about 100 square feet of space, wherein at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% of the population comprises at least 10 CFUs, 100 CFUs, 1,000 CFUs, 10,000 CFUs or more of the facultative fungal endophyte of the invention. In yet another embodiment, the population occupies at least about 100 square feet of space, wherein at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% of the population comprises the facultative fungal endophyte of the invention.

In one embodiment, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95% or more of the seeds in the population, contains a viable endophyte or endophytes disposed on the surface of the seeds. In a particular embodiment, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95% or more of the seeds in the population contains at least 10 CFU, for example, at least 30 CFU, at least 100 CFU, at least 300 CFU, at least 1,000 CFU, at least 3,000 CFU, at least 10,000 CFU or more, of the endophyte or endophytes coated onto the surface of the seed.

In a further aspect for certain of these methods and synthetic combinations, the endophytes that are native to the agricultural plant and whose colonization frequencies or ratios are altered may belong to phylum Ascomycota or Basidiomycota. In yet another aspect, the endophytes that are native to the agricultural plant may be of class Leotiomycetes, Dothideomycetes, Eurotiomycetes, Saccharomycetes, Sordariomycetes, Agaricomycetes, Microbotryomycetes, Tremellomycetes. In yet another aspect, the native endophytes may belong to order *Capnodiales, Pleosporales, Chaetothyriales, Eurotiales, Saccharomycetales, Diaporihales, Hypocreales, Ophiostomatales, Sordariales, Trichosphaeriales, Xylariales, Cantharellales, Corticiales, Polyporales, Russulales, Sporidiobolales*, or *Tremellales*. In a further aspect, the native endophytes may belong to genus *Davidiellaceae, Mycosphaerellaceae, Pleosporaceae, Didymellaceae, Sporormiaceae, Chaetothyriaceae, Trichocomaceae, Saccharomycetaceae, Gnomoniaceae, Cordycipitaceae, Nectriaceae, Hypocreaceae, Plectosphaerellaceae, Ophiostomataceae, Chaetomiaceae, Lasiosphaeriaceae, Trichosphaeriaceae, Ceratobasidiaceae, Corticiaceae, Coriolaceae, Peniophoraceae, Sporidiobolaceae*, or *Tremellaceae*. In a further aspect, the endophytes that are native to the agricultural plant may be a species from Table 2, namely *Cladosporium* sp., *Cladosporium cladosporioides, Davidiella* sp., *Cercospora* sp., *Cercospora beticola, Alternaria* sp., *Alternaria alternata, Alternaria citri, Alternaria tenuissima, Cochliobolus* sp., *Curvularia* sp., *Exserohilum* sp., *Lewia* sp., *Lewia infectoria, Pyrenophora* sp., *Pyrenophora tritici-repentis, Pleospora* sp., *Phoma americana, Preussia africana, Penicillium* sp., *Thermomyces* sp., *Thermomyces lanuginosus, Candida* sp., *Candida quercitrusa, Candida tropicalis, Cyberlindnera* sp., *Cyberlindnera jadinii, Kluyveromyces* sp., *Kluyveromyces maxianus, Gnomoniopsis* sp., *Beauveria bassiana, Cordyceps* sp., *Cordyceps bassiana, Fusarium* sp., *Gibellulopsis nigrescens, Hypocrea* sp., *Hypocrea lixii, Hypocrea vixens, Trichoderma* sp., *Trichoderma tomentosum, Verticillium* sp., *Ophiostoma* sp., *Ophiostoma dendifundum, Chaetomium* sp., *Chaetomium globosum, Thielavia hyrcaniae, Taifanglania* sp., *Taifanglania inflata, Schizothecium inaequale, Nigrospora* sp., *Rhizoctonia* sp., *Phanerochaete* sp, *Trametes* sp., *Trametes hirsuta, Trametes villosa, Rhodotorula* sp., *Rhodotorula mucilaginosa, Cryptococcus* sp, *Cryptococcus skinneri*, or *Tremella* sp.

In a further aspect for certain of these methods and synthetic combinations, the benefit provided by the facultative fungal endophyte to the agricultural plant is an improved agronomic property selected from the group consisting of increased biomass, increased tillering, increased root mass, increased flowering, increased yield, increased water use efficiency, reduction of yield loss, altered plant height, decreased time to emergence, increased seedling height, increased root length, increased chlorophyll levels, retention of developing flowers, retention of developing fruits, altered phytohormone levels, and enhanced resistance to environmental stress relative to a reference plant. In some aspects, the benefit provided is the alteration of levels of at least two phytohormones. In some aspects, the environmental stress is selected from the group consisting of drought stress, cold stress, heat stress, nutrient deficiency, salt toxicity, aluminum toxicity, grazing by herbivores, insect infestation, nematode infection, and fungal infection, bacterial infection and viral infection. In some aspects, the benefit to agricultural plants derived from the seed is increased yield in a population of said plants by about 5%, 10%, 15%, 20%, 30%, 40%, or 45% relative to a reference population of plants. In other aspects, the benefit to agricultural plants derived from the seed is a reduction of yield loss in a population of said plants by more than 40%, 30%, 20%, 10%, 5%, or 1% relative to a reference population of plants. In some aspects, treatment of seeds with facultative fungal endophytes may decrease thrip damage, decrease fleahopper damage, increase canopy temperature, increase drought tolerance, increase above ground biomass, and increase below ground biomass in the plants grown from the treated seeds.

In a further aspect for certain of these methods and synthetic combinations, the facultative fungal endophyte is present in the synthetic combination in an amount effective to obtain at least 50% colonization of the leaves, stems or roots of an agricultural plant grown from the seed.

In a further aspect for certain of these methods and synthetic combinations, the facultative fungal endophytes are capable of producing substances that are detrimental to pests. In certain aspects, the pest may be a nematode and/or an insect, for example, a root knot nematode, a aphid, a lygus bug, a stink bug, or combinations thereof.

In a further aspect for certain of these methods and synthetic combinations, the synthetic combination may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 facultative fungal endophytes. In one aspect, the invention provides a synthetic combination of a cotton plant or seed and a fungal endophyte comprising at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 endophytes selected from those in Table 1, wherein the cotton or seed is a host of the endophyte.

In another aspect, a seed coating is provided comprising a fungal endophyte comprising at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 endophytes from Table 1; and at least one sticker, wherein the fungal endophyte is in contact with the sticker. In certain aspects, the sticker may comprise, for example, alginic acid, carrageenan, dextrin, dextran, pelgel, polyethelene glycol, polyvinyl pyrrolidone, methyl cellulose, polyvinyl alcohol, gelatin, or combinations thereof. In certain aspects, the sticker may have a weight ratio between fungal endophyte and sticker of 1:1-10, 1:10-50, 1:50-100, 1:100-500, 1:500-1000, or 1:1000-5000. The seed coating may be a solid or fluid. In certain aspects, the seed coating is a powder. In certain aspects, the fungal endophyte may comprise fungal spores. In various aspects, the seed coating may comprise about 1, 2, 5, 10, 50, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, or 10^9 or more colony forming units per gram or spores per gram.

In certain embodiments, compositions for foliar or soil application may comprise a fungal endophyte comprising at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 endophytes from Table 1, and at least one carrier, surfactant or diluent. In certain aspects, the compositions may comprise may comprise about 1, 2, 5, 10, 50, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, or 10^9 or more colony forming units per gram or spores per gram. In various aspects, the composition may comprise water, a detergent, Triton X, insecticides, fungicides, or combinations thereof, for example. In further embodiments, seed compositions comprise a plant seed and the above-described seed coating. In certain aspects, the plant seed comprises a cotton seed, a seed of an agronomically elite plant, a dicot plant seed, and/or a monocot plant seed. In certain aspects, the seed composition may be resistant to a pest comprising an insect and/or a nematode.

In yet another aspect, the invention provides methods for preventing pest infestation or increasing yield, which may comprise treating a plant, plant seed, or the rhizosphere of said plant or seed with the endophyte containing compositions described herein. In certain aspects, the method may also comprise identifying a plant or seed as in need of endophyte treatment. The pest may comprise, for example, a nematode and/or insect. In certain aspects, the pest may comprise a root knot nematode, a aphid, a lygus bug, a stink bug, or combinations thereof.

In still yet another aspect, methods for preventing pest infestation are provided comprising obtaining a seed described herein and planting the seed. The method may further comprise identifying a need of preventing pest infestation. In certain aspects, the pest may comprise a nematode and/or a insect; and/or the pest may comprise a root knot nematode, a aphid, a lygus bug, a stink bug, or combinations thereof.

In a further embodiment, a method for treating a pest infestation comprises identifying a plant suspected of being infected with a pest, applying an above-described composition to the plant, whereby an endophyte-treated plant is generated. In certain aspects, the pest may comprise a nematode and/or an insect; and/or the pest may comprise a root knot nematode, a aphid, a lygus bug, a stink bug, or combinations thereof.

In still yet another aspect, a method of manufacturing pest-resistant seeds is provided comprising providing a fungal endophyte composition comprising at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 endophytes from Table 1, providing seeds; and combining the seeds with the endophyte composition, whereby pest-resistant seeds are generated. In certain aspects, the method increases the percentage of colonization with the endophyte of the plant developing from the seed.

In still yet another aspect, methods of increasing a yield of a crop or a reduction of loss are disclosed comprising providing a fungal endophyte composition comprising at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 endophytes from Table 1; and applying the endophyte composition to a seed, plant or part thereof, whereby the yield of the crop increases. In certain aspects, the crop may be cotton, and the increase of yield may be at least about 2%, 3% 5%, 15%, 20%, or 25% relative to a crop to which no endophyte composition has been applied. In certain aspects, the increase of yield is about 2%-5%, 3%-5%, 5%-10%, 10%-15%, or greater than about 20%, 30%, or more relative to a crop to which no endophyte composition has been applied. In certain aspects, the crop is cotton and the increase of yield comprises reduced boll damage. In certain aspects, the reduction of loss comprises reduction of loss due to insect infestation or drought, and the loss is less than 50%, 40%, 30%, 20%, 10%, 5%, or 5% relative to a crop to which no endophyte composition has been applied.

Also described herein are commodity plant products comprising a plant or part of a plant (including a seed) and further comprising the facultative fungal endophyte described above that is present in a detectable level, for example, as detected by the presence of its nucleic acid by PCR. In another aspect, disclosed is a method of producing a commodity plant product, comprising obtaining a plant or plant tissue from the synthetic combination described above, and producing the commodity plant product therefrom. The commodity plant product can be produced from the seed, or the plant (or a part of the plant) grown from the seed. The commodity plant product can also be produced from the progeny of such plant or plant part. The commodity plant product can be is selected from the group consisting of grain, flour, starch, seed oil, syrup, meal, flour, oil, film, packaging, nutraceutical product, an animal feed, a fish fodder, a cereal product, a processed human-food product, a sugar or an alcohol and protein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**: The colonization efficiencies demonstrate that endophytes can be manipulated in the field. Depicted are the mean+/−SE endophytic colonization frequencies of cotton seedlings under field conditions inoculated by seed treatments with different spore concentrations of either (left) *Paecilomyces lilacinus* or (right) *Beauveria bassiana.*

FIG. **2**: The endophytic fungus *Paecilomyces lilacinus* negatively affects root knot nematode (*Meloidogyne incognita*) reproduction when present as an endophyte in cotton. At high nematode inoculum levels (10,000 eggs), the endophyte reduced egg production in plants following treatment of seeds with solutions containing either 10^6 or 10^7 spores/

ml when compared to untreated control seeds. At field inoculum levels (2000 eggs), the presence of the endophyte significantly reduced both galls and egg production at both seed treatment concentrations.

FIG. **3**: Endophytic *Chaetomium globosum* negatively affects root-knot nematode reproduction. Negative effects of endophytic *Chaetomium globosum* on root-knot nematode gall formation and egg production following cotton seed soaking treatments in solutions of 0 (untreated controls), 10^6 and 10^8 spores/ml. Seedlings were inoculated with 1000 nematode eggs and grown in the greenhouse. Egg production by hatching nematodes that successfully infected the seedlings was quantified 60 days later.

FIG. **4**A: The effect of endophytic fungi on cotton aphids (*Aphis gossypii*) reproduction. FIG. **4**A demonstrates that the presence of *Beauveria bassiana* in cotton negatively affects the reproduction of cotton aphids.

FIG. **4**B: The effect of endophytic fungi on cotton aphids (*Aphis gossypii*) reproduction. FIG. **4**B demonstrates that the presence of *Paecilomyces lilacinus* in cotton negatively affects the reproduction of cotton aphids.

FIG. **5**: Effects of *Chaetomium globosum* on cotton aphids. Endophytic *Chaetomium globosum* in cotton negatively affects cotton aphid population growth rates as evidenced by reduced reproduction after 14 days on endophyte-colonized versus control plants. Cotton plants were grown from seeds treated by soaking in spore solutions of 0 (control), 10^6 (low) and 108 (high) spores/ml.

FIG. **6**A: The effect of the endophytic fungi *Beauveria bassiana* and *Paecilomyces lilacinus* on western tarnished plant bugs *Lygus hesperus* (Miridae). FIG. **6**A demonstrates that *Beauveria bassiana* and *Paecilomyces lilacinus* negatively affect host plant selection of western tarnished plant bugs when present as an endophyte in cotton.

FIG. **6**B: The effect of the endophytic fungi *Beauveria bassiana* and *Paecilomyces lilacinus* on western tarnished plant bugs *Lygus hesperus* (Miridae). FIG. **6**B demonstrates that *Beauveria bassiana* and *Paecilomyces lilacinus* negatively affect host plant selection behavior of western tarnished plant bugs when present as an endophyte in cotton.

FIG. **7**A: The effect of the endophytic fungi *Beauveria bassiana* and *Paecilomyces lilacinus* on southern green stink bugs (*Nezara viridula* (Pentatomidae). FIG. **7**A demonstrates that *Beauveria bassiana* and *Paecilomyces lilacinus* negatively affect host plant selection of southern green stink bugs when present as an endophyte in cotton.

FIG. **7**B: The effect of the endophytic fungi *Beauveria bassiana* and *Paecilomyces lilacinus* on southern green stink bugs (*Nezara viridula* (Pentatomidae). FIG. **7**B demonstrates that *Beauveria bassiana* and *Paecilomyces lilacinus* negatively affect host plant selection behavior of southern green stink bugs when present as an endophyte in cotton.

FIG. **8**: A reduction in cotton boll damage was observed during field trials. Relative to control plants, levels of insect-related boll damage were lower among plants that were treated by soaking seeds in spore solutions of *Beauveria bassiana* and *Paecilomyces lilacinus* at concentrations of 10^6 and 10^8 spore/ml.

FIG. **9**: Foliar application of cotton in the field with spores of endophytic entomopathogenic fungi improves plant performance. Cotton (variety FM1740B2F) seeds treated with a variety of typical fungicide (Metalaxyl, Triadimenol, Trifloxystrobin, 2-(Thiocyanome-thylthio) benzothioazole) and insecticide (Thiodicarb, Imidacloprid, Chloropyrifos) seed treatments were planted and grown under field conditions. The plants were sprayed at the 5th true leaf stage with aqueous solutions of *Beauveria bassiana* and *Paecilomyces fumosoroseus*. Sucrose was included (1% wt/vol) as an additional nutritional resource for the fungi. Significantly higher first position boll (developing fruit) retention was observed in plants sprayed with *Beauveria bassiana* without sucrose and *Paecilomyces fumosoroseus* plus sucrose.

FIG. **10**A: Positive effects of fungal endophytes on cotton plant performance under field conditions. FIG. **10**A demonstrates an early season trend for higher square retention in the treated versus untreated plants.

FIG. **10**B: Positive effects of fungal endophytes on cotton plant performance under field conditions. FIG. **10**B demonstrates that significantly more bolls were retained in the endophyte treatment groups later in the season, relative to control. This is demonstrated with both endophyte species used and with both seed treatment concentration employed (Repeated measures ANOVA: Time, $P<0.001$; Time*Endophyte, $P=0.045$, Endophyte, $P=0.003$).

FIG. **11**: Positive effects of fungal endophytes on cotton yields under field conditions. The data demonstrate that endophyte treatments achieved 25% higher yields in treated cotton plants.

FIG. **12**: Positive effects of fungal endophytes on *sorghum* (a) plant height and (b) total fresh biomass under growth chamber seedling assays. Data shown is average plant height (cm) and total fresh biomass (g) of n=10 independent replicates. Error bars represent ±1 standard error. All three fungal endophytes improve both traits relative to the untreated control.

FIG. **13**: The in-field modulation of the colonization of endogenous cotton endophytes in (a, b) stems and (c, d) roots when treated with fungal endophytes *Paecilomyces lilacinus* (a, c) and *Beauveria bassiana* (b, d). Data shown is a percentage change in colonization relative to the corresponding untreated control and plant tissue.

FIG. **14**: Average percent difference in yield between endophyte treated and control cotton plants (n=6 replicate plots in a dryland field, College Station, Tex.) for 15 facultative fungal endophytes in the Phytogen (PHY 499WRF) cultivar.

FIG. **15**: Aggregated average percent difference in yield between endophyte treated and control cotton plants (n=6 replicate plots in a dryland field, College Station, Tex.) for 15 facultative fungal endophytes and two cotton cultivars; Delta Pine (DP 0912B2RF) and Phytogen (PHY 499WRF). Bars represent a 95% confidence interval around the mean.

FIG. **16**: Average percent difference in thrip damage (A) and fleahopper damage (B) between endophyte treated and control cotton plants. The thrip damage was assessed in the Delta Pine (DP 0912B2RF) cultivar (n=6 replicate plots in a dryland field, College Station, Tex.) for 15 facultative fungal endophytes. 12 out of the 15 facultative fungal endophytes tested showed a decrease in thrip damage relative to the untreated cotton plants. The fleahopper damage was assessed in cotton plants of the Phytogen (PHY 499WRF) cultivar (n=6 replicate plots in a dryland field, College Station, Tex.) for 15 facultative fungal endophytes. 6 out of the 15 facultative fungal endophytes tested showed an average decrease in fleahopper damage as compared to untreated cotton plants.

FIG. **17**: Mid-season field-trait measured in June at the dryland trial of (A) root length and (B) belowground weight. Data presented is the average of n=10 independent replicates and error bars represent ±one standard error.

FIG. **18**: Mid-season field-trait measured in July at the dryland trial of canopy temperature (Celsius) for the (blue bars) Delta Pine and (green bars) Phyton cultivars. Data presented is the block-controlled average of n=10 independent replicates, relative to the control plot and error bars represent ±one standard error.

FIG. **19**: Mid-season field-trait measured in August at the dryland trial of NDVI for the (blue bars) Delta Pine and (green bars) Phyton cultivars. Data presented is the block-controlled average of n=10 independent replicates, relative to the control plot and error bars represent ±one standard error.

FIG. **20**: Mid-season field-trait measured in August at the dryland trial of first position square retention for the (blue bars) Delta Pine and (green bars) Phyton cultivars. Data presented is the block-controlled average of n=10 independent replicates, relative to the control plot and error bars represent ±one standard error.

FIG. **21**: Mid-season field-trait measured in August at the dryland trial of plant height (cm) for the (blue bars) Delta Pine and (green bars) Phyton cultivars. Data presented is the block-controlled average of n=10 independent replicates, relative to the control plot and error bars represent ±one standard error.

FIG. **22**: Mid-season field-trait measured in July at the dryland trial of plant height (cm) for the (blue bars) Delta Pine and (green bars) Phyton cultivars. Data presented is the block-controlled average of n=10 independent replicates, relative to the control plot and error bars represent ±one standard error.

FIG. **23**: Picture showing increased biomass in the plants treated with endophytes (right half of the image) compared to untreated control (left half of the image).

FIG. **24**: Table showing the time to wilt following drought stress in days for plants grown from seeds treated with fungal endophytes and control.

FIG. **25**: Table showing the time to death following drought stress in days for plants grown from seeds treated with fungal endophytes and control.

DETAILED DESCRIPTION OF THE INVENTION

Endophytic fungi are ubiquitous in nature, infecting virtually all plants in both natural and agronomic ecosystems. Plants commonly harbor a diversity of fungi living within their tissues as asymptomatic endophytes that can provide protection from a range of biotic and abiotic stressors. The present disclosure describes certain fungal endophytes that can be pathogens, parasites or antagonists to plant pathogens, insects, and nematode pests, thereby providing health and performance benefits to crop plants. The symbiotic endophyte-host relationships can provide several general health and fitness benefits to the host plant, such as enhancement of nutrition, increased drought tolerance and/or chemical defense from potential herbivores and often enhanced biomass production. Root-colonizing mycorrhizae survive on photosynthetic carbohydrates from the plant, and in return, aid in the solubilization and uptake of water and minerals to the host, which can lead to the promotion of seed germination and plant growth. Additionally, the association of a fungal endophyte with a host plant often provides protection from pathogens or tolerance to a variety of biotic and abiotic stresses, such as insect infestation, grazing, water or nutrient deficiency, heat stress, salt or aluminum toxicity, and freezing temperatures. Host growth and fitness promotion and protection are thought to be achieved through multiple beneficial properties of the endophyte-host association.

These fungal endophytes provided in Table 1 were originally collected as fungal endophytes of cotton. These endophytic fungi can be inoculated to live within cotton using either seed, soil or foliar applications and exhibited surprisingly beneficial effects by providing protection from pest infestation. Pests can be nematode and/or insect pests. In addition, these endophytic fungi have an unexpected beneficial effect on cotton yield.

Described is the application of beneficial fungi to establish endophytically within crop plants to improve plant performance and yield while conferring protection against insect and nematode pests. In this regard, the present invention overcomes the limitations of the prior art such as the susceptibility of the fungi to degradation by UV light, desiccation or heat after exposure to the environment following application as an inundative soil or foliar biopesticide. Inoculation and endophytic establishment of the fungi within the plant protects the fungi from UV light, desiccation, and unfavorable temperatures, while harboring the fungi in the very plant tissues they are intended to protect. Introducing fungi to live endophytically within plants requires no genetic modification of the plant or microorganisms, and the fungi themselves can be a source for natural products. In various embodiments, the fungal inoculant can be formulated and applied, for example, as treatment of seeds, in furrow applications, before or during planting, or as foliar application after plant germination, and after inoculation, the fungal endophytes provide season-long protective effects and higher crop yields (approximately 25% higher). In certain embodiments, the increase of yield is about 5%, 10%, 15%, 20%, 30%, 40%, 45%, 50%, or greater than 50% relative to a crop to which no endophyte composition has been applied. In further embodiments, the increase of yield is the result of reduction of loss that comprises reduction of loss due to insect infestation or drought and the loss is less than 50%, 40%, 30%, 20%, 10%, 5%, or 5% relative to a crop to which no endophyte composition has been applied. In certain embodiments, the crop is cotton and the reduction of loss comprises reduced boll damage.

Thus, in one aspect, the invention provides a combination (also termed a “symbiotum”) of a host plant and an endophyte that allows for improved agronomic properties of host plants. The combination may be achieved by artificial inoculation, application, or other infection of a host plant or seeds thereof, such as a cotton plant or seed thereof, or host plant tissues, with a fungal endophyte strain of the present invention. Thus, a combination achieved by such an inoculation is termed a “synthetic” combination, synthetic composition, synthetic seed coating, and/or synthetic pest-resistant seed composition. The fungal endophyte may be present in intercellular spaces within plant tissue, such as the root. Its presence may also occur or may also be maintained within a plant or plant population by means of grafting or other inoculation methods such as treating seeds, plants or parts thereof with endophyte mycelia, or endophyte spores. In certain embodiments, the plant, part of the plant, roots, seed, or leaves are sterilized to remove microorganisms before applying the endophyte. In particular embodiments, seeds are sterilized to remove native endophytes before adding the endophyte compositions herein described. In certain aspects, the ability of the seed to germinate is not affected by the sterilization.

The invention also provides methods for detecting the presence of the fungal endophyte of the present invention within a host plant. This may be accomplished, for instance, by isolation of total DNA from tissues of a potential plant-endophyte combination, followed by PCR, or alternatively, Southern blotting, western blotting, or other methods known in the art, to detect the presence of specific nucleic or amino acid sequences associated with the presence of a fungal endophyte strain of the present invention. Alternatively, biochemical methods such as ELISA, HPLC, TLC, or fungal metabolite assays may be utilized to determine the presence of an endophyte strain of the present invention in a given sample of crop tissue. Additionally, methods for identification may include microscopic analysis, such as root staining, or culturing methods, such as grow out tests or other methods known in the art (Deshmukh et al. 2006). In particular embodiments, the roots of a potential grass plant-endophyte combination may be stained with fungal specific stains, such as WGA-Alexa 488, and microscopically assayed to determine fungal root associates.

In certain embodiments, the agronomic qualities may be selected from the group consisting of: increased biomass, increased tillering, increased root mass, increased flowering, increased seed yield, and enhanced resistance to biotic and/or abiotic stresses, each of these qualities being rated in comparison to otherwise identical plants grown under the same conditions, and differing only with respect to the presence or absence of a fungal endophyte. The synthetic combinations and methods of the present invention may be applied to respond to actual or anticipated stresses. Such stresses may include, for instance, drought (water deficit), cold, heat stress, nutrient deficiency, salt toxicity, aluminum toxicity, grazing by herbivores, insect infestation, nematode infection, and fungal, bacteria or viral infection, among others.

The present disclosure provides, in one embodiment, fungal endophytes selected from those in Table 1 that negatively affect the reproduction of insect herbivores feeding on leaves above ground (cotton aphids, *Aphis gossypii*) and plant parasitic nematodes attacking roots below ground (root knot nematodes, *Meloidogyne incognita*). In addition, improved plant performance and yields in colonized versus uncolonized control plants may be observed in field trials employing seed treatment with such endophytes. Plant growth enhancement and increased resistance to root knot nematodes was demonstrated in cotton, for example, employing *Chaetomium globosum* as an endophyte in greenhouse trials. In addition and as a further non-limiting illustrative example, using *Beauveria bassiana* as an endophyte in cotton, reductions in insect (cotton aphid) reproduction was demonstrated in both greenhouse and field trials. The endophytic presence of *Paecilomyces lilacinus* and *Beauveria bassiana* also had negative effects on the host selection behavior of key sucking bug pests (*Lygus hesperus* and *Nezara viridula*) that attack developing flowers and fruits in cotton. Furthermore, in field trials using *Beauveria bassiana* as an endophyte in cotton positive effects on plant performance and higher yields in endophyte colonized versus uncolonized control plants was demonstrated.

Metabolomic differences between the plants can be detected using methods known in the art. For example, a biological sample (whole tissue, exudate, phloem sap, xylem sap, root exudate, etc.) from the endophyte-associated and reference agricultural plants can be analyzed essentially as described in Fiehn et al., (2000) Nature Biotechnol., 18, 1157-1161, or Roessner et al., (2001) Plant Cell, 13, 11-29. Such metabolomic methods can be used to detect differences in levels in hormones, nutrients, secondary metabolites, root exudates, phloem sap content, xylem sap content, heavy metal content, and the like.

In another embodiment, the present invention contemplates methods of coating the seed of a plant with a plurality of endophytes, as well as seed compositions comprising a plurality of endophytes on and/or in the seed. The methods according to this embodiment can be performed in a manner similar to those described herein for single endophyte coating. In one example, multiple endophytes can be prepared in a single preparation that is coated onto the seed. The endophytes can be from a common origin (i.e., a same plant). Alternatively, the endophytes can be from different plants.

Where multiple endophytes are coated onto the seed, any or all of the endophytes may be capable of conferring a beneficial trait onto the host plant. In some cases, all of the endophytes are capable of conferring a beneficial trait onto the host plant. The trait conferred by each of the endophytes may be the same (e.g., both improve the host plant's tolerance to a particular biotic stress), or may be distinct (e.g., one improves the host plant's tolerance to drought, while another improves phosphate utilization). In other cases the conferred trait may be the result of interactions between the endophytes.

DEFINITIONS

In the description and tables herein, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, the following definitions are provided. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

When a term is provided in the singular, the inventors also contemplate aspects of the invention described by the plural of that term. The singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes one or more cells, including mixtures thereof.

The term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and agriculturally acceptable carriers. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for applying the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

Biological control: the term "biological control" and its abbreviated form "biocontrol," as used herein, is defined as control of a pest, pathogen, or insect or any other undesirable organism by the use of at least one endophyte.

A "composition" is intended to mean a combination of active agent and at least another compound, carrier or composition, inert (for example, a detectable agent or label or liquid carrier) or active, such as a pesticide.

As used herein, an "agricultural seed" is a seed used to grow plants in agriculture (an "agricultural plant"). The seed may be of a monocot or dicot plant, and is planted for the production of an agricultural product, for example grain, food, fiber, etc. As used herein, an agricultural seed is a seed that is prepared for planting, for example, in farms for growing. Agricultural seeds are distinguished from commodity seeds in that the former is not used to generate products, for example commodity plant products.

As used herein, a "commodity plant product" refers to any composition or product that is comprised of material derived from a plant, seed, plant cell, or plant part of the present invention. Commodity plant products may be sold to consumers and can be viable or nonviable. Nonviable commodity products include but are not limited to nonviable seeds and grains; processed seeds, seed parts, and plant parts; dehydrated plant tissue, frozen plant tissue, and processed plant tissue; seeds and plant parts processed for animal feed for terrestrial and/or aquatic animal consumption, oil, meal, flour, flakes, bran, fiber, and any other food for human or animal consumption; and biomasses and fuel products. Any such commodity plant product that is derived from the plants of the present invention may contain at least a detectable amount of the specific and unique DNA corresponding to the endophytes described herein. Any standard method of detection for polynucleotide molecules may be used, including methods of detection disclosed herein.

As used herein, the phrase "agronomically elite plants" refers to a genotype or cultivar with a phenotype adapted for commercial cultivation. Traits comprised by an agronomically elite plant may include biomass, carbohydrate, and/or seed yield; biotic or abiotic stress resistance, including drought resistance, insect resistance, fungus resistance, virus resistance, bacteria resistance, cold tolerance, and salt tolerance; improved standability, enhanced nutrient use efficiency, and reduced lignin content.

In certain embodiments, cotton agronomically elite plants include, for example, known cotton varieties AM 1550 B2RF, NG 1511 B2RF, NG 1511 B2RF, FM 1845LLB2, FM 1944GLB2, FM 1740B2F, PHY 499 WRF, PHY 375 WRF, PHY 367 WRF, PHY 339 WRF, PHY 575 WRF, DP 1252 B2RF, DP 1050 B2RF, DP 1137 B2RF, DP 1048 B2RF, and/or DP 1137 B2RF.

As used herein, the phrase "culture filtrate" refers to broth or media obtained from cultures inoculated with a strain of fungi and allowed to grow. The media is typically filtered to remove any suspended cells, leaving the nutrients, hormones, or other chemicals.

As used herein, the term "endophyte" refers to an organism capable of living within a plant or plant tissue. An endophyte may comprise a fungal organism that may confer an increase in yield, biomass, resistance, or fitness in its host plant. Fungal endophytes may occupy the intracellular or extracellular spaces of plant tissue, including the leaves, stems, flowers, or roots.

The phrase "pest resistance" refers to inhibiting or reducing attack from pests. Pest resistance provides at least some increase in pest resistance over that which is already possessed by the plant.

As used herein, the term "genotypes" refers to the genetic constitution of a cell or organism.

As used herein, the term "phenotype" refers to the detectable characteristics of a cell or organism, which characteristics are either the direct or indirect manifestation of gene expression.

As used herein, the phrase "host plant" refers to any plant that an endophytic fungi colonizes. In certain embodiments, the host plant comprises progeny of colonized plant.

As used herein, the phrase "increased yield" refers to an increase in biomass or seed weight, seed or fruit size, seed number per plant, seed number per unit area, bushels per acre, tons per acre, kilo per hectare, carbohydrate yield, or cotton yield. Such increased yield is relative to a plant or crop that has not been inoculated with the endophyte. In certain embodiments, the increase yield is relative to other commonly used pest treatments or other methods of addressing the biotic or abiotic stress.

As used herein, the phrase "biomass" means the total mass or weight (fresh or dry), at a given time, of a plant tissue, plant tissues, an entire plant, or population of plants, usually given as weight per unit area. The term may also refer to all the plants or species in the community (community biomass).

As used herein, "sticker" refers to compounds to enhance binding of spores to the seed surface. Non-limiting examples of such compounds are alginic acid, carrageenan, dextrin, dextran, pelgel, polyethelene glycol, polyvinyl pyrrolidone, methyl cellulose, polyvinyl alcohol, or gelatin.

As used herein, an "agriculturally acceptable" excipient or carrier is one that is suitable for use in agriculture without undue adverse side effects to the plants, the environment, or to humans or animals who consume the resulting agricultural products derived therefrom commensurate with a reasonable benefit/risk ratio.

As used herein, the term "synthetic" or the phrase "synthetic combination" refers to an artificial combination that includes mycelia and/or spores of a endophyte that is or leads to an endophytic fungal-host relationship (also termed a "symbiotum") of a host plant and an endophyte. The synthetic combination may be achieved, for example, by artificial inoculation, application, or other infection of a host plant, host plant seeds, or host plant tissues with the endophyte. In addition, the combination of host plant and an endophyte may be achieved by inoculating the soil or growth media of the plant.

The present invention contemplates the use of "isolated" microbe. As used herein, an isolated microbe is a microbe that is isolated from its native environment, and carries with it an inference that the isolation was carried out by the hand of man. An isolated microbe is one that has been separated from at least some of the components with which it was previously associated (whether in nature or in an experimental setting) or occurs at a higher concentration, viability, or other functional aspect than occurring in its native environment. Therefore, an "isolated" microbe is partially or completely separated from any other substance(s) as it is found in nature or as it is cultured, propagated, stored or subsisted in naturally or non-naturally occurring environments. Specific examples of isolated microbes include partially pure microbes, substantially pure microbes and microbes cultured in a medium that is non-naturally occurring.

As used herein, a microbe is considered to be "native" to a plant or a portion of the plant, and is said to be "natively" present in the plant or a portion of plant, if that plant or portion of the plant contains the microbe, for example, in the absence of any contacting with the microbe preparation, or contains the microbe at much lower concentrations than the contacting with the microbe preparation would provide.

Some of the methods described herein allow the colonization of plant seeds by microbes. As used herein, a microbe is said to "colonize" a plant or seed when it can exist in a symbiotic or non-detrimental relationship with the plant in the plant environment, for example on, in close proximity to or inside a plant, including the seed.

A "population" of plants, as used herein, refers to a plurality of plants that were either grown from the seeds treated with the endophytes as described herein, or are progeny of a plant or group of plants that were subjected to the inoculation methods. The plants within a population are typically of the same species, and/or typically share a common genetic derivation.

EXAMPLES

Example 1

Creating Spore Suspensions and Treatment of Seeds

Cultivation of plants and endophytic fungi strains: The cotton seed variety used in particular embodiments was variety LA122 (available from All-Tex Seed, Inc., Levelland, Tex. 79336). *Paecilomyces lilacinus* and *Chaetomium globosum* were obtained from cotton plants as described (Ek-Ramos et al. 2013, PLoS ONE 8(6): e66049. doi: 10.1371/journal.pone.0066049). Persons of ordinary skill in the art can obtain endophytes suitable for performing the various embodiments of the present invention by performing the procedures described therein. In short, plant samples were rinsed in tap water and surface sterilized by immersion in 70% ethanol for 5 min, 10% bleach solution for 3 min, and rinsed twice with autoclaved distilled water. Samples were blotted dry using autoclaved paper towels. Five individual surface sterilized leaves, squares and bolls (N=15 total samples) were randomly selected and imprinted onto fresh potato dextrose agar (PDA) and V8 media as a way to monitor surface sterilization efficiency. For endophyte isolation, leaves were cut in small fragments of approximately 1 cm^2. Squares and bolls were cut in six pieces. Any fiber present was removed and cut into six smaller pieces. Leaf fragments were placed upside down on PDA and V8 medium plates in triplicate. Each plate contained 3 leaf fragments for a total of 9 fragments assayed per plant. For squares collected early in the season, 3 slices per square were plated on PDA and V8 media as with the leaf fragments. Because of similarity in size and location within a plant, when collected later in the season, squares and bolls from a given plant were plated together on petri dishes containing two square slices, two boll slices and two pieces of fiber. Antibiotics Penicillin G (100 Units/mL) and Streptomycin (100 µg/mL) (Sigma, St Louis, Mo., USA) were added to the media to suppress bacterial growth. All plates were incubated in the dark at room temperature for, in average, two weeks until growth of fungal endophyte hyphae from plant tissues was detected.

An inclusive combination of morphological and molecular fungal endophyte identification was employed for identification. Once fungal hyphae were detected growing from the plant material, samples were taken to obtain pure fungal isolates. For identification by PCR, genomic DNA was extracted from mycelium of each isolated fungal strain, following a chloroform:isoamyl alcohol 24:1 protocol and fungal specific primers were used to amplify the ITS (Internal Transcribed Spacer) region of nuclear ribosomal DNA. This region is the primary barcoding marker for fungi and includes the ITS1 and ITS2 regions, separated by the 5.8S ribosomal gene. In order to avoid introducing biases during PCR (taxonomy bias and introduction of mismatches), it has been suggested to amplify the ITS1 region only, therefore the primers ITS1 (5' TCC GTA GGT GAA CCT GCG G 3') (SEQ ID NO:5) and ITS2 (5' GCT GCG TTC TTC ATC GAT GC 3') (SEQ ID NO:6) were used to amplify and sequence the ~240 bp ITS1 region of each one of the isolated fungal strains. The resulting sequences were aligned as query sequences with the publicly available databases GenBank nucleotide, UNITE and PlutoF. The last two are specifically compiled and used for fungi identification. Table 1 provides a list of endophytes identified and useful in the present invention. All of these endophytes belong to phylum Ascomycota, subphylum Pezizomycotina, except for *Phanerochaete crassa*, which belongs to phylum Basidiomycota, subphylum Agaricomycotina, and *Pseudozyma* sp, which belongs to phylum Basidiomycota, subphylum Ustilaginomycotina. Table 1 shows the species/genus, family, order, subclass, class, and the SEQ ID NO corresponding to the ~240 bp ITS1 region for each one of the isolated fungal strains, except for *Beauveria bassiana, Aspergillus parasiticus, Lecanicillium lecanii*, and *Paecilomyces lilacinus*, where the sequences shown includes the ITS1, ITS2, 5.8S, 18S, and 285 sequences and were obtained from the UNITE database for GenBank numbers JF837090, JX857815, FJ643076, and EU553283, respectively.

TABLE 1

endophytes identified and useful in the present invention

Genus/Species	Family	Order	Subclass	Class	SEQ ID NO.
Acremonium alternatum	Incertaesedis	Hypocreales	Hypocreomycetidae	Sordariomycetes	7
Alternaria alternata	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	8
Alternaria brassicae	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	9
Alternaria compacta	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	10
Alternaria dianthi	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	11
Alternaria longipes	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	12
Alternaria mali	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	13
Alternaria sesami	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	14
Alternaria solani	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	15
Alternaria sp.	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	16
Alternaria tenuissima	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	17
Bipolaris spicifera	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	18
Cercospora canescens	Mycosphaerellaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	19
Cercospora capsici	Mycosphaerellaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	20
Cercospora kikuchii	Mycosphaerellaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	21

TABLE 1-continued

endophytes identified and useful in the present invention

Genus/Species	Family	Order	Subclass	Class	SEQ ID NO.
Cercospora zinnia	Mycosphaerellaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	22
Chaetomium globosum	Chaetomiaceae	Sordariales	Sordariomycetidae	Sordariomycetes	23
Chaetomium piluliferum	Chaetomiaceae	Sordariales	Sordariomycetidae	Sordariomycetes	24
Chaetomium sp.	Chaetomiaceae	Sordariales	Sordariomycetidae	Sordariomycetes	25
Cladosporium cladosporioides	Cladosporiaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	26
Cladosporium sp.	Cladosporiaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	27
Cladosporium uredinicola	Cladosporiaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	28
Cochliobolus sp	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	29
Phanerochaete crassa	Phanerochaetaceae	Polyporales	Incertae sedis	Agaricomycetes	30
Phoma americana	Incertae sedis	Pleosporales	Pleosporomycetidae	Dothideomycetes	31
Phoma subherbarum	Incertae sedis	Pleosporales	Pleosporomycetidae	Dothideomycetes	32
Phomopsis liquidambari	Diaporthaceae	Diaporthales	Sordariomycetidae	Sordariomycetes	33
Phomopsis sp.	Diaporthaceae	Diaporthales	Sordariomycetidae	Sordariomycetes	34
Pleospora sp.	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	35
Pleosporaceae sp.	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	36
Preussia africana	Sporormiaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	37
Preussia sp.	Sporormiaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	38
Pseudozyma sp.	Ustilaginaceae	Ustilaginales	Ustilaginomycetidae	Ustilaginomycetes	39
Pyrenophora teres	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	40
Colletotrichum capsici	Glomerellaceae	Incertae sedis	Sordariomycetidae	Sordariomycetes	41
Coniolariella gamsii	Incertae sedis	Xylariales	Xylariomycetidae	Sordariomycetes	42
Coniothyrium aleuritis	Coniothyriaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	43
Coniothyrium sp.	Coniothyriaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	44
Corynespora cassiicola	Corynesporascaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	45
Diaporthe sp.	Diaporthaceae	Diaporthales	Sordariomycetidae	Sordariomycetes	46
Diatrype sp.	Diatrypaceae	Xylariales	Xylariomycetidae	Sordariomycetes	47
Drechslerella dactyloides	Orbiliaceae	Orbiliales	Orbiliomycetidae	Orbiliomycetes	48
Embellisia indefessa	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	49
Epicoccum nigrum	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	50
Epicoccum sp.	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	51
Exserohilum rostratum	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	52
Fusarium chlamydosporum	Nectriaceae	Hypocreales	Hypocreomycetidae	Sordariomycetes	53
Fusarium sp.	Nectriaceae	Hypocreales	Hypocreomycetidae	Sordariomycetes	54
Gibellulopsis nigrescens	Plectosphaerellaceae	Incertae sedis	Hypocreomycetidae	Sordariomycetes	55
Gnomoniopsis sp.	Glomerellaceae	Incertae sedis	Hypocreomycetidae	Sordariomycetes	56
Lewia infectoria	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	57
Mycosphaerella coffeicola	Mycosphaerellaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	58
Mycosphaerellaceae sp.	Mycosphaerellaceae	Capnodiales	Dothideomycetidae	Dothideomycetes	59
Nigrospora oryzae	Incertae sedis	Trichosphaeriales	Incertae sedis	Sordariomycetes	60
Nigrospora sp.	Incertae sedis	Trichosphaeriales	Incertae sedis	Sordariomycetes	61
Nigrospora sphaerica	Incertae sedis	Trichosphaeriales	Incertae sedis	Sordariomycetes	62
Paecilomyces sp.	Trichocomaceae	Eurotiales	Eurotiomycetidae	Eurotiomycetes	63
Penicillium citrinum	Trichocomaceae	Eurotiales	Eurotiomycetidae	Eurotiomycetes	64
Retroconis sp.	Incertae sedis	Incertae sedis	Incertae sedis	Incertae sedis	65
Rhizopycnis sp.	Incertae sedis	Incertae sedis	Incertae sedis	Dothideomycetes	66
Schizothecium inaequale	Lasiosphaeriaceae	Sordariales	Sordariomycetidae	Sordariomycetes	67
Stagonospora sp.	Phaeosphaeriaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	68
Stemphylium lancipes	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	69

TABLE 1-continued

endophytes identified and useful in the present invention					
Genus/Species	Family	Order	Subclass	Class	SEQ ID NO.
Thielavia hyrcaniae	Chaetomiaceae	Sordariales	Sordariomycetidae	Sordariomycetes	70
Thielavia sp.	Chaetomiaceae	Sordariales	Sordariomycetidae	Sordariomycetes	71
Ulocladium chartarum	Pleosporaceae	Pleosporales	Pleosporomycetidae	Dothideomycetes	72
Verticillium sp.	Plectosphaerellaceae	Incertae sedis	Hypocreomycetidae	Sordariomycetes	73
Beauveria bassiana	Cordycipitaceae	Hypocreales	Hypocreomycetidae	Sordariomycetes	74
Aspergillus parasiticus	Trichocomaceae	Eurotiales	Eurotiomycetidae	Eurotiomycetes	75
Lecanicillium lecanii	Cordycipitaceae	Hypocreales	Hypocreomycetidae	Sordariomycetes	76
Paecilomyces lilacinus	Trichocomaceae	Eurotiales	Eurotiomycetidae	Eurotiomycetes	77

TABLE 1 List of endophytes:

Acremonium alternatum, Alternaria alternata, Alternaria brassicae, Alternaria compacta, Alternaria dianthi, Alternaria longipes, Alternaria mali, Alternaria sesami, Alternaria solani, Alternaria sp., *Alternaria tenuissima, Ascomycota* sp., *Bipolaris spicifera, Cercospora canescens, Cercospora capsici, Cercospora kikuchii, Cercospora zinnia, Chaetomium globosum, Chaetomium piluliferum, Chaetomium* sp., *Cladosporium cladosporioides, Cladosporium* sp., *Cladosporium uredinicola, Cochliobolus* sp, *Phanerochaete crassa, Phoma americana, Phoma subherbarum, Phomopsis liquidambari, Phomopsis* sp., *Pleospora* sp., *Pleosporaceae* sp., *Polyporales* sp., *Preussia africana, Preussia* sp., *Pseudozyma* sp., *Pyrenophora teres, Colletotrichumcapsici, Coniolariella gamsii, Coniothyrium aleuritis, Coniothyrium* sp., *Corynespora cassiicola, Diaporthe* sp., *Diatrype* sp., *Drechslerella dactyloides, Embellisia indefessa, Epicoccum nigrum, Epicoccum* sp., *Exserohilum rostratum, Fusarium chlamydosporum, Fusarium* sp., *Gibellulopsis nigrescens, Gnomoniopsis* sp., *Lewia infectoria, Mycosphaerella coffeicola, Mycosphaerellaceae* sp., *Nigrospora oryzae, Nigrospora* sp., *Nigrospora sphaerica, Paecilomyces* sp., *Penicillium citrinum, Retroconis* sp., *Rhizopycnis* sp., *Schizothecium inaequale, Stagonospora* sp., *Stemphylium lancipes, Thielavia hyrcaniae, Thielavia* sp., *Ulocladium chartarum, Verticillium* sp., *Beauveria bassiana, Aspergillus parasiticus, Lecanicillium lecanii, Paecilomyces lilacinus.*

Beauveria bassiana was cultured from a commercially obtained strain (available from Botanigard). *Beauveria bassiana, Paecilomyces lilacinus*, and *Chaetomium globosum* were cultured on potato dextrose agar media (PDA). Stock spore concentration solutions of each fungi were made by adding 10 ml of sterile water to the fungi plates and scraping them free of the agar with a sterile scalpel. The resulting mycelia and spores obtained were then filtered into a sterile beaker utilizing a cheese cloth to filter out the mycelia, thereby creating stock solutions. A haemocytometer was used to measure and calculate spore concentrations of the stock solutions. The desired concentrations were created by dilution, and seeds were placed into spore suspensions with the desired spore concentrations. In various embodiments, the final treatment concentrations can be about 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, or 10^9 spores/ml which can be reached by serial dilutions in sterile water or in an appropriate solution or buffer.

For seed inoculation, the seeds were surface sterilized prior to soaking them in spore suspensions with the desired concentration by immersion the seeds in 70% ethanol for 3 minutes with constant shaking followed by incubation in 2% NaOCl for 3 minutes; followed by three washes in sterile water. The third sterile water wash was plated onto potato dextrose agar media (PDA) to confirm that surface sterilization was effective. Seeds were then soaked for 24 hours in beakers containing spore suspensions with two different concentrations of fungi. Control group seeds were treated with sterile water only. Spore concentrations for *Beauveria bassiana* were zero (control), 1×10^6 (treatment 1) and 1×10^9 (treatment 2) and for *Paecilomyces lilacinus* or *Chaetomium globosum* were zero (control), 1×10^6 (treatment 1) and 1×10^7 (treatment 2). These beakers were incubated for 24 hours at 32° C. in a culture chamber until next day for planting (24 hr).

Soaked seeds were planted in L22 mix soil (Borlaug Institute, Texas A&M). All plants were grown in a laboratory greenhouse at ~28° C. with a natural light photoperiod. There was no fertilization of the plants, and watering was done consistently across all treatments as needed.

Direct seed inoculation: In particular embodiments, individual seeds and the surrounding soil can be directly inoculated with the spore solution (10^2-10^3, 10^3-10^4, 10^4-10^5, 10^6-10^7, or 10^7-10^8 spores/ml) at planting before covering the seed with soil.

In various embodiments, any seed or plant treatments that are suitable for application of biological agents to seeds or plants and known to persons having ordinary skill in the art can be employed.

Example 2

Application of Endophyte Spores as a Dry Powder Composition

In addition to application of a spore solution for seed treatment, the endophytes or endophyte spores can also be applied as dry powder or using a sicker such as methyl cellulose for seed treatment. In certain embodiments, the concentration may be at least 10^5, 10^6, 10^7, 10^8, 10^9, or higher colony forming units or spores/g dry weight.

In certain embodiments, endophytes can be grown in fungi cultivation media in a fermenter. Endophytic mycelial fragments or spores can be collected, dried and ground. A sticker such as caboxymethyl cellulose may also be added to the ground endophytic material.

In certain embodiments the weight ratio between endophytic material and sticker may be between 1:10-50, 1:50-

100, 1:100-500, or 1:500-1000 to obtain the seed coating or seed inoculation material. This seed inoculation material can be applied to seeds. In various embodiments, the weight ratio between seed inoculation material and seed may be 1:10-50, 1:50-100, 1:100-500, 1:500-1000, or 1:1000-5000.

Example 3

Soil (in Furrow) Endophyte Treatments

Soil drench (in furrow) application may be performed by applying an endophyte composition to the surface of the soil and/or seed during planting. In particular embodiments, the endophyte composition may comprise an endophyte suspension or an endophyte dry powder formulation. In various embodiments the endophyte may comprise mycelia and/or spores. In particular embodiments, the soil drench application may comprise applying the endophyte composition to the surface of the soil directly above each seed. In certain embodiments, the endophyte composition may comprise 0.01-0.1, 0.1-1, or 1-10 ml endophyte suspension, which may be a endophyte spore suspension.

Soil inoculation: In certain embodiments, seeds can be planted into inoculated soil. The inoculum can be obtained by multiplying the endophyte on fungal growth media. The fungal growth media can be potato dextrose agar media (PDA). In other embodiments the fungal growth media can be as wheat grain. In a non-limiting example, 100 g of wheat grain can be washed and soaked overnight in sterile water. Excess water can be drained, seeds dried on paper towel, packed in a 500 ml conical flask and autoclaved at 15 psi for 1 h. One milliliter of the endophytic fungal spore suspension (10^7 spores/ml) can be inoculated to the flask, and the cultures can be incubated at 25° C. for 2 weeks. To avoid clumping, the flasks can be shaken vigorously to separate the grain and break the mycelial mat. Approximately 5 g of inoculum can be placed in soil at planting. In certain embodiments, the inoculum can be placed in the soil at the same time or within 1 month of planting the seeds. In certain embodiments, the seeds may comprise sterilized seeds.

Example 4

Foliar Endophyte Treatments

Plants were inoculated via foliar application at the third true leaf stage by spraying the surface of fully expanded leaves to run-off with a spore suspension (10^8 spores/ml) using a hand-held plastic sprayer (1 L). In certain embodiments, endophyte spore suspensions were made in water. In certain embodiments, the water was supplemented with a detergent. In a particular non-limiting example, the spore suspension contained 0.02% Triton X 100 as a detergent.

Foliar endophyte treatment may be performed using any suitable method known to a person having ordinary skill in the art. In particular, foliar endophyte treatment may be performed using a sprayer by directly spraying leaves with an endophyte suspension, which may be a endophyte spore suspension.

FIG. **9** demonstrates that foliar application of cotton in the field with spores of endophytic entomopathogenic fungi improved plant performance. Cotton (variety FM1740B2F) seeds were treated with a variety of typical fungicide (Metalaxyl, Triadimenol, Trifloxystrobin, 2-(Thiocyanome-thylthio) benzothioazole) and insecticide (Thiodicarb, Imidacloprid, Chloropyrifos), and seed treatments were planted and grown under field conditions. The plants were sprayed at the 5th true leaf stage with aqueous solutions of *Beauveria bassiana* and *Paecilomyces fumosoroseus*. Sucrose was included (1% wt/vol) as an additional nutritional resource for the fungi. Significantly higher first position boll (developing fruit) retention was observed in plants sprayed with Beauveria *bassiana* without sucrose and *P. fumosoroseus* plus sucrose.

Example 5

Confirmation of Plant Colonization by Endophytic Fungi

Plants were individually placed in plastic bags, which were labeled with plant number, treatment, and final aphid number, and stored in 4° C. until the next day for endophyte confirmation. Half of each plant was utilized for plating on PDA agar and the other half was freeze-dried for to conduct diagnostic PCR assays for endophyte confirmation. The surface sterilization protocol and plating of third sterile water wash on PDA to test for surface contamination was conducted as described above. For diagnostic PCR assays, plant tissue was freeze-dried and DNA was extracted utilizing the CTAB protocol (Doyle & Doyle, 1987, Phytochemistry Bulletin 19:11-15). The oligonucleotide primer sequences synthesized were based upon a NCBI BLAST search corresponding to the laboratory culture sequence results isolated (Ek-Ramos et al., 2013). Sense and antisense oligonucleotide sequences for *Beauveria bassiana* were: 5'-CGGCGGACTCGCCCCAGCCCG-3' (SEQ ID NO:1) and CCGCGTCGGGGTTCCGGTGCG-3' (SEQ ID NO:2) respectively. The oligonucleotides used to amplify *Paecelomyces lilacinus* were: 5' CTCAGTTGCCTCGGCGGGAA 3' (SEQ ID NO:3) and 5' GTGCAACTCAGAGAAGAAATTCCG **3'** (SEQ ID NO:4).

The PCR protocol consisted of a denaturation step at 95° C. for 5 min, followed by alignment of oligonucleotides at 56° C. for 2 min and an extension step of 7 min at 72° C. with a total of 35 cycles. The PCR products were visualized in a 2% agarose gel containing 1% ethidium bromide. Electrophoresis was performed at 70 volts for 30 min.

Example 6

Endophytic Fungi can be Manipulated in the Field

A field trial using isolates of *Paecilomyces lilacinus* and Beauveria *bassiana* was conducted during the summer. A randomized block design with five replicate plots that were planted with seeds that were inoculated by soaking for 9 hr in three different aqueous spore concentrations (0, 10^6, or 10^8 spores/ml) of the candidate endophyte (such as *Paecilomyces lilacinus* or *Beauveria bassiana*). Each plot consisted of four 15.24 m (40 ft) rows, each separated by 101.6 cm (40 in).

Colonization efficiency: At the first true leaf stage, four plants from each plot for a total of 20 plants per treatment were randomly sampled and tested for colonization by each of the candidate endophytes. Colonization frequencies were determined by incubating surface sterilized root, stem and leaf fragments on PDA media and observing for fungal growth. Colonization frequencies are reported as the number of plants per treatment group with at least one positively colonized plant fragment.

The high endophytic colonization frequency of seedlings by *Paecilomyces lilacinus* or *Beauveria bassiana* demonstrates that the presence of specific endophytes can be manipulated under field planting conditions (FIG. **1**).

Example 7

Cotton Aphid Reproduction Test

A colony of *A. gossypii* was reared on cotton in cages in a greenhouse kept at approximately 28° C. with natural light photoperiod. Second instar nymphs were placed directly onto endophyte-treated cotton plants and control plants. Ten plants were utilized per treatment group and ten aphids were placed per plant. After plants were inoculated with the aphids, the plants were placed in individual plastic 45×20 cm cups and sealed with no-see-um mesh (Eastex products, NJ) to avoid aphid movement from plant to plant. In one embodiment, the plants used were 13 days old, approximately in the first true leaf stage, and aphids were left to reproduce for seven days under greenhouse conditions. In another embodiment, aphids were left to reproduce for 14 days on plants initially 20 days old at the beginning of the experiment, approximately in the third true leaf stage. At the end of each embodiment, aphid numbers were counted and recorded per individual plant. The presence of *Beauveria bassiana* or *Paecilomyces lilacinus* as an endophyte in cotton significantly reduced the reproduction of cotton aphids on endophyte treated plants versus untreated control plants (FIG. **4**A, **4**B, and FIG. **5**)

Example 8

Fungal Endophytes Reduce Nematode Reproduction

Plants were germinated from treated and untreated control seeds in an environment chamber and then transplanted to soil in pots 11 days after planting. Two replicate seedlings per treatment were sampled to examine the endophyte colonization efficiency by surface sterilization and plating on PDA agar. Nematode treatment group seedlings were treated with either 2,000 or 10,000 eggs/plant at day six after transplanting. Plants were harvested and processed 6 weeks after nematode inoculation. The numbers of galls per gram of root tissue and total egg numbers in the population for each plant were quantified to compare nematode performance between endophyte-treated and untreated (control) plants.

FIGS. **2** and **3** demonstrate that the endophytic fungi *Paecilomyces lilacinus* and *Chaetomium globosum* negatively affected root knot nematode (*Meloidogyne incognita*) reproduction when present as an endophyte in cotton. At high nematode inoculum levels (10,000 eggs), *Paecilomyces lilacinus* reduced egg production in plants following treatment of seeds with solutions containing either 10^6 or 10^7 spores/ml when compared to untreated control seeds. At field inoculum levels (2000 eggs), the presence of *Paecilomyces lilacinus* significantly reduced both galls and egg production at both seed treatment concentrations. Endophytic *Chaetomium globosum* negatively affects root-knot nematode reproduction. Negative effects of endophytic *Chaetomium globosum* on root-knot nematode gall formation and egg production were demonstrated following cotton seed soaking treatments in solutions of 0 (untreated controls), 10^6 and 10^8 spores/ml.

Example 9

Effect of Fungal Endophytes on Insects

Endophyte-treated and control plants were grown from non-transgenic cotton seeds (*Gossypium hirsutum*)(variety LA122, AllTex Seed Co.). Seeds were soaked for 24 hours in beakers containing 10^8 spores/ml solutions of the fungi utilized plus sterile water-only as a control. The beakers were placed in a 32° C. culture chamber overnight (approx. 9 h) until planting the next day. The plants were grown under both greenhouse and field conditions. Greenhouse plants were first germinated in seedling trays and then transferred to 30 cm pots. Field grown plants were concurrently planted and grown.

Behavioral assays: No-choice and choice behavioral assays were conducted to compare the response of western tarnished plant bugs (*L. hesperus*) and green stink bugs (*N. viridula*) to squares and bolls from endophyte-treated and untreated plants. The assays were conducted at 30° C. in 10 cm diameter petri dishes with a thin layer of 2% agar on the bottom to provide moisture for the squares (*L. hesperus* assays) and bolls (*N. viridula* assays) from experimental plants offered to the insects during the observations. For no-choice assays, a single square or boll was inserted by the base into the agar in the center of the dish. A single young adult (1-7 days post molt) insect was placed in each dish and covered with the top. A total of 30 insects were observed in each trial with N=10 insects each in the *Beauveria bassiana, Paecilomyces lilacinus* and control treatment groups. The *L. hesperus* no-choice trials were replicated four times (N=40 per treatment) with squares from greenhouse grown plants used in all but one trial. The *N. viridula* no-choice trials were replicated three times (N=20 per treatment) with bolls from greenhouse grown plants used in one trial.

Choice tests were conducted under the similar conditions using the same arenas, but with two equal sized squares (*L. hesperus*) or bolls (*N. viridula*) placed 4 cm apart in the center of the petri dish. The two squares or bolls per arena were from an untreated control plant and either a *Beauveria bassiana* or *Paecilomyces lilacinus* treated plant. A total of 20 insects were observed in each trial, with N=10 each in the Beauveria *bassiana* vs. control and *Paecilomyces lilacinus* vs. control treatment groups. The *L. hesperus* and *N. viridula* choice trials were both replicated twice (N=20 per treatment) with squares from field-grown plants in all trials.

Insects were observed for 6 hours per trial using a point sampling procedure for both the no-choice and choice assays. Preliminary observations indicated that the insects of both species were more active at the beginning of the assay, thus staged sampling schedule was adopted with observations recorded at 5 minute intervals early in the assay (0-60 min), 15 minute intervals in the middle (61-180 min) and 30 minute intervals late (181-360 min) in the assay. At each sampling interval, the insects were recorded as either off the square/boll or feeding or roosting upon the square/boll.

Data analysis: In the no-choice assays, the proportion of insects observed either feeding or resting upon cotton squares (*L. hesperus*) or bolls (*N. viridula*) was compared between treatment groups at each observation point across the duration of the assay using the Wilcoxon Signed Ranks Test. To test for variation in responses over time, for each individual the proportion of observations either feeding or upon the plant sample was calculated for early (0-60 min), middle (61-180 min) and late (181-360 min) periods of the assay and compared across treatment groups using a repeated measures analysis of variance (ANOVA) with the endophyte treatment group as the main factor and time as the repeat effect. The observed frequency of individuals failing to make contact with squares or bolls from endophyte-treated plants was compared to the expected frequency of individuals failing to do so based on the control group using a X2 test. Among the insects that did make contact with either a square or boll, the time to first contact (latency) was compared among treatment groups using a one-way ANOVA. All analyses including tests of normality and homogeneity of variances were conducted in SPSS 21 (SPSS Inc.).

Results of the *L. hesperus* no-choice assays: Over the duration of the assay, a significantly higher proportion of *L. hesperus* individuals over time was observed in contact with and feeding upon squares from untreated control plants relative to those from either of the *Beauveria bassiana* or *Paecilomyces lilacinus* endophyte treatment groups (Wilcoxon Signed Ranks test, P<0.0001 for both comparisons) (FIG. **6**A). Repeated measures ANOVA indicated a significant effect of time ($F_{1,116}$=86.175; P<0.001) with a higher proportion of insects contacting the square as the assay progressed (FIG. **6**B). There was also a significant effect of endophyte treatment ($F_{2,116}$=4.929; P=0.009) with no significant time×endophyte treatment interaction ($F_{2,116}$=1.015; P=0.366). Of the 40 insects in each treatment group, 12.5% of the control group failed to make contact with the square over the course of the assay, while a significantly higher 35% and 32.5% the *Beauveria bassiana* and *Paecilomyces lilacinus* treatment group insect respectively failed to make contact (X2 test, P<0.0001). Among the insects that did make contact with a square, there was significant difference in the latency to first contact among the treatment groups ($F_{2,85}$=7.225; P<0.0001) with the control group exhibiting a shorter latency to contact than either the *Beauveria bassiana* (posthoc LSD test; P=0.001) or *Paecilomyces lilacinus* endophyte treatment groups (posthoc LSD test; P=0.006 (FIG. **6**A).

Results of the *L. hesperus* choice assays: In simultaneous choice tests, *L. hesperus* individuals selected squares from untreated control plants more often than those from endophyte-treated plants. Response ratios were significantly greater than 0.5 over the duration of the assays, indicating that the insects non-randomly selected bolls from control plants over bolls from plants endophytically colonized by either (A) Beauveria *bassiana* (P<0.0001; Wilcoxon Signed Ranks test) or (B) *Paecilomyces lilacinus* (P<0.0001; Wilcoxon Signed Ranks test)(FIG. **6**B).

Results of the *N. viridula* no-choice assays: Over the duration of the assay, a significantly higher proportion of *N. viridula* individuals over time was observed in contact with and feeding upon bolls from untreated control plants relative to those from either of the *Beauveria bassiana* or *Paecilomyces lilacinus* endophyte treatment groups (Wilcoxon Signed Ranks test, P<0.0001 for both comparisons)(FIG. **7**A). Repeated measures ANOVA indicated a significant effect of time ($F_{1,116}$=86.175; P<0.001) with a higher proportion of insects contacting the square as the assay progressed (FIG. **1**), There was also a significant effect of endophyte treatment ($F_{2,116}$=4.929; P=0.009) with no significant time×endophyte treatment interaction ($F_{2,116}$=1.015; P=0.366). Of the 40 insects in each treatment group, 12.5% of the control group failed to make contact with the square over the course of the assay, while a significantly higher 35% and 32.5% the *Beauveria bassiana* and *Paecilomyces lilacinus* treatment group insect respectively failed to make contact (X2 test, P<0.0001). Among the insects that did make contact with a square, there was significant difference in the latency to first contact among the treatment groups ($F_{2,85}$=7.225; P<0.0001) with the control group exhibiting a shorter latency to contact than either the *Beauveria bassiana* (posthoc LSD test; P=0.001) or *Paecilomyces lilacinus* endophyte treatment groups (posthoc LSD test; P=0.006 (FIG. **7**B).

Example 10

More Bolls are Retained after Endophyte Treatment

During the field trial, cotton phenology and development was quantified using a plant mapping and information system developed specifically for cotton to track fruit development and retention by the plant as a means of monitoring plant development and stress (COTMAN™, Cotton Inc.). One measure of cotton stress is the retention of developing flowers (squares) and fruits (bolls) in the first fruiting position on branches. First position squares and bolls were measured on 5 plants per row in two rows in each of the five replicate plots (N=10 plants per plot) for each treatment group.

FIG. **10** demonstrates that early in the growing season as flowers begin to develop, a trend for higher square retention in the endophyte-treated plants relative to controls was observed. This trend continued later in the season as evidenced by significantly higher boll retention among the endophyte treatment groups relative to the untreated control plants.

FIG. **8** demonstrates reduction in cotton boll damage during field trials. Relative to control plants, levels of insect-related boll damage were lower among plants that were treated by soaking seeds in spore solutions of *Beauveria bassiana* and *Paecilomyces lilacinus* at concentrations of 10^6 and 10^8 spore/ml. Positive effects of fungal endophytes on cotton plant performance under field conditions.

Example 11

Endophyte Treatment Increases Yield

At the end of the field trial employing endophyte treatment and treatment plants, plots were machine harvested with a 1-row picker. Surprisingly, the final yields at harvest were significantly higher than expected (25% higher than the untreated controls). Unexpectedly, treatment with *Paecilomyces lilacinus* or *Beauveria bassiana* resulted in higher yields than untreated control plants with regardless of the initial seed treatment concentration. (FIG. **11**)

Example 12

Endophyte Treatment of *Sorghum* Increased Growth in the Greenhouse

The effect of the described microbial compositions on *sorghum* was tested in a seedling assay. *Sorghum bicolor* seeds were surface sterilized using ethanol and bleach as described in Example 1 for cotton. Three strains (*B. bassiana, P. fumosoroseus*, and *P. lilacinus*) were prepared as conidia suspensions at 10^7 conidia/ml, and coated on the *sorghum* seeds as described in Example 1. Control seeds were soaked in sterile water instead of a conidia suspension. Planted seeds were held in constant growth chamber conditions for two weeks at a replication of 10. At the end of two weeks, the plants were removed from the growth chamber and the plant height and biomass were measured. FIG. **12**A shows the increase in plant height when applied with the described microbial composition relative to the control (p<0.05). FIG. **12**B shows the increase in plant biomass in plants grown from seed that were treated with the described microbial composition relative to the control (p<0.05).

Example 13

Treatment with Fungal Endophytes Modulates the Colonization Frequencies of Native Endophytes

To determine whether endophyte seed treatments could alter the microbiome of the plant grown from the seed, cotton seeds were treated with spore suspensions of *Paecilomyces lilacinus* or *Beauveria bassiana*. Plants were grown in the field as part of a field trial planted and maintained under standard agricultural practices. Endophytic fungi were isolated on PDA media separately from surface-sterilized above-ground stem/leaf and below-ground root tissue to assess changes in the microbial community. The comparison shown in FIG. **13** is relative to the fungal endophyte communities in untreated control plants. The results show that these treatments can alter the colonization rates of native fungal endophytes.

Fungal endophyte treatments may alter the colonization frequencies of any of the fungal endophytes naturally present in plants. To determine what other native endophytes may be affected by seed treatments with fungal endophytes, the identity of cotton fungal endophytes isolated from plants of two commercial cotton varieties, CG3787B2RF and PHY499WRF, were assessed. The samples were obtained during a variety trial near Lubbock, Tex., USA identified as Lubbock-RACE. One single healthy leaf was collected from each of nine individual plants sampled per variety across multiple replicate plots arranged in a randomized block design to control for spatial variation in the field. To identify the fungal endophyte species, whole genomic DNA was extracted and the ribosomal DNA internal transcribed spacer (ITS) region was amplified as a barcode for 454 pyrosequencing using ITS1F forward and ITS2 reverse universal fusion primers. The fungal endophytes identified in this experiment, along with those shown in FIG. **13**, are listed in Table 2.

TABLE 2

Native fungal endophytes that may be altered by seed treatments with other fungal endophytes

Phylum	Class	Order	Family	Genus species
Ascomycota	Leotiomycetes			
	Leotiomycetes			*Geomyces auratus*
	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Macrophomina* sp.
	Dothideomycetes	Capnodiales	Davidiellaceae	
	Dothideomycetes	Capnodiales	Davidiellaceae	*Cladosporium* sp.
	Dothideomycetes	Capnodiales	Davidiellaceae	*Cladosporium cladosporioides*
	Dothideomycetes	Capnodiales	Davidiellaceae	*Davidiella* sp.
	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Cercospora* sp.
	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Cercospora beticola*
	Dothideomycetes	Pleosporales		
	Dothideomycetes	Pleosporales	Pleosporaceae	
	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria* sp.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria alternata*
	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria citri*
	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria porri*
	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria tenuissima*
	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus* sp.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Curvularia* sp.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Epicoccum* sp.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Exserohilum* sp.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Lewia* sp.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Lewia infectoria*
	Dothideomycetes	Pleosporales	Pleosporaceae	*Pyrenophora* sp.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Pyrenophora triticirepentis*
	Dothideomycetes	Pleosporales	Pleosporaceae	*Pleospora* sp.
	Dothideomycetes	Pleosporales	Didymellaceae	*Phoma americana*
	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia africana*
	Eurotiomycetes	Chaetothyriales		
	Eurotiomycetes	Chaetothyriales	Chaetothyriaceae	
	Eurotiomycetes	Eurotiales	Trichocomaceae	
	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus* sp.
	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium* sp.
	Eurotiomycetes	Eurotiales	Trichocomaceae	*Thermomyces* sp.
	Eurotiomycetes	Eurotiales	Trichocomaceae	*Thermomyces lanuginosus*
	Saccharomycetes	Saccharomycetales		
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Candida* sp.
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Candida quercitrusa*
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Candida tropicalis*
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Cyberlindnera* sp.
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Cyberlindnera jadinii*
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Kluyveromyces* sp.
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Kluyveromyces marxianus*

TABLE 2-continued

Native fungal endophytes that may be altered by seed treatments with other fungal endophytes				
Phylum	Class	Order	Family	Genus species
	Sordariomycetes			
	Sordariomycetes	Diaporthales	Gnomoniaceae	*Gnomoniopsis* sp.
	Sordariomycetes	Hypocreales	Cordycipitaceae	*Beauveria bassiana*
	Sordariomycetes	Hypocreales	Cordycipitaceae	*Cordyceps* sp.
	Sordariomycetes	Hypocreales	Cordycipitaceae	*Cordyceps bassiana*
	Sordariomycetes	Hypocreales	Nectriaceae	
	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium* sp.
	Sordariomycetes	Hypocreales	Hypocreaceae	
	Sordariomycetes	Hypocreales	Hypocreaceae	*Gibellulopsis nigrescens*
	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypocrea* sp.
	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypocrea lixii*
	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypocrea virens*
	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma* sp.
	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma tomentosum*
	Sordariomycetes	Hypocreales	Plectosphaerellaceae	*Verticillium* sp.
	Sordariomycetes	Ophiostomatales	Ophiostomataceae	
	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Ophiostoma* sp.
	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Ophiostoma dendifundum*
	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium* sp.
	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium globosum*
	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia hyrcaniae*
	Sordariomycetes	Sordariales	Chaetomiaceae	*Taifanglania* sp.
	Sordariomycetes	Sordariales	Chaetomiaceae	*Taifanglania inflata*
	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Schizothecium inaequale*
	Sordariomycetes	Trichosphaeriales	Trichosphaeriaceae	*Nigrospora* sp.
	Sordariomycetes	Xylariales	Amphisphaeriaceae	*Truncatella angustata*
Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Rhizoctonia* sp.
	Agaricomycetes	Corticiales	Corticiaceae	
	Agaricomycetes	Corticiales	Corticiaceae	*Phanerochaete* sp
	Agaricomycetes	Polyporales	Coriolaceae	
	Agaricomycetes	Polyporales	Coriolaceae	*Trametes* sp.
	Agaricomycetes	Polyporales	Coriolaceae	*Trametes hirsuta*
	Agaricomycetes	Polyporales	Coriolaceae	*Trametes villosa*
	Agaricomycetes	Russulales	Peniophoraceae	
	Microbotryomycetes	Sporidiobolales		
	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodotorula* sp.
	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodotorula mucilaginosa*
	Tremellomycetes			
	Tremellomycetes	Tremellales		
	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus* sp
	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus skinneri*
	Tremellomycetes	Tremellales	Tremellaceae	*Tremella* sp.

Example 14

Fungal Endophyte Seed Treatment Leads to Modulation of Phytohormone Levels in Plants Grown from the Seed

To determine whether fungal endophyte seed treatment affects phytohormone levels in plants grown from the seed, tissue was harvested from the root or third true leaf of cotton plants inoculated with either endophytic *Beauveria bassiana* or *Paecilomyces lilacinus*. The experiment was done with three endophyte treatments (uncolonized control, *B. bassiana* or *P. lilacinus*) and, for *Beauveria bassiana*, two herbivory treatments (no aphids, or aphid herbivory for either 1, 4, 8, 24 or 48 hours). Phytohormone levels for abscisic acid (ABA), tuberonic acid (12-OH-JA, an oxidation product of JA-Ile) (TA), ascorbic acid (AA), 12-Oxophytodienoic acid (a JA precursor) (OPDA), JA isoleucine (JA-Ile), and salicylic acid (SA) were assessed by LC-MS in leaf and root tissues separately. All phytohormone level comparisons were made versus plants in the uncolonized control group with significance at $P<0.05$. Phytohormone levels in plants grown from seed treated with *Beauveria bassiana* are shown in Table 3, and phytohormone levels in plants grown from seed treated with *Paecilomyces lilacinus* are shown in Table 4.

TABLE 3

Phytohormone levels in plants grown from seed treated with *Beauveria bassiana*					
Herbivory	Phytohormone	Tissue	Upregulated/downregulated	Tissue	Upregulated/downregulated
Yes	ABA	Leaves	Down at 8 hours of feeding	Roots	Upregulated at 48 hrs of feeding
No			Not significant		Upregulated

TABLE 3-continued

Phytohormone levels in plants grown from seed treated with *Beauveria bassiana*					
Herbivory	Phytohormone	Tissue	Upregulated/downregulated	Tissue	Upregulated/downregulated
Yes	TA	Leaves	Not significant	Roots	Upreguated at 48 hrs of feeding
No			Not significant		Not significant
Yes	AA	Leaves	Down at 4 hrs up at 24 hrs	Roots	Up at 8 hrs down at 48 hrs
No			Not significant		Upregulated
Yes	OPDA	Leaves	Not significant	Roots	Up at 4 hrs and 8 hrs
No			Not significant		Upregulated
Yes	JA-Ile	Leaves	Up at 48 hrs	Roots	Up at 48 hrs
No			Not significant		Upregulated
Yes	SA	Leaves	Up at 1 hr, 8 hr, 24 and 48 hr	Roots	Down at 4 hr the rest n.s
No			Not significant		Not significant

TABLE 4

Phytohormone levels in plants grown from seed treated with *Paecilomyces lilacinus*					
Yes	ABA	Leaves	Down at 48 hrs	Roots	Up at 1 hr and 8 hrs
Yes	TA	Leaves	down at 4 and 8 hrs	Roots	up at 4 hrs
Yes	AA	Leaves	down at 4 and 8 hrs	Roots	up at 4 hrs
Yes	OPDA	Leaves	down at 4 and 8 hrs	Roots	Up at 4 and 48 hrs, down at 24 hrs
Yes	JA-Ile	Leaves	Down at 8 and 48 hrs	Roots	Up at 4 and 24 hrs
Yes	SA	Leaves	Up at 1 and 4 hr, down at 8 hrs	Roots	Up at 1, down at 8 hrs

Example 15

Fungal Endophyte Seed Treatments Alter Traits in Certain Cotton Cultivars in Field Trials

The 2014 field trials were executed in a similar fashion as described in Example 6. A field trial using isolates of listed below was conducted during the summer. Each plot consisted of four 15.24 m (40 ft) rows, each separated by 101.6 cm (40 in), and there were 6 replicate plots per treatment. Yield from plots treated with the described microbial compositions was compared relative to the untreated control plots. For *thrips*, this damage assessment was on a scale of 0-5; 0=no damage, 1=noticeable feeding scars, but no stunting, 2=noticeable feeding and 25% stunting, 3=feeding with blackened leaf terminals and 50% stunting, 4=severe feeding and 75% stunting, and 5=severe feeding and 90% stunting. For fleahoppers, the number of insects per plant were quantified and reported as an average for each plot. FIG. **14** shows the yield improvement of crops when treated with the described microbial compositions, for Delta Pine and Phytogen cultivars, respectively. FIG. **15** shows the aggregated yield improvement of the microbes across the two cultivars. Bars represent 95% confidence intervals. FIG. **16**A shows the beneficial effect of 12 out of 15 microbial compositions tested on thrip damage in the Delta Pine cultivar. In the Phytogen cultivar, only 2 out of the 15 microbial compositions tested showed a benefit by reducing thrip damage. FIG. **16**B shows the beneficial effect of reducing fleahopper damage in the Phytogen cultivar, where 6 out of the 15 facultative fungal endophytes tested showed an average decrease in fleahopper damage as compared to untreated cotton plants. In the Delta Pine cultivar, only one microbial composition showed a beneficial effect on fleahopper damage.

A number of other mid-season plant traits were also assessed in the field to determine the effect of the described fungal endophyte compositions. FIG. **17**A shows the beneficial increase of the described microbial compositions on mid-season mean root length. FIG. **17**B shows the beneficial increase of the described fungal endophyte compositions on mid-season below ground weight. FIG. **18** shows the beneficial increase of the described fungal endophyte compositions on mid-season canopy temperature for both Delta Pine and Phyton cultivars. FIG. **19** shows the beneficial increase of the described fungal endophyte compositions on mid-season NDVI (Normalized Difference Vegetation Index) for both Delta Pine and Phytogen cultivars. NDVI is a measure of chlorophyll content. FIG. **20** shows the beneficial increase of the described fungal endophyte compositions on mid-season first-position square retention for both Delta Pine and Phytogen cultivars. FIG. **21** and FIG. **22** show the modulation (up in July and down in August) of mid-season plant height when treated with the described fungal endophyte compositions for both Delta Pine and Phytogen cultivars. FIG. **23** shows increased biomass in the plants treated with endophytes (right half of the image) compared to untreated control (left half of the image).

In FIGS. **15** through **22**, TAM505 is *Acremonium* sp., TAM32 is *Epicoccum nigrum*, TAM534 is *Cladosporium urdinicola*, TAM244 is *Cladosporium* sp., TAM514 is *Cladosporium urdinicola*, TAM474 is *Cladosporium cladosporoides*, TAM554 is *Chaetomium globosum*, TAM15 is *Exserohilum* sp., TAM488 is *Epicoccum nigrum*, TAM452 is *Cladosporium* urdinicola, TAM490 is *Paecilomyces lilacinus*, TAMBB is *Beauveria bassiana*, TAM105 is *Cochliobolus* sp., TAM189 is *Bipolaris* sp., and TAM47 is *Epicoccum nigrum.*

Example 16

Fungal Endophyte Seed Treatments Provide Drought Tolerance in Cotton Cultivars in Greenhouse Trials

Cotton plants were germinated from endophyte-treated and untreated control seeds in the greenhouse. All seeds watered for 7 days or until cotyledon stage using predetermined soil saturation volume of water per plant. At 7 DAP, water was withheld from water stressed plants while controls continued to be watered. Time to wilt and time to death were measured at a max of 21 DAP. The data in FIG.

24 shows the mean time to wilt, and the data in FIG. 25 shows the mean time to death. Endophyte treatment increased the survival of plants subjected to drought stress in both the Delta Pine (DTP) and the Phytogen (PHY) cultivars. In FIGS. 24 and 25, endophyte number 194 is *Epicoccum nigrum,* 249 is *Cladosporium* cladosporioides, 355 is *Chaetomium globusum,* 46 is *Epicoccum* sp., 463 is *Cladosporium* sp., 534 is *Cladosporium uredinicola,* 554 is *Chaetomium globosum,* 58 is *Epicoccum nigrum*, and control is no endophyte treatment.

Example 17

Identification of Fungal Endophytes with at Least 97% Identity to Those in Table 1

All known fungal endophytes with 97% identity to SEQ ID NO:7 through SEQ ID NO:77 were identified and are listed here by accession number: FJ425672, AY526296, JQ760047, UDB014465, KC662098, HQ649874, JQ764783, EU881906, KF251285, JQ862870, AB019364, AB594796, JF773666, JN034678, KC343142, EU707899, AB627855, GU138704, JN695549, DQ279491, HM776417, AB361643, DQ782839, AF222826, EU682199, DQ782833, EU054429, FJ025275, AY354239, AF222828, GU721921, GU721920, DQ093715, AJ309335, FR774125, JQ747741, EF042603, KC968942, HE584924, AY740158, FJ645268, HQ692590, GQ203786, AY233867, HE579398, AB777497, KF435523, DQ420778, JQ649365, AJ271430, GQ996183, EF070423, FJ172277, AF483612, JX675127, EF070420, EF070421, AB741597, JN225408, DQ019364, KF251279, EF194151, EU977196, JX981477, EU686115, JX021531, FJ527863, AJ302451, AJ302455, JN975370, EU754952, AF284388, KF296855, AF502785, JX317207, AF502781, DQ278915, EU686867, KC179120, HM991270, AF284384, DQ632670, JQ759806, JQ747685, EU885302, GU721781, EF434047, EF505854, JQ666587, JQ619887, GQ919270, KF531831, AB627854, DQ914679, DQ914681, HQ599592, DQ279490, DQ660336, JX069862, AB607957, HE820869, FJ859345, JX966567, GU910230, AB627850, JX144030, DQ914723, HM595556, KC771473, DQ849310, EU179868, KF312152, JN890447, JX042854, EU554174, JN198518, HM992813, JQ845947, KF251310, JQ758707, AM930536, KF296912, JN865204, JN943512, GQ921743, EU245000, EU977304, EU144787, HE579322, HE579402, GU910171, HE792919, KC960885, DQ485941, JN604449, HQ607913, AF502620, DQ468027, JX944132, JN207338, JQ922240, JN207336, JX559559, JN207330, JN207333, HE820882, JX969625, HQ339994, JF744950, HE584937, JN120351, JX298885, DQ872671, AJ877102, JQ081564, DQ019391, AF071342, EF104180, JQ759755, GU827492, JN418769, GU324757, JX984750, JX256420, KF436271, JX205162, JN712450, KF435911, GU367905, JX416919, KC315933, JQ736648, AY904051, AF404126, FJ466722, HE584965, JN890282, HE584966, HQ166312, KC305124, HE977536, KC305128, AY907040, JF710504, AF483609, AJ302460, AJ302461, AJ302462, AY969615, EU685981, U75615, AJ302468, FJ210503, GU237860, JX960591, JX143632, HM044649, EU164404, HE584824, HQ116406, DQ156342, JX416911, U75617, GU721359, KC427041, EU254839, JX262800, KC179307, HQ107993, KF361474, GU721420, HM053659, EF619702, EU686156, HE820839, HQ634617, GU721810, AB277211, AJ302417, KC315945, JQ002571, AM237457, AF009805, JX489795, EU680554, KC507199, FJ236723, HQ692618, JN846717, JX944160, JQ585672, KF435573, EU520590, HM581946, DQ250382, JX243908, KC343184, KC485454, GQ479695, GU237760, KF147147, EF619849, GU237767, GU237766, AB818997, AF502847, EU683672, KF225801, KC965743, AJ488254, DQ825983, JN031007, DQ825985, KF028765, AB818999, HQ238268, EU685984, KC966180, HE998711, HQ533007, AM113729, KF251637, FN394692, KF435172, JN207307, JQ814305, HM770988, KC145175, AB511813, EU552102, AJ309344, EU645686, JQ936328, JN038492, DQ875349, EU977228, JQ814357, KF040480, JX317350, DQ401548, DQ318195, DQ318194, GU721776, KF193449, AF178544, AM262354, AB540567, AY627787, HE792907, HE579333, EU445372, AF362069, GU973687, HM053663, AB374284, DQ062977, GU237797, JQ760783, EF029240, HM751829, FM200445, AY953383, AY233922, JF742784, HM626650, FN610871, JX155902 JX006065, AB566289, AF163078, AY344976, AB566287, AF282089, AY251441, AF395693, JQ761899, AJ315835, HQ187633, KC287233, AJ315831, HE820745, JN418779, M13906, JQ761896, AJ315838, AY536373, HQ328035, JX838793, JQ758986, HQ166357, JN163855, KC965595, JN545789, JN545788, GU944558, HE579247, KF296900, EF377335, KC965954, GU269703, AB095511, EF419913, DQ993641, AB325678, HQ223035, AY513945, FJ197013, FR799277, HM071900, JN207293, FJ025268, JQ758966, GU138733, GU138730, DQ267595, GQ919269, JF770450, GU138734, DQ279488, DQ279486, DQ242472, EU164804, EF104177, GU366726, KF212243, DQ923534, GU079598, JX987761, JX984765, AY585343, JQ769260, GU721919, DQ923538, EU686756, EU040222, U75626, GU004264, EU686753, JQ765651, JX270629, JN943408, EF042604, AJ271588, HE579386, GQ479556, JQ759962, JX317413, EU516867, DQ780361, JQ905644, HQ649792, JQ247355, FN386296, AY004778, DQ102374, KF251383, GU237835, DQ383642, FN868479, GU237814, KC343032, JN943394, HQ450001, KC800573, AB217793, GQ851883, EU330630, JF309198, AY489281, GU325687, JX399008, AB164703, EF159407, AJ302429, UDB008141, UDB008140, FM200496, AJ302426, AJ302422, AJ302423, JQ683725, KF193481, JQ683727, HE792931, AB220252, FJ013057, DQ286207, JQ759811, JF414842, JX088707, JN415754, AY787715, JX559577, K C776206, GU166440, KC460867, FJ515595, KF056850, DQ118964, KC806227, KC631802, EU823315, AY528970, HQ116401, JX317516, KF251313, KC800565, AF502705, AF502810, JQ747697, AY527407, EU680518, AJ621773, AB374285, HQ832827, GU174316, DQ974750, JN198507, JF749806, JQ782739, HQ023202, AY616234, KC965315, AB743781, EU554161, KC507201, HM036624, EF464164, JX391942, AB743995, FJ415474, AY647237, KC965503, AB540553, HQ377280, JX898571, JN969419, DQ166962, HM123519, GU237881, AB683953, AY681487, EU498738, EU687037, AB540550, 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Example 18

Endophytes and Combination Thereof

The protocols as described in Examples 1-16 are used in connection with the endophytes of Table 1 to confirm beneficial properties on plant health, such as yield and/or past resistance, for example. In particular, endophytes from Table 1 are employed in a synthetic combination with a plant as described herein with crop plants, such as cotton. Any single or combination of endophytes listed in Table 1 can also be used in this manner, employing for example seed coatings or foliar, soil, or rhizosphere applications. A seed composition may comprise seeds and any combination of endophytes listed in Table 1. Endophytes listed in Table 1 or combinations thereof are thus employed in methods for preventing pest infestation, increased yield, treating a pest infestation, manufacturing pest-resistant seeds; or increasing a yield or reducing loss of a crop according to the methods of Examples 1-15.

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 1

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<210> SEQ ID NO 2
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 2

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<210> SEQ ID NO 3
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 3

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<210> SEQ ID NO 4
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
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agtgtatttc tgagtggcat aagcaaataa atcaaaactt tcagcaacgg atctcttggt 180

tctggcatcg atgaagaacg cagcaggact aacgtgtgtc gacgg 225

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ggacaaacat aaaccttttg taattgcaat cagcgtcagt aacaaattaa taattacaac 180

tttcaacaac ggatctcttg gttctggcat cgatgaagaa cgcacagtca gtgtgaaatc 240

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aaacataaac cttttgtaat tgcaatcagc gtcagtaaca aattaataat tacaactttc    180

aacaacggat ctcttggttc tggcatcgat gaaaaacgca tcaa                     224


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atcattacac aggtatgaag gcgggctgga atctctcggg gttacagcct tgctgaatta    180

ttcacccgtg tcttttgcgt acttcttgtt tcctgggtgg gttcgcccac caccaggacc    240

aaccataaac cttttgtaat tgcaatcagc gtcagtaaca aaataataat tacaact       297


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taaacctttt gtaattgcaa tcagcgtcag taacaaatta ataattacaa ctttcaacaa    180

cggatctctt ggttctggca tcgatgaaga acgcagcaaa ttaatgccgg ctggaacgcc    240

tctgggata                                                            249


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attacaactt tccacaacgg gatctcttgg gttctggcat cgctagc                  227


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tggttctggc atcgatgaag aacgcagcaa                                     210


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taaacctttt gtaattgcaa tcagcgtcag taacaaatta ataattacaa ctttcaacaa 180

cggatctctt ggttctggca tcgatgaaga acgcagcgaa atgcgataag tagtgtgaat 240

tgcagaattc agtaat 256

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tgcaatcagc gtcagtaaca aattaataat tacaactttc aacaacggat ctcttggttc 180

tggcatcgat gaagaacgca gctaaataca tatgaaggcg ggctggaacg tcccgcggtt 240

gcagacttgc tgacttattc acc 263

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<213> ORGANISM: Alternaria tenuissima

<400> SEQUENCE: 17

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cgtacttctt gtttccttgg tgggttcgcc caccactagg acaaacataa accttttgta 120

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tctggcatcg atgaagaacg cagc 204

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agcgtcagta taacaaatgt aaatcattta caactttcaa caacggatct cttggttctg 180

gcatcgatga agaacgcagc aatacacact caataaaaaa cgaaggccgt tcgcggacgg 240

acta 244

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tcgatgaaga acgcagcaga aatgcgataa gtaatgtgaa ttgcagaatt cagtgaatca 240

tcgaatcttt gaacgcacat tgcgcccctt ggtattccga 280

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ccgtaggtga acctgcggag ggatcattac tgagtgaggg ccttcgggct cgacctccaa 180

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gtaaatttaa ttaataaatt aaaactttta acaacggatc tcttggttct ggcatcgatg 180

aagaacgcag ccaaaccagc aaacccggtc taacccccgg gatgttcatg accctttgtt 240

gtccgactct gaggc 255

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gtgcactttt tgtaggctct ggttgaaagg cgttgcttca cttcggtgtg gtaatcgctg 120

gaagacctgg tctatgtttt attacaaacg cttcagttat acaatgttta tctgcgtata 180

acgcatttat atacaacttt cagcaacgga tctcttggct ctcgcatcga tgaagaacgc 240

agctcgagt 249

<210> SEQ ID NO 31
<211> LENGTH: 267
<212> TYPE: DNA
<213> ORGANISM: Phoma americana

<400> SEQUENCE: 31

cgtacgctac atggaagtaa aagtagtaac aaggtttccg taggtgaacc tgcggaagga 60

tcattaccta gagttgtagg ctttgcctgc tatctcttac ccatgtcttt tgagtacctt 120

cgtttcctcg gcgggtccgc ccgccgattg gacaatttaa accatttgca gttgcaatca 180

gcgtctgaaa aaacttaata gttacaactt tcaacaacgg atctcttggt tctggcatca 240

atgaaaaacg cagcaacaca aaattac 267

<210> SEQ ID NO 32
<211> LENGTH: 205
<212> TYPE: DNA
<213> ORGANISM: Phoma subherbarum

<400> SEQUENCE: 32

tacgtgcagc gctttgcctg ctatctctta cccatgtctt ttgagtacct tcgtttcctc 60

ggcgggtccg cccgccgatt ggacaattta aaccatttgc agttgcaatc agcgtctgaa 120

aaaaacttaa tagttacaac tttcaacaac ggatctcttg gttctggcat cgatgaagaa 180

cgcagcttac ctagagaatg cgtgt 205

<210> SEQ ID NO 33
<211> LENGTH: 264
<212> TYPE: DNA
<213> ORGANISM: Phomopsis liquidambari

<400> SEQUENCE: 33

aggcgcaccc agaaaccctt tgtgaactta taccttactg ttgcctcggc gcatgctggc 60

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ccccctcgggg tcccctggag acagggagca ggcacgccgg cggccaagtt aactcttgtt    120

tttacactga aactctgaga aaaaaacaca aatgaatcaa aactttcaac aacggatctc    180

ttggttctgg catcgatgaa gaacgcacaa gtggagggcc ccaggcgccc ccccaaaacc    240

ttttttgagt tattacttac tgtt                                           264


<210> SEQ ID NO 34
<211> LENGTH: 222
<212> TYPE: DNA
<213> ORGANISM: Phomopsis sp.

<400> SEQUENCE: 34

ccggcgcacc cagaaaccct ttgtgaactt atacctactg ttgcctcggc gcaggccggc     60

cttttgtcaa aaaaggcccc ctggagacag ggagcagccc gccggcggcc aaccaaactc    120

ttgtttctac agtgaatctc tgaggaaaaa acataaatga atcaaaactt tcaacaacgg    180

atctcttggt tctggcatcg atgaagaacg cagcatgctg gc                       222


<210> SEQ ID NO 35
<211> LENGTH: 255
<212> TYPE: DNA
<213> ORGANISM: Pleospora sp.

<400> SEQUENCE: 35

cgggttggga cctcacctcg gtgagggctc cagcttgtct gaattattca cccatgtctt     60

ttgcgcactt cttgtttcct gggcgggttc gcccgccacc aggaccaaac cataaacctt    120

tttgtaattg caatcagcgt cagtaaacaa tgtaattatt acaactttca acaacggatc    180

tcttggttct ggcatcgatg aagaacgcag cgaaatgcga tacgtagtgt gaattgcaga    240

attcagtgat tttgc                                                     255


<210> SEQ ID NO 36
<211> LENGTH: 349
<212> TYPE: DNA
<213> ORGANISM: Pleosporaceae sp.

<400> SEQUENCE: 36

ggatcattac acaatatgaa ggcgggctgg aacctctcgg ggttacagcc ttgctgaatt     60

attcaccctt gtcttttgcg tacttcttgt ttccttggtg ggttcgccca ccactaggac    120

aaacataaac cttttgtaat tgcaatcagc gtcagtaaca aattaataat taccactttc    180

aacaacggga tctcttggtt ctgggcatcg agcagaaaaa cgcagaattg aaggcgggct    240

gggaacctct tcgggggggt ccagggcttt ggtgaattat tcaccccttg cctttgcgta    300

cgttgtttgt tttccttggg gggggtaggc acacaaaaaa agaaaacgg                349


<210> SEQ ID NO 37
<211> LENGTH: 222
<212> TYPE: DNA
<213> ORGANISM: Preussia africana

<400> SEQUENCE: 37

aagtaccatt atcgtagggc ttcggccctg tcgagataga acccttgcct ttttgagtac     60

cttttcgttt cctcggcagg ctcgcctgcc aatggggacc ccaacaaaca ctttgcagta    120

cctgtaaaca gtctgaacaa acttttaaaa attaaaactt tcaacaacgg atctcttggt    180

tctggcatcg atgaagaacg cagcgaaatg cgataaaacg tg                       222
```

<210> SEQ ID NO 38
<211> LENGTH: 218
<212> TYPE: DNA
<213> ORGANISM: Preussia sp.

<400> SEQUENCE: 38

ttcgagatac acccttgcct ttttgagtac cttttcgttt cctcggcagg ctcgcctgcc 60

aacggggacc cttcaaaacg ctttgtaata cctgtaactg tctgatataa caagcaaaaa 120

tcaaaacttt caacaacgga tctcttggtt ctggcatcga tgaagaacgc agcaatcgtt 180

gggcttcggc ccattagaga taacaccctt gccttttt 218

<210> SEQ ID NO 39
<211> LENGTH: 254
<212> TYPE: DNA
<213> ORGANISM: Pseudozyma sp.

<400> SEQUENCE: 39

acatctcgcg gttcagcctt gttgagtatt caccottgtt ttttgcggag aaatgtttgg 60

gtggagggag caccagcacc aagacaaatc taaacctttt gcaattgcaa tacgggcgac 120

atttacctta ataattgttg atttcataag attatatctt ggttgaaact ccactggtaa 180

tgccatcgtc taaaatctaa aaacaacttt tggcaacgga tctcttggtt ctcgcatcga 240

tgaagaacgc agcc 254

<210> SEQ ID NO 40
<211> LENGTH: 277
<212> TYPE: DNA
<213> ORGANISM: Pyrenophora teres

<400> SEQUENCE: 40

aggcagattg ggtagtcccc gcttttgggg tttgcccatt ctggcgccat attcacccat 60

gtcttttgcg tactacttgt ttccttggcg ggttcgcccg ccaattggac tttattcaac 120

cctttttttt ttattgcaat cagcgtcagc aaaacaatgt aatcaattta caactttcaa 180

caacggatct cttggttctg gcatcgatga aaaacgcagc cacaatatga tggccgatgg 240

ggcaggcctc ttttggggtt gccccctctg gcgccct 277

<210> SEQ ID NO 41
<211> LENGTH: 289
<212> TYPE: DNA
<213> ORGANISM: Colletotrichum capsici

<400> SEQUENCE: 41

gctcatcacc ctttgtgaca taccttaact gttgcttcgg cgggtaggcg tcccctgaaa 60

aggacgtctc ccggccctct cccgtccgcg ggtggggcgc ccgccggagg ataaccaaac 120

tctgatttaa cgacgtttct tctgagtgac acaagcaaat aatcaaaact tttaacaacg 180

gatctcttgg ttctggcatc gatgaagaac gcagcaatta ttggggtgtt gctcatcatc 240

ctttgtggtg aaccttaact gttgctgcgg cggggggcgg cgtcccctg 289

<210> SEQ ID NO 42
<211> LENGTH: 216
<212> TYPE: DNA
<213> ORGANISM: Coniolariella gamsii

<400> SEQUENCE: 42

tgacactccc aaaacccctg tgaacatacc gtacgttgcc tcggcggggg ggcgctcccc 60 ccccgccggc ggcccacgaa actctgtttt gccctgaatc tctgaaacga caaactaaat 120

cagttaaaac tttcaacaac ggatctcttg gttctggcat cgatgaagaa cgcagcgaaa 180

tatagaagtg acccaactcc taaccactgt gaacaa 216

<210> SEQ ID NO 43
<211> LENGTH: 268
<212> TYPE: DNA
<213> ORGANISM: Coniothyrium aleuritis

<400> SEQUENCE: 43

tagacttcac taaagcttgt agacttcggt ctgctacctc ttacccatgt cttttgagta 60

ccttcgtttc ctcggcgggt ccgcccgccg attggacaac attcaaaccc tttgcagttg 120

caatcagcgt ctgaaaaaac ataatagtta caactttcaa caacggatct cttggttctg 180

gcatcgatga agaacgcagc gaaatgcgat aagtagtgtg aattgcagaa ttcagtgaat 240

catcgaatct ttgaacgcac attgcgcc 268

<210> SEQ ID NO 44
<211> LENGTH: 210
<212> TYPE: DNA
<213> ORGANISM: Coniothyrium sp.

<400> SEQUENCE: 44

gggctggatc tctcggggtt acagccttgc tgaattattc acccttgtct tttgcgtact 60

tcttgtttcc ttggtgggtt cgcccaccac taggacaaac ataaaccttt tgtaattgca 120

atcagcgtca gtaacaaatt aataattaca actttcaaca acggatctct tggttctggc 180

atcgatgaag aacgcagcaa cactaatatg 210

<210> SEQ ID NO 45
<211> LENGTH: 218
<212> TYPE: DNA
<213> ORGANISM: Corynespora cassiicola

<400> SEQUENCE: 45

cgccccttcg agatagcacc ctttgtttat gagcacctct cgtttcctcg gcaggctcgc 60

ctgccaacgg ggacccacca caaacccatt gtagtacaag aagtacacgt ctgaacaaaa 120

caaaacaaac tatttacaac tttcaacaac ggatctcttg gttctggcat cgatgaagaa 180

cgcagcggat atcgtagggg ccgcgccccc ttccagat 218

<210> SEQ ID NO 46
<211> LENGTH: 204
<212> TYPE: DNA
<213> ORGANISM: Diaporthe sp.

<400> SEQUENCE: 46

accctttgtg aacttatacc taccgttgcc tcggcgcagg ccggcccccc tcaccggggg 60

ccccccggag acggggagca gcccgccggc ggccaaccaa actcttgttt cttagtgaat 120

ctctgagtaa aaatcataaa tgaatcaaaa ctttcaacaa cggatctctt ggttctggca 180

tcgatgaaga acgcagcaag ttgc 204

<210> SEQ ID NO 47
<211> LENGTH: 281
<212> TYPE: DNA
<213> ORGANISM: Diatrype sp.

<400> SEQUENCE: 47

ccatgtgaac ttacctttgt tgcctcggcg ggagagccta cccggtacct accctgtagt 60

tacccgggag cgagctaccc tgtagcccgc tgctggccga cccgccggtg gacagtaaaa 120

ctcttgtttt ttagtgatta tctgagtgtt tatacttaat aagttaaaac tttcaacaac 180

ggatctcttg gttctggcat cgatgaagaa cgcagccaat acagagttat cttctcccag 240

cccatgtgaa cttacctttg ttgccccggc gggagagcct a 281

<210> SEQ ID NO 48
<211> LENGTH: 338
<212> TYPE: DNA
<213> ORGANISM: Drechslerella dactyloides

<400> SEQUENCE: 48

ggttagaaac tgttgtttcg gcgggatctc tgccccgggg gcgtcgcagc cccggaccaa 60

gggggccgcc ggaggaccaa ccaaaactct ttttgtatac cccctcgcgg gttttttta 120

taatctgagc cttctcggcg cctctcgtag gcgtttcgaa aatgaatcaa aacttttaaa 180

aacggatctc ttggttctgg catcggatga agaacgcaga gaaatgcgat aagtaatgtg 240

aattgcagaa ttcactgaat catctaatct ttgaacggac attgcgcccg ccagttttct 300

ggcgggcatg cctgtccgag cgtcatttca accctcga 338

<210> SEQ ID NO 49
<211> LENGTH: 247
<212> TYPE: DNA
<213> ORGANISM: Embellisia indefessa

<400> SEQUENCE: 49

gcatcgatac ctgatccgag gtcaaaagtt gaaaaaaggc tttgtggatg ctgaccttgg 60

ctggaagaga gcgcgacttg tgctgcgctc cgaaaccagt aggccggctg caatgacttt 120

aaggcgagtc tccagcgaac tggagacaag acgcccaaca ccaagcaaag cttgagggta 180

caaatgacgc tcgaacaggc atgccctttg gaataccaaa gggcgcaatg tgcgttcaaa 240

aaaagca 247

<210> SEQ ID NO 50
<211> LENGTH: 207
<212> TYPE: DNA
<213> ORGANISM: Epicoccum nigrum

<400> SEQUENCE: 50

ttgtagactt cggtctgcta cctcttaccc atgtcttttg agtaccttcg tttcctcggc 60

gggtccgccc gccgattgga caacattcaa accctttgca gttgcaatca gcgtctgaaa 120

aaacataata gttacaactt tcaacaacgg atctcttggt tctggcatcg atgaaaaacg 180

catcacctag agtttgtaga cttcggt 207

<210> SEQ ID NO 51
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Epicoccum sp.

<400> SEQUENCE: 51

gtacttacct acgatttgtg gagttcggtc tgctacctct tacccatgtc tttttaagta 60

ccttcgtttc ctcggcgggt ccgcccgccg gttggacaac attcaaaccc tttgcagttg 120

caatcagcgt ctgaaaaaac ttaatagtta caactttcaa caacggatct cttggttctg 180 gcatcgaaca caaacgcagc agcttttagg gacctaccgt ctcctcctct tacc 234

<210> SEQ ID NO 52
<211> LENGTH: 237
<212> TYPE: DNA
<213> ORGANISM: Exserohilum rostratum

<400> SEQUENCE: 52

gctaatttcc ccaccaaact tgtagggtgt ggtttgctgg caacagcgaa ccgccccaag 60

tatttttcac ccatgtcttt tgcgcacttt ttgtttcctg ggccagttcg ctcgccacca 120

ggacccaacc ataaaccttt ttttatgcag ttgcaatcag cgtcagtata ataattcaat 180

ttattaaaac tttcaacaac ggatctcttg gttctggcat cgatgaagaa cgcacaa 237

<210> SEQ ID NO 53
<211> LENGTH: 223
<212> TYPE: DNA
<213> ORGANISM: Fusarium chlamydosporum

<400> SEQUENCE: 53

tcccaacccc tgtgacatac ctatacgttg cctcggcgga tcagcccgcg ccccgtaaaa 60

cgggacggcc cgcccgagga cccctaaact ctgtttttag tggaacttct gagtaaaaca 120

aacaaataaa tcaaaacttt caacaacgga tctcttggtt ctggcatcga tgaagaacgc 180

agctcgatga agaacgcagc cccctcccca cgggtgggaa cat 223

<210> SEQ ID NO 54
<211> LENGTH: 258
<212> TYPE: DNA
<213> ORGANISM: Fusarium sp.

<400> SEQUENCE: 54

cactcccaac ccatgtgaac ttatctcttt gttgcctcgg cgcaagctac ccgggacctc 60

gcgccccggg cggcccgccg gcggacaaac caaactctgt tatcttagtt gattatctga 120

gtgtcttatt taataagtca aaactttcaa caacggatct cttggttctg gcatcgatga 180

agaacgcagc aaatcattac agaattatcc aactcccaaa cccatgtgaa cttttctttt 240

tgttgcctcg gcgcaagc 258

<210> SEQ ID NO 55
<211> LENGTH: 236
<212> TYPE: DNA
<213> ORGANISM: Gibellulopsis nigrescens

<400> SEQUENCE: 55

atactcataa ccctttgtga ccttcatacc tgttgcttcg gcggcgcgcc tctcggggcg 60

tgcccgccgg cattatcaga atctctgttc gaacccgacg atacttctga gtgttctaag 120

cgaactgtta aaactttcaa caacggatct cttggctcca gcatcgatga agaacgcagc 180

aaatgagggg tactactctc accccccttt ggcctcttcc cacttgttgc ttcggc 236

<210> SEQ ID NO 56
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: Gnomoniopsis sp.

<400> SEQUENCE: 56

cgggtgctac ccagaaaccc tttgtgaatt attctcattg ttgcctcggc attgactggc 60

ctcttctgga ggtccctttt ccttcgggga aaggagcagg tcggccggtg gccctataaa 120 ctctttgttt ttacagtgta tcttctgagt aaacaactat aaatgaatca aaacttttaa 180

caacggatct cttggttctg gcatcgatga agaacgcagc aatggaacaa acgccctccg 240

ggg 243

<210> SEQ ID NO 57
<211> LENGTH: 251
<212> TYPE: DNA
<213> ORGANISM: Lewia infectoria

<400> SEQUENCE: 57

gcgggctgga cacccccagc cgggcactgc ttcacggcgt gcgcggctgg gccggccctg 60

ctgaattatt cacccgtgtc ttttgcgtac ttcttgtttc ctgggtgggc tcgcccgcca 120

tcaggaccaa ccacaaacct tttgcaatag caatcacggt cagtaacaac gtaattaatt 180

acaactttca acaacggatc tcttggttct ggcatcgatg aagaacgtag cgaaatgcga 240

tacgtagtgt g 251

<210> SEQ ID NO 58
<211> LENGTH: 210
<212> TYPE: DNA
<213> ORGANISM: Mycosphaerella coffeicola

<400> SEQUENCE: 58

aagtcgtact ggcttcgggc tcgacctcca ccctttgtga acacaacttg ttgcttcggg 60

ggcgaccctg ccgtttcgac ggcgagcgcc cccggaggcc ttcaaacact gcatctttgc 120

gtcggagttt aagtaaatta aacaaaactt tcaacaacgg atctcttggt tctggcatcg 180

atgaagaacg cagcggtctg cacacatcag 210

<210> SEQ ID NO 59
<211> LENGTH: 213
<212> TYPE: DNA
<213> ORGANISM: Mycosphaerellaceae sp.

<400> SEQUENCE: 59

gaccacggcc ggccgcgcca gcgataatcc tttgtgcccc gacattgttg cctgcctttt 60

gaccctgcct tggggcgggg gctccgggtg gacacttaaa ctcttgcgta actttgcagt 120

ctgagtaaac ttaattaata aattaaaact tttaacaccg gatctcttgg ttctggcatc 180

gatgacaaaa cgcaacaaac gcagcagtta acc 213

<210> SEQ ID NO 60
<211> LENGTH: 227
<212> TYPE: DNA
<213> ORGANISM: Nigrospora oryzae

<400> SEQUENCE: 60

ctcccaaccc atgtgaactt atctctttgt tgcctcggcg caagctaccc gggacctcgc 60

gccccgggcg gcccgccggc ggacaaacca aactctgtta tcttcgttga ttatctgagt 120

gtcttattta ataagtcaaa actttcaaca acggatctct tggttctggc atcgatgaag 180

aacgcagcaa aaaacgcagc attatcccac tcccaaaccc gtgggaa 227

<210> SEQ ID NO 61
<211> LENGTH: 216
<212> TYPE: DNA
<213> ORGANISM: Nigrospora sp.

<400> SEQUENCE: 61

cccatgtgaa catatctctt tgttgcctcg gcgcaagcta cccgggacct cgcgccccgg 60

gcggcccgcc ggcggacaaa ccaaactctg ttatcttcgt tgattatctg agtgtcttat 120

ttaataagtc aaaactttca acaacggatc tcttggttct ggcatcgatg aagaacgcag 180

cagaaacgct cagccaactc ccagacccgt gtgaag 216

<210> SEQ ID NO 62
<211> LENGTH: 249
<212> TYPE: DNA
<213> ORGANISM: Nigrospora sphaerica

<400> SEQUENCE: 62

actcccaaac ccatgtgaac atatctcttt gttgcctcgg cgcaagctac ccgggacctc 60

gcgccccggg cggcccgccg gcggacaaac caaactctgt tatcttcgtt gattatctga 120

gtgtcttatt taataagtca aaactttcaa caacggatct cttggttctg gcatcgatga 180

agaacgcagc aaaaaaaaaa atattccact ccccaagccg gggggaaaa ttttttttt 240

tttttttgg 249

<210> SEQ ID NO 63
<211> LENGTH: 223
<212> TYPE: DNA
<213> ORGANISM: Paecilomyces sp.

<400> SEQUENCE: 63

aatgcggact cccaaaccac tgtgaacata cccgtaccgt tgcctcggcg ggcggcccca 60

gggcggggcc gcagcctccc cagcggaggc gcccgccgca ggtcgcaaaa ctataactat 120

atttagtggc atctctgagt aacttccaaa caatcaaaac tttcaacaac ggatctcttg 180

gttctggcat cgatgaagaa cgcagccaat acagaacttc gcg 223

<210> SEQ ID NO 64
<211> LENGTH: 205
<212> TYPE: DNA
<213> ORGANISM: Penicillium citrinum

<400> SEQUENCE: 64

aagtacgtga acggggcaaa cctcccaccc gtgttgcccg aacctatgtt gcctcggcgg 60

gccccgcgcc cgccgacggc ccccctgaac gctgtctgaa gttgcagtct gagacctata 120

acgaaattag ttaaaacttt caacaacgga tctcttggtt ccggcatcga tgaagaacgc 180

agcatctggc atcggctgca attcg 205

<210> SEQ ID NO 65
<211> LENGTH: 213
<212> TYPE: DNA
<213> ORGANISM: Retroconis sp.

<400> SEQUENCE: 65

gctatcccaa ccattgtgaa cctacctaca accgttgctt cggcgggcgg ccccgggtct 60

ccccgggcgc ccctccggcc cctcgcgggg gcccgccgga ggtacgcaac cctctgtatt 120

tgcatggcct ctctgagtct ctgtactgaa taagtcaaaa ctttcaacaa cggatctctt 180

ggttctggca tcgatgaaga acgcagcagc tac 213

<210> SEQ ID NO 66
<211> LENGTH: 253

<212> TYPE: DNA
<213> ORGANISM: Rhizopycnis sp.

<400> SEQUENCE: 66

gaaatattgg gggtaagttt acgcttaacc aaaccgttcc gtaggtgaac ctgcggaagg 60

atcattatcg atttcggttt acaccgtttt ctacctttgt ctatgcgtac cacacgttcc 120

ctcggggggc ttggccccca ctaggaccaa acataaacct ttggtaatgg caatcggggt 180

ctgaaataat ttaattatta caactttaaa caacggatct ctgggttctg gcatcggtaa 240

aaaaacacag gaa 253

<210> SEQ ID NO 67
<211> LENGTH: 210
<212> TYPE: DNA
<213> ORGANISM: Schizothecium inaequale

<400> SEQUENCE: 67

tgcaactccc aaccattgtg aacctacctc accgttgcct cggcgggtgg cccccacccg 60

ggccgcgccg gccccaccgg gccggcaacc cgtcagagga ccgcaactct tagtcatcat 120

tggcctctct gagtaactta tacaataagt caaaactttc aacaacggat ctcttggttc 180

tggcatcgat gaagaacgca gcaagtctaa 210

<210> SEQ ID NO 68
<211> LENGTH: 237
<212> TYPE: DNA
<213> ORGANISM: Stagonospora sp.

<400> SEQUENCE: 68

ctagctactg gcatggggac tgttagtctg catggtatca ctaccgatga gcagcaggtc 60

ccctgtctat acccttgttt tttgcgtacc tattgtttcc tcggcgggct tgctcgccgg 120

ctggacaaaa tctataacct ttttttaatc ttcaatcagc gtctgaaatt atacataata 180

attacaactt tcaacaacgg atctcttggt tctggcatcg atgaaaaacg cagccaa 237

<210> SEQ ID NO 69
<211> LENGTH: 223
<212> TYPE: DNA
<213> ORGANISM: Stemphylium lancipes

<400> SEQUENCE: 69

aaatgtggcg ccctttggta ttccaaaggg catgcctgtt cgagcgtcat ttgtaccctc 60

aagctttgct tggtgttggg cgtctttgtc tctcacgaga ctcgccttaa aatgattggc 120

agccgaccta ctggtttcgg agcgcagcac aattcttgca ctttgaatca gccttggttg 180

agcatccatc aagaccacat ttttttaact ttttaccgta cta 223

<210> SEQ ID NO 70
<211> LENGTH: 209
<212> TYPE: DNA
<213> ORGANISM: Thielavia hyrcaniae

<400> SEQUENCE: 70

ctaaaccatt gtgaacctac cttctaccgt tgcttcggcg ggcgggcccc agcgcccccc 60

ccggcccccc gcgggcgccc gccggaggat acccaaactc ttgacattag tggcctctct 120

gagtattctt tactgaataa gtcaaaactt tcaacaacgg atctcttggt tctggcatcg 180

atgaagaacg cagcaattta cagagttgc 209

<210> SEQ ID NO 71
<211> LENGTH: 252
<212> TYPE: DNA
<213> ORGANISM: Thielavia sp.

<400> SEQUENCE: 71

aaccattgtg acgttacctt caaaccgttg cttcggcggg cggcccgggt ccgcccggtg 60

cccccctggcc ccctcgcggg gcgcccgccg gaggaaaccc aactcttgat acattatggc 120

ctctctgagt cttctgtact gaataagtca aaactttcaa caacggatct cttggttctg 180

gcatcgatga agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat 240

catcgaatct tt 252

<210> SEQ ID NO 72
<211> LENGTH: 217
<212> TYPE: DNA
<213> ORGANISM: Ulocladium chartarum

<400> SEQUENCE: 72

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ttggctctgg catcgatgaa gaacgcagcg aaatgcgata agtaatgtga attgcagaat 240

ccagtgaatc atcgaatctt tgaacgcaca ttgcgcccgc cagcattctg gcgggcatgc 300

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taaactttca acaatggatc tcttggttcc gggatcaatg agcaacccaa caaaatgcga    240

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tgtgttgggt cgtcgtcccc tctccggggg ggacgggccc caaacgcagc tgaggcaccg    420

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actttcaaca acggaacctc ttgggtttcg ggcatcgatg aagaacgcag cgaaatgcga    180

taagtaatgt gaattgcaga attcagtgaa tcatcgaatc tttgaacgca cattgcgccc    240

gccagcattc tggcgggcat gcctgttcga gcgtcatttc aaccctcgac ttccctttgg    300

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ccccggaaac aggcgcccgc cggaggactc aaactctgta ttttttcttg ttttagtgta    180

tactatctga gtaaaaaaca atataatgaa tcaaaacttt caacaacgga tctcttggtt    240

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actgtccttt cgccgccccc taaatgactt ggcggcctcg tcgcggccct cctctgcgta    480
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What is claimed is:

1. A method for improving a trait in a cotton plant, the method comprising:

contacting a cotton a seed of said cotton plant with a formulation comprising purified filamentous, spore-forming, facultative fungal endophytes of at least one species, wherein the facultative fungal endophytes are Dothideomycetes capable of producing substances that are beneficial to plants or detrimental to pests or both, and are present in the formulation in an amount effective to decrease the colonization frequencies of endophytes of genus *Alternaria* that are native to the cotton plant and to provide a benefit to the cotton plant compared to a reference cotton plant grown from a seed untreated with the Dothideomycetes facultative fungal endophytes, wherein the benefit is selected from the group consisting of increased square retention, increased boll retention, increased biomass, increased root length, increased root mass, enhanced resistance to drought stress and increased yield.

2. The method of claim 1, wherein the increased square retention is measured in first fruiting position on branches as flowers begin to develop.

3. The method of claim 1, wherein the increased biomass is measured at mid-season in a field.

4. The method of claim 1, wherein the enhanced resistance to drought stress is assessed by withholding water from 7-day old seedlings of the cotton plant grown in the greenhouse, wherein the seedlings have increased time to wilt or time to death as compared to a seedling grown from a reference seed.

5. The method of claim 1, wherein yield is increased by at least about 2%.

6. The method of claim 1, wherein the formulation contains at least 100 (10ˆ2) spores/ml or 100,000 (10ˆ5) spores/g dry weight of the facultative fungal endophytes.

7. The method of claim 1, wherein the benefit is increased square retention.

8. The method of claim 1, wherein the benefit is increased boll retention.

9. The method of claim 1, wherein the benefit is increased biomass.

10. The method of claim 1, wherein the benefit is increased root length.

11. The method of claim 1, wherein the benefit is increased root mass.

12. The method of claim 1, wherein the benefit is enhanced resistance to drought stress.

13. The method of claim 1, wherein the benefit is increased yield.

14. A synthetic combination of a cotton seed and purified filamentous, spore-forming facultative fungal endophytes of at least one species, wherein the facultative fungal endophytes are Dothideomycetes capable of producing substances that are beneficial to plants or detrimental to pests or both and are present in an amount effective to decrease the colonization frequencies of endophytes of genus *Alternaria* that are native to the cotton plant grown from the seed and to provide a benefit to the cotton plant grown from the seed compared to a reference cotton plant grown from a seed untreated with the Dothideomycetes facultative fungal endophytes, wherein the benefit is selected from the group consisting of increased square retention, increased boll retention, increased biomass, increased root length, increased root mass, enhanced resistance to drought stress and increased yield.

15. The synthetic combination of claim 14, wherein the facultative fungal endophytes are present in an amount effective to provide the benefit of increased yield by about 2%.

16. The synthetic combination of claim 14, wherein the facultative fungal endophytes are present at a concentration of at least 100 (10ˆ2) spores/seed on the surface of the seed.

17. The synthetic combination of claim 14, wherein the facultative fungal endophytes are present at a concentration of at least 1,000 (10ˆ3) spores/seed on the surface of the seed.

18. The synthetic combination of claim 14, wherein the facultative fungal endophytes are present at a concentration of at least 10,000 (10ˆ4) spores/seed on the surface of the seed.

19. The synthetic combination of claim 14, wherein the facultative fungal endophytes are in spore form.

20. The synthetic combination of claim 14, comprising at least 2 species of facultative endophytes.

21. The synthetic combination of claim 14, wherein the facultative fungal endophyte is native to the cotton plant grown from the seed.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,545,111 B2
APPLICATION NO. : 14/964440
DATED : January 17, 2017
INVENTOR(S) : Gregory A. Sword

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Column 73, Line 16, Claim 1, replace "contacting a cotton a seed of said cotton plant with a" with --contacting a cotton seed of said cotton plant with a--

Signed and Sealed this
Fifth Day of December, 2017

Joseph Matal

Joseph Matal
Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office