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ScienceDirect
Biological Control 42 (2007) 308–315
Biological Control
www.elsevier.com/locate/ybcon

Utilization of chemical inducers of resistance and *Cryptococcus flavescens* OH 182.9 to reduce Fusarium head blight under greenhouse conditions ☆

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Received 6 February 2007; accepted 24 May 2007
Available online 12 June 2007

Abstract

Four chemicals [salicylic acid (SA), sodium salt of salicylic acid (NaSA), isonicotinic acid (INA), and DL-β-amino-*n*-butyric acid (BABA)] and the yeast antagonist *Cryptococcus flavescens* (=*C. nodaensis* nomen nudum) OH 182.9 were evaluated separately or together for the ability to reduce Fusarium head blight (FHB) of wheat in the greenhouse. When sprayed onto wheat heads at 3 days prior to pathogen challenge with *Gibberella zeae*, NaSA and INA at 10 mM significantly reduced FHB severity compared to the non-treated disease control. Applied at concentrations of 1 and 5 mM at 3 days before pathogen challenge, NaSA or INA in combination with OH 182.9 did not significantly reduce FHB severity compared to either treatment alone, though the lowest disease severity values frequently were associated with the combination treatments. When sprayed onto wheat heads just beginning to emerge from boot at 10 days prior to pathogen inoculation, NaSA, INA, and BABA at 1 mM significantly reduced FHB severity indicating that induced systemic resistance was at least partially responsible for the reduction of FHB disease. Induced FHB resistance was achieved by treating wheat with INA at concentrations as low as 0.1 mM. In only one instance was 100-kernel weight affected by any chemical or combination of chemicals with OH 182.9 treatment. Data from our studies in the greenhouse suggest that chemical inducers can induce resistance in wheat against FHB, and that further efforts are warranted to explore the potential of improved control of FHB disease by incorporating chemical inducers with the FHB biocontrol agent OH 182.9.
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Keywords: *Gibberella zeae*; Induced localized resistance; Systemic acquired resistance; *Triticum aestivum*; Wheat scab

1. Introduction

Fusarium head blight (FHB)[1] is a devastating disease of wheat (*Triticum aestivum*) worldwide causing significant losses in yield and quality when infections occur at or soon after flowering (Bai and Shaner, 2004). In the 1990s, for instance, FHB outbreaks in the United States caused over $6.0 billion of total losses including direct losses of yield and accumulative economic impact (Johnson et al., 2003). In China, losses of wheat yield due to FHB epidemics have

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[1] *Abbreviations used:* SA, salicylic acid; NaSA, sodium salt of salicylic acid; BABA, DL-β-amino-*n*-butyric acid; INA, isonicotinic acidl; FHB, Fusarium head blight.

1049-9644/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2007.05.020

accumulated to more than 1 million tons (Bai et al., 2003; Lu et al., 2001). In addition, *Gibberella zeae* (Schw.) Petch (anamorph = *Fusarium graminearum* Schwabe), the primary causal agent of FHB, commonly produces mycotoxins during the infection including the estrogenic toxin zearalenone and the vomitoxin deoxynivalenol (DON) (Bai and Shaner, 2004), which in sufficient concentration can render grain unacceptable for human or animal consumption.

Reducing the impact of FHB on wheat production and quality remains an intractable problem. Practices such as the use of tillage management (Miller et al., 1998; Dill-Macky and Jones, 2000) to reduce pathogen inoculum, fungicides (Wilcoxson, 1996; Mesterhazy, 2003), and moderately resistant varieties (Bai and Shaner, 2004) provide some but not adequate control of Fusarium head blight. Therefore, it is important to develop additional means for controlling FHB disease in wheat.

Biological control and induced disease resistance by chemicals and microbes have potential to be important components of an integrated pest management approach to enhance the level of FHB reduction achieved. Significant success in biocontrol of FHB has been demonstrated (Stockwell et al., 1997; Yuen et al., 2003; Khan et al., 2004). Microbial antagonists isolated from wheat anthers show effective biological control of FHB on different wheat cultivars under greenhouse and field conditions (Khan et al., 2001; Schisler et al., 2002b). The yeast *Cryptococcus flavescens* (=*C. nodaensis* nomen nudum) OH 182.9 has been one of the most effective strains against FHB in multiple field trials (Schisler et al. 2002a; Khan et al., 2004). Frozen biomass of strain OH 182.9 significantly reduced FHB when results were averaged across 15 field sites in the US (Milus et al., 2001).

Over the last 30 years a number of chemical compounds have been shown to increase disease resistance of plants. Salicylic acid (SA), and its functional analogs [isonicotinic acid (INA), benzothidiazoles (BTH)] and β-amino-*n*-butyric acid (BABA) are among the most thoroughly studied compounds for inducing disease resistance. These compounds activate resistance in many crops against plant diseases that are incited by a variety of pathogens including oomycetes, fungi, bacteria, viruses, and nematodes (Kessmann et al., 1994; Cohen, 2001; Oostendorp et al., 2001). Though some reports have demonstrated that infection by *F. graminearum* (Pritsch et al., 2001) or treatment of wheat with some chemical inducers (Görlach et al., 1996; Ruess et al., 1996) can incite a resistance response in wheat, little is known about the chemicals that can induce resistance against FHB. To our knowledge, no studies have addressed the possibility of combining biological and induced resistance methodologies to reduce diseases on monocot crops. The objectives of this research were to determine whether known chemical inducers such as SA, NaSA, INA, and BABA can induce resistance to FHB of wheat, and to investigate the effects of these chemicals on FHB severity under greenhouse conditions when they were combined individually with the biocontrol agent *C. flavescens* OH 182.9.

2. Materials and methods

2.1. Inoculum of C. flavescens OH 182.9

The yeast strain utilized in this study, *C. flavescens* OH 182.9 (NRRL Y-30216), was originally isolated from the anthers of field-grown wheat in Ohio and was recently renamed from *C. nodaensis* nomen nudem (Dunlap et al., 2007). A stock culture of strain OH 182.9 was stored in 10% (v/v) glycerol at −80 °C and streaked on 1/5 Tryptic soy broth agar (TSBA/5) (Difco Laboratories, Detroit, MI) to ensure purity. A single colony was streaked on TSBA/5 and incubated at 25 °C for 48 h before cells were harvested in weak phosphate buffer (PO_4 buffer, 0.004% KH_2PO_4 buffer with 0.019% $MgCl_2$, Aid Pack, Gloucester, MA). Inoculum of OH 182.9 for greenhouse tests then was prepared by inoculating 50 ml of semi-defined complete liquid medium (SDCL, Slininger et al., 1994) in 250-ml Erlenmeyer flasks with the concentrated cell suspension to an optical density of 0.1 at the wavelength of 620 nm and shaking at 250 rpm, 2.0-cm eccentricity, at 25 °C for 48 h prior to use. In all experiments, a 48 h culture diluted to 1/4-strength, i.e., 25% fully colonized OH 182.9 culture [5×10^7 colony-forming units (CFU)/ml] was applied at 3 days prior to pathogen challenge with conidia of *F. graminearum*.

2.2. Chemical inducers

The chemical inducers tested alone or in combination with OH 182.9 were salicylic acid (SA, Aldrich Chemical Company, Inc., Milwaukee, WI), sodium salt of salicylic acid (NaSA, Aldrich Chemical Company, Inc., Milwaukee, WI), DL-β-amino-*n*-butyric cid (BABA, Sigma–Aldrich Co., St. Louis, MO), and isonicotinic acid (INA, Sigma–Aldrich Co., St. Louis, MO). Aqueous solutions were prepared in deionized water at room temperature (25 °C), or on a hot plate with continuous stirring in the case of INA.

2.3. Wheat plants, growth, and greenhouse conditions

Two wheat seedlings of the cultivar Norm were grown in a 19-cm diameter plastic pot containing air-steam pasteurized (60 °C for 30 min) potting mix (Terra-lite Redearth mix, W.R. Grace, Cambridge, MA) in a growth chamber (25 °C, 14 h light/day, 600 μmol/(m^2/s)) for 7–8 weeks prior to use. Pots were fertilized after 1 week and weekly thereafter with 50 ml of a solution containing 1.25 g/L Peters 20-20-20 (Grace-Sierra Horticultural Products, Milpitas, CA) and 0.079 g/L iron chelate (Sprint 330, Becker Underwood, Inc., Ames, IA).

All experiments were conducted in a climate-controlled greenhouse using wheat plants initially grown for 7–8 weeks in a growth chamber as described previously (Zhang et al., 2005). Temperatures in the greenhouse ranged from 23 to 28 °C during the day and 15 to 20 °C at night.

Natural sunlight was supplemented by high-pressure sodium lights for 14 h/day during the winter season.

2.4. Application of chemical inducers

To evaluate chemical resistance inducers for the efficacy to reduce FHB disease, 10 ml of chemical inducers (1 and 10 mM) per replicate were sprayed onto wheat heads at start of flowering (Feeke's growth stage 10.5.1; Large, 1954) at 3 days before pathogen challenge. In order to determine whether FHB reduction was due at least partially to induced systemic resistance by chemical treatments, a separate experiment was conducted in which chemical solutions at 1 and 10 mM were applied to foliage when wheat heads were nearing the stage of splitting the flag leaf sheath (Feeke's 10.0, late boot), at 10 days before pathogen inoculation. Chemical solutions (40 ml) were sprayed onto four plants representing four replicates with a total of 12–16 heads in each treatment. Treated plants were arranged in a completely randomized design, and each experiment was performed twice.

2.5. Application of the biocontrol agent OH 182.9 in combination with chemical inducers

The experiment was conducted to determine whether incorporation of a chemical inducer with OH 182.9 would enhance the efficacy of FHB control when the combination was applied at 3 days before pathogen challenge. Chemical inducers were evaluated for reducing FHB at various concentrations alone or in combination with 25% fully colonized OH 182.9 cultures. NaSA and INA were tested at 1, 3, 5, 7, and 10 mM and BABA at 1, 5, 7, and 10 mM. The spray solutions were obtained by diluting chemical inducers, fully colonized OH 182.9 cultures (to 25%, approx. 5×10^7 CFU/ml), or a mixture of a chemical inducer and strain OH 182.9 with weak PO_4 buffer and adding 0.036% Tween 80 (Sigma Chemical Co., St. Louis, MO). Samples were collected from mixtures of chemical inducers and OH 182.9 to evaluate the effect of the chemicals on OH 182.9 cell viability before application (0 h) and after being stored at room temperature for 24 h. Three samples were taken from each treatment solution. Serial dilutions were made for each sample and plated on TSBA/5 medium. Plates were incubated at 25 °C for 2 days before determining colony counts. Chemical solutions or mixtures with OH 182.9 (40 ml) were used to inoculate four plants representing four replicates with a total of 12–16 heads in each treatment. Heads sprayed with 25% fully colonized OH 182.9 cultures served as an additional control. Treatments were arranged in a completely randomized design, and the experiment was performed twice.

Treatments with INA alone or in combination with OH 182.9 were found to reduce FHB to the greatest extent. To determine if FHB control could be achieved in a more economical way by combining chemical inducers and OH 182.9, the efficacy of INA at lower concentrations (0.1, 0.5, and 1 mM) alone or in combination with OH 182.9 was evaluated when INA was applied at 10 days, and 25% OH 182.9 cultures at 3 days before pathogen challenge. The experiment had the same experimental design as stated above.

2.6. Preparation, inoculation of F. graminearum inoculum and data collection

Inoculum of *F. graminearum* was prepared from isolate Z-3639 by producing macroconidia on clarified V8 agar (CV8 agar) under a regime of 12 h/day fluorescent light for 7 days at 24 °C, flooding the surface of colonized CV8 agar with weak PO_4 buffer and removing conidia with a sterile inoculation loop.

For all the experiments described earlier, after treatment with chemical inducers or cells of OH 182.9, wheat heads at or soon after anthesis (Feeke's growth stage 10.5.1 or 10.5.2) were challenged with 12 ml of an inoculum containing conidia of *F. graminearum* (1.5×10^4 conidia/ml) in weak PO_4 buffer with 0.036% Tween 80. Wheat heads inoculated only with weak PO_4 buffer and Tween 80, and then with a suspension of *F. graminearum* served as disease controls. Inoculated plants were misted lightly with water and incubated in a plastic humidity chamber at 20–25 °C for 3 days before being transferred to greenhouse benches. The severity of FHB was visually estimated using a 0–100% scale (Stack and McMullen, 1995) at 10–14 days after inoculation. Wheat heads were then allowed to mature and dry, hand threshed, and 100-kernel weights were determined for each replicate of treatments in all experiments.

2.7. Statistical analysis

Disease severity data were normalized using the arcsine transformation before analysis of variance. Disease data from 2 or more experimental repeats were pooled after statistical analysis demonstrated that experiment by treatment interactions were not significant ($P > 0.05$). Data were then subjected to analysis of variance (ANOVA) (JMP PC Windows Version 5.0, SAS Institute, Cary, NC). One-way ANOVAs were performed for each OH 182.9 or OH 182.9 with SAR inducer combination. The significance of treatment effects was determined by the magnitude of the F value ($P = 0.05$). Separation of means was accomplished using Fisher's protected LSD test.

3. Results

3.1. Effect of chemical inducers on FHB disease severity when applied at 3 days prior to pathogen challenge

NaSA and INA at 10 mM significantly ($P \leqslant 0.05$) reduced FHB severity compared to the non-treated control (Fig. 1). None of the treatments at 1 mM had a significant effect on FHB reduction. With the exception of treatment with NaSA at 10 mM, there were no effects of the

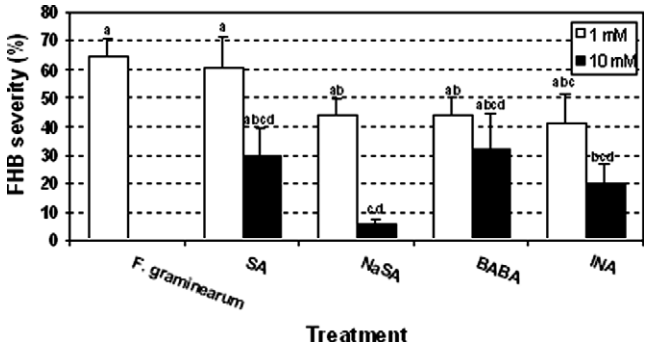

Fig. 1. Effect of chemical resistance inducers on FHB when applied to wheat heads at 3 days before pathogen challenge with *F. graminearum*. Chemical resistance inducers (SA, NaSA, BABA, and INA) at 1 or 10 mM were sprayed onto heads at anthesis. Three days after treatment with chemicals, heads were inoculated with conidia of *F. graminearum* (1.5×10^4 conidia/ml). Wheat heads inoculated only with weak PO_4 buffer, and then with a suspension of *F. graminearum* served as a non-treated disease control. Data presented are averages of FHB disease severity from two experimental repeats. Error bars represent standard errors of the mean. Bars not marked with the same letter are significantly different (Fisher's protected LSD test, $LSD_{0.05} = 36$).

treatments on 100-kernel weight compared to the non-treated control ($P \leqslant 0.05$), with values ranging from 2.4 to 2.9 g among treatments (data not shown).

3.2. Effect of chemical inducers on FHB disease severity when applied at 10 days prior to pathogen challenge

When chemical inducers were sprayed onto the flag leaf sheath that enclosed the heads at 10 days before the pathogen challenge, all treatments at 1 mM except for SA significantly ($P \leqslant 0.05$) reduced FHB disease compared to the non-treated control (Fig. 2). Disease severity was reduced by 42–57%. None of the treatments at 10 mM had a significant effect on FHB reduction, though reduction of FHB severity in these treatments was 38–51%, relative to the non-treated control. There were no effects on 100-kernel weight in any of the treatments in this experiment compared to the control, with values ranging from 2.0 to 2.5 g among treatments (data not shown).

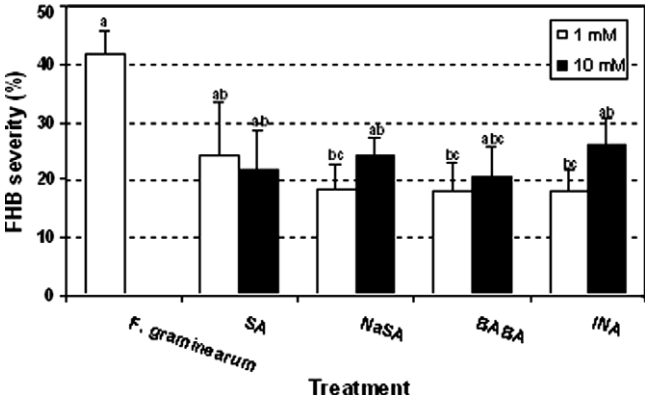

Fig. 2. Effect of chemical inducers on FHB when applied to the leaf sheath surrounding the heads at 10 days before pathogen challenge with *F. graminearum*. Chemical resistance inducers (SA, NaSA, BABA, and INA) at 1 and 10 mM were sprayed onto foliage at boot stage. Ten days after treatment with chemicals, heads were inoculated with conidia of *F. graminearum*. Wheat heads inoculated only with weak PO_4 buffer, and then with a suspension of *F. graminearum* served as a non-treated control. Data presented are averages of FHB disease severity from two experimental repeats. Error bars represent standard errors of the mean. Bars not marked with the same letter are significantly different (Fisher's protected LSD test, $LSD_{0.05} = 24$).

3.3. Efficacy of chemical inducers alone or in combination with OH 182.9 against FHB when both applied at 3 days prior to pathogen challenge

Because SA did not significantly reduce FHB severity at 1 or 10 mM, SA was not tested in this experiment. Chemical inducers alone at the highest concentrations significantly ($P \leqslant 0.05$) reduced FHB disease compared to the non-treated controls when applied at 3 days prior to

Table 1
Efficacy of chemical resistance inducers alone or in combination with *C. flavescens* OH 182.9 for controlling FHB in the greenhouse[a]

Chemical inducer	Concentration (mM)	OH 182.9	FHB severity (%)[b]
NaSA	1	–	31 ab
	1	+	12 b
	3	–	23 ab
	5	–	29 ab
	5	+	23 ab
	7	–	15 b
	10	–	16 b
OH 182.9		+	32 ab
Disease control			49 a
$LSD_{0.05}$			30
INA	1	–	18 ab
	1	+	5 b
	3	–	8 b
	5	–	10 b
	5	+	3 b
	7	–	11 ab
	10	–	4 b
OH 182.9		+	7 b
Disease control			26 a
$LSD_{0.05}$			16
BABA	1	–	21 ab
	1	+	29 ab
	5	–	27 ab
	5	+	25 ab
	7	–	22 ab
	10	–	13 b
OH 182.9		+	8 b
Disease control			45 a
$LSD_{0.05}$			32

[a] Chemical inducers at 1, 3, 5, 7, and 10 mM and/or 25% fully colonized OH 182.9 cultures (5×10^7 CFU/ml) was sprayed onto wheat heads at 3 days before pathogen challenge with *F. graminearum*. Wheat heads inoculated only with weak PO_4 buffer, and then with a suspension of *F. graminearum* served as a disease control.

[b] Data from two repeated experiments and after arcsine transformation. Means followed by a common letter in a column are not significantly different ($P \leqslant 0.05$, Fisher's protected LSD).

pathogen inoculation (Table 1). Plants treated with NaSA at 7 and 10 mM, INA at 3, 5, and 10 mM and BABA at 10 mM had significantly lower FHB disease compared to the non-treated controls. NaSA and INA at low concentrations (1 and 5 mM) combined with 25% fully colonized OH 182.9 culture did not significantly reduce FHB severity compared to either treatment alone. Treatment with NaSA at 1 mM and OH 182.9 significantly decreased the disease severity of FHB compared to the non-treated control, while NaSA or OH 182.9 treatments alone did not. Similarly, wheat plants treated with INA at 1 or 5 mM and OH 182.9 had a significant lower FHB severity compared to the non-treated control plants. Treatments with INA at 1 or 5 mM in combination with OH 182.9 were among the best treatments for FHB disease reduction, as was treatment with INA at 10 mM. Incorporation of BABA at 1 or 5 mM with OH 182.9 did not have a significant effect on FHB reduction, though OH 182.9 alone significantly suppressed FHB compared to the control. The population of OH 182.9 cells was not affected by any of the chemical inducers at the concentrations tested (1 and 5 mM, Table 2). None of the treatments increased 100-kernel weight compared to the control, with values ranging from 2.2 to 3.0 g among treatments (data not shown).

3.4. Efficacy of INA at low concentrations alone or in combination with OH 182.9 when INA and OH 182.9 were applied at 10 and 3 days prior to pathogen challenge, respectively

All treatments significantly ($P \leqslant 0.05$) protected wheat plants from FHB disease compared to the non-treated control (Fig. 3). Combining INA at the test concentrations and OH 182.9 did not enhance the level of disease reduction compared to either treatment alone. Although the treatments that significantly reduced FHB disease severity generally also were associated with higher 100-kernel weight, none of these treatments, either with chemical inducers alone or in combination with OH 182.9, had a significant effect ($P \leqslant 0.05$) on 100-kernel weight compared to the non-treated control, with values ranging from 2.2 to 2.5 g among treatments (data not shown).

4. Discussion

Our results demonstrated that the chemical inducers tested in this study significantly reduced FHB severity in greenhouse-grown wheat. Treatment with some lower concentrations of the chemicals NaSA or INA in combination with the yeast biocontrol agent *C. flavescens* OH 182.9 did not significantly reduce FHB severity compared to either treatment alone. In many cases the combined treatment reduced FHB severity to a modestly greater extent than either treatment alone, albeit not significantly. More efforts are needed to explore the potential for reducing FHB by incorporating chemical inducers with the FHB biocontrol agent OH 182.9.

Chemical inducers tested at a relatively higher concentration (10 mM) rather than at lower concentrations generally reduced FHB severity when applied at 3 days prior to the challenge inoculation with *F. graminearum*, though the effect of SA and BABA was not significant compared to the control. A dose response was not clearly demonstrated for any of the chemical inducers tested. Pajot et al. (2001) demonstrated a clear dose response using 58% potassium phosphonate (K_2HPO_3) in water to reduce downy mildew of lettuce, though concentrations of the inducer varied over a wider range (0–87 ppm) than used in the present study. When the time between chemical treatments and challenge with conidia of *F. graminearum* increased to 10 days, NaSA, INA, and BABA at 1 mM, but not 10 mM significantly induced resistance to FHB. While the longer period between treatment with a lower concentration of a chemical inducer and challenge with pathogen inoculum may have resulted in a greater cumulative induced resistance response in plants, it is uncertain why this response did not occur in the treatments with 10 mM chemical inducer.

Although there are numerous reports on chemical-induced disease resistance, very few products have been commercially developed due in part to the potential for these compounds to be phytotoxic to some crops (Siegrist et al., 2000). For instance, application of BABA caused small necrotic lesions on treated leaves when sprayed on tobacco leaves at 1 mM (Cohen, 1994). In another study on tobacco by Siegrist et al. (2000), a rapid induction of

Table 2
Effect of chemical inducers on the population ($\log_{10}$ CFU/ml) of *C. flavescens* OH 182.9 cells *in vitro*[a]

Concentration (mM)	0 h			24 h			% Change after 24 h		
	NaSA	INA	BABA	NaSA	INA	BABA	NaSA	INA	BABA
0	7.42 ± 0.83 a	7.41 ± 0.78 a	7.51 ± 0.94 ab	7.34 ± 0.67 a	7.34 ± 0.79 a	7.45 ± 0.66 ab	−1.1 a	−1.0 a	−0.8 a
1	7.42 ± 0.75 a	7.35 ± 0.84 a	7.43 ± 0.69 b	7.37 ± 0.70 a	7.34 ± 0.86 a	7.39 ± 0.80 b	−0.7 a	−0.1 a	−0.5 a
5	7.47 ± 0.91 a	7.38 ± 0.72 a	7.56 ± 0.77 a	7.44 ± 0.78a	7.33 ± 0.76 a	7.53 ± 0.70 a	−0.4 a	−0.7 a	−0.4 a
$LSD_{0.05}$	0.09	0.12	0.10	0.16	0.13	0.12	1.8	1.5	1.1

[a] Samples were collected from a suspension containing a chemical inducer and 25% fully colonized OH 182.9 culture. Serial dilutions were made for each sample and plated on TSBA/5 medium. Plates were incubated at 25 °C for 2 days until quantifying the colony-forming units (CFU). Data presented are averages ± standard deviations from two repeated experiments. Within a column, means with different letters are significantly different ($P \leqslant 0.05$) based on Fisher's protected LSD test.

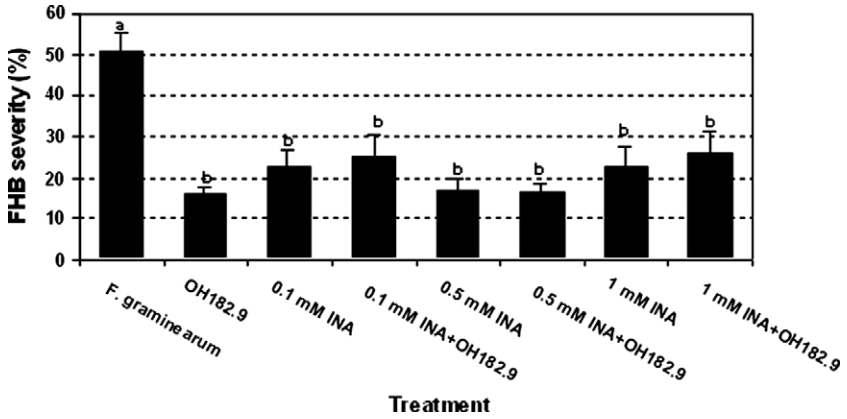

Fig. 3. Efficacy of isonicotinic acid (INA) at low concentrations in combination with *C. flavescens* OH 182.9 against FHB. INA at 0.1, 0.5, and 1 mM and/or OH 182.9 were sprayed onto leaf sheaths surrounding heads and onto heads that had emerged from leaf sheaths at 10 and 3 days before pathogen challenge with *F. graminearum* OH 182.9, respectively. Wheat heads inoculated only with weak PO_4 buffer, and then with a suspension of *F. graminearum* served as a non-treated control. Data presented are averages of FHB disease severity from two identical experiments. Error bars represent standard errors of the mean. Bars not marked with the same letter are significantly different (Fisher's protected LSD test, $LSD_{0.05} = 23$).

necrotic lesions was observed after foliar treatment with BABA at 10 mM. Formation of necrotic lesions was accompanied by induction of reactive oxygen species, lipid peroxidation, and callose around the lesion. It is apparent that disease protection in tobacco induced by BABA was a result of induced local resistance (ILR) (Hammerschmidt et al., 2001). Under our experimental conditions, no visible phytotoxicity in wheat was observed for any of the chemicals tested at either concentration. However, we cannot discount the possibility that microscopic necrotic lesions formed in wheat tissues in response to foliar treatment with 10 mM chemical inducers led to ILR to FHB. It is possible that treatments with chemical inducers at the higher concentration (10 mM) elicit ILR, while at lower concentration (1 mM) induced systemic resistance (ISR) was triggered, which requires enough time to be expressed. In our case, when applied at 3 days prior to pathogen challenge, the chemicals directly contacted the heads. FHB decrease may be a result of ILR triggered by chemical treatments at 10 mM as ISR with treatments at 1 mM was not allowed to express due to the short time between pathogen challenge and chemical treatments. With the case in which chemicals were applied to foliage (flag leaf sheath) surrounding wheat heads at 10 days before pathogen inoculation, reduction of FHB severity may be a manifestation of ISR by treatments at 1 mM, while ILR triggered at 10 mM was confined in the leaf sheath, not in heads of wheat where FHB occurs. This may, to some extent, explain the reasons that chemical inducers behaved differently at two concentrations in reduction of FHB in wheat.

One of the most prominent induced responses upon treatment with elicitors including chemical inducers is the accumulation of pathogenesis-related (PR) proteins (Linthorst, 1991). Some of these PR proteins have antifungal or antibacterial activities, suggesting that PR proteins play a role in the enhanced protection against disease in induced plants. In addition, phytoalexin synthesis and cell wall strengthening are defense responses that are induced by chemical treatment. Increases in activities of oxidative enzymes are involved in these responses. For the FHB suppression by chemical inducers, little is known about the mechanisms by which these chemicals exert disease control effect. Research is needed to determine how chemical treatment induces resistance to FHB in wheat.

The mode of action of antagonistic yeasts for controlling plant diseases may include competition for space and nutrients (Roberts, 1990), attachment and production of lytic enzymes (Wisniewski et al., 1991; El-Ghaouth et al., 1998) and induction of host responses (Droby et al., 2002). The biocontrol activity of yeast strains against plant diseases may be the result of interactions of different factors. The ability to multiply rapidly and competition for nutrients are likely the primary mechanisms by which *Cryptococcus laurentii* controls gray mold of apple (Roberts, 1990). The same mechanisms by which *C. flavescens* OH 182.9 exerts biocontrol activity against FHB were suggested by Core et al. (2002), who investigated the population dynamics of OH 182.9 cells after inoculating wheat heads at anthesis in the field. The population of OH 182.9 cells increased 80- and 84-fold by 6 and 10 days, respectively, after spraying. In our preliminary study, OH 182.9 did not demonstrate any antagonistic effect on conidia of *F. graminearum* regardless of which substrate was used for this test, either on Tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) or water agar (data not shown). In the greenhouse, treating wheat foliage, but not heads, with OH 182.9 cells at 3 and 10 days before pathogen inoculation of heads did not result in lower FHB disease relative to the non-treated control plants (data not shown), indicating that the induction of systemic resistance in the host is not a predominant mechanism of FHB control by OH 182.9.

While our study produced the first example of the potential benefit of combining antagonist and chemical inducers,

e.g., at 1 and 5 mM, to combat a pathogen on monocots, a similar phenomenon has been described in dicot pathosystems. Enhancement of biocontrol efficacy of an antagonistic yeast *Rhodotorula glutinis* was achieved against fruit decay caused by *Penicillium expansum* and *Alternaria alternata* in sweet cherry by addition of SA (Qin et al., 2003). Improved control of *Rhizoctonia solani* in bean and cucumber has been reported when any one of five inducers, including acetylsalicylic acid, 2,6-dichoroisonicotinic acid, and 2-aminoisobutyric acid was applied in a mixture with an antagonistic strain of *Pseudomonas fluorescens* (Kataria et al., 1997). A mixture of BABA and a plant growth-promoting rhizobacterial (PGPR) strain *Bacillus cereus* C1 greatly enhanced protection of peanut against late leaf spot (Zhang et al., 2001). In tomato, Fakhouri et al. (2000) reported induced synergistic disease resistance against fungal and bacterial pathogens by application of acibenzolar-*S*-methyl (ASM) in combination with fluorescent pseudomonad isolates G309 and CW2. As discussed earlier, the ability to multiply rapidly and to maintain a high population on wheat heads appears to be critical for OH 182.9 to exert biocontrol activity against FHB. Therefore, when considering combining cells of OH 182.9 with other disease-reducing treatments, it is important that the multiplication ability of OH 182.9 not be inhibited. At the concentrations tested, none of the chemical inducers showed significant adverse effect *in vitro* on cells of *C. flavescens* OH 182.9 (Table 1).

Improving the consistency of disease control using antagonists will greatly enhance their acceptance in the marketplace. Biocontrol consistency can be enhanced by manipulation of the environment, the use of mixtures of microorganisms, and by combining microorganisms with fungicides (Janisiewicz and Korsten, 2002). In the past several years, promising research has been conducted for improving disease control by combining antagonists with chemical resistance inducers (Kataria et al., 1997; Fakhouri et al., 2000; Zhang et al., 2001; Qin et al., 2003; Kloepper et al., 2004). Data from our greenhouse experiments do not discount the possibility of enhancing control of FHB by incorporating known chemical inducers with the yeast biocontrol agent OH 182.9. Further research should clarify how to improve FHB control by optimizing the appropriate concentrations and application timing of chemical inducers. Field testing of combinations of chemical inducers and the yeast *C. flavescens* strain OH 182.9 along with studies to develop formulations that enhance delivery and maintenance of these products on wheat plants will determine whether such combinations can provide a useful tool for FHB management.

Acknowledgments

The authors thank Jennifer Sloan for producing the wheat plants used in this study. This research was supported by the US Department of Agriculture.

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