IN VITRO SENSITIVITY OF *FUSARIUM OXYSPORUM* f. sp. *VASINFECTUM* TO SEVERAL FUNGICIDES

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Abstract

Laboratory studies were conducted to determine the in vitro sensitivity of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) to the fungicides azoxystrobin, flutriafol, iprodione and pyraclostrobin. Inhibition of hyphal growth was observed for all fungicide concentrations evaluated. The response of *Fov* to increasing concentrations of flutriafol and iprodione (log_{10}-transformed) exhibited the typical s-shaped dose-response relationship with EC_{50} values of 0.0004 and 0.0012 µg a.i. per ml, respectively. Overall, the fungus appears to be more sensitive to pyraclostrobin than azoxystrobin; however, realistic EC_{50} estimates could not be determined from these data. The addition of salicylhydroxamic acid (which is used to inhibit the action of the alternative oxidase respiratory pathway) *in vitro* was found to adversely affect growth of the fungus by 21.8±1.9%. Additional studies are needed to better quantify the sensitivity of *Fov* to fungicides, especially the strobilurins before they should be evaluated in the field.

Introduction

The Fusarium wilt – Root-knot nematode complex (caused by *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) and *Meloidogyne incognita*, respectively) is an important disease of cotton (*Gossypium hirsutum*) throughout the United States (Blasingame et al., 2008, Starr et al., 1989). In the southern High Plains of Texas, the disease is most prevalent on light-textured sandier soils. The initial symptoms appear on the foliage and above-ground portions of the plant. Infected plants will begin to wilt, especially during warmer weather, and exhibit yellowing or browning of lower leaf margins. These symptoms generally occur early in the season typically 30 to 45 days after planting, but may appear throughout the entire growing season. Plant death may occur in the case of severe infections. A general field diagnostic test for wilt diseases is to examine the vascular system. Healthy plants will have a white vascular system, whereas, the vascular system of diseased plants will be discolored.

Management of Fusarium wilt is achieved through the use of cultivars that are resistant to the fungus or the nematode (Shepherd et al., 1986; Shepherd et al., 1986). Other management options consist of rotation with crops that are a poor host for the nematode, fumigation and at plant nematicides (Colyer et al., 1997). Economically viable non-host crops are limited, and fumigation is seldom used in this region. Aldicarb is often used to reduce damage caused by *M. incognita*; however, the pending loss of this nematicide will greatly impact Fusarium wilt management in the future. Several fungicides are common components of cotton seed treatments some of which can be applied in the seed furrow at planting. Other fungicides commercially available in other cropping systems are being evaluated in cotton. The objective of this study was to evaluate the *in vitro* sensitivity of *Fov* to such fungicides.

Materials and methods

***In vitro* sensitivity assays.** Pathogen sensitivity to the fungicides azoxystrobin, flutriafol, iprodione and pyraclostrobin (Table 1) was det. Quarter strength potato dextrose agar (QPDA) was amended with 0.001, 0.01. 0.1, 1.0 and 10.0 µg a.i. / ml using a commercial formulation of each fungicide (Table 1). Molten media was cooled to 50 ± 5 °C prior to the addition of fungicides. Salicylhydroxamic acid (SHAM) at 100 µg / ml was added to agar amended with azoxystrobin and pyraclostrobin to inhibit the action of the alternative oxidase respiratory pathway (Broders et al., 2007; Vincelli and Dixon, 2002). Two controls were included for comparison with the strobilurin fungicides; the first was non-amended QPDA and the second was QPDA amended with SHAM at 100 µg / ml.

The experimental design was a randomized complete block with a split-plot arrangement of treatments and five replications. Fungicide concentration served as main plots and *Fov* isolate served as sub-plots. Petri plates were maintained at room temperature in the dark to minimize photo-degredation. The experiment was repeated once

resulting in a total of 10 replications. Hyphal growth was measured from the edge of agar plugs in two directions perpendicular to each other. Measurements were taken 5 days after inoculation (DAI).

Statistical analysis. Data were subjected to analysis of variance the general linear model procedure (PROC GLM) in SAS (SAS Institute, Cary, NC, version 9.1). Means were separated using Fisher's Protected LSD. Data (mean colony diameter) were used to fit exponential decay models. The percent inhibition of hyphal growth was calculated by dividing the average colony diameter by the average colony diameter on the non-fungicide amended media, multiplied by 100. Percent inhibition values were converted to a proportion, probit transformed and linearly regressed on $\log_{10}$-transformed fungicide concentration. Fungicide sensitivity expressed as EC_{50} and EC_{95} (the effective concentration to inhibit hyphal growth by 50 and 95%, respectively) were estimated from linear regressions (Stevenson et al., 2004). Differences in activity between fungicides (irpodione vs. flutriafol and azoxystrobin vs. pyraclostrobin) were determined using a simple t-test. Comparisons between QPDA and QPDA amended with SHAM were based on area under hyphal growth curve values.

Results and discussion

Variation in growth of the four *Fov* isolates was observed with isolate *Fov*2010.AB1 exhibiting a faster growth rate (data not shown). Despite such subtle differences the overall effect of increasing fungicide concentrations were similar, thus data were combined for analysis.

The growth responses of *Fov* to increasing concentrations of azoxystrobin and pyraclostrobin were described by negative exponential functions (Fig. 1). In general, *Fov* appears to be more sensitive to pyraclostrobin than azoxystrobin with hyphal growth at 1.0 µg/ml being inhibited by 100 and 87.8%, respectively (Fig. 2). SHAM at 100 µg / ml was found to negatively impact hyphal growth for all *Fov* isolates with differences in growth on amended and non-amended QPDA being observed 1 DAI (Fig. 3). When comparing hyphal growth over time (7 DAI), the addition of SHAM inhibited growth of the fungus by 19.1 to 23.3% (Table 2). Estimates of EC_{50} and EC_{95} values for both pyraclostrobin and azoxystrobin do not appear realistic (Table 3). This could be a function of the adverse effects of SHAM *in vitro* in conjunction with the response of the fungus to the concentrations evaluated. Higher concentrations (Wise et al., 2008) in addition to a more observations between concentrations (Stevenson et al., 2004) as well as an increased number of isolates may be required to better determine sensitivity of the fungus to strobilurin fungicides. Furthermore, additional studies may be required to quantify the impact of SHAM on *Fov* (Broders et al., 2007).

As with pyraclostrobin and azoxystrobin the growth responses of *Fov* to iprodione and flutriafol were best described by negative exponential functions (Fig. 4). Hyphal growth was completely inhibited at 0.1 µg/ml for flutriafol (Fig. 4-A); however, this level of control was not achieved with any of the iprodione concentrations evaluated (Fig. 4-B). Overall, *Fov* was found to be more sensitive flutriafol than iprodione (Fig. 5). Iprodione is labeled for use in cotton as an in-furrow treatment for seedling disease; however, the fungicide has limited activity on *Fusarium* spp. (Jones, 2000). According to Isakeit et al. (2010) flutriafol has activity toward *Phymatotrichopsis omnivora*, the cotton root rot pathogen and is currently being evaluated in cotton. Information regarding the sensitivity of *Fusarium* spp. to flutriafol is lacking; however, results from this study suggest that *Fov* is quite sensitive to the fungicide with EC_{50} and EC_{95} values of 0.0004 and 0.09, respectively (Table 3). Previous studies have shown that triazole fungicides have activity towards *Fusarium* spp. (Burlakoti et al., 2010; Jones, 2000). While numerous fungicides exhibit activity toward a pathogen *in vitro*, the problem appears to be getting the fungicide to the target. Additional testing is needed to determine if any of the fungicides evaluated in this study can be used for management of Fusarium wilt in the field.

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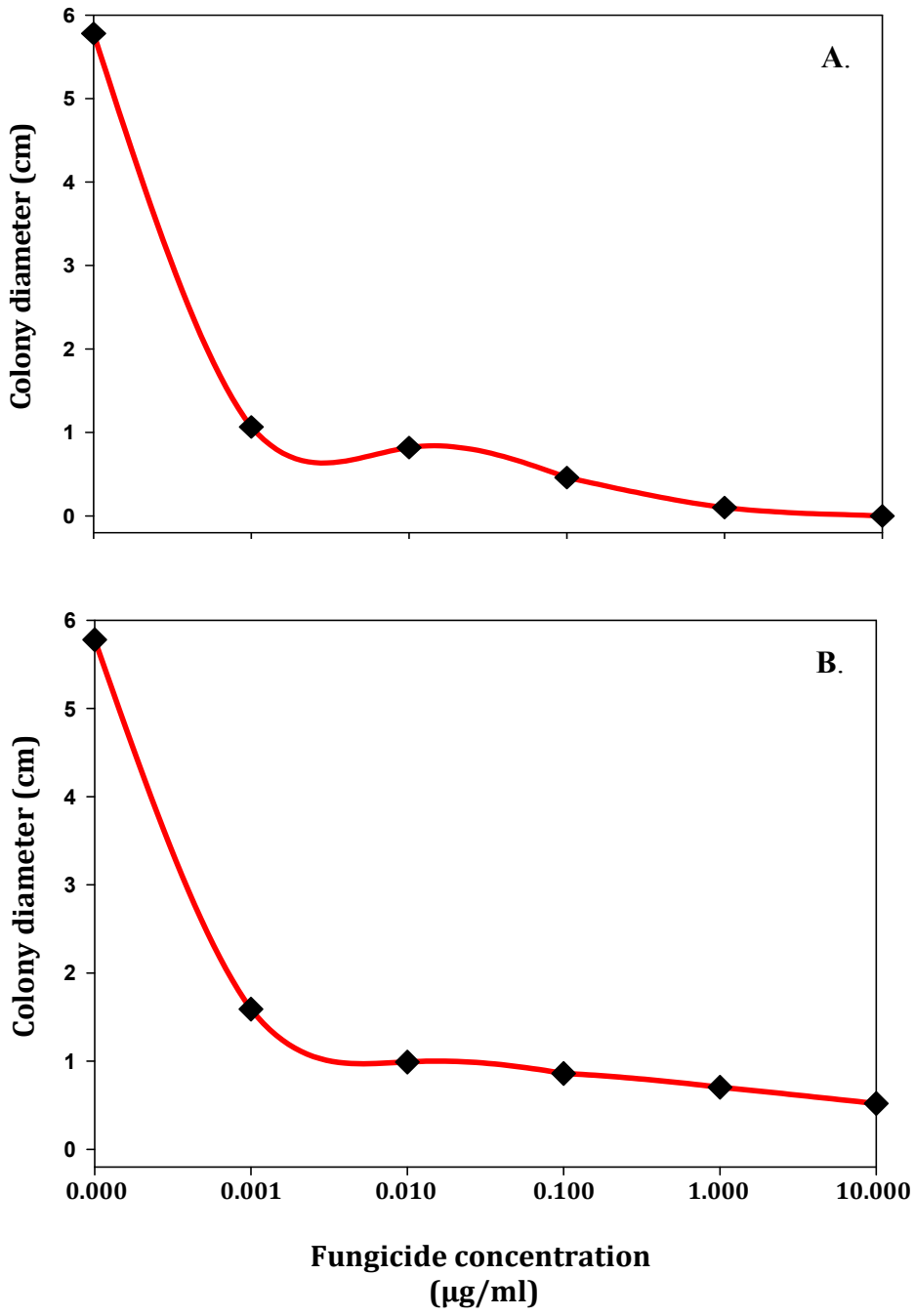

Figure 1. Colony diatemeter of *Fusarium oxysporum* f. sp. *vasinfectum* (the mean of four isolates) to increasing concentrations of (A) pyraclostrobin and (B) azoxystrobin. Data were combined from two experiments (n=10).

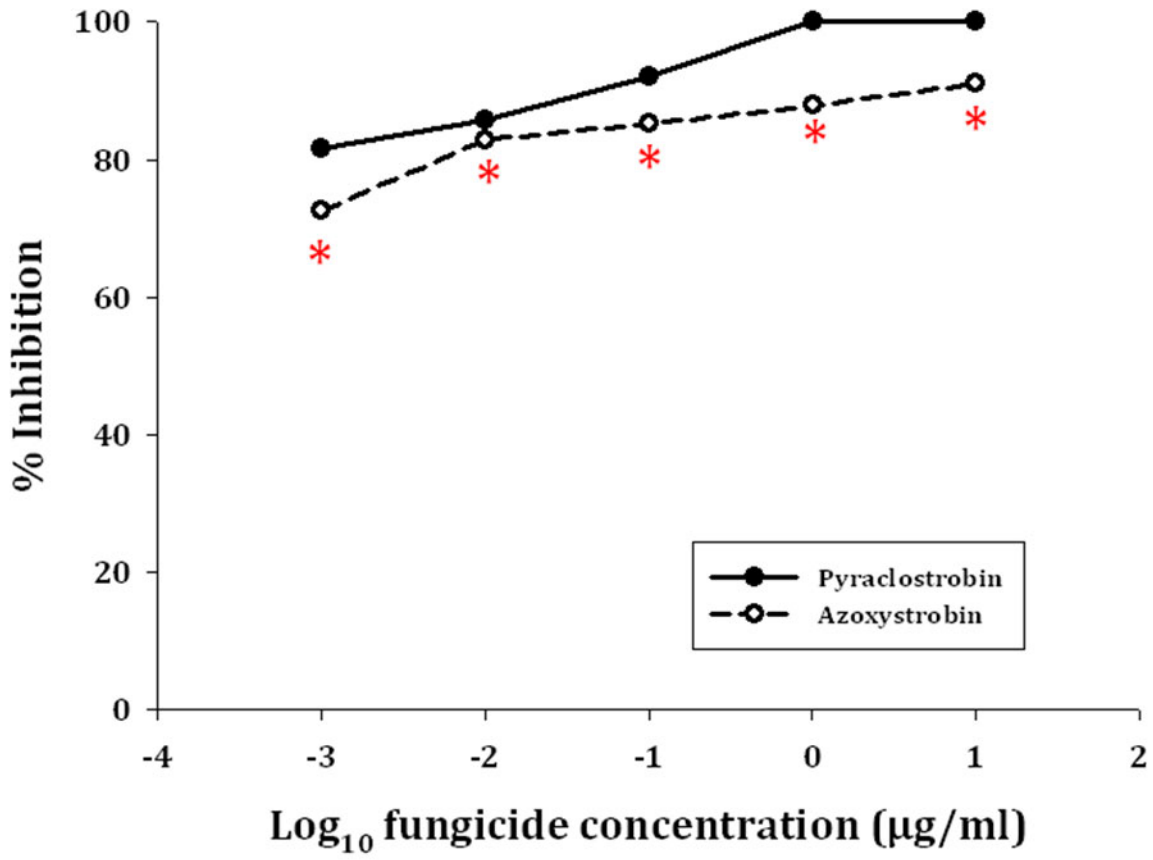

Figure 2. Dose response curves of *Fusarium oxysporum* f. sp. *vasinfectum* (the mean of four isolates) to increasing concentrations of pyraclostrobin and azoxystrobin. Data were combined from two experiments (n=10). Aesteriks idicate diffrences in inhibition of the two fungicides at the given concentration.

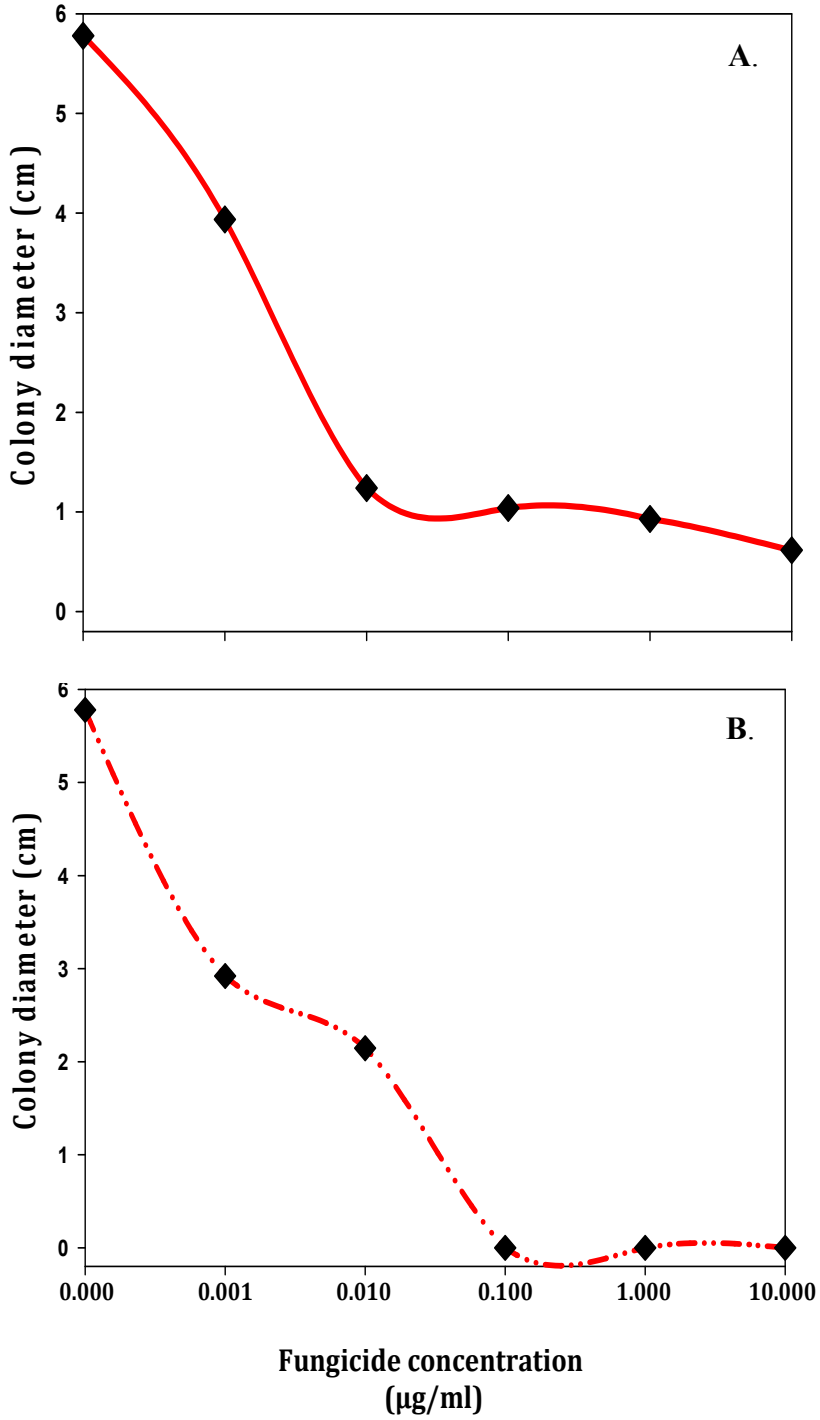

Figure 3. Colony diatemeter of four isolates of *Fusarium oxysporum* f. sp. *vasinfectum* to increasing concentrations of (A) iprodione and (B) flutriafol. Data were combined from two experiments and represent the means of 10 observations per concentration.

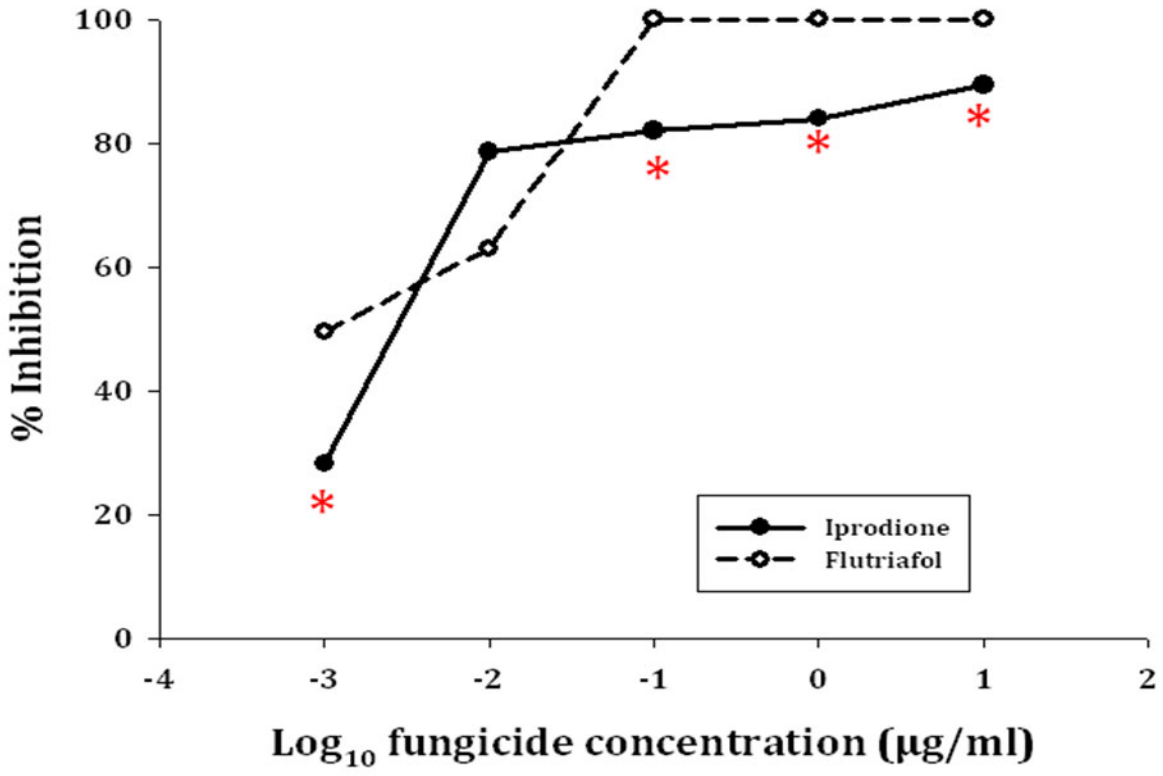

Figure 4. Dose response curves of *Fusarium oxysporum* f. sp. *vasinfectum* (the mean of four isolates) to increasing concentrations of iprodione and flutriafol. Data were combined from two experiments (n=10). Aesteriks idicate diffrences in inhibition of the two fungicides at the given concentration

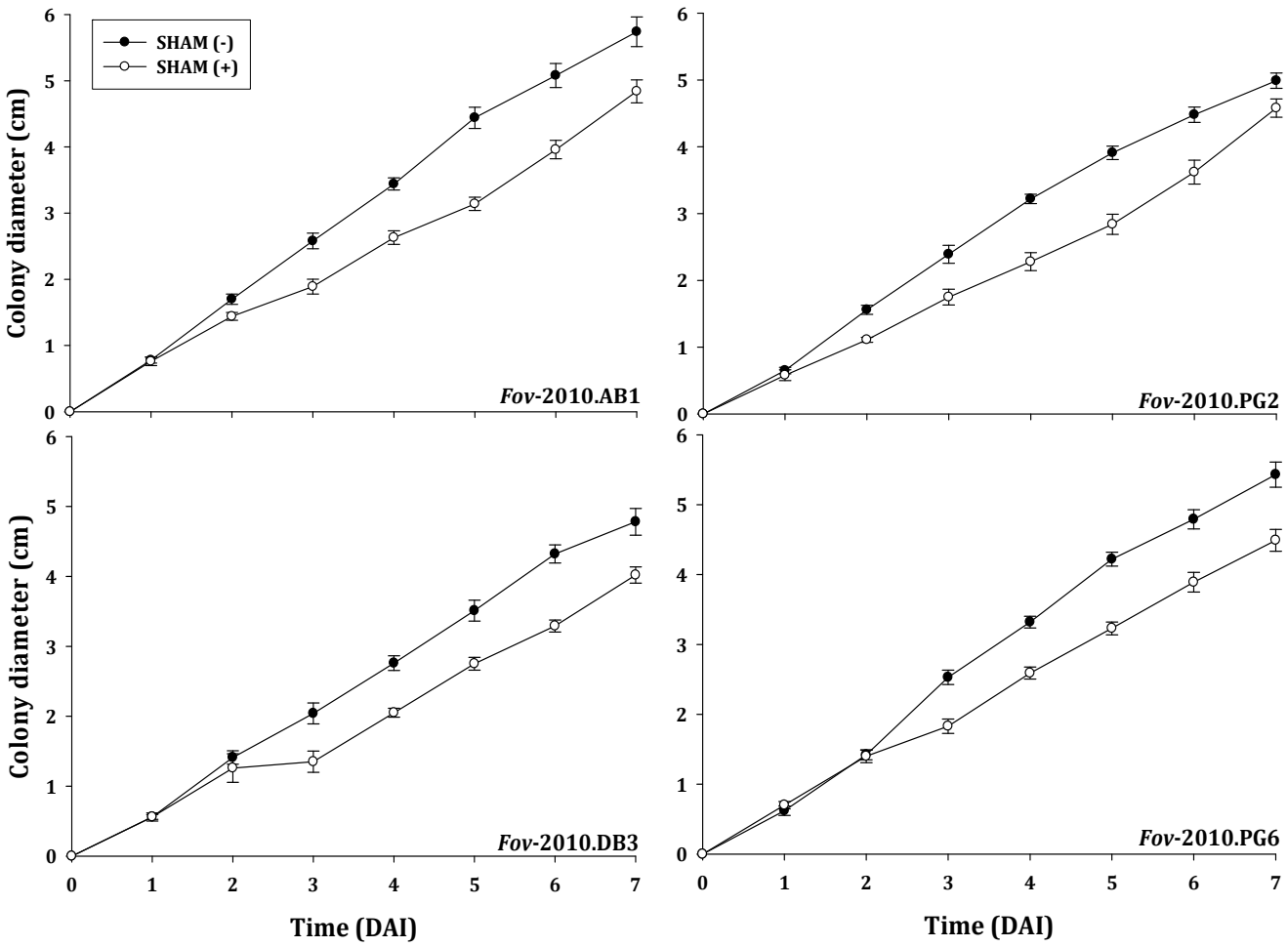

Figure 5. Effect of salicylhydroxamic acid (SHAM, 100 µg/ml) on hyphal growth of four isolates of *Fusarium oxysporum* f. sp. *vasinfectum* over time (days after inoculation, DAI). Data points represent the mean and standard error from two separate experiments (n=10).

Table 1. List of fungicides evaluated in *in vitro* sensitivity assays

Fungicide	Brand name	Manufacturer	Group
Azoxystrobin	Abound 2.08F	Syngenta	Strobilurins (11)
Flutriafol	Topguard 1.04F	Cheminova	Triazoles (3)
Iprodione	Rovral 4F	Bayer	Dicarboximide (2)
Pyraclostrobin	Headline 2.09EC	BASF	Strobilurins (11)

Table 2. Effect of salicylhydroxamic acid on hyphal growth of four isolates of *Fusarium oxysporum* f. sp. *vasinfectum*[a]

Isolate	No SHAM	SHAM	% Inhibition
1	358.8 (7.1)	277.8 (10.2)	22.6
2	321.7 (8.9)	246.7 (7.9)	23.3
3	291.5 (6.1)	226.8 (10.6)	22.2
6	336.8 (7.9)	272.5 (11.1)	19.1

[a] Data represent the area under hyphal growth curves. Values are the means of ten replications with standard errors in parenthesis.

Table 3. Effective concentrations (EC) of fungicides to reduce hyphal growth of *Fusarium oxysporum* f. sp. *vasinfectum* by 50 and 95 percent[a]

Fungicide	EC_{50}	EC_{95}
	---------- (µg a.i. / ml)---------	
Azoxystrobin	nd	nd
Flutriafol	4.0×10^{-4}	0.09
Iprodione	1.2×10^{-3}	6.57
Pyraclostrobin	nd	nd

[a] Values were estimated from linear regressions. nd indicates that the estimated values were not realistic.