

# Studies on the Efficacy of Non-Pathogenic *Fusarium Oxysporum* Isolate to Control *Fusarium* Wilt of Tomato Plants in Saudi Arabia

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**Abstract:** Nonpathogenic *Fusarium oxysporum* endophytes from tomato rhizosphere were evaluated for ability to reduce *Fusarium* wilt of tomato. Isolation from tomato, green bean and squash diseased plants revealed the association of one or more of the following fungal species, i.e., *Alternaria tenuis*, *Aspergillus flavus*, *A. niger*, *A. tamarii*, *Cephalosporium maydes*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Penicillium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii*. Upon testing the pathogenicity of isolates belong to *Fusarium oxysporum* on tomato, green bean and squash plants were found more or less able to attack plants causing pre and post-emergence damping-off. Tomato plants was highly vulnerable to attack by isolate of *F. oxysporum* (F.o-T2) caused a pre-emergence (57.50%), post emergence (61.60%) and 72.50% death of the survival tomato plants. The data also show that tomato plants inoculated with *F. oxysporum* (F.o-T5) isolate did not show symptoms of vascular disease and developed normally as plants in the control treatment. The in-vitro antagonistic effect of nonpathogenic of *F. oxysporum* isolate (F.o-T5) showed different level of antagonistic effects against 4 fungal isolates of plant pathogens. The non pathogenic isolate of *F. oxysporum* isolate (F.o-T5) was highly antagonistic to the pathogenic isolate of F.o-T2. Tomato seedling grown in soil inoculated with both the pathogenic and non-pathogenic *F. oxysporum* isolates, the non-pathogenic isolate F.o-T5 was efficient in controlling the disease incidence, as the disease incidence was significantly reduced by the non-pathogenic *F. oxysporum* (F.o-T5) from 80 to 28% (65.0% reduction) in soil infested with wilt pathogen and non-pathogenic respectively. Hence, the non-pathogenic *F. oxysporum* offer a very good prospects for integrated management of root disease in greenhouse crops

**Keywords:** Tomato, Rhizosphere, *Fusarium oxysporum*, Biological control.

## 1. Introduction

Soil borne plant pathogens continue to cause major problems agriculture throughout the world, reducing yield and quality of many crops. Fungi are an extremely versatile class of organisms comprised mostly of saprophytes, thriving on dead organic material. There is concept that manipulating the rhizosphere in such a way that beneficial microorganisms with antagonistic and/or eliciting properties are favored would protect roots from the deleterious effect of soil borne pathogens (Handelsman & Stabb, 1996; Weller, 1988). Support for this concept came from the discovery of suppressive soils in which the active microflora naturally controls the disease-causing activities of pathogen populations (Alabouvette et al., 1985 and 1987).

The soil borne fungus *Fusarium oxysporum* is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide (Ortoneda et al., 2004; Joshi et al., 2013).

The tomato plant (*Lycopersicon esculentum* Mill) is one of the world's most cultivated vegetable crops. Tomato plants are affected by several diseases including root-rot caused by *Rhizoctonia solani*, *Fusarium solani*, *Sclerotium rolfsii* which cause serious diseases and finally reduced crop yield and quality (Abdel-Monaim, 2011; Saad, 2006; El-Mohamedy, 2014) and *Fusarium* wilt caused by *F. oxysporum* (Ignjatova et al., 2012).

The use of biocontrol agents is a viable alternative for minimizing the yield loss. However, the survivability of a

biocontrol agent, especially in tropical countries, where the soil are in general, poor in organic matter has to address properly. Therefore, use of biocontrol agent of the same nature as pathogen could be an alternative. Non-pathogenic antagonistic *Fusarium* is a viable alternative (Minuto et al., 1995; da Silva & Bettiol, 2005 and Joshi et al., 2013). These saprophytic species of non-pathogenic *Fusarium* have been found to be effective and play a critical role in reducing diseases caused by pathogenic *F. oxysporum* in tomato (Rouxel et al., 1979; Garibaldi et al., 1987; Minuto et al., 1995; Larkin & Fravel, 1999; He et al., 2002; Reid et al., 2002; Silva & Wagner, 2005; Joshi et al., 2013) and colonize the plant rhizosphere and roots without inducing any symptoms (Benhamou & Grand, 2001).

The non-pathogenic antagonistic *F. oxysporum* have the same characteristic as pathogenic, except that they are not disease causing and hence are important because these organisms can sustain up to the crop duration (Joshi et al., 2013). Some strains of *F. oxysporum* have shown the ability to suppress the growth of several fungal plant pathogens such as *Phytophthora erythroseptica* and *Pythium ultimum* (Park, 1963; Benhamou et al., 2002) and to affect the germination of *S. sclerotiorum sclerotia* (Zizzerini & Tosi, 1985). Other species of *Fusarium* have been evaluated both against *Penicillium ultimum* (Ishimoto et al., 2004) and as a potential biocontrol agent against *S. sclerotiorum* in the rhizosphere (Zhou & Boland, 1998). A little is known about the antagonism related with antifungal metabolite production by nonpathogenic *F. oxysporum* (Fravel et al., 2003).

Therefore, present study was conducted to specify the pathogens causing root-rot and wilt diseases of tomato, green bean and squash in Saudi Arabia. Also, to evaluate the efficiency of non-pathogenic *F. oxysporum* isolate for biological control of tomato wilt caused by *F. oxysporum lycopersici*. In addition to the antagonism between the non-pathogenic *F. oxysporum* isolate and the main pathogen in-vitro and in-vivo under greenhouse conditions was studied.

## 2. Materials and Methods

### 2.1 Source of Plant Samples

The tomato (*Lycopersicon esculentum*), green bean (*Phaseolus vulgaris*) and squash (*Cucurbita pepo*) plants showing symptoms of root-rot and wilt were obtained from Huda Al-Sham agricultural farm located at 110 km north-east Jeddah, Saudi Arabia and brought to the laboratory.

### 2.2 Isolation of Fungi

The infected samples were rinsed in tap water and the necrotic portions were excised and cut into 2 mm pieces, surface sterilized with 2.0 % sodium hypochlorite for one minute and rinsed 3 successive changes of sterilized water. Test pieces were then plated on PDA medium and incubated for 7 days at 25± 2°C under 12 hrs photoperiod. Fungi growing were isolated, purified and identified according to Gilman (1957), Barnett & Hunter (2000), Domsch et al., (2007) and Samson et al., (2010).

### 2.3 Pathogenicity of *F. oxysporum* isolates

The pathogenicity tests were carried out by sowing surface sterilized tomato, green bean and squash seeds with calcium hypochlorite (2%) in infesting pasteurized clay soil at the rate of 1% by weight (Fahim et al., 1981) in pots. Other pots were left without inoculation served as control. The inoculum was prepared by growing each tested *Fusarium* isolates on sand: barely: water (1:3:3, w: w: w) for two weeks. Plants were kept in green house at 22°C under 12hrs periods of fluorescent light. A set of 4 pots were used for each isolate, as well as for the un-infested control soil. Disease assessment for the percentage of pre-and post emergence damping off were recorded 15 and 60 days after planting respectively.

### 2.4 In-vitro antagonistic assay between pathogenic and non-pathogenic *Fusarium* isolates

Based on the pathogenicity test of tomato crop, the non pathogenic isolate of *F. oxysporum* isolate (*F.o-T5*) which did not give any symptom of pre and post emergence damping-off disease was used against most virulent pathogenic *F. oxysporum* isolates (*F.o-T1*, *F.o-T2*, *F.o-T3* and *F.o-T4* ). To check the antagonistic property of non-pathogenic *F. oxysporum* isolate against pathogenic strain, 5mm disc of fully grown PDA plate of the pathogenic and non-pathogenic *F. oxysporum* were cut and inoculated at the opposite ends of the PDA plates. Both pathogenic and non-pathogenic isolates were also inoculated on Petri plates separately which served as control. Four replicates were used

for each treatment. After incubation at 27±2°C for 7 days, the dual culture in each dish was examined for the formation of inhibition zone.

### 2.5 In-vivo test

Pot experiment was conducted in the greenhouse to test the efficacy of the non-pathogenic *F. oxysporum* isolate (*F.o-T5*) as a biological agent in controlling tomato wilt pathogen. The inoculums of the pathogen and non-pathogenic *F. oxysporum* isolates were prepared as described by Ziedan (1998) and mixed separately with the upper layer of the soil as mentioned before in pathogenicity test. The infested soil was watered and mixed thoroughly several times over a week to ensure a good distribution of the fungal inoculums. Two weeks later tomato (cv. Super red) seedlings (30-days old) were transplanted above prepared pots (5 seedlings / pot) and observed over 90 days. Disease incidence as percentage of post emergence wilt was calculated during 30-90 days. At the end of the experiment the percentage of disease severity in the roots and shoots was recorded according to Woltz & Arthur (1973).

### 2.6 Statistical analysis

The statistical analysis of experiments were done using one way ANOVA and results were compared using least significant difference (LSD) test at P≤5%.

## 3. Results and Discussion

### 3.1 Isolation of Associated Fungi

Isolation from tomato diseased plants revealed the association of one or more of the following 8 fungal species, i.e., *Alternaria tenuis*, *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *F. solani*, *Penicillium spp.*, *Rhizoctonia solani* and *Sclerotium rolfsii* (Table, 1). Among the isolated fungi that may cause some of them to wilt and root rot diseases in tomatoes plants. *Fusarium oxysporum* and *Fusarium solani* isolates were found by 8.51%, while isolates of *Rhizoctonia solani* and *Sclerotium rolfsii* by 4.26%. These fungi were previously reported to be associated with root-rot and wilt diseases of tomato plants (Abdel-Monaim, 2011; Saad, 2006; El-Mohamedy, 2014).

Isolation from root-rotted or wilt of green bean plants revealed also the association of 9 fungal species, i.e., *Alternaria tenuis*, *Aspergillus flavus*, *A. niger*, *A. tamarii*, *Cephalosporium maydes*, *Fusarium moniliforme*, *F. solani*, *F. oxysporum* and *Rhizoctonia solani* (Table, 1). Among the isolated fungi some may cause wilt and root-rot in green bean plants. *Fusarium* isolated fungi were existed with range between 3.13-9.38%. These fungi were previously reported to be associated with root-rot and wilt of legume plants (Aly, 1967; Wu, 1998, Haggag & Saber 2000; Nawar, Lobna, 2008).

Isolation from diseased squash plants revealed the association of 5 fungal species., *Alternaria tenuis*,

*Aspergillus flavus*, *A. niger*, *Fusarium solani* and *F. oxysporum* (Table, 1). Among the isolated fungi some may cause wilt and root-rot in squash plants, *Fusarium oxysporum* and *F. solani* were found by 11.54 and 15.38% respectively. These fungi were previously reported to be associated with root-rot and wilt of squash plants (Martyn & Mclaughin, 1983; Pushpa et al., 1999; Nawar, Lobna, 2007).

Upon testing the pathogenicity of isolates belong to *Fusarium oxysporum* on tomato, green bean and squash were found more or less able to attack plants causing pre and post-emergence damping-off (Table,2). The data show that tomato plants were highly vulnerable to attack by isolate of *F. oxysporum* (*F.o*-T2) caused a pre-emergence (57.50%), post emergence (61.60%) and 72.50% death of the survival tomato plants. The data also show that tomato plants inoculated with *F. oxysporum* (*F.o*-T5) isolate did not show symptoms of vascular disease and developed normally as plants in the control treatment. This is important because the same non-pathogenic *F. oxysporum* isolate can be useful for biological control in tomato and for other hosts, as demonstrated by Garibaldi et al., (1990); da silva & Bettli (2005).

Concerning, green bean plants, upon testing the pathogenicity of *F. oxysporum* isolates, all of them were found or less able to attack plants at any stages of plant growth (Table, 2). Isolates numbers of *F.o*-G1 and *F.o*-G2 were the most aggressive fungus without significant difference compared with control treatment.

Concerning infection of squash plants inoculated with *F. oxysporum* isolates, the dead plants recorded at the end of experiment were found within the range of 14.58 and 100%. Isolate of *F.o*-S1 was the most aggressive followed by isolates of *F.o*-S8 and *F.o*-S2 causing 70.0, 50.0 and 35.0 % pre-emergence and 85.42, 30.0 and 28.57% post-emergence respectively.

The isolate having maximum disease incidence on tomato plant (*F.o*-T2) and also the non-pathogenic isolate (*F.o*-T5) were further used in the following experiments. Taking into account the symptoms observed on tomato plants, it was confirmed that the four isolates having nos. of *F.o*-T1, *F.o*-T2, *F.o*-T3, and *F.o*-T4 were *F.oxysporum f.sp. lycopersici*, while isolate no. *F.o*-T5 was not pathogenic.

**Table 1:** Fungi isolated from diseased tomato, green bean and squash collected from Saudi Arabia

Plant	Isolated fungi	No. of isolates	Frequency occurrence %
Tomato	<i>Alternaria tenuis</i>	10	21.28
	<i>Aspergillus flavus</i>	10	21.28
	<i>A. niger</i>	12	25.53
	<i>Fusarium oxysporium</i>	4	8.51
	<i>Fusarium solani</i>	4	8.51
	<i>Penicillium spp.</i>	3	6.38
	<i>Rhizoctonia solani</i>	2	4.26
	<i>Sclerotium rolfsii</i>	2	4.26
Total		47	-
Green bean	<i>Alternaria tenuis</i>	12	18.75
	<i>Aspergillus flavus</i>	13	20.31

	<i>A. niger</i>	15	23.44
	<i>tamaritii</i>	4	6.25
	<i>Cephalosporium maydes</i>	4	6.25
	<i>Fusarium moniliforme</i>	2	3.13
	<i>F. solani</i>	6	9.38
	<i>Fusarium oxysporium</i>	6	9.38
	<i>Rhizoctonia solani</i>	2	3.13
Total		64	-
Squash	<i>Alternaria tenuis</i>	12	23.08
	<i>Aspergillus flavus</i>	12	23.08
	<i>A. niger</i>	14	26.92
	<i>Fusarium oxysporium</i>	8	15.38
	<i>F. solani</i>	6	11.54

### 3.2 Characterization of *Fusarium oxysporum* isolates

*Fusarium spp.* isolates were identified on the basis of colony morphology, morphological characteristic of macro, microconidia and chlamyospores and conidial measurement.

**Table 2:** Pathogenicity of isolates representing fungal species causing root –rot and wilt diseases isolated from tomato, green bean and squash plants

Plants and Fungal isolates	Serial No.	**Pre-emergence Damping - off %	***Post-emergence Damping - off %	Dead plants %
Tomato				
<i>Fusarium oxysporum</i>	<i>Fo</i> -T1	*31.25 B	19.83 BC	78.83 A
<i>F. oxysporum</i>	<i>Fo</i> -T2	57.50 A	61.60 A	72.50 A
<i>F. oxysporum</i>	<i>Fo</i> -T3	38.75 B	23.86 B	26.18 B
<i>F. oxysporum</i>	<i>Fo</i> -T4	6.25 C	13.52 BCD	13.21 BC
<i>F. oxysporum</i>	<i>Fo</i> -T5	0.00 C	0.00 D	0.00 C
Control	Non infested	3.75 C	6.79 CD	0.00 C
L.S.D. at 5%		8.95	13.52	21.36
Green bean				
<i>Fusarium oxysporum</i>	<i>Fo</i> -G1	38.75 B	30.51 B	32.49 B
<i>F. oxysporum</i>	<i>Fo</i> -G2	58.75 A	57.29 A	50.00 A
<i>F. oxysporum</i>	<i>Fo</i> -G3	27.50 C	12.14 CD	13.52 CD
<i>F. oxysporum</i>	<i>Fo</i> -G 4	11.25 D	17.08 BCD	11.96 CD
<i>F. oxysporum</i>	<i>Fo</i> -G 5	15.00 D	18.32 BC	22.50 BC
<i>F. oxysporum</i>	<i>Fo</i> -G 6	63.75 A	31.24 B	58.33 A
Control	Non infested	0.00 E	0.00 D	0.00 D
L.S.D. at 5%				
Squash				
<i>Fusarium oxysporum</i>	<i>Fo</i> -S1	70.00 A	85.42 A	100.00 A
<i>F. oxysporum</i>	<i>Fo</i> -S2	35.00 BC	28.57 BC	42.08 B
<i>F. oxysporum</i>	<i>Fo</i> -S3	42.50 BC	36.25 BC	18.75 BC
<i>F. oxysporum</i>	<i>Fo</i> -S4	12.50 DE	47.92 B	14.58 BC
<i>F. oxysporum</i>	<i>Fo</i> -S5	47.50 B	26.49 BC	30.85 BC
<i>F. oxysporum</i>	<i>Fo</i> -S6	15.00 DE	23.01 C	23.81 BC
<i>F. oxysporum</i>	<i>Fo</i> -S7	27.50 CD	39.61 BC	26.69 BC
<i>F. oxysporum</i>	<i>Fo</i> -S8	50.00 B	30.00 BC	37.08 B
Control	Non infested	0.00 E	0.00 D	0.00 C
L.S.D. at 5%		17.17		
Tomato	<i>Fo</i> -S1	70.00 A	85.42 A	100.00 A

\*Four replicates were used for each treatment.

\*\* Pre emergence at 15 days after sowing

\*\*\* Post -emergence at 60 days after sowing

-Values followed by the same letter are not significantly different at  $P \geq 0.05$  according to Duncan's multiple range tests.

The most distinguishing characteristics were the presence of chlamydospores and microconidia borne in false heads on short monophialides. Microconidia are abundant, single celled kidney and oval shaped measuring  $2.5-3 \mu\text{m} \times 6-10 \mu\text{m}$ . Macroconidia are abundant, slightly sickle shaped, thin walled with an attenuated apical cell and a foot shaped basal cell. Macroconidia three septate,  $25-59 \mu\text{m}$ , five septate,  $35-71 \mu\text{m}$ . Growth on PDA medium is rapid with white aerial mycelium, cottony appearance which may become tinged with violet to purple color. Such findings go in accordance with recorded about the species of *F. oxysporum* Schlecht.

### 3.3 Antifungal activity between pathogenic and non-pathogenic *Fusarium* spp.

Agar plates inoculated with pathogenic fungal isolates of *F. oxysporum* and the non-pathogenic isolate (Fig,1) revealed the presence of clear antagonistic action between them. The non pathogenic isolate of *F. oxysporum* isolate (*F.o-T5*) showed different level of antagonistic effects against 4 fungal isolates of plant pathogens. The non pathogenic isolate of *F. oxysporum* isolate (*F.o-T5*) was highly antagonistic to the pathogenic isolate of *F.o-T2*, whereas the non pathogenic *F. oxysporum* showed only weak or none antagonistic effects to the 3 isolates of *Fusarium* pathogens.

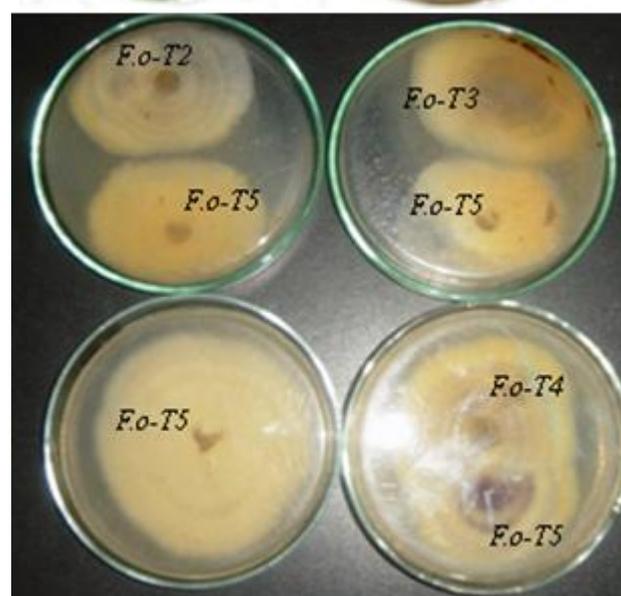
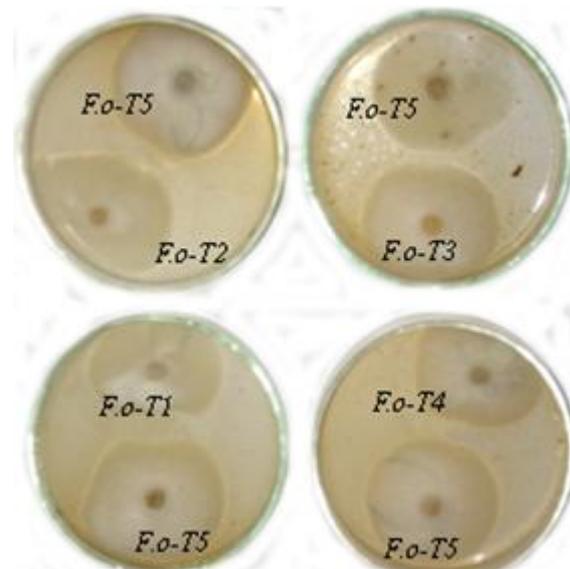
The antagonistic activity of the non-pathogenic *Fusarium oxysporum* against the phytopathogenic fungi in-vitro by dual cultures were studied by several investigators (Park, 1963; Benhamou et al., 2002; Rodriguez et al., 2006). Little is known about the antagonism related with antifungal metabolite production by nonpathogenic *F. oxysporum*

(Fravel et al., 2003). However, Rodriguez et al., (2006) found that cyclosporin produced by non-pathogenic *F. oxysporum* caused both growth inhibition and suppression of sclerotia formation of *Sclerotinia sclerotiorum* in-vitro.

### 3.4 In-vivo test

Data in Table(3) showed that the pathogenic isolate of *F. oxysporum* (*F.o-T2*) was most virulent, causing 80% disease incidence in plants grown in infested soil. These results agree with those of Andrade & Micherref (2000), who demonstrated that tomato plants inoculated with the pathogenic isolate of *F. oxysporum* f.sp. *lycopersici* race 2 showed 50% disease incidence.

Tomato seedlings cultivated in soil inoculated with the non-pathogenic *F. oxysporum* isolate of *F.o-T5* did not show symptoms of vascular diseases and developed normally. This is important because the same non-pathogenic *F. oxysporum* isolates can be useful for other hosts as demonstrated by Minuto et al., (1995) for cyclamen and basil by Garibaldi et al., (1990) for melon and radish.



**Figure 1:** In-vitro inhibition of 4 virulent pathogenic *F. oxysporum* isolates (*F.o-T1*, *F.o-T2*, *F.o-T3* and *F.o-T4*) and the non pathogenic *F. oxysporum* isolate (*F.o-T5*)

When tomato seedling grown in soil inoculated with both the pathogenic and non-pathogenic *F. oxysporum* isolates, the non-pathogenic isolate *F.o-T5* was efficient in controlling the disease incidence, as the disease incidence was significantly reduced by the non-pathogenic *F. oxysporum* (*F.o-T5*) from 80 to 28% (65.0% reduction) in soil infested with wilt pathogen and non-pathogenic respectively. These results agree with Garibaldi et al., (1987), Postma & Rattink (1992); Minuto et al., (1995); da Silva et al., (2005). They reported that the non-pathogenic *Fusarium* spp. isolates were efficient in colonizing the rhizosphere and controlling Fusarium wilt.

Several investigators proposed mechanisms of biological control for wilt disease by non-pathogenic *F. oxysporum* isolate involved (1)- competition for infection sites (Mandeeel & Baker, 1991; Schneider, 1984), (2)- for nutrients (Couteaudier, 1992, & Larkin and Fravel, 1999) and (3)- by induction of resistance (Mandeeel & Baker, 1991; Yamaguchi et al., 1992). In conclusion, because of the remarkable of the antimicrobial activity of some non-pathogenic *Fusarium*

*oxysporum* isolates, it given good prospects for integrated management of root disease in greenhouse crops.

**Table 3:** *in-vivo* effect of non- pathogenic *Fusarium oxysporum* isolate (*F.o-T5*) on disease incidence and severity caused by pathogenic isolate of *F. oxysporum* (*F.o-T2*)

Treatment	Disease incidence %	**Disease scale (1-4)		Symptoms
		Root	Shoot	
Control	8.00 C	0.40 C	1.20 B	-
<i>F. oxysporum</i> non pathogenic ( <i>F.o-T5</i> )	0.00 C	0.00 C	1.20 B	-
The pathogen isolate ( <i>F.o-T2</i> )	80.00A	3.65 A	3.42 A	wilt
The pathogen + non pathogenic <i>F. O.</i>	28.00 B	1.40 B	1.54 B	wilt
L.S.D. at 5%	16.30	0.59	1.54	

\*\* Disease scale was calculated as follows:

- 0= normal light color
- 1= very light brown
- 2= brown
- 3= dark brown
- 4= very dark brown

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