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REGULAR ARTICLE

Cross-protection of cotton against *Verticillium* wilt by *Verticillium nigrescens*

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ABSTRACT

Two species of plant pathogenic fungi causing *Verticillium* wilt of cotton, *Verticillium dalhiae* Kleb. and *V. nigrescens* Pethybr., proved aggressive and weakly pathogen of cotton, respectively, and were used in this study. Cotton cultivars cvs 4S and Stoneville 453, susceptible to *Verticillium* wilt disease, were treated with both organisms in order to determine possible cross protection effects of *V. nigrescens* against *V. dahliae*. Results showed that the cotton plants which were inoculated on the same day as *V. dahliae* prior to inoculation by *V. nigrescens* were showing aggressive wilt symptoms of *Verticillium* wilt of cotton. However, the plants inoculated on the same day as *V. nigrescens* prior to inoculation by *V. dahliae* resulted low to mild wilt symptoms. Control of *Verticillium* wilt of cotton was obtained when *V. nigrescens* was inoculated four days prior to inoculation by *V. dalhiae.* Those promising results where further explained by data obtained from the transpiration rates of the mass flow of sap in cotton stems and demonstrated that conidia of *V. nigrescens* could protect the cotton plants from virulent conidia of *V. dahliae.*

Keywords: Biocontrol; Vascular diseases; Defense mechanisms induced by fungi

INTRODUCTION

Verticillium wilt caused by *Verticillium dalhiae* Kleb., is a well-known serious disease of many economical crops, especially of cotton where it can reduce cotton yield greatly under certain environmental conditions (Pegg, 1984; Bhat and Subbarao, 1999). The fungus is widespread in most cotton cultivated areas and is one of the greatest threats to cotton production worldwide (Erdogan and Benlioglum, 2010) as well as in cotton areas in Greece where this disease is one of the main constraints to cotton production especially in the region of Thessaly (Tjamos et al., 2000).

Most cotton plants are asymptomatic at initial infection (Shen, 1992; Xiao et al., 1998; Ma, 2007), thus, cotton yield losses can be reduced effectively when cotton *Verticillium* infection is predicted early and accurately, and prophylactic measures are properly taken (Jing and Huang, 2010).

However, according to Paplomatas et al., (1992) when infection occurs at later stages infected plants usually exhibit symptoms of marginal necrosis in leaves, discoloration of the stem vascular bundles, decrease in photosynthesis and increase in respiration, resulting in a significant reduction of the plant biomass and significant loss in yield.

The study of the symptoms on the infected plants depends mainly on the manual investigation and sampling in fields which is time and effort consuming and difficult to practice for large areas. Thus, correlation analysis of leaf spectral reflectance and disease severity of cotton *Verticillium* has shown that there are a number of sensitive bands that spread in several spectrums (Ning et al., 2013).

In general, different methods of controlling *Verticillium* wilt of cotton plant like heat treatment, fumigation with broad-spectrum biocides and eco-friendly friendly alternative methods like rhizobacteria (Weller, 1998; Fravel and Larkin, 2000; Landa et al., 2004) mycoparasites (Grunden et al., 2001; Rekanovic et al., 2007; Zheng et al., 2011) and endophytes (Yang et al., 2013) have been well-reported.

Furthermore, according to Xue et al., (2013) shown that the application of powdered biocontrol agent containing living actinomycetes could be useful for biocontrol of the plant pathogen in agricultural production. The isolates

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successfully colonized the cotton rhizosphere in a wilt-sick field study when the powdered biocontrol agent was applied either as a seed coating or as a soil inoculation.

Moreover, planting resistant varieties is a possible strategy for protection of cotton from *V. dahliae*, but few upland cotton cultivars showed resistance towards *Verticillium* wilt (Bolek et al., 2005; Gore et al., 2009; Gao et al., 2011).

Furthermore, organic amendments in applied soil also reduced disease severity in both inoculated pots and naturally infested cotton field plots. According to Huang et al., (2006) the most effective control was achieved with crab shell (chitin), soybean stalk and alfalfa.

The aim of this study was to to determine possible cross protection effects of *V. nigrescens* against *V. dahliae* in susceptible cotton cultivars in the region of Thessaly, Greece.

MATERIALS AND METHODS

In the present study the severe pathogen (*V. dalhiae*) was isolated, in the laboratory of plant protection of Technological Educational Institute of Thessaly (T.E.I.), several times from cotton plants showing symptoms of *Verticillium* wilt at several locations in central Greece. In order to determine protection against a virulence isolate of *V. dalhiae*, an isolate of *V. nigrescens*, a comparatively weaker vascular pathogen, recovered from cotton plants showing weak wilt symptoms, was tested. Following that we tested cross protection effects in cotton against disease induced by a virulent isolate of *V. dalhiae* with the weaker vascular pathogen *V. nigrescens* four days prior to inoculation with a challenger strain based on the idea presented (Melouk and Horner, 1975). Moreover, that cross protection effects were tested in process biochemical mechanisms, antifungal mechanisms, such as phytoalexins of cotton plants in response to inoculums of the aggressive pathogen (*V. dalhiae*) as represented in previous research work (Zaki et al., 1972).

Both pathogens were isolated from cotton vascular tissues and grown on PDA plates which were incubated in darkness at 22°C for 20 days. Pathogenicity tests were determined by immersing cotton plant roots (shoot tip cutting) for 45 min in a *Verticilliums* solution, which contained 5×10^5 conidia/ml. This high spore suspension can affect plant tissues and cause *Verticillium* disease symptoms (Pegg and Brady, 2002). Treated plants were transplanted in pots and kept in a glasshouse with 28-30°C for 45 days. Two cotton (*Gossypium hirsutum* L.) cultivars, "4S" and "Stoneville 453", susceptible to Verticillium wilt were used. The experiment was designed with seven treatments and 21 replications, as follows:

a) Immersing the root cut ends into *V. dahliae* (*Vd*), in conidia suspension (5×10^5 conidia/ml), for 45 min (Melouk and Horner, 1975).
b) Immersing the root cut ends into *V. nigrescsens* (*Vn*), in conidia suspension (5×10^5 conidia/ml), for 45 min.
c) Immersing the root cut ends into *V. nigrescens*, in conidia suspension (5×10^5 conidia/ml), for 45 min and subsequently, on the same day, inoculation with *V. dahliae* (*Vn + Vd*), by adding spore suspension ($5x10^5$ spores/ml) in the pot (10 ml/pot).
d) Immersing the root cut ends into *V. nigrescens*, in conidia suspension ($5x10^5$ conidia/ml), for 45 min and after 4 days inoculation with *V. dahliae (Vn + 4Vd),* by adding spore suspension ($5x10^5$ conidia/ml) in the pot (10 ml/pot).
e) Immersing the root cut ends into *V. dahliae*, in conidia suspension ($5x10^5$ conidia/ml), for 45 min and subsequently, on the same day, inoculation with *V. nigrescens (Vd + Vn)*, by adding spore suspension ($5x10^5$ conidia/ml) in the pot (10 ml/pot).
f) Immersing the root cut ends into *V. dahliae*, in conidia suspension (5×10^5 conidia/ml), for 45 min and after 4 days inoculation with *V. nigrescens (Vn → 4Vd),* by adding spore suspension ($5x10^5$ conidia/ml) in the pot (10 ml/pot).
g) Immersing the root cut ends into sterile water (Control plants).

Number of leaf discoloration (foliar damage by 0-5 rating scale where 0: no foliar symptoms; 3: 25% of plants showing evidence of leaf yellow; 5: severe foliar damage), stem vascular staining (darker in color by 1-4 rating scale where 1: light vascular staining to 4: vascular staining darker in color), stem height, diameter, fresh weight and dry weight of inoculated plants were assessed at the end of the experiment, after 45 days of incubation. All data were analysed by analysis of variance (ANOVA) and presented with graphs using Microsoft Excel.

Xylem sap flow investigation in cotton plants infected with or without *Verticillium* species was determined as follows. The study was carried out after 40 days of inoculation in eight periods (30 to 240 min, Fig. 4), using two random plants for each period. The probes of phytometric system LPS-03 Phytomonitor (PhyTech Ltd) was established into the stem up to a height of 20 cm from soil surface to measure sap flow density.

RESULTS AND DISCUSSION

The results showed that cotton plants inoculated with only *V. nigrescens* were symptomless, obtained significant high

values for stem height, diameter, fresh and dry weight and significant less values for number of leaf discoloration and stem vascular staining compared to those cotton plants inoculated with only *V. dalhiae* (Figs. 1-3). When inoculation with *V. nigrescens* precedent *V. dalhiae,* mild wilt symptoms occurred in all cotton tested plants, agreeing with Korolev and Katan, (1999). Plants which were inoculated on the same day as *V. nigrescens* before the inoculation with *V. dahliae* were statistically different from those inoculated on the same day as *V. dahliae* before the inoculation with *V. nigrescens*, in number of leaf discoloration, stem vascular staining, diameter, fresh and dry weight (Figs. 1-3).

When *V. nigrescens* was inoculated four days after *V. dalhiae,* significant wilt symptoms were observed as measurements such as stem diameter, stem height, foliar damage, stem discolouration, stem dry weight and stem fresh weight were done (Figs. 1-3).

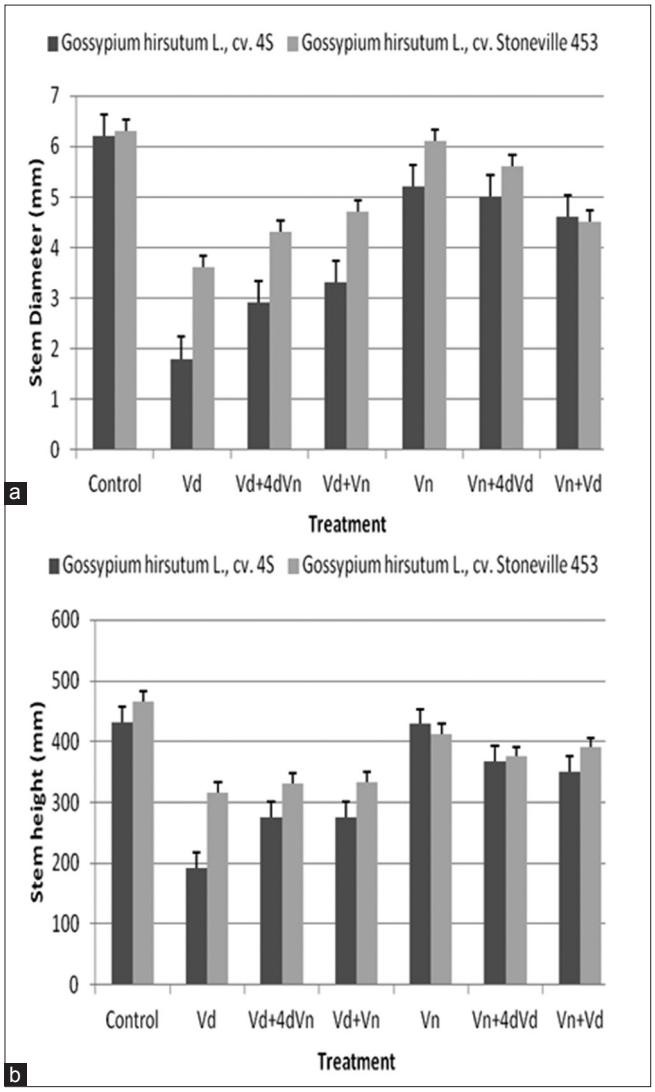

Fig 1. Cotton stems diameter (a) and height (b), in inoculated plants with *V. nigrescens* and *V.dahliae*.

Moreover, the results of Figs. 1-3 also showed that when *V. dalhiae* was inoculated four days after *V. nigrescens,* a significant less wilt symptoms was observed, and we can say that *V. nigrescens* induced a crop protection effect against *V. dalhiae* when introduced first in cotton plant rhizosphere. As determined by measurement the transpiration rates of mass flow of sap in the cotton stem (Figs. 4), the rate of mass flow of sap increased significantly in plants inoculated with only *V. nigrescens* and in plant when *V. dalhiae* was inoculated four days after *V. nigrescens.* This is in agreement with previous research work, where cross protection effects were observed in peppermint when inoculation with *V. nigrescens* preceded inoculation with *V. dahliae* by two days (Melouk and Horner, 1975).

CONCLUSION

In the present investigation results show that it is possible to reduce disease induced by a virulent isolate of *V. dalhiae,* when inoculated first with a weak pathogen, *V. nigrescens*. It is believed that cross-protection of cotton to a severe isolate of *V. dalhiae* was possible due to accumulation into xylem of antifungal secondary metabolites in response to

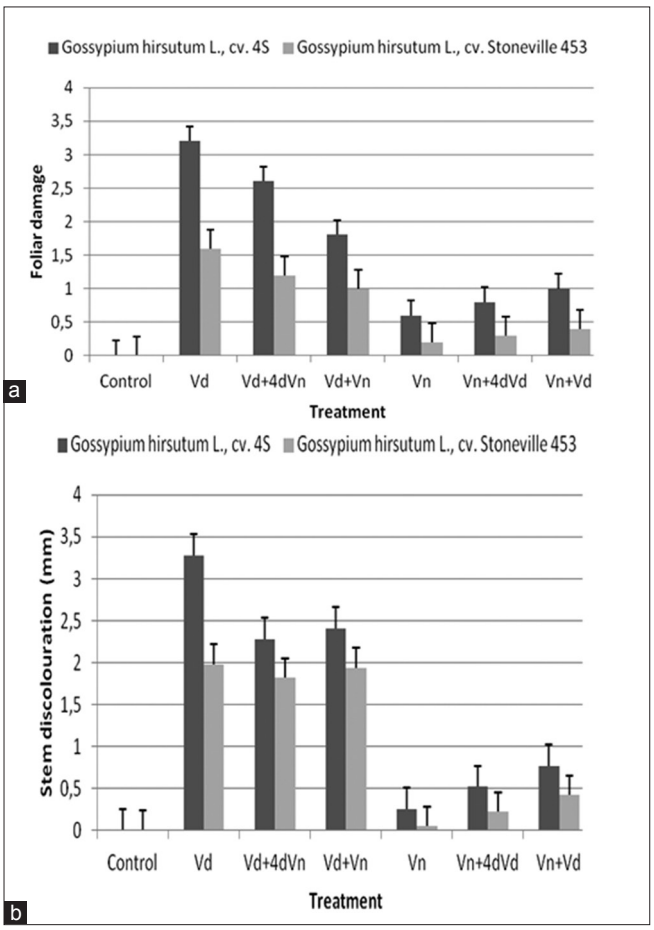

Fig 2. Cotton foliar damage (a) and stem discolorations (b), in inoculated plants with *V. nigrescens* and *V.dahliae*.

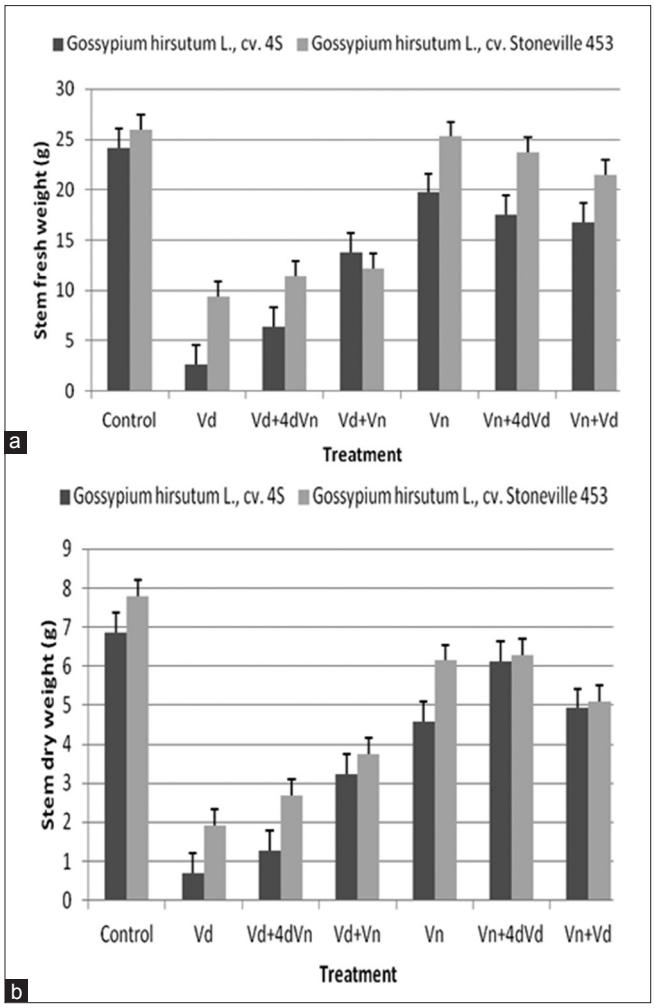

Fig 3. Cotton stems fresh weight (a) and dry weight (b), in inoculated plants with *V. nigrescens* and *V.dahliae*.

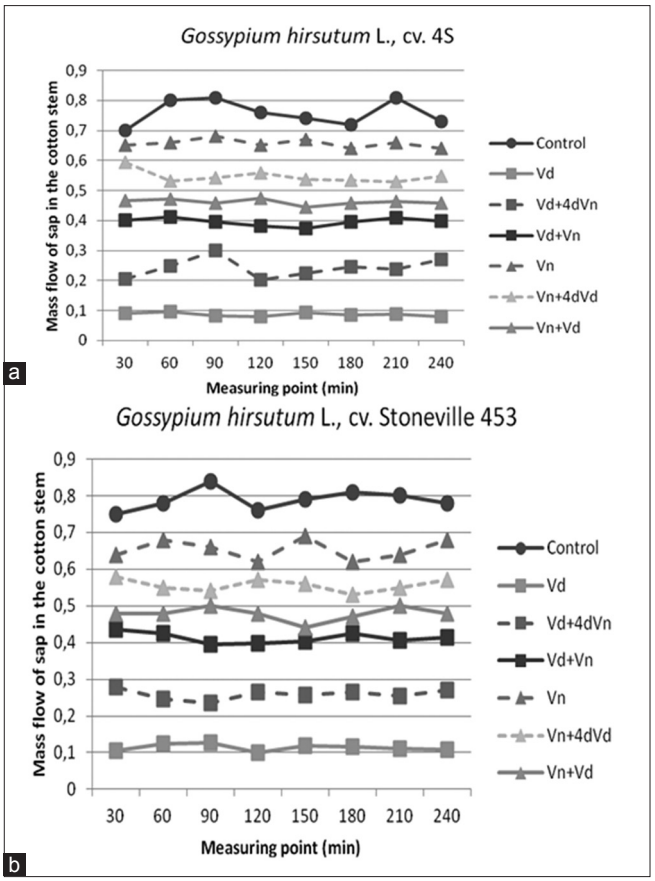

Fig 4. Mass flow of sap in the cotton Stems inoculated with *V. nigrescens* and *V.dahliae*.

inoculation with *V. nigrescens*, (Zaki et al., 1972; Schnathorst and Mathre, 1996). Finally, we can concluded that the cross-protection phenomenon reported in the present study is a form of a biological control occurred in nature and may have application in control of wilt diseases in plant (Price and Sackston, 1989; Huertas-Gonzalez et al., 1999; Erdogan et al., 2013).

Author contributions

The work is a product of the intellectual environment of both authors which have contributed in various degrees to the analytical methods used, to the research concept, and to the experiment design. Dr. I. Vagelas designed the study, developed the methodology, performed the analysis while Dr. S. Leontopoulos collected the data, and made the isolations. Both authors contributed in writing the manuscript.

REFERENCES

Bhat, R. G. and K.V. Subbarao. 1999. Host range specificity in *Verticillium dahliae*. Phytopathology. 89: 1218-1225.

Bolek, Y., K. M. El-Zik and A. E. Pepper. 2005. Mapping of *Verticillium* wilt resistance genes in cotton. Plant Sci. 168: 1581-1590.

Erdogan, O. and K. Benlioglu. 2010. Biological control of *Verticillium* wilt on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. Biol. Cont. 53: 39-45.

Erdogan, O., S. Nemli, T. Oncu and B. Tanyolac. 2013. Genetic variation among pathotypes of *Verticillium dahliae* Kleb. from cotton in western Turkey revealed by AFLP. Can. J. Plant Path. 35**:** 354-362.

Fravel, D. R. and R. P. Larkin. 2000. Effect of sublethal stresses on microsclerotia of *Verticillium* dahliae. In: Tjamos, E. C., R. C. Rowe., J. B. Heale and D. R. Fravel., (Eds.). Advances in *Verticillium* Research and Disease Management. American Phytopathological Society (APS) Press, St. Paul, Minessota., Pp. 301-306.

Gao, X., T. Wheeler., Z. Li., C.M. Kenerley., P. He. and L. Shan. 2011. Silencing GhNDR1 and GhMKK2 compromised cotton resistance to *Verticillium* wilt. Plant J. 66: 293-305.

Gore, E. M., O. K. Caner., N. Altın., M. H. Aydın., O. Erdogan., F. Filizer and A. Buyukdogerlioglu. 2009. Evaluation of cotton cultivars for resistance to pathotypes of *Verticillium dahliae*. Crop Prot. 28: 215-219.

Grunden, E., W. Chen and J. L. Crane. 2001. Fungi colonizing microsclerotia of *Verticillium dahliae* in urban environments. Fungal Divers. 8: 129-141.

Huanga, J., H. Lib and H. Yuan. 2006. Effect of organic amendments on *Verticillium* wilt of cotton. Crop Prot. 25: 1167-1173.

Huertas-Gonzalez, M. D., M. C. Ruiz-Roldan., A. Di Pietro and

M. I. G. Roncero. 1999. Cross protection provides evidence for race-specific avirulence factors in *Fusarium oxysporum*. Phys. Mol. Plant Path. 54: 63-72.

Jing, X., W. J. Huang and Y. Ju. 2010. Remote sensing monitoring severity level of cotton *Verticillium* wilt based on partial least squares regressive analysis. Trans. CSAE. 26: 229-235.

Jin, N., W. Huang., Y. Ren., J. Luo., Y. Wu., Y. Jing and D. Wang. 2013. Hyperspectral identification of cotton *Verticillium* disease severity. Optik. 124: 2569-2573.

Landa, B. B., J. A. Navas-Cortés and R. M. Jiménez-Díaz. 2004. Influence of temperature on plant: Rhizobacteria interactions related to biocontrol potential for suppression of *Fusarium* wilt of chickpea. Plant Pathol. 53: 341-352.

Ma, C. 2007. Studying on *Verticillium* wilt and *Fusarium* wilt of cotton. In: Jian, G. L., J. R. Yang., J. Feng and J. X. He. (Eds.). Breeding of Cotton Resistant Varieties. Vol. 1. Chinese Agricultural Press, Beijing, Pp. 185-246.

Melouk, H. A. and C. A. Horner. 1975. Cross protection in mints by *Verticillium nigrescens* against *V. dahliae*. Phytopathology. 65: 767-769.

Paplomatas, E. J., D. M. Bassett., J. C. Broome and J. E. Devay. 1992. Incidence of *Verticillium* wilt and yield losses of cotton cultivars (*Gossypium hirsutum*) based on soil inoculum density of *Verticillium dahliae*. Phytopathology. 82: 1417-1420.

Pegg, G. F. 1984. The impact of Verticillium diseases in agriculture. Phytopathol. Mediterr. 23: 176-192.

Pegg, G. F. and B. L. Brady. 2002. Verticillium Wilts. CABI Publishing, Oxon, UK.

Price, D. and W. E. Sackston. 1989. Cross protection among strains of *Verticillium dahliae* on sunflower. Vascular wilt diseases of plants. NATO ASI Series. 28: 229-235.

Rekanovic, E., S. Milijasevic., B. Todorovic and I. Potocnik. 2007. Possibilities of biocontrol and chemical control of *Verticillium* wilt in pepper. Phytoparasitica. 35: 436-441.

Shen, Q. Y. 1992. Cotton disease-basic research and protection. In: Deng, Y. S., Q. J. Li and Q. Y and Chen, Q. Y. (Eds.). Identification of the Resistance of Cotton Varieties. Vol. 1. Science Press, Beijing, Pp. 268-269.

Schnathorst, W. C. and D. E. Mathre. 1996. Cross protection in cotton with strains of *Verticillium albo-atrum*. Phytopathology. 56: 1204-1209.

Tjamos, E. C., R.C. Rowe., J. B. Heale and D. R. Fravel. 2000. Advances in *Verticillium* Research and Disease Management. APS Press. The American Phytopathological Society, St. Paul, MN, p. 378.

Weller, D. M. 1998. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol. 26: 379-407.

Xiao, C. L., K. V. Subbarao., K. F. Schulbach and S. T. Koike. 1998. Effects of crop rotation and irrigation on *Verticillium dahliae* microsclerotia in soil and wilt in cauliflower. Phytopathology. 88: 1046-1055.

Xue, L., Q. Xue, Q. Chen, C. Lin, G. Shen and J. Zhao. 2013. Isolation and evaluation of rhizosphere actinomycetes with potential application for biocontrol of *Verticillium* wilt of cotton. Crop Prot. 43: 231-240.

Yang, P., Z. Sun., S. Liu., H. Lu., Y. Zhou and M. Sun. 2013. Combining antagonistic endophytic bacteria in different growth stages of cotton for control of *Verticillium* wilt. Crop Prot. 47: 17-23.

Zaki, A. I., N. T. Keen and D. C. Erwin. 1972. Implication of vergosin and hemigossypol in the resistance of cotton to *Verticillium albo-atrum*. Phytopathology. 62: 1402-1406.

Zheng, Y., Q. Y. Xue., L. L. Xu., Q. Xu., S. Lu., C. Gu and J. H. Guo. 2011. A screening strategy of fungal biocontrol agents towards *Verticillium* wilt of cotton. Biol. Cont. 56: 209-216.