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# Effects of Foliar Application of *Bacillus subtilis* Osu-142 on the Yield, Growth and Control of Shot-Hole Disease (Coryneum blight) of Apricot

## Einfluß der Blattbehandlung mit *Bacillus subtilis* Osu-142 auf Ertrag, Wachstum und Kontrolle der Schrotschusskrankheit bei Aprikose

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## Abstract

From 2000 to 2001, experiments were conducted to determine the effects of foliar application of the bacterial strain *Bacillus subtilis* OSU-142 on yield, growth and control of shot-hole disease of apricot trees grown in Malatya province of Turkey. In 2000 apricot trees cv. 'Hacıhaliloglu' were sprayed with suspensions of the bacterial strain at full bloom, and 30 and 60 days after full bloom stage. Based on results of the first year, the full bloom stage was proven to be the best time for bacterial application. Significant differences were found in yield, shoot development and reduction of shot-hole disease. In 2001 application of bacteria was performed only during the full bloom stage.

The data show that the average increase in yield in 2000 and 2001 was about 30 % and 90 %, respectively as compared with the untreated control. Similarly, reduction in disease incidence and severity was 52 and 71 % in 2000 and 15 and 41 % in 2001, respectively. Development in shoot length and diameter were significantly higher when trees were treated with OSU-142. The results of this study indicate an increase in quality and quantity of apricot fruit production by application of OSU-142 at full bloom stage.

## Zusammenfassung

In den Jahren 2000 und 2001 wurden in der türkischen Provinz Malatya Versuche durchgeführt, um die Wirkung von Blattapplikationen des *Bacillus subtilis*–Stammes OSU-142 auf den Ertrag, das Wachstum und die Ausprägung der Schrottschusskrankheit bei Aprikosen zu untersuchen. Aprikosen der Sorte 'Hacıhaliloglu' wurden 2000 während der Vollblüte und 30 bzw. 60 Tage danach mit einer Suspension des Bakterien-Stammes besprüht. Bei diesem Versuchsansatz erwies sich die Vollblüte als wirkungsvollster Zeitpunkt für die Applikation der Bakterien. Es zeigten sich signifikante Unterschiede hinsichtlich Ertrag, Triebentwicklung und Reduktion der Schrotschuss-Symptome. Deshalb wurden die Bakterien 2001 nur noch während der Vollblüte ausgebracht.

Der durchschnittliche Ertragszuwachs lag 2000 und 2001 im Vergleich zur unbehandelten Kontrolle bei 30 bzw. 90 %. Die verminderte Häufigkeit und Schwere der Krankheitsausprägung betrug 2000 52 bzw. 71 % und 2001 15 bzw. 41 %. Auch die Werte für Länge und Durchmesser der Triebe waren bei OSU-142 behandelten Bäumen signifikant höher. Die Ergebnisse dieser Untersuchung zeigen einen deutlichen Zuwachs hinsichtlich Qualität und Quantität der erzeugten Aprikosen durch die Anwendung von OSU-142 zum Zeitpunkt der Vollblüte.

## Introduction

Apricot is the most important fruit crop grown in Anatolia, with approximately 500.000 tons of fruit produced annually and Turkey takes the first place in apricot production in the world (ANON. 2000). High fruit quality is a prerequisite in marketing of dried apricots produced mostly in the province of Malatya, where apricots find the best ecological conditions. The main apricot cultivar grown there is 'Hacıhaliloğlu' (GÜLERYÜZ et al. 1999).

Intensive farming practices, that achieve high quality and quantity yield, require chemical fertilizers, which are not only costly but may also create environmental problems. Recently, there has been a resurgence of interest in environmental friendly sustainable agricultural practices (O'CONNELL 1992, ORSON 1996). In the development and implementation of sustainable agriculture techniques, bio-fertilization is of great importance in order to alleviate deterioration of natural and environmental pollution (O'CONNELL 1992).

Another important factor that causes economic yield and quality losses in apricot fruit production are diseases, particularly shot-hole (Coryneum blight), a fungal disease, caused by *Wilsonomyces carpophilus* L. This is one of the most destructive diseases on apricot cv 'Hacıhaliloğlu' grown in Malatya, Turkey. Control of the disease is commonly achieved by cultural practices and by chemical control, which is not well accepted due to negative side effects such as cost, development of fungicide resistance in the pathogen, environmental pollution and disturbance of agroecosystem. Therefore, nonchemical management strategies like biological control, need to be developed and used for effective

control of shot-hole disease on apricot. A number of bacterial strains identified in previous studies were reported as potential biocontrol agents against many fungal and bacterial pathogens (MC LOUGHLIN et al. 1992, DUIJFF et al. 1993, VICEDO et al. 1993, KORSTEN et al. 1997, RAMAMOORTHY et al. 2001). One of which *Bacillus* OSU-142 was reported to have great potential with antagonistic activity against plant pathogenic bacteria and fungi as well as growth promoting effect. However, the effect of OSU-142 application on yield increase and control of shot-hole disease in apricot has not yet been reported.

The objective of this study was to investigate biological control effect of OSU-142 against *Wilsonomyces carpophilus* L. and growth promoting effect on apricot on the basis of disease prevalence, fruit weight, yield and quality, shoot length and diameter via leaf application.

## Material and Method

*Bacterial strain, culture conditions, media and treatment*

The bacterial strain used in all experiment in the study was *Bacillus* OSU-142 obtained from Dr. Fikrettin Şahin (Atatürk University, Department of Plant Protection). OSU-142 is a biocontrol and growth promoting agent (KOTAN et al. 1999, CUPPELS et al. 1999, ŞAHIN et al. 2000, ÇAKMAKCI et al. 2001). The bacterial strain was maintained for long-term storage in nutrient broth with 15 % glycerol at –80 °C. For this experiment, the bacterial strain was grown on nutrient agar (beef extract 3.0 g $l^{-1}$, peptone 5.0 g $l^{-1}$, glucose 2.5 g $l^{-1}$ and agar 15.0 g $l^{-1}$). A single colony was transferred to 500 ml flasks containing nutrient broth (NB) (beef extract 3.0 g $l^{-1}$ and peptone 5.0 g $l^{-1}$), and grown aerobically in flasks, on a rotating shaker (150 rpm) overnight at 27 °C (Merck KGaA, Germany). Bacteria-grown nutrient broth was then diluted in sterile distilled water to a final concentration of $10^9$ CFU $ml^{-1}$. The resulting bacterial suspensions were used to treat apricot trees.

*Field experiments*

In 2000 and 2001, field experiments were conducted on 10 year old apricot trees cv. 'Hacıhaliloğlu' grown in an orchard in the province of Malatya, Turkey. An orchard was selected with no previous application of conventional fertilizer or pesticides. Only manure (1 t $da^{-1}$) has been applied to the orchard at regularly 3 years intervals. The first year of the field experiment, 72 apricot trees in total were selected and divided into 4 application groups including 6 trees with 3 replicates in a completely randomized design. Control trees were sprayed with sterile water, and trees in other application groups were sprayed at full bloom stage, 30 days after full bloom and 60 days after full bloom, with a bacterial suspension ($10^9$ CFU $ml^{-1}$) of OSU-142 until run off.

At the end of the growing season in 2000, fruits were harvested and evaluated for yield increase, and shot-hole disease reduction for each treatment. Data of the first year showed that the application at full bloom stage significantly increased yield and decreased disease severity and incidence. In the second year (2001), the field experiment was conducted in the same orchard using the same apricot trees. However, two applications performed after full bloom in the first year were not included in the second year. Growth promoting effects of bacterial treatments were evaluated by determining average weight per fruit (g/fruit), total harvested fruit weight (kg/tree), total soluble solid (TSS) and acidity, and average length and diameter (cm) of 100 annual shoots (Tables 1 and 2). In addition, the effect of the

Table 1. The effect of *Bacillus* OSU-142 on average fruit weight, yield, TSS, acidity, shoot length and diameter, and shot-hole disease incidence and severity in apricot cv. '*Hacıhaliloğlu*' in 2000.
*Der Einfluss von Bacillus OSU-142 auf durchschnittliches Fruchtgewicht, Ertrag, Gehalt an löslichen Kohlehydraten, Säuregehalt, Trieblänge und -durchmesser sowie Häufigkeit und Schwere der Symptomausprägung der Schrotschusskrankheit bei Aprikosen der Sorte 'Hacıhaliloğlu' im Jahr 2000.*

| Treatment | Fruit weight (g) | Yield (kg/tree) | Total soluble solid (%) | Acidity (%) | Shoot length (cm) | Shoot diameter (cm) | Disease incidence (%) | Disease severity |
|---|---|---|---|---|---|---|---|---|
| Control | 27.43 | 31.6 b | 22.10 | 0.25 | 22.74 b | 0.38 b | 10.33 a | 9.16 a |
| A | 30.12 | 41.0 a | 21.80 | 0.28 | 28.61 a | 0.49 a | 5.00 b | 2.63 c |
| B | 28.39 | 33.2 b | 21.70 | 0.28 | 24.35 b | 0.47 a | 7.67 ab | 4.72 bc |
| C | 28.52 | 33.0 b | 22.06 | 0.28 | 23.66 b | 0.43 ab | 10.00 a | 7.61 ab |
| SED | NS | 1.99*** | NS | NS | 3.86** | 0.07** | 3.35* | 4.15** |

Spraying at A= Full Bloom, B= 30 days after full bloom, C= 60 days after full bloom
NS = Not Significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Table 2. The effect of *Bacillus* OSU-142 on average fruit weight, yield, TSS, acidity, shoot length and diameter, and shot-hole disease incidence and severity in apricot cv. '*Hacıhaliloğlu*' in 2001.
*Der Einfluss von Bacillus OSU-142 auf durchschnittliches Fruchtgewicht, Ertrag, Gehalt an löslichen Kohlehydraten, Säuregehalt, Trieblänge und -durchmesser sowie Häufigkeit und Schwere der Symptomausprägung der Schrotschusskrankheit bei Aprikosen der Sorte 'Hacıhaliloğlu' im Jahr 2001.*

| Treatment | Fruit weight (g) | Yield (kg/tree) | Total soluble solid (%) | Acidity (%) | Shoot length (cm) | Shoot diameter (cm) | Disease incidence (%) | Disease severity |
|---|---|---|---|---|---|---|---|---|
| Control | 26.61 | 30.83 b | 21.73 | 0.27 | 19.11 b | 0.357 b | 29.00 | 5.81 a |
| OSU-142 | 28.49 | 58.50 a | 20.00 | 0.27 | 25.24 a | 0.475 a | 24.67 | 3.43 b |
| SED | NS | 11.46*** | NS | NS | 4.56** | 0.096** | NS | 1.83* |

NS = Not Significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

bacterial treatments on the suppression of shot-hole disease was evaluated by estimating the percent of fruits with lesions (disease incidence) and average number of lesions on per fruit (disease severity), respectively (Tables 1, 2).

### *Data analysis*

Data were evaluated by analysis of variance, and means were separated by Duncan's multiple range tests.

## Results and Discussion

### *Growth promoting effect of OSU-142 treatment in apricots*

Data obtained for each treatment in both years are shown in Tables 1 and 2. In 2000, the results showed that average fruit weight and yield increased by bacterial treatments at full bloom, 30 days after full bloom and 60 days after full bloom. Significant yield increase was obtained with the bacterial treatments at full bloom stage (41 kg/tree) as compared with the other treatments (33.2–33.0 kg/tree) and control (31.6 kg/tree). However, there were no significant differences between the treatments in terms of average fruit weight, TSS and acidity. In addition, mean shoot length and diameter increased by all applications with Bacillus OSU-142 ($p<0.001$). The best shoot development with 28.61 cm in length and 0.49 cm in diameter was obtained with the application of bacteria at full bloom stage (Table 1).

In the second year of the experiment (2001), only one bacterial treatment at full bloom stage was performed. Like the first year's results, application of *Bacillus* OSU-142 increased yield (58.5 kg/tree), length (25.24 cm) and diameter (0.475 cm) of shoots as compared with the control (Table 2). The average percentage of yield increase was 90 % when *Bacillus* OSU-142 was applied in 2001 (Table 2). Similarly, there were no significant differences between treatments concerning the average fruit weight and other fruit properties (TSS and acidity) tested although there was a numerical increase in average fruit weight of the bacterial treatments (Table 2).

This is the first study where plant growth promoting rhizobacteria (PGPR) have been reported on a perennial plant such as a stone fruit. De Silva et al. (2000) reported that the leaf area and stem diameter of high bush blueberry were increased by the application of *Pseudomonas fluerescens* Pf 5. Results of the study with sugar beet and barley confirmed the finding in this study. The use of OSU-142 growth promoting bacteria in sugar beet and barley showed that OSU-142 significantly affected yield, yield parameters and quality parameters in both crops (Çakmakçı et al. 2001). However, there are very few studies about the application of those bacteria on the phyllospher including trees, except a study on *Azotobacter, Azosprillum* and *Beijerinckia* sprayed on the leaves of mulberry increasing leaf yield and quality (Sudhakar et al. 2000).

Positive effects of OSU-142 on yield of some other crops such as tomatoes, sugar beet and barley were explained by $N_2$-fixation ability and antimicrobial substance production. However, in our study OSU-142 stimulated fertilization and fruit setting, which could be due to auxin production. This can be excepted since some other studies demonstrated that OSU-142 application promoted auxin production, $N_2$-fixation (Çakmakçı et al. 2001) and antimicrobial substance production (Şahin et al. 2000, Abbasi et al. 2001). Although auxin levels were not analyzed before and after bacteria application, it is well known that members of the auxin group have positive effects on fruit set in different plant species, for examples in strawberry, squash, fig, tomato, rose, tobacco, eggplant, citrus and olive (Leopold and Kriedemann 1975, Ryugo 1986, Greene 1989, Arteca 1996, Talon et al. 1998). In our study, higher yield ratio obtained by bacterial application of full bloom stage may be explained by auxin stimulating effect of OSU-142. Increase in yield did not influence the average fruit weight. The yield increase ratio in the second year was higher than in the first year. This may be explained by the repeated use of trees in second year experiment having residue effect of *Bacillus* OSU-142 from the first year application. This can be assumed, as the yield in control plants did not change with the year of the experiments.

### *Effects of biocontrol bacteria on the suppression of shot-hole disease of apricot*

The data of disease severity and incidence on the fruits harvested in 2000 are summarized in Table 1. The results show that the highest disease incidence (10 %) is determined in the control and the application of bacteria 60 days after full bloom. It is followed by application of bacteria 30 days after full bloom (7.67 %) and treatment at full bloom (5 %). The average number of lesions on fruits of the control, 30 days and 60 days after full bloom and at full bloom stage were 9.2, 4.7, 7.6 and 2.6, respectively (Table 1). The data obtained from the first year of the experiment showed that application at full bloom stage had significantly reduced both shot-hole disease severity and incidence on apricot fruits. The preliminary results suggested that the best time for the treatment with the biocontrol agent to control shot-hole disease was the full bloom stage. The data of treatments applied at full bloom in the second year's experiments confirm the findings of the first year's experiment. The means incidence of the control (29 %) was higher than that of the bacterial treatment (24.7 %). Similarly means of the disease severity in bacterial treatment (3.4) was significantly less than that of control (5.8). Effective control of shot-hole disease in this study may be explained by the antagonistic capability of the bacterium OSU-142 to inhibit spore germination and penetration of the fungus *Wilsonomyces carpophilus* on the foliage, in particular leaves and fruits of apricot. This hypothesis may be supported by the evidence that in some other studies OSU-142 had a broad spectrum of antimicrobial activity against many plant pathogenic fungi and bacteria species in in vitro and in in vivo tests (Miller et al. 1998, Dönmez et al. 1999, Kotan et al. 1999, Cuppels et al. 1999, Dönmez et al. 2000). Comparing the data of control treatments in both years, there was a higher disease pressure in the second year, which could be explained by the frequency and the amount of precipitation being higher in the second year.

## Conclusion

This is the first study demonstrating that the *Bacillus* strain OSU-142 had the capacity to increase yield (approximately 41.0–58.5 kg/tree) and quality by reducing shot-hole disease severity (2.63–3.43) and incidence (5.0–24.67 %) in apricot. The results also suggested that OSU-142 might be used as biological fertilizer to spray apricot at blooming stage for better yield. Furthermore, the data also show that OSU-142 has an antagonistic effect against *Wilsonomyces carpophilus* and could be applied to control shot-hole disease on apricot and other alternative host species. The data in this study confirm the results of previous studies. OSU-142 had a great potential of bio-control activity and a wide range of pathogen and growth promoting effects on different plant species. In conclusion, OSU-142 was found to be effective a bacterial strain with a good potential to be used in sustainable and ecological agricultural systems as a bio-fertilizer and a bio-pesticide.

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