A New Approach to Fire Blight Control: Mycorrhiza

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Additional index words. *Glomus intraradices*, fire blight, apple, control, *Malus x domestica*

Abstract. The first outbreak of fire blight incited by *Erwinia amylovora* (Burr.) Winslow et al. occurred on pome fruits in Turkey in 1985, and it is now one of the most serious diseases of pear, apple, quince, and loquat (Oktem and Benlioglu, 1988). In this study, experiments were conducted in Konya Province to evaluate the efficacy of *Glomus intraradices* Schenck & Smith vesicular-arbuscular mycorrhiza (VAM) and bactericides for control of the shoot blight phase of fire blight and control of shoot growth on the different apple cultivars (Gala, Red Elstar, Pinova, Jonagored) on M9 rootstock in 2002 to 2003. Streptomycin provided 84.38% to 95.24% and 85.28% to 89.97% disease control in 2002 and 2003, respectively. Copper complex was not so effective against shoot blight phase of the disease, and it reduced disease by 16.18% to 27.75% and 14.48% to 19.06% in 2002 and 2003, respectively. Results of VAM application were encouraging, indicating a reduction of fire blight by *Glomus intraradices* of between 9.7% and 50.5% in 2002 and between 23.9% and 48.4% in 2003, respectively.

Fruit growing is one of the main sectors of Turkish agriculture. Almost 3 million tons of pome fruit is harvested annually, which is approximately 23% of the total fruit production (Anonymous, 2001).

Apple cultivation on dwarf rootstock has become widespread, and cultivars such as Fuji, Braeburn, Gala, Elstar, and Jonagold are preferred by Turkish growers (Gunduz, 1997).

Fire blight, a destructive disease of pome fruits incited by *Erwinia amylovora*, was the first plant disease known to be incited by a bacterium (Van der Zwet and Keil, 1979).

Apples grown on M26 and M9 rootstock were shown to be extremely susceptible to fire blight (Norelli and Aldwinckle, 2000; Travis et al., 1999).

Dehne (1982) and Graham and Menge (1982) pointed out that VAMs increased the uptake of phosphorus and other nutrients as well as resistance of plants to disease.

The aim of this work is to determine the effects of a VAM (*Glomus intraradices*) on

shoot growth and fire blight development on dwarf apple cultivars and to compare the disease reduction with streptomycin and a copper compound.

Materials and Methods

Soil and leaf analyses

The soil used in the experiments was taken from 0 to 20-cm depth of the Selcuk University Experimental Fields strained through 4-mm mesh sieves and analyzed physically and chemically (sand, silt, clay, and textural class, Bouyoucos, 1951; pH [H₂O, 1;2.5] and EC (H₂O, 1; 2.5), Jackson, 1962; organic matter (%), Bayrakli, 1986; carbonates (%); Hizalan and Unal, 1966; CEC [cmol kg⁻¹]; Anonymous, 1954; Available P [mg kg⁻¹], Olsen et al., 1954). After approximately 2 months from the inoculation of the plants by *E. amylovora* (when the symptom development stopped), 40 samples of randomly selected leaves from each apple cultivar were collected. Leaves were passed through 0.1% HCl and rinsed in distilled water. Samples were prepared by a wet-ashing technique and nutrients were measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Varian-Vista model) and atomic absorption spectrophotometer (Bayrakli, 1986).

Plant material and growing conditions

The widely grown apple cultivars Gala, Pinova, Red Elstar (a variant of 'Elstar'), and Jonagored (a variant of 'Jonagold'), which are getting widespread in Turkey and are

becoming on the fire blight-susceptible M9 rootstocks, were selected and used in the experiments. The experiments were conducted under natural conditions in Konya, Turkey, in the year 2002 to 2003 by using 3-year-old healthy, uniformly grown apple saplings.

Inoculum of *G. intraradices* (100 spores/g soil) was spread on top of 2 kg of soil placed in the bottom of the pots earlier. Then the saplings were trimmed and placed on the inoculum and buried with soil up to scion-rootstock graft union (Gianinazzi et al., 1989). The potted plants were placed on the ground 1 m apart at a field location in Konya and watered when necessary throughout the growing season.

E. amylovora isolate

Pathogenicity of the available *Erwinia amylovora* isolates was tested according to the method of Norelli et al. (1984), and the most virulent one (EAI) was used in the experiments (Bastas and Maden, 2004).

The chemicals and their application

Two bactericides, streptomycin (streptomycin sulfate, powder, 100%, I.E. Ulagay) and Tenn Cop 5E (copper salts of fatty and rosin acids, 51.4%, liquid, Hektas A.S.), which is commonly used against the disease, were used at rates of 59 g/100 L water and 250 mL/100 L water, respectively. Tenn Cop 5E was applied 3 times when the shoot lengths were 6 to 12 cm, 15 to 20 cm, and 25 to 30 cm, and streptomycin was applied twice, 1 d before and 1 d after the inoculation (Momol et al., 1999).

After transplantation, the trees were fertilized (1 g/pot) with ammonium sulfate 25 g/pot, diammonium phosphate 25 g/pot, potassium sulfate 25 g/pot, and humic acid (humic acid 55%, fulvic acid 30%, potassium hydroxide 8%; powder 20 g/100 L) twice a week by supplying 50 mL of a liquid fertilizer having Mn, Cu, Zn, B, Mo at less than 0.05% once a week (Kacar and Katkat, 1999). In addition, sulfur dust was applied for powdery mildew control.

Experimental setup and design

The experiment was set up in a completely randomized block design with 3 replicates. The average of the treatments at 3 shoots of a sapling was counted as a replication. Every treatment was applied to 2 groups of plants, the first group of plants being treated by mycorrhizal fungus and the chemicals and inoculated with *E. amylovora* to see the effects of the treatments and the second group was treated as mentioned previously and was not inoculated with the pathogen to see the effect of treatments on shoot growth of apples (Duzgunes et al., 1987).

Inoculation of the shoots with the bacterium

The shoots were inoculated when they reached 25 to 30 cm in length (approximately 3 months after *G. intraradices* inoculation)

Received for publication 12 Dec. 2005. Accepted for publication 17 Mar. 2005. This work was supported by the Coordinatorship of Selcuk University's Scientific Research Projects. We thank Prof. Dr. Ibrahim Ortac from the Soil Science Department of Agricultural Faculty of Çukurova University for providing *G. intraradices* and Prof. Dr. Hüseyin Basim from the Plant Protection Department of Agricultural Faculty of Akdeniz University for *Erwinia amylovora* isolate EAI. Journal article HCS 01-314.

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by cutting two fully expanded leaves with a pair of scissors dipped in a suspension of *E. amylovora* prepared from a 48-h culture grown on 5% SNA medium (Norelli et al., 1984; Norelli et al., 1986).

Evaluation of disease severity and plant growth

Evaluation of the disease was made after symptom development stopped, approximately 2 months after inoculation, by measuring the total shoot length and the length of the necrotic area on shoots. The average of 9 shoots was considered one replicate.

Based on this evaluation; disease severity was calculated as below:

$$\text{Disease severity (\%)} = (a/b) 100$$

in which a is the length of the necrotic area (cm) and b is the whole length of the shoot (cm) (Fernando and Jones, 1999). Percent effectiveness of the applications (A) was calculated according to the following formula of

$$A = (B - C/B) 100$$

in which B is the percent disease severity in the controls and C is percent disease severity in treated shoots. Percent effectiveness of the treatments on reduction of shoot growth (D) was calculated in a similar way,

$$D = (E - F/E) 100$$

in which E is the mean shoot length in the controls and F is the length of treated shoots (Anonymous, 1996). MSTAT software was used for statistical calculations, and the differences between the treatments were determined by Duncan's multiple range analysis.

Results

Soil properties

The soil used in the experiments was a loamy soil, which had 42.3% sand, 32.0% silt, 25.7% clay, a moderate concentration of organic matter (2.25%), and a high concentration of lime (29.23). It was slightly alkaline (pH = 7.78), containing very low usable phosphorus (Olsen: 3.14 ppm P), 14.14 me/100 g changeable cations, 0.65 me/100 g soluble cations, and 204 μ mhos/cm salt.

Nutrient contents of leaves

Nutrient analyses of the leaves were carried out only in 2002 and the results were presented at Table 1. There were significant differences between the nutrient leaf contents of trees inoculated and noninoculated with *Glomus intraradices* trees except Na. 'Red Elstar' inoculated with *G. intraradices* had an increase in all the nutrient elements.

Disease severity and plant growth

There were significant differences between the apple cultivars Gala, Red Elstar, Pinova, and Jonagored treated differently in terms of disease severity and shoot growth in 2002 and 2003 (Tables 2 and 3).

In 2002, the differences between the treatments were significant both in terms of disease severity and shoot growth, and there was an interaction between the cultivars and treatments ($P < 0.01$). In 2003, only the difference between the treatments was significant and there was cultivar $\times$ treatment interaction on disease severity. Treatments differed significantly when shoot growth was considered. Streptomycin gave the highest rate of protection in both years and this was followed by *G. intraradices* treatment on 3 cultivars (Red Elstar, Jonagored, and Pinova). Disease reduction in mycorrhiza application ranged from 9.68% to 50.46% and 23.86% to 48.39% in 2002 and 2003, respectively. There was no significant difference in shoot growth between the 2 years, but there was an interaction between disease severity and cultivar and treatment in 2 years.

Mycorrhiza applications did not cause negative effects on shoot growth of apple varieties in 2002 and 2003.

Discussion

The mycorrhizal fungus-plant association provides carbohydrates and some organic materials for the fungus, and improves plant access to water and mineral intake (Bolan et al., 1987; Li et al., 1991a; Rhodes, 1980). There are many reports indicating that VAMs increase phosphorus, zinc, copper, iron, calcium, potassium, and nitrogen uptake (Hayman, 1982), but Rhodes and Gerdemann (1978) found out that calcium uptake and transport was low in VAM-infected plants. We also found low levels of Ca in VAM-infected plants. *G. intraradices* stimulated

growth on apple (Plenchette et al., 1981) and pear (Gardiner and Christensen, 1991) saplings in orchard soils in greenhouse conditions. Fortuna et al. (1996) inoculated apples grafted on micropropagated MM 106 rootstocks and Mr. S. 2/5 plums with *G. mossae* and *G. intraradices* and observed increased apical shoot growth along with P uptake. They pointed out that chemical fertilizer input could be reduced in this way. The reason *G. intraradices* was selected for our experiments is its widespread use on apple species among the *Glomus* species.

There are many papers dealing with positive effects of *G. intraradices* on the growth of many plant species (Aquilera-Gomez et al., 1999; Blee and Anderson, 1996; Frey and Ellis, 1997; Varma and Schüepp, 1994) along with its effect on fungal diseases on some apple varieties as well as other fruit and vegetable species (Caron et al., 1986; Graham and Egel, 1988; Kjoller and Rosendahl, 1997; St.-Arnaud et al., 1997). Despite its protection against nematodes (Pinochet et al., 1998; Price et al., 1995) and elimination of the negative effects of pesticides (Graham et al., 1986; Hamel et al., 1994; Perrin and Plenchette, 1993), there does not appear to be a report of *G. intraradices* on bacterial diseases.

Soil conditions such as soil type, soil moisture, pH, and nutrient content affect tree growth and their resistance to fire blight. Nutrient levels found favoring fire blight are N, 2.2% to 2.6%; P, 0.13% to 0.33%; K, 1.35% to 1.85%; Ca, 1.3% to 2%; Mg, 0.35% to 0.5%; B, 35–50 ppm; Zn, 35–50 ppm; Cu, 7–12 ppm; Mn, 50–150 ppm; and Fe, 50+ ppm (Van der Zwet and Beer, 1995). Koseoglu et al. (1996) found a relation between fire blight onset and K and Mn contents of the leaves and shoots on 'Santa Maria' pears in natural conditions. Disease severity decreased when the K levels in the leaves increased and Mn acted similarly in the shoots. George et al. (1992) and Marschner and Dell (1994) also found higher K levels in mycorrhiza treated apples. In our study, the lowest rate of disease was obtained on Red Elstar cv., and K and Mn content of this variety were higher after mycorrhiza treatment. This shows that high levels of K and Mn reduce fire blight development. In another research carried out in New York, it was determined that in poorly drained soils with low K content, fire blight severity was higher, and this was reversed in well-drained soils (Van der Zwet and Keil, 1979). Treatment with *G. intraradices* caused a negative effect on nutrient uptake in 'Gala', and a higher rate of disease severity on this cultivar could be linked with that. Hence, mycorrhizal interaction may change based on plant species, plant varieties, and mycorrhiza species as well. Guillemain et al. (1992) also found out the presence of a selectivity of VAMs in terms of stimulation of plant growth. For the best disease control, different mycorrhiza species should be tested on different apple cultivars. Results also showed that there is a relation between nutrient content and fire blight intensity in the plants.

Table 1. Nutrient contents of leaves of various apple cultivars grafted on M9 rootstocks and inoculated with mycorrhiza in 2002.

Cultivars	Elements								
	K (%)	P (%)	N (%)	Ca (%)	Mg (%)	Na (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)
Gala (² C)	1.560	0.224	1.624	0.840	0.319	0.193	1.274	64.143	20.270
Gala	1.561	0.176	2.099	0.449	0.183	0.155	0.626	38.64	18.990
Red Elstar (C)	1.680	0.172	2.099	0.329	0.183	0.161	1.130	31.672	5.296
Red Elstar	1.894	0.181	2.275	0.412	0.196	0.134	1.450	44.389	13.418
Jonagold (C)	1.391	0.184	1.902	0.415	0.202	0.105	1.645	43.520	5.790
Jonagold	1.627	0.214	2.169	0.377	0.211	0.134	3.180	81.757	7.852
Pinova (C)	1.159	0.446	1.749	0.325	0.206	0.164	5.934	62.930	28.859
Pinova	1.447	0.263	1.892	0.539	0.236	0.183	4.707	63.946	10.306

²C = noninoculated control.

Table 2. Effects of biologic and chemical treatments on fire blight disease incited by *Erwinia amylovora* and on shoot growth of 4 apple cultivars on M9 rootstock in 2002.

Treatments	Apple cultivars											
	Gala			Red Elstar			Pinova			Jonagored		
	Disease severity (%)	Shoot length (cm)	ET (%)	Disease severity (%)	Shoot length (cm)	ET (%)	Disease severity (%)	Shoot length (cm)	ET (%)	Disease severity (%)	Shoot length (cm)	ET (%)
<i>Glomus intraradices</i> ^z	36.51 aB ^x	—	11.76	17.18 bC	—	50.46	28.30 bB	—	34.21	34.31 aAB	—	9.68
<i>Glomus intraradices</i> ^y	—	39.18	0.65	—	42.69	0.00	—	41.04	0.00	—	37.32	6.18
Tenn Cop 5 E ^z	32.18 bA ^w	—	22.23	28.46 aA	—	17.93	31.94 bA	—	27.75	31.84 aA	—	16.18
Tenn Cop 5 E ^y	—	37.79	4.18	—	36.95	3.12	—	37.68	3.16	—	38.56	3.06
Streptomycin ^z	6.46 cA	—	84.38	1.65 cA	—	95.24	3.75 cA	—	91.28	5.60 bA	—	85.25
Streptomycin ^y	—	38.54	2.28	—	38.36	0.00	—	37.96	2.44	—	39.68	0.25
Control ^z	41.38 aAB	—	—	34.68 aB	—	—	43.02 aA	—	—	37.99 aAB	—	—
Control ^y	—	39.44	—	—	38.14	—	—	38.91	—	—	39.78	—

^zInoculated plants with *Erwinia amylovora*.

^yNoninoculated plants.

^xFigures are averages of 3 replications, each consisting of 3 shoots.

^wET, Effectiveness of treatment.

^vThe upper case letters show the difference between the cultivars, whereas the lower case letters show the difference between the treatments in a cultivar. The difference between the years was significant.

Table 3. Effects of biologic and chemical treatments on fire blight disease incited by *Erwinia amylovora* and on shoot growth of 4 apple cultivars on M9 rootstock in 2003.

Treatments	Apple cultivars											
	Gala			Red Elstar			Pinova			Jonagored		
	Disease severity (%)	Shoot length (cm)	ET (%)	Disease severity (%)	Shoot length (cm)	ET (%)	Disease severity (%)	Shoot length (cm)	ET (%)	Disease severity (%)	Shoot length (cm)	ET (%)
<i>Glomus intraradices</i> ^z	24.31 cAB ^x	—	37.10	19.09 cB	—	48.39	26.07 bA	—	23.86	18.62 bB	—	48.23
<i>Glomus intraradices</i> ^y	—	41.88	0.00	—	43.62	0.00	—	38.64	0.00	—	41.31	0.00
Tenn Cop 5 E ^z	31.28 bA ^w	—	19.06	30.26 bA	—	18.19	27.79 abA	—	18.83	30.76 aA	—	14.48
Tenn Cop 5 E ^y	—	39.50	3.25	—	39.98	0.00	—	35.77	5.79	—	38.49	0.54
Streptomycin ^z	5.11 dA	—	86.77	3.71 dA	—	89.97	5.04 cA	—	85.28	4.02 cA	—	88.82
Streptomycin ^y	—	38.47	5.78	—	38.58	1.93	—	38.33	0.00	—	39.20	0.00
Control ^z	38.65 aA	—	—	36.99 aA	—	—	34.24 aA	—	—	35.97 aA	—	—
Control ^y	—	40.83	—	—	39.34	—	—	37.97	—	—	38.70	—

^zInoculated plants with *Erwinia amylovora*.

^yNoninoculated plants.

^xFigures are averages of 3 replications, each consisting of 3 shoots.

^wThe upper case letters show the difference between the cultivars, whereas the lower case letters show the difference between the treatments in a cultivar. The difference between the years was significant.

^vET, Effectiveness of treatment.

Plenchette et al. (1981) and Gianinazzi et al. (1989) reported increase of shoot growth in apples inoculated with mycorrhiza. Although we did not have a statistically significant difference on shoot growth of apples, effects of VAMs on crop quality and yield should be studied.

Increases in copper uptake were also reported in many places (Li et al., 1991b; Marschner and Dell, 1994), including our experiments, carried out in Konya conditions.

Utkhede and Smith (2000) tested the effects of the mixture of *Glomus intraradices* + *Enterobacter agglomerans* Beijerinck + metham sodium on Gala variety grafted M9 rootstock and found that this treatment reduced infections incited by *Phytophthora cactorum* and *Pythium ultimum* and increased yield. This approach might be considered for fire blight control as well.

Morandi et al., (1984) determined that the concentrations of phytoalexin like isoflavonoid compounds increased in VAM-treated plants. Changes in the metabolism of phytoalexins and phenolic compounds that play a role in fire blight resistance (Rademacher,

2000; Rademacher et al., 1999) should also be studied in relation to VAM-treated plants.

Timing of inoculation with VAM is so important for their success. Inoculation of many micropropagated fruit trees gave hopeful results (Blal and Gianinazzi-Pearson, 1989; Ravolanirina et al., 1989). However, Azcon-Aguilar et al. (1992) obtained the best results when avocado plants were inoculated 4 weeks after transplantation. In addition, selection of appropriate fungal species and substrate are also necessary for maximum benefit. Soil conditions such as pH, soil nutrient levels, pesticide applications, irrigation, and drainage should also be considered.

Success in mycorrhizal relationship depends on VAM species, pathogen species, and nutrients, synergistic and antagonistic effects of other soil microorganisms to VAMs. Despite all of these unknown factors, reduction of fire blight with VAM is comparable that with a copper compound. In addition to economic and environmental advantages of using VAM fungi, the prospect of gaining a new tool in fire blight management should encourage us to do more

detailed studies on VAM as related to this disease.

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