Romanian Biotechnological Letters *Vol. 14, No. 1, 2009, pp. 4123-4129*

ORIGINAL PAPER

Evaluations of apricot trees infected by *Candidatus* Phytoplasma prunorum for horticultural characteristics

Received for publication, September 15, 2008
Accepted, November 12, 2008

MONA GAZEL[1], KADRIYE CAGLAYAN[1], CIGDEM ULUBAS SERCE[1], LEVENT SON[2]
[1]*Mustafa Kemal University, Agriculture Faculty, Plant Protection Department Hatay, Turkey*
[2]*Mersin University, Mut Vocational School, Icel, Turkey*
Correspondence to: monagazel@hotmail.com

Abstract

Apricot, Prunus armeniaca L., is an important stone fruit species in Turkey. The Mediterranean coastal area in Turkey has advantageous climatic conditions for early table apricot production. New orchards with both local and foreign cultivars are being established in this region. The disease caused by phytoplasma is a critical threat for apricot growers. In this study, pomological data were collected from three apricot trees (cv. 'Precoce de Tyrinthe') that are infected by Candidatus Phytoplasma prunorum disease and from healthy plants under field conditions. The variables evaluated included yield, fruit width, length, height and weight, seed weight, seed/fruit ratio, soluble solids and acidity between 2004 and 2007. Analyses of variance indicated that all infected trees had lower yield when compared to the uninfected trees for four experimental years. The reduction in yield reached up to 77% in some cases. Significant differences were also recovered for variables for infected versus control comparisons. In some cases, the means of the trees also differed among infected ones. The results suggested that Ca. Phytoplasma prunorum negatively affects the 'Precoce de Tyrinthe' apricot for both yield and pomological characteristics in different ratios.

Keywords: Apricot, European stone fruit yellows phytoplasma, pomological characters, yield.

Introduction

Turkey's favorable climatic and soil conditions are advantageous for growing a broad range of crops due to its favorable climatic and soil conditions. The apricot is one of the most important crops in Turkey with the annual production rate of 860,000 tons [1]. The apricot growers in the east Mediterranean region of Turkey have complained of a dieback in apricot trees since 1999 [2]. Symptomological and molecular tests have confirmed that the causal agent of this dieback is as *Candidatus* Phytoplasma prunorum (formerly named European stone fruit yellows phytoplasma; ESFY) [3, 4, 5]. In many European countries, *Ca.* Phtytoplasma prunorum has been identified as one of the most prevalent problems facing apricot trees [6, 7, 8].

Ca. Phytoplasma prunorum causes serious economic losses in cultivated *Prunus* species. Susceptible young apricot and plum trees infected with *Ca.* Phytoplasma prunorum die quickly (within 1-2 years after infection), and the pathogen also causes yield and quality losses on trees older than five years [9]. The age of the trees, environmental conditions and the rootstocks influence the severity of symptoms and the progress of the disease. European plums have been determined to be tolerant to *Ca.* Phytoplasma prunorum, whereas Japanese plums are highly susceptible [10].

Apricot variety significantly affects the response of fruit yield and several quality parameters of tree infected with *Ca.* Phytoplasma prunorum. To our knowledge, the susceptibility or resistance to *Ca.* Phytoplasma prunorum of various rootstocks and scion

varieties has been investigated by several researchers, but the effect of this phytoplasma on fruit yield and quality has not been examined. The major objective of this study is to investigate the effects of *Ca.* Phytoplasma prunorum infections on fruit yield and on the quality of 'Precoce de Tyrinthe' apricot trees infected by this pathogen under field conditions.

Materials and Methods

Three apricot trees infected by *Ca.* Phytoplasma prunorum [5] were observed during 2004-2007 under field conditions. The trees were grafted on seedling-grown apricot rootstocks, and they were three years old when the experiment started. Two uninfected trees in the same orchard were selected as a negative control.

The plants were tested to verify the presence of phytoplasma by PCR and RFLP. Total DNAs from all the stone fruit plant samples, including the healthy control, were extracted according to Doyle and Doyle [11]. Phytoplasma specific primers for direct PCR P1/ P7 (P1: 5'- AGA GTT TGA TCC TGG CTC AGG A- 3'; P7: 5'- CGT CCT TCA TCG GCT CTT- 3') [12, 13] and for nested- R16F2n/R2 (R16F2n: 5'- GAA ACG ACT GCT AAG ACT GG- 3'; R2: 5'- TGA CGG GCG GTG TGT ACA AAC CCC G- 3') [14] were used for amplification. The following amplification conditions were used: for the first cycle 94º C for 1 min (30 sec for nested PCR) denaturation, 55º C for 2 min (30 s for nested PCR) annealing, 72º C for 3 min (1 min for nested PCR) extension and the final extension 5 min at 72º C. PCR products were analyzed in 1.5 % agarose gels, stained with ethidium bromide and visualized with UV transilluminator. Nested PCR products were separately digested with *Rsa*I and *Ssp*I restriction endonucleases (MBI Fermentas, GmbH, Germany). The digested products were analyzed by electrophoresis using 2% agarose gel.

The pomological analyses were carried out on three replicates with 10 fruits in each replicate. Fruit width, length and height were measured using a digital caliper. Total soluble solids content (TSS) and titratable acidity (TA) were assessed in the juice obtained. Fruit and seed weight were determined using a scale sensitive to 0.1 g. TSS content was determined with a refractometer (Atago, Model ATC-1E) and TA by titration of 5 ml of fruit juice with 0.1 N NaOH to pH 8.1. The harvest date was recorded, and yield was measured by harvesting all of the fruits from each tree. Therefore, the yield did not have tree replications.

Statistical analyses were conducted using SAS program and procedures [15]. GLM procedure was used to construct analysis of variance (ANOVA) tables, and means were calculated using TABULATE. The main effect of genotypes was separated by Tukey at $P < 5\%$.

Results and Discussion

The trees under investigation flowered in late January instead of in March. Following the whole leaf set, the first symptoms manifested in May as a severe upward longitudinal rolling of the leaves which were thicker and more brittle than normal (Fig. 1). These leaf symptoms were unevenly distributed throughout the branches. This off-season flowering symptom was typical for infected trees in the east Mediterranean region, whereas quick dieback was obvious in other regions and cultivars [5]. PCR analyses revealed phytoplasma incidence in these trees (Fig. 2). Nested-PCR amplifications yielded a characteristic band of approximately 1.2 kb from the three apricot trees tested and the reference strain of *Ca.* Phytoplasma prunorum. No amplifications were obtained from the reaction mixture without nucleic acid template or containing DNAs from healthy GF 305. In RFLP analysis, PCR amplified three apricot samples had restriction profiles with *Rsa*I enzyme identical to those of

reference Ca. Phytoplasma prunorum. Digestions with *Ssp*I of the *Ca.* Phytoplasma prunorum infected apricot DNA were not given any profile (Fig. 2).

Considerable differences of horticultural characters were observed between the infected and control trees. Although it was not determined quantitatively, the infection adversely affected the fruit set (Fig. 3). The yield was negatively affected as well. The lack of replication did not allow us to test if these differences were significant; however, on average the infected trees had 50, 14, 33 and 33% less yield than the uninfected controls (Table 1). In 2004, tree 6 (18.66 kg / tree) has the lowest yield followed by 5 (731.66 kg / tree) and 1 (72.33 kg / tree). Flowering years had similar patterns. Average yield was ranged from 45.88 (2005) to 64.32 (2006).

The *Ca.* Phytoplasma prunorum infection significantly affected fruit size measurements for trees 5 and 6 (Table 1). Fruit width, length and height means of tree 5 and 6 were significantly lower than the control for all experimental years. These measurements significantly differed for tree 1 in 2006 and 2007 but not in the first two experimental years. Excepting tree 1 in 2005, the fruit weight of the infected trees was significantly lower than the control. Among the infected trees, tree 1 had significantly higher fruit weight than others. This may have played a role in the higher yield obtained from tree 1. The fruits of *Ca.* Phytoplasma prunorum infected trees are undersized and sometimes bumpy, and they ripened and dropped prematurely as reported by Nemeth [9]. In the current study, we have also noticed undersized fruits on cv. 'Precoce de Tyrinthe' (Fig 4).

Figure 1. Unrolling leaf symptoms of *Ca.* Phytoplasma prunorum on Precoce de Tyrinthe apricot cultivar.

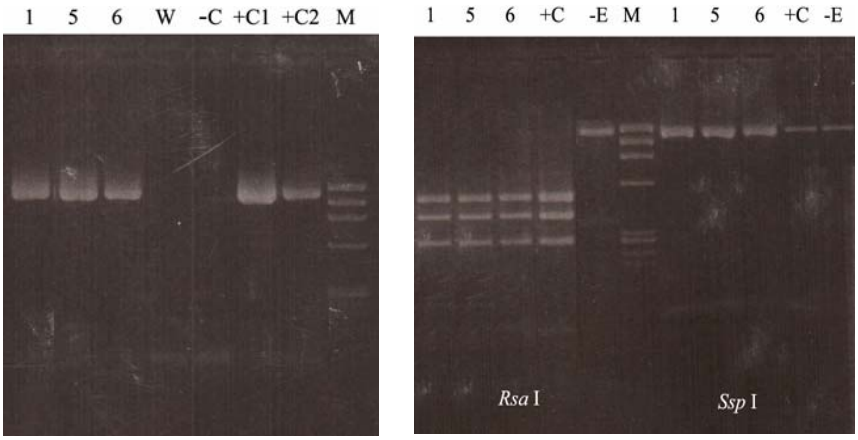

Figure 2. PCR amplification of *Ca.* Phytoplasma prunorum infected Precoce de Tyrinthe apricot cultivar trees (left). Restriction enzyme digestions of nested PCR products amplified with R16F2n/R2 primers (right). Numbers 1, 5 and 6 indicated *Ca*. Phytoplasma prunorum infected trees; W water control; -C Healthy control; +C1, +C2 and +C *Ca*. Phytoplasma prunorum reference strains; -E control DNA without enzyme; M DNA ladder ΦX174 DNA/BsuRI (HaeIII) Marker (MBI Fermentas, GmbH, Germany).

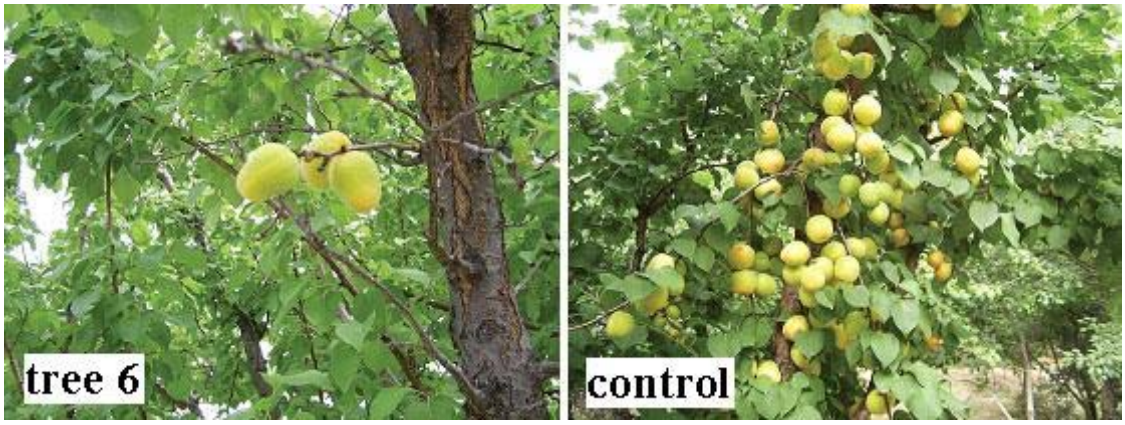

Figure 3. Fruit set of *Ca.* Phytoplasma prunorum infected (tree 6) and control Precoce de Tyrinthe apricot cultivar.

Figure 4. The fruits of the trees infected with *Ca.* Phytoplasma prunorum on infected (A, B and C) and control trees of Precoce de Tyrinthe apricot cultivar.

Seed weight had varying patterns during there research period (Table 1). For example, in 2006 infected trees had significantly lower seed weight while only tree 6 and the control had significant differences in 2007. The flesh/seed ratio also exhibited varying patterns. It appears that *Ca.* Phytoplasma prunorum did not have a consistent effect on TSS and TA. In 2005, control trees had the highest SS (10.33%) while the same plants had the lowest TA (1.90%). The next year, however, control trees had only differed from tree 5 for TSS and TA. Tree 5 and control had significant differences in 2004, while tree 1 and 6 had similar results. In 2007, the control tree had the lowest TA, and the highest TSS means were obtained from trees 1 and control.

Table 1. Several pomological characteristics of Precoce de Tyrinthe apricot cultivar between 2004 and 2007. The numbered trees were infected by *Ca.* Phytoplasma prunorum.

Source	Fruit width (mm)	Fruit length (mm)	Fruit height (mm)	Fruit weight (g)	Seed weight (g)	Flesh / seed ratio	Soluble solids (%)	Acidity (%)	Harvest date	Yield (kg/tree)
2004										
1	43.64a	44.85a	45.11a	50.17b	3.44a	13.58b	10.73a	1.92a	17.05.2004	72.33
5	39.88b	40.01c	40.94c	36.74d	3.04b	11.08c	9.26b	1.85b	14.05.2004	31.66
6	41.3b	42.67b	43.52b	43.33c	3.06b	13.12b	10.66a	1.93a	15.05.2004	18.66
Control	44.98a	45.47a	45.99a	55.33a	3.49a	14.83a	10.93a	1.92a	17.05.2004	81.00
$D_{5\%}$	1.46	1.41	0.94	2.5	0.08	0.79	0.3	0.05	-	--
2005										
1	43.28a	45.10 a	44.68ab	48.59a	3.37b	13.38a	10.06b	2.04ab	18.05.2005	52.91
5	39.17c	39.07c	40.00c	39.74c	3.11c	11.75b	9.06c	1.98b	17.05.2005	40.20
6	41.47b	42.94b	43.82b	45.55b	3.12c	13.59a	9.93b	2.08a	17.05.2005	39.03
Control	43.81a	45.52a	45.49a	50.15a	3.50a	13.57a	10.33a	1.90c	19.05.2005	51.36
$D_{5\%}$	0.54	0.7	0.94	1.64	0.07	0.51	0.18	0.07	-	--
2006										
1	45.49b	47.81b	46.96b	51.25b	3.55b	13.45a	10.2ab	2.08a	18.05.2006	72.18
5	39.88b	40.01c	40.94c	36.74d	3.04b	11.08c	9.26b	1.85b	16.05.2006	51.54
6	41.30b	42.67b	43.52b	43.33c	3.06b	13.12b	10.66a	1.93a	15.05.2006	47.66
Control	44.98a	45.47a	45.99a	55.33a	3.49a	14.83a	10.93a	1.92a	20.05.2006	85.88
$D_{5\%}$	1.55	1.83	0.98	2.38	0.11	0.58	0.33	0.04	-	--
2007										
1	42.96 b	45.03b	44.26b	48.55b	3.30ab	13.46a	10.06a	2.06a	21.05.2006	64.08
5	39.56d	41.29c	42.61c	42.40d	3.33ab	11.8b	9.66b	1.99b	20.05.2006	45.89
6	41.41c	41.97c	42.86c	44.41c	3.04b	13.7a	9.46b	2.09a	20.05.2006	40.92
Control	46.77a	48.34a	48.41a	53.26a	3.75a	13.38a	10.2a	1.86c	23.05.2006	75.06
$D_{5\%}$	1.15	1.39	1.15	1.15	0.57	0.33	0.26	0.06	-	--

In a previous work focused on germplasm and commercial orchards in Turkey [5], *Ca.* Phytoplasma prunorum infected plants declined and died within two years after the symptoms' first appearance. However, the present work reveals that during the observation period (2004-2007) the phytoplasma infected trees survived and gave fruit. The survival of ESFY infected trees might result from the scion variety or ESFY strain. Great differences in susceptibility to ESFY of the apricot varieties with different genotypes were observed. Audergon et al. [16] identified great differences between cultivars and selections of apricot, and Carraro et al. [10] observed that Japanese plum cv. 'Ozark Premier' is more susceptible than cv. 'Shiro'. They noted severe symptoms and a high percentage of mortality on 'Ozark Premier', but in spite of having phytoplasma symptoms, cv. 'Shiro' survived and produced a normal yield. Graft inoculation of trees on different rootstocks revealed that great differences in the virulence of *Ca.* Phytoplasma prunorum [17]. Kison and Seemüller [17] reported that the most virulent strains killed all trees, whereas the mild strains did not cause mortality but induced mild foliar symptoms and slightly reduced vigor. The virulence of the strain became more severe when the scion was the same cultivar with the original host of the pathogen.

Conclusion

The economic impact of ESFY on stone fruit trees is still unknown. However, Lichou [18] described ESFY is one of the factors that limit the culture of apricot Japanese plum in France. The yield and quality damage caused by this phytoplasma in stone fruit trees need further investigation. ESFY is, in practice, not curable. Therefore, control by prevention is necessary. In areas with a high natural presence of ESFY, the cultivation of tolerant or resistant species instead of sensitive ones is advisable.

References

1. ANONYMOUS, Tarımsal yapı (üretim, fiyat, değer). T.C. Başbakanlık Devlet İstatistik Enstitüsü, 2005.
2. CAGLAYAN, K., GAZEL. M., Primary studies for viroid and phytoplasma problems of stone fruits in East Mediterranean Area of Turkey. XIVth Int. Plant Protection Cong. (IPPC) Jerusalem, Israel, July 25-30. p.16, 1999.
3. CAGLAYAN, K., GAZEL, M., ULUBAS, C., EMBER, I. Doğu Akdeniz Bölgesinde sert çekirdekli meyve ağaçlarında Sert Çekirdekli Meyve Ağacı Sarılığı (European Stone Fruit Yellows = ESFY) fitoplazmasının yaygınlık durumunun PCR/RFLP yöntemiyle saptanması. Türkiye I. Bitki Koruma Kongresi Bildiri Özetleri, 8-10 Eylül, Samsun. 141, 2004.
4. SERTKAYA, G., MARTINI, M., ERMACORA, P., MUSETTI, R., OSLER, R. Detection and characterization of phytoplasmas in diseased stone fruits and pear by PCR-RFLP analysis in Turkey. *Phytoparasitica* **33**, 380-390, 2005.
5. ULUBAS SERCE, C., GAZEL, M., CAGLAYAN, K., BAS, M., SON, L. Phytoplasma diseases of fruit trees in germplasm and commercial orchards in Turkey. *Journal of Plant Pathology* **88**, 175-181, 2006.
6. JARAUSCH, W., JARAUSCH-WEHRHEIM, B., DANET, J.L., BROQUAIRE, J.M., DOSBA, F., SAILLARD, C., GARNIER, M. Detection and identification of European stone fruit yellows and other phytoplasmas in wild plants in the surroundings of apricot chlorotic leaf roll-affected orchards in southern France. European J. *Plant Pathology* **107,** 209-217, 2001.
7. NAVRATIL, M., VALOVA, P., FIALOVA, R., PATROVA, K. Survey for stone fruit phytoplasmas in the Czeck Republic. *Acta Hort*., **550**, 377-382, 2001.
8. TORRES, E., MARTIN, M.P., PALTRINIERI, S., VILA, A., MASALLES, R., BERTACCINI, A. Spreading of ESFY phytoplasmas in stone fruit in Catalonia (Spain). *J. Phytopathology* **152**, 432-437, 2004.
9. NEMETH, M. Virus, mycplasma and rickettsia diseases of fruit trees. Martinus Nijhoff Publishers, the Netherlands and Akademia Kiado, Budapest, Hungary, 840 p.p., 1986.

10. CARRARO, L., OSLER, R., LOI, N., ERMACORA, P., REFATTI, E. Transmission of European stone fruit yellows phytoplasma by Cacopsylla pruni. *Journal of Plant Pathology* **80**, 233-239, 1998.
11. DOYLE, J.J., DOYLE, J.L. Isolation of plant DNA from fresh tissue. *Focus*, **12,** 13-15, 1990.
12. DENG, S., HIRUKI, C. Genetic relatedness between two non-culturable mycoplasmalike organisms revealed by nucleic acid hybridization and polymerase chain reaction. *Phytopathology* **81,** 1475-1479., 1991.
13. SMART, C.D., SCHNEIDER, B., BLOMQUIST, C.L., GUERRA, L.J., HARRISON, N.A., AHRENS, U., LORENZ, K.H., SEEMULLER, E., KIRKPATRICK, B.C. Phytoplasma-specific PCR primers based on sequences of 16S-23S rRNA spacer region. *Applied Environmental Microbiology* **62,** 2988-2993, 1996.
14. LEE, I.M., DAVIS, R.E., SINCLAIR, W.A., DEWITT, N.D., CONTI, M. Genetic relatedness of mycoplasmalike organisms detected in Ulmus spp. in USA and Italy by means of DNA probes and polymerase chain reactions. *Phytopathology* **83**, 829-833, 1993.
15. SAS. Institute Inc. SAS users guide; SAS/STAT, version 6. SAS Inst. Inc., Cary, N.C., 1990.
16. AUDERGON, J. M., CASTELAIN, C., MORVAN, G., CASTELLIÉRE, M.G. Behaviour of 150 apricol varieties after an apricot chlorotic leaf roll inoculation. *Acta Hortic*. **293**, 593-598, 1991.
17. KISON, H., SEEMÜLLER, E. Differences in strain virulence of the European stone fruit yellows Phytoplasma and susceptibility of stone fruit trees and various rootstocks to this pathogen. *J. Phytopathology* **149,** 533-541. 2001.
18. LICHOU, J. Main reasons for the decline of apricot. *Klosterneuburger Mitteilungen*, **49**, 209-210, 1999.