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Determination of the Effect of Fosetyl-Al Against Citrus Gummosis Disease Caused By *Phytophthora citrophthora* (Smith and Smith) Leonian

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Abstract: In this study the effect of fosetyl-Al against citrus root not and gummosis caused by *Phytophthora citrophthora* was investigated. The results showed that fosetyl-Al protected citrus from the disease at least for one year. It was found that two fungicide applications sufficiently protected the trees against citrus gummosis. No significant differences were obtained were obtained at two fungiciede dosages, 160 g a.i./100L and 200 g a.i./100L, in all applications. When the pathogen was inoculated two weeks before fosetyl-Al, the infection area was diminished and with time had complete recovery with callus formation. Thus, fosetyl-Al was shown to have a curative effect as well as a protective effect on citrus gummosis.
Key Words: citrus, citrus root rot and gummosis, fosetyl-Al, induced resistance.

Turunçgillerde Zamklanma Hastalığı (*Phytophthora citropthora* (Smith and Smith) Leonian)'na Karşı Fosetyl-Al'un Etkisinin Belirlenmesi

Özet: Bu çalışmada turunçgillerde zamklanma ve kök çürüklüğü etmeni *Phytophthora citrophthora*'a karşı fosetyl-Al'un etkinliği araştırılmıştır. Deneme sonuçları fosetyl-Al'un turunçgil ağaçlarını en az bir yıl süreyle hastalıktan koruduğunu göstermiştir. Bu fungisitin iki kez uygulamasının ağaçları zamklanma hastalığına karşı etkin bir şekilde koruduğu bulunmuştur. Fungisitin iki uygulama dozu, 160 g a.i./100L and 200 g a.i./100L arasında hastalık üzerine etki yönünden önemli bir farklılık bulunmamıştır. Patojen inokulasyonunun fosetyl-Al uygulamasından iki hafta önce yapıldığı zaman infeksiyon alanının azaldığı ve zamanla kallus gelişimi ile kapandığı görülmüştür. Bu da fosetyl-Al'un turunçgil zamklanma hastalığına karşı koruyucu etkisi yanında iyileştirici etkisinin de olduğunu göstermektedir.
Anahtar Sözcükler: turunçgil, turunçgil kök çürüklüğü ve zamklanma, fosetyl-Al, teşvik edilmiş dayanıklık.

Introduction

Phytophthora root rot and gummosis caused by *Phytophthora citropthora* (Sm. and Sm.) Leonian are major problems in citrus orchards worldwide (1). This disease was first reported in Turkey in 1958 (2), and it has affected lemon, mandarin, orange, grapefruit and sour orange cultivars (3). Survery results in the 1970's the disease incidence in lemon orchards in the provinces of İçel and Adana to be 24% and 22.4%, respectively, (4). Some cultural practices such as bud unions at soil surface level or less than 15 cm above soil line, overwatering, poor drainage, soil structure and high clay levels enhance the incidence of the disease. Methods of controlling Phytophthora root rot are generally preventative, e.g., cultural practices and fungicide application to trunk and lower branches. When infection does occur, removal of the infected bark is recommended; however, bark removal diminishes tree performance and fruit yield. This technique is also laborious and uneconomical when attempted on a large scale.

Recently, several new systemic fungicides have been developed to control Oomycet fungi. Among these, metalaxyl and fosetlyl-Al have yielded the best results (5, 6, 7, 8, 9). Toker and Biçici (10) studied the effects of a combination of fosetyl-Al and benomyl on citrus postharvest diseases such as brown rot, blue and green mold. They observed that when combined fungicides were applied, the rate of diseased fruit diminished by 14.4%, 15.3% and 44.5% in 2-month ambient conditions, and 2- and 4-month cold storage conditions, respectively. Although bioassay studies for fungicide registration have been conducted in Turkey, no research up to now has been carried out to determine the biological effects of fosetyl-Al on the susceptible lemon cultivars Molla Mehmed and Kütdiken, local varieties in the provinces of İçel and Adana. Both are major export

products of Turkey (11). Therefore, the purpose of this study was to determine the effectiveness of fosetyl-Al on *P. citrophthora* and applicability of the fungicide in practice.

Material and Methods

Locations

The two lemon orchards selected for the test were each 15 years old and showed no signs of gummosis. The following sites were used: **1)** Cultivar Molla Mehmed orchard on sour orange (*Citrus aurantium L.*) rootstock planted on a spacing of 8x6 m in Icel; **2)** Cultivar Kütdiken on sour orange rootstock planted on a spacing of 8x6 m in Karataş, Adana. Both orchards were located near the Mediterranean sea.

Treatments

Two recommended dosages of fosetyl-Al (Aliette 80 WP) were applied as foliar sprays: 160 g a.i./100 L and 200 g a.i./100 L (recommended dosage). Fungicide application dates were in spring (2nd of April), early summer (20th of June) and autumn (19th of September) when shoot length reached 10-15 cm in each flush period. Fungicide treatments at both locations were carried out on 12 trees arranged in a randomised complete block design. Tree replicate blocks were used and one untreated row was left between the treated rows. Blocks of 12 trees contained three trees treated three times each with fosetyl-Al (200 g a.i./100L) one year before and three untreated control trees.

Pathogen inoculations

Pathogen inoculations were done in three different combinations: **1)** inoculation two weeks before fungicide application; **2)** inoculation simultaneous with fungicide application; **3)** inoculation two weeks after fungicide application. The isolate of *P. citrophthora* used was isolated from diseased lemon trees and its pathogenicity was confirmed by inoculation of lemon seedlings. Inoculations were carried out by placing 10-day old PDA culture disks 6.0 mm in diameter, under the bark, fungal side downward one on the trunk and four on the main branches. In order to provide the moisture required, infection sites were covered with cotton embedded in sterile distilled water and wrapped in aluminium foil and polyethylene sheets. Barks were stripped off the trunks 40 days after the first fungicide application, off the first 2 branches 40 days after the second application, and off the second 2 branches 40 days after the third application. The discoloured lesions on the wood surface of each infection side were then measured. Lesion-area data were obtained from different inoculations. Fungicide application combinations were analysed by LSD test. The bark pieces were used for reisolation of the fungus.

Results and Discussion

Disease development pathogen inoculation two weeks before fungicide application

Disease development in comparison with untreated trees was diminished after the first fungicide applictaion at all dosages in both cultivars (Fig. 1A and 2A). Lesion areas of *P. citrophthora* on control trees gradually increased with time, whereas they decreased up to zero level on treated trees of both cultivars at all fungicide dosages. The maximum effectiveness of the first fungicide application two weeks after pathogen inoculation at a dosage of 160 g a.i./100 L in the cultivars Molla Memed and Kütdiken were 62.9% and 38.7%, respectively (Table 1). The effectiveness rose to 92.5% and 87.9%, respectively, after the second fungicide applications, suggesting that the first treatment was still effective on the disease development. Although the third fungicide application yielded a higher effectievness, two fosetyl-Al applications wer more economical than three applications and yielded sufficient protection against citrus gummosis. The previous year, fosetyl-Al treatment+200 g a.i./100 L yielded the best results in this combination as well. When the previous years's application+200 g a.i./100 L was compared with fosetyl-Al applications at dosages of 160 and 200 g a.i./100L, the effectiveness of the former was much higher, 90.3% and 74.7% for Molla Memed and Kutdiken, respectively, than that of the latter. This effectiveness reached 100% after the second application, suggesting that sufficient phytoalexin accumulation had resulted. However, at dosages of 160 and 200 g a.i./100 L, adequate effectiveness was not obtained, but about 90% effectiveness was determined after the second application. This indicates that the lesion area developed after the inoculation can be decreased by the second fosetyl-Al application.

Disease development pathogen inoculation simultaneous with fungicide application

In this combination, the lesion area diminished after the first fungicide applications at all dosages (Fig. 1B and 2.B). After the third application, the lesion areas of the control Molla Memed and Kutdiken cultivars were 250 and 90 cm^2, respectively, but the trees treated with fosetyl-Al developed calli on the infection area and the lesion size was measured as zero. After the first

application, the effectiveness of the fungicide on the infection area at dosages of 160, 200 g. a.i./100 L. and previous treatment+200 g a.i./100 L were 78.5%, 93.9% and 95.9%, respectively, in Molla Memed, whereas the effectiveness rates in Kütdiken were 59.9%, 59.0% and 89.3%, respectively (Table 2). Results obtained from previous fungicide treatment+200g. a.i./100 L applicaitons indicate that the fungicide is effective for at least one year. After the second fosetyl-Al application, the infection area of the disease was less than 2.6 cm^2 with more than 95% effectiveness (Table 2). These results indicate that two fosetyl-Al treatments are adequate to control the gummosis caused by *P. citrophthora*. As expected, the infection areas had

Table 1. Percentage of effectiveness of fosetyl-Al application at different dosages on the development of gummosis disease of lemon cultivars, Molla Memed and Kütdiken, when pathogen was inoculated 2 weeks before fosetyl-Al application.

Variety	Applications	Fosetyl-Al dosages (g/100 L)		
		160	200	+200[z]
		x		y
Molla	first	62.9 b	55.3 b	90.3 b*
Memed	second	92.5 b	95.9 b	99.7 b*
	third	95.8 a	97.7 a	100.0 a*
	first	38.7 b	38.5 c	74.7 c
Kütdiken	second	87.9 a	87.0 b	97.0 b*
	third	99.4 a	98.9 a	99.7 a

x: Mean percentage in the same row for each cultivar followed by the same letter are not significantly different as determined by LDS at P=0.05 level

y* dosages are significant at P=0.05 level

z: three times fosetyl-Al application (200 g/100L) one year before+ 200 g/100L

Table 2. Percentage of effectiveness of fosetyl-Al application at different dosages on the development of gummosis disease of lemon cultivars, Molla Memed and Kutdiken, when pathogen inoculation and fosetyl-Al application were simultaneous.

Variety	Applications	Fosetyl-Al dosages (g/100 L)		
		160	200	+200[z]
		x		y
Molla	first	78.5 c	93.9 b	95.9 b
Memed	second	98.9 b	99.4 b	99.7 b
	third	100.0 a	100.0 a	100.0 a
	first	59.9 b	59.0 b	89.3 b*
Kütdiken	second	94.5 a	95.8 a	100.0 a
	third	100.0 a	100.0 a	100.0 a

x, y and z: similar to Table 1

complete recovery with callus formation after the third treatment, resulting in 100% effectiveness.

Disease development-pathogen inoculation two weeks after fungicide application

Fosetyl-Al application significantly affected the disease development when the fungicide was applied two weeks before *P. citrophthora* inoculation (Fig. 1C and 2C).

Even the first treatment showed 92.5% and 62.7% effectiveness at a dosage of 160 g a.i./100 L in Molla Memed and Kütdiken, respectively (Table 3), indicating that fosetyl-Al induces a defence mechanism in citrus, preventing the pathogen from becoming established. A previous study on the effects of fosetyl-Al on phytoalexin synthesis of citrus showed similar results (7). In this combination, the best result was obtained from fosetyl-Al application one year in advance+200 g a.i./100L, as in other combinations. As can be seen from Table 2, two

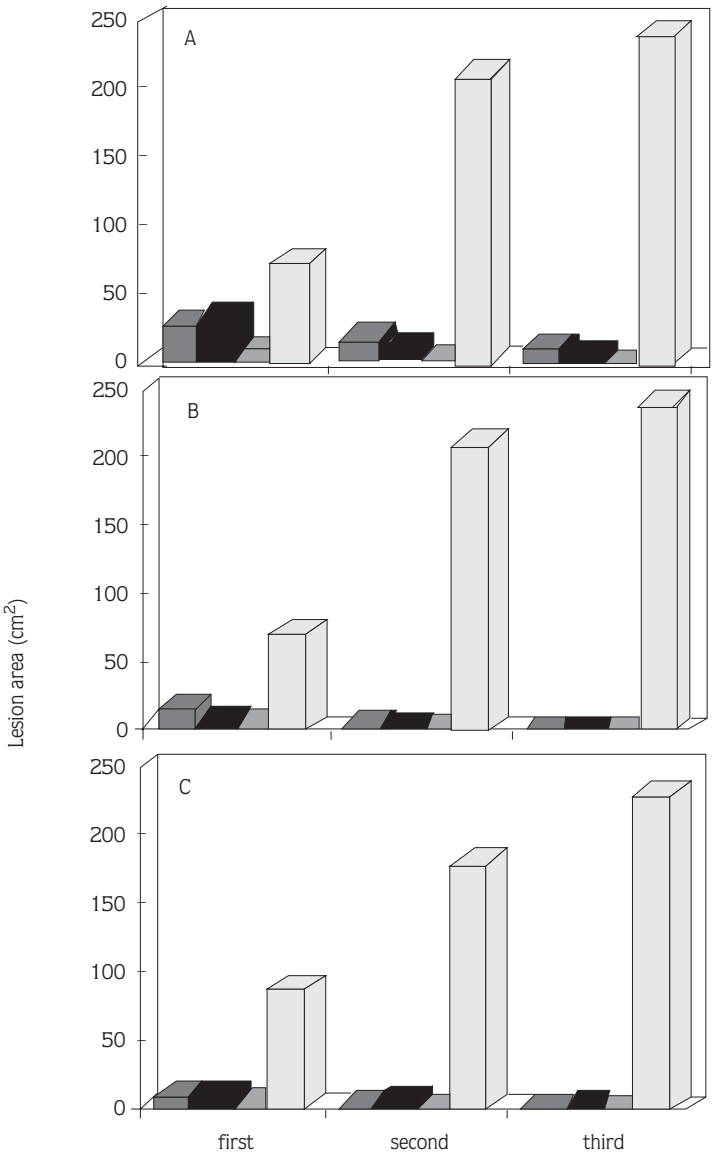

Figure 1. Lesion area in barks of the cultivar Molla Memed at three fosetyl-Al applications. A. Inoculation 2 weeks before application; application; B. Inoculation and fungicide application at the same time; C. Inoculation 2 weeks after application.:

160 g a.i./100 L,
200 g a.i./10
application 1 year advance+20 a.i./100L,
untreated control.

fungicide applications are enough for protection against the disease.

The results show that Fosetyl-Al application protected the citrus against root rot and brown rot caused by *P. citrophthora* for at least one year. Afek and Sztenjnberk (7, 12) reported that fosetyl-Al controlled diseases caused by *Phytophthora* spp. by inducing host deference mechanism. They found that fosetyl-Al treatment induced scoporone, a phytoalexin asociated with resistance of citrus to *P. citrophthora*. Matheron and Matejka (6) reported that sporangium production by *P. citrophthora* decreased at least 90% at three days after soil treatment with as much as 3000 mg/ml of fosetyl-Al. They suggested that fosetyl-Al affected *P. citrophthora* in vivo in two ways: at low level treatment it increases the host defence mechanism, and higher levels it acts directly as a fungistat. Observations of other researchers have confirmed that fosetyl-Al has little direct effectiveness against mycelial growth of Oomycetes fungi (6, 8).

In this studys no significant differences were obtained at two fungicide dosages, 160 g a.i./100L and 200 g a.i./100L, the in all applications, suggesting that a lower dosage would be more economical. When the pathogen was inoculated two weeks before fosetyl-Al, the infection area was diminished and with time completely recovered. This shows that fosetyl-Al also has a curative effect on citrus gummosis. In conclusion, two applications are necessary for proper control, and correct timing improves effectiveness. However, if fosetyl-Al is applied one year before, a single spring application the following year

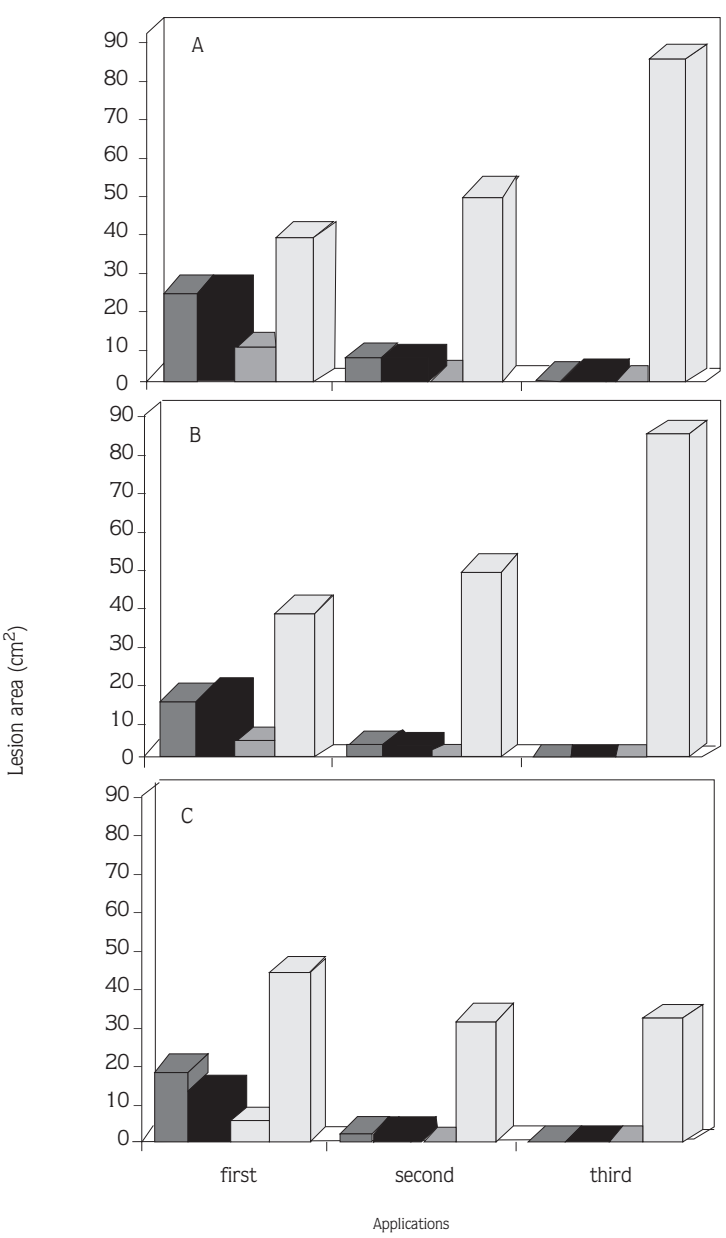

Figure 2. Lesion area in barks of the cultivar Kutdiken at three fosetyl-Al applications. A, B, C similar to Fig. 1.

Table 3. Percentage of effectiveness of fosetyl-Al application at different dosages on the development of gummosis disease of lemon cultivars, Molla Memed and Kütdiken, when pathogen was inoculated 2 weeks after fosety-Al application.

Variety	Applications	Fosetyl-Al dosages (g/100 L)		
		160	200	+200[z]
		x		y
Molla	first	92.5 b	92.8 b	97.3 c
Memed	second	99.4 a	98.6 a	99.7 b
	third	99.7 a	99.5 a	100.0 a
	first	62.7 c	63.5 b	88.3 b*
Kütdiken	second	93.9 b	95.1 a	98.8 a
	third	99.6 a	97.7 a	100.0 a

x, y and z: similar to Table 1

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