

## **Integrated Control of Verticillium Wilt of Olive with Cryptonol in Combination with a Solar Chamber and Fertilizer**

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A study was conducted during 2 years to determine the effects of three different control measures on the development of *Verticillium* wilt on olive trees cv. 'Nabali' at Al-Hallabat, Jordan. The causal agent of the wilt was *Verticillium dahliae* Kleb. Treating diseased trees with Cryptonol (8-hydroxyquinoline sulfate) in a soil drench, or covering trees with a solar chamber for 15 days, was effective in suppressing disease development. The fertilizer treatment (NPK, 15:15:30) decreased disease severity and percent infection. All decreases are in comparison with the untreated control, and as recorded during the active phase of the pathogen. The treatments did not differ significantly from each other, and disease incidence in treated trees remained lower than in the control throughout the examined period.

KEY WORDS: *Verticillium dahliae*; olive; solar heating.

### INTRODUCTION

*Verticillium* wilt of olive is considered one of the most difficult phytopathological problems in tree pathology (13). The impact of the disease is severe in groves during the first year after their establishment, but tends to become rather mild with aging. Olive trees possess a superficial root system and are always vulnerable to the pathogen, particularly in irrigated groves of susceptible olive cultivars (2,12,14).

The fungus is present in the aboveground and underground parts of infected trees and in the surrounding soil. This makes its control a difficult task (1). Chemical control by either soil drenching or trunk injection has been tested by Tawil *et al.* (8) and Karajah (11). A solar chamber is an innovative technique developed by Al-Ahmad (1) for controlling *Verticillium* wilt in the aboveground parts of olive trees. Fertilization by KCl solution has been tested by Ashworth *et al.* (3). None of the previous studies gave satisfactory and complete control of this disease in olive groves. The present research was conducted to study the effects of: soil drenching with Cryptonol, solar chamber, NPK fertilizer, and combined treatments as integrated control, on development of *Verticillium dahliae*.

### MATERIALS AND METHODS

The *Verticillium* pathogen has a relatively slow growth rate on common media compared with most pathogenic and saprophytic fungi, which often overrun the plates

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before *Verticillium* colonies can be detected. Thus, a selective medium described by Ausher *et al.* (4) was used for isolation of *V. dahliae* from host tissue of olive and from soil surrounding the roots. All materials used in the preparation of selective media, except ethanol and streptomycin, were mixed together and autoclaved at 121°C at 15 Psi for 30 min. Ethanol and streptomycin were mixed with autoclaved cooled medium before being poured into plates. The ingredients (per liter of water) of the above selective medium used for isolation of *V. dahliae* were: sucrose, 7.5 g; ethanol, 5 ml; NaNO<sub>3</sub>, 2.0 g; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5 g; K<sub>2</sub>HPO<sub>4</sub>, 1 g; FeSO<sub>4</sub>.4H<sub>2</sub>O, 0.01 g; pentachloronitrobenzene, 0.05 g; chloramphenicol, 1 g; agar, 20 g.

**Isolation of the fungal pathogen from host tissue** Six twigs were taken at random from a *Verticillium*-infected tree. The branches were cut into 0.5–1.0-cm pieces. A slant cut of each section was made with the purpose of exposing the pith cavity to contact with the selective medium. Plant pieces were dipped in sodium hypochlorite (0.5%) for 2 min (10). The pieces were rinsed in sterile distilled water, dried on sterile filter paper, and plated on the selective medium in petri dishes. Plates were incubated for 2 weeks at 20°C. Isolations were conducted to determine whether the trees were infected with the *V. dahliae* pathogen prior to conducting the treatments.

**Assessment of the development of Verticillium wilt** A naturally infected grove of olive cv. 'Nabali' at Al-Hallabat in the Al-Zarqa region of Jordan was selected for assessment of disease severity and the percentage of *Verticillium*-infected trees. The soil in the grove is a silt loam, and the trees are irrigated twice a week. Four diseased trees were selected at random for each treatment.

Infection percentage was determined monthly for each tree as follows:

$$\% \text{ Infection} = \frac{\text{number of infected branches}}{\text{total number of examined branches}} \times 100$$

A method of visual estimation of disease severity was developed by Tjamos *et al.* (14) and the same scale was used in this study. It is based on foliar symptoms such as leaf yellowing, leaf rolling and defoliation of the twigs, as follows: 0, tree free of infection; 1, very slight infection; 2, slight infection; 3, moderate infection; 4, severe infection; 5, nearly dead or dead tree. The overall assessment of disease severity was calculated by the following equation:

$$\text{Severity of disease} = \frac{\text{sum of all numerical ratings}}{\text{total number of plants}} \times \frac{100}{5}$$

This experiment was initiated in June 1998. Percent infection and disease severity were evaluated monthly. Isolation of the fungus was done to identify the naturally infected trees before treatment applications. The isolation was done once a month, in the last week of the month. The experiment was laid out in a completely randomized block design, with trees chosen according to a randomization distribution table. There were four replicates (one tree serving as a replicate) per treatment; least significant difference (LSD) was used for means separation.

**Chemical control** A soil drench treatment was given to 5–8-year-old diseased olive trees at Al-Hallabat during the course of the study. A circular trench (15 cm deep and 1 m in diameter) was dug in the soil around the tree trunk after elimination of the weeds; then the

fungicide solution was placed in the trench. Cryptonol (C) was used at the commercial rate of application, 80 ml / 40 l water / tree. The first application was given on August 1, 1998, and the second on April 10, 1999.

**Solar chamber** The solar chambers (S) consisted of a wooden framework covered with transparent plastic sheeting (60  $\mu$ m thick). In this experiment a 2.5-m pyramid-shaped solar chamber was used. Before the solar chambers were placed on trees, weeds were eradicated mechanically and the top 5 cm of soil around each tree was removed to the periphery of the projection of the tree canopy. The solar chamber was placed on a diseased tree. A layer of soil was placed on the edge of the plastic around the treated tree to stabilize the chamber and to prevent external air from entering the solar chamber. The temperature in the chamber was 55°C in summer; it was not checked in winter. Four trees were covered with solar chambers for 15 days from August 1 to 15, 1998, and again from April 10 to 25, 1999.

**Fertilization** Fertilization with NPK, 15:15:30, at the rate of 150 g/tree, was done in three applications at 3-week intervals. Each spray comprised 50 g/tree, dissolved in 2 l of water, applied with a manual sprayer. N was given as both NO<sub>3</sub> and NH<sub>4</sub>. Treatments in the first year started on August 1, 1998, and those in the second year on April 10, 1999. There were four replicates of each treatment.

**Integrated control** Integrated treatments were given to diseased trees to evaluate the combined action of different treatments. The treatments comprising each combination treatment were given just like the individual treatments, and replicated four times. All treatments were applied in August 1998 and repeated, on the same trees, in April 1999.

## RESULTS

**Isolation and identification of the olive wilt pathogen** White cottony mycelium growth was observed after 7 days of incubation of plant material on the selective medium. The color of the cottony mycelium turned black within 2 weeks after plating. The isolations from diseased trees indicated that *Verticillium dahliae* Kleb. was the causal agent of olive wilt at Al-Hallabat. Table 1 shows the percent infection of all trees before the application of treatments. It varied from 75% to 91.6% in June and from 62.5% to 87.5% in July. Disease severity for the same trees varied from 30% to 65% in June and from 35% to 60% in July (Table 2).

Fertilized trees did not suffer from either potassium or phosphorus deficiency.

**Effect of treatments on development of *Verticillium* wilt after first application** The first application of treatments (August 1998) reduced percent infection to various levels, compared with the untreated control (Table 1), the combined fertilizer + Cryptonol + solar chamber (F+C+S) treatment being the most effective in most cases. Disease severity in the untreated control did not change much during the examined period (45–70%), whereas starting from August 1998 it was significantly lower in the various treatments in most cases (Table 2). Disease severity in treated trees remained lower than in control trees throughout the examined period; the treatments did not differ significantly from each other.

**Effect of treatments on development of *Verticillium* wilt after second application** The second application of treatments (April 1999) significantly lowered the incidence of infection in treated compared with untreated control trees, in which infection remained high

TABLE 1. Effect of individual and combined treatments on percent Verticillium infection of olive trees after the first application (August 1998)

Treatment <sup>z</sup>	Infection (%)											
	June '98 <sup>y</sup>	July '98 <sup>y</sup>	Aug. '98	Sept.'98	Oct. '98	Nov. '98	Dec. '98	Jan. '99	Feb. '99	March '99		
<b>C</b>	79.1 a <sup>x</sup>	66.7 ab	8.4 b	12.5 b	12.5 ab	70.8 ab	20.8 b	37.5 ab	37.5 ab	37.5 b		
<b>S</b>	91.6 a	70.8 ab	12.5 b	29.2 ab	29.1 ab	62.5 abc	66.7 ab	41.7 ab	41.7 ab	33.3 b		
<b>F</b>	75.0 a	70.8 ab	16.7 b	12.5 b	0.0 b	20.8 bcd	45.8 ab	29.2 b	29.2 b	33.3 b		
<b>C+S</b>	83.3 a	83.3 ab	4.2 b	16.7 b	0.0 b	66.7 ab	50.0 ab	37.5 ab	45.9 ab	41.7 ab		
<b>F+C</b>	91.6 a	75.0 ab	16.7 b	12.5 b	41.6 ab	50.0 abcd	50.0 ab	41.7 ab	62.5 ab	66.7 ab		
<b>F+S</b>	75.0 a	79.1 ab	20.8 ab	4.2 b	50.0 ab	12.5 cd	45.8 ab	45.8 ab	41.7 ab	41.7 ab		
<b>F+C+S</b>	91.6 a	87.5 a	0.0 b	0.0 b	25.0 ab	8.3 d	37.5 b	20.8 b	33.3 b	25.0 b		
<b>Ct</b>	87.5 a	62.5 b	41.7 a	54.2 a	58.3 a	87.5 a	95.8 a	87.5 a	87.5 a	87.5 a		

<sup>z</sup> C, Cryptonol; S, Solar chamber; F, Fertilizer; Ct, Control. In combined treatments, the order of treatments was as listed in the column.

<sup>y</sup> Isolations were done before treatment application.

<sup>x</sup> Within columns, means followed by a common letter do not differ significantly ( $P>0.05$ ). Average of four replicates.

TABLE 2. Effect of individual and combined treatments on *Verticillium* disease severity on olive trees after the first application (August 1998)

Treatment <sup>z</sup>	Disease severity (%)											
	June '98 <sup>y</sup>	July '98 <sup>y</sup>	Aug. '98	Sept.'98	Oct. '98	Nov. '98	Dec. '98	Jan. '99	Feb. '99	March '99		
<b>C</b>	45 b <sup>x</sup>	40 b	15 b	15 b	15 b	10 b	15 b	20 b	15 b	20 b		
<b>S</b>	40 bc	35 b	10 bc	20 b	15 b	15 b	20 b	25 b	20 b	15 b		
<b>F</b>	50 b	50 ab	20 b	15 b	5 b	20 b	30 b	20 b	25 b	20 b		
<b>C+S</b>	65 a	60 a	10 bc	10 b	10 b	10 b	15 b	15 b	20 b	25 b		
<b>F+C</b>	30 c	35 b	15 b	20 b	20 b	15 b	20 b	15 b	20 b	20 b		
<b>F+S</b>	40 bc	40 b	20 b	15 b	15 b	10 b	15 b	20 b	15 b	20 b		
<b>F+C+S</b>	45 b	40 b	5 c	5 b	5 b	10 b	10 b	10 b	15 b	15 b		
<b>Ct</b>	65 a	60 a	45 a	50 a	50 a	60 a	65 a	70 a	70 a	60 a		

<sup>z</sup> C, Cryptonol; S, Solar chamber; F, Fertilizer; Ct, Control. In combined treatments, the order of treatments was as listed in the column.

<sup>y</sup> Isolations were done before treatment application.

<sup>x</sup> Within columns, means followed by a common letter do not differ significantly ( $P>0.05$ ). Average of four replicates.

TABLE 3. Effect of individual and combined treatments on percent *Verticillium* infection of olive trees after the second application (April 1999)

Treatment <sup>z</sup>	Infection (%)					
	April '99	May '99	June '99	July '99	Aug. '99	Sept. '99
<b>C</b>	33.3 bc <sup>y</sup>	41.7 bc	62.5 ab	33.3 ab	16.7 bc	29.2 b
<b>S</b>	25.0 bc	20.8 cd	0.0 d	12.5 b	0.0 c	4.2 c
<b>F</b>	29.2 bc	33.3 bcd	33.3 bcd	0.0 b	8.3 bc	12.5 bc
<b>C+S</b>	0.0 c	0.0 d	16.7 cd	0.0 b	8.3 bc	20.8 bc
<b>F+C</b>	54.1 ab	58.3 ab	54.1 abc	16.7 b	20.8 b	12.5 bc
<b>F+S</b>	25.0 b	4.2 d	12.5 d	16.7 b	4.2 bc	8.3 bc
<b>F+C+S</b>	16.7 bc	0.0 d	12.5 d	12.5 b	0.0 c	4.2 c
<b>Ct</b>	83.3 a	83.3 a	91.6 a	62.5 a	62.5 a	79.2 a

<sup>z</sup>C, Cryptonol; S, Solar chamber; F, Fertilizer; Ct, Control. In combined treatments, the order of treatments was as listed in the column.

<sup>y</sup> Within columns, means followed by a common letter do not differ significantly ( $P > 0.05$ ). Average of four replicates.

TABLE 4. Effect of individual and combined treatments on *Verticillium* disease severity on olive trees after the second application (April 1999)

Treatment <sup>z</sup>	Disease severity (%)					
	April '99	May '99	June '99	July '99	Aug. '99	Sept '99
<b>C</b>	25 b <sup>y</sup>	20 b	25 b	15 b	10 c	15 b
<b>S</b>	15 b	15 b	20 b	20 b	15 bc	20 b
<b>F</b>	15 b	20 b	20 b	20 b	20 b	10 b
<b>C+S</b>	20 b	20 b	15 b	15 b	10 c	15 b
<b>F+C</b>	25 b	15 b	20 b	20 b	15 bc	10 b
<b>F+S</b>	30 b	25 b	25 b	15 b	15 bc	15 b
<b>F+C+S</b>	15 b	15 b	10 b	5 b	5 c	5 b
<b>Ct</b>	65 a	65 a	65 a	60 a	60 a	65 a

<sup>z</sup>C, Cryptonol; S, Solar chamber; F, Fertilizer; Ct, Control. In combined treatments, the order of treatments was as listed in the column.

<sup>y</sup> Within columns, means followed by a common letter do not differ significantly ( $P > 0.05$ ). Average of four replicates.

(62-92%) throughout the examined period (Table 3). At the end of the examination period (Sept. 1999), incidence of infection in the treated trees ranged from 4% to 29%, compared with 79% in the untreated control.

All treatments reduced disease severity significantly compared with the control. The triple treatment (F+C+S) resulted in the lowest disease severity, especially from June 1999, although not significantly lower than other treatments (Table 4). The lower tree infection resulting from the F+C+S treatment was reflected in a similar reduction in disease severity (Table 4). Thus, while disease severity ranged from 60% to 65% in the untreated control, it was still low in the F+C+S treatment at the last reading in Sept. 1999 (5–20%). Disease level generally remained constant in both treated and untreated trees. With few exceptions, there were no significant differences between treatments.

## DISCUSSION

Soil drench with Cryptonol was efficient in reducing disease development compared with untreated trees, especially immediately after the application of treatments. Using the solar chamber for 15 days during August 1998 and April 1999 was effective in reducing

disease development significantly compared with the control. The heating resulting from the solar chamber did not affect trees adversely, apparently because they have a strong structure and can tolerate the effect of heat. The same findings were reported by Al-Ahmad (1), who could not isolate the fungus from infected trees after 15–20 days of treatment. Extension of the solarization period gave better results but continuous covering should not be practiced, to avoid tree burning.

Fertilization was found to be an important cultural practice in strengthening the trees and increasing their tolerance to *V. dahliae* infection. Fertilized trees did not suffer from either potassium or phosphorus deficiency, in agreement with the findings of Ashworth *et al.* (3) and Hafez *et al.* (7). Nonfertilized trees were vulnerable to infection, and K-deficiency reduces root growth and makes the plant more susceptible to infection than thrifty trees at low inoculum density (5). Olive trees in general are slow-growing and long-lived.

The combined action of several treatments gave additive effects, with Cryponol affecting the pathogen in the soil, the solar chamber affecting the disease in the soil and branches, and the fertilizer strengthening the tree. *V. dahliae* is normally present in the soil, roots, trunk, branches and leaves and is very difficult to control. Integrated control using more than one control measure is highly effective and advisable in complicated cases of plant pathology. Such a control method avoids the development of resistance and environmental pollution problems, as reported by El-Zik (6).

It is assumed that the fluctuation of disease development after the applications in the 2 years of the study was due to environmental (*viz.*, temperature, rainfall and humidity) conditions. Disease development increased during the winter months apparently due to the rise in soil moisture and the decline in air and soil temperatures; this in turn may increase the fungal activity in the soil and plant parts. This was followed by an intermediate stage in spring and autumn, whereas in summer decreased disease development was due to the decline in soil moisture and the rise in air and soil temperatures, which led to reduced fungus activity in the soil and tree. These findings agree with those reported by Naser (9).

The treatments did not differ significantly from each other, and disease incidence in treated trees remained lower than in the controls throughout the examined period.

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