European Journal of Plant Pathology (2007) 117:123–127
DOI 10.1007/s10658-006-9076-6

Effect of heat-stress predisposition on the development of Scytalidium wilt of 'Star Ruby' grapefruit, caused by *Scytalidium lignicola*

A. Sadowsky[1,*], Z. Solel[2], and A. Sztejnberg[3]
[1]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan, 50250, Israel;* [2]*Department of Plant Pathology, Agricultural Research Organization, Bet Dagan, 50250, Israel;* [3]*Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot, 76100, Israel; *Author for Correspondence (Phone: +97239485324; Fax: +97239485878; E-mail: avisad@shaham.moag.gov.il)*

Accepted 9 October 2006

Key words: *Citrus paradisi*, disease, environment, plant pathogen

Abstract

Scytalidium wilt, caused by *Scytalidium lignicola*, has become prevalent on 'Star Ruby' grapefruit in orchards in the Jordan Valley, an area with a warm climate in the north of Israel. It occurs in the summer in certain years, soon after extreme hot and dry weather conditions have prevailed for several consecutive days, but not in other years with regular summer temperatures. The effect of temperature conditions before and after inoculation with *S. lignicola* on disease development on 'Star Ruby' was studied in greenhouse chambers with three day/night temperature regimes: 'Very Hot' (47 °C/34 °C); 'Hot' (36 °C/28 °C); and 'Moderate' (30 °C/20 °C). Among the pre-inoculation regimes, 'Very Hot' was most conducive to infection, whereas the 'Hot' regime sustained canker development only when followed by a 'Very Hot' post-inoculation regime. The moderate pre-inoculation conditions appeared to have a negligible, if any, effect on canker development, even with a 'Very Hot' post-inoculation regime. Wilt developed in infected saplings if they were exposed to the 'Very Hot' temperature regime either pre- or post-inoculation, but did not develop under the cooler conditions. Saplings of 'Star Ruby' exposed to a 'Very Hot' regime developed heat-stress symptoms, similar to those observed on 'Star Ruby' in the Jordan Valley. Under a constant 'Very Hot' regime, both canker expansion and subsequent foliar wilt developed on 'Flame', but not on 'Marsh Seedless' or 'Rio Red' grapefruit. The study confirmed an hypothesis that predisposition induced by extremely hot temperature is a prerequisite for infection of susceptible hosts by *S. lignicola*.

Introduction

In the summer of 1998, an outbreak of an unknown wilt occurred on 'Star Ruby' grapefruit trees (*Citrus paradisi*) in orchards along the Jordan Valley, in the north of Israel (Oren et al., 2001). Thousands of trees were affected at the same time, and many of them died. The wilt was accompanied by oozing of gum from the affected branches. The epidermis sloughed off easily revealing a mass of black powder, resulting from copious sporulation of dark conidia of *Scytalidium lignicola* (Oren et al., 2001). Both the bark and xylem were intensively colonized with mycelium. Koch's test confirmed the pathogenic nature of the isolate of *S. lignicola* (Oren et al., 2001). Scytalidium wilt occurred again in the same region in the summers of 1999, 2000, 2002 and 2004 (unpublished data). The disease was always observed about 3 weeks after extreme hot and dry weather conditions, collectively termed Hamsin, when day temperatures above 45 °C and a relative humidity of 15% or less, had prevailed for 3–10 consecutive days. Soon after each event heat stress symptoms

became apparent on the foliage. The incidence of the disease was not rated, but it was estimated in some orchards to affect 10–50% of the trees. In the summers of 2001, 2003 and 2005, with regular temperatures, the disease did not occur. 'Star Ruby' grapefruit is grown in various climatic regions in Israel, but Scytalidium wilt developed predominantly in the Jordan Valley, a region below sea level with a warm Mediterranean climate. It was postulated that the affected trees were predisposed by heat stress, which triggered their infection (Oren et al., 2001). Sometimes, comprehensive pruning preceding the hot spell further enhanced this predisposition effect (Oren et al., 2001).

The predisposing effect of heat stress to enhance susceptibility to weak pathogens was reviewed by Schoenweiss (1975). Recently it was reported from Oman (Elshafie and Ba-Omar, 2001) that in the summer of 1998, after a period of heat (up to 45 °C) and shortage in water, a dieback of *Albizia lebbeck* and other trees was associated with *S. dimidiatum*, and it was suggested that the pathogen was able to kill trees especially under stress conditions. This organism is morphologically similar to *S. lignicola*.

The objective of the present study was to examine the hypothesis that predisposition to extremely hot temperature is a prerequisite for infection of susceptible hosts by *S. lignicola*.

Materials and methods

Plants, inoculation procedure and disease assessment

Saplings of 'Star Ruby', 'Marsh Seedless', 'Rio Red' and 'Flame' grapefruit, grafted on Volkamer (*Citrus limonia*, synonym *C. volkameriana*), and grown in 8 l plastic bags, were 10 to 30 months-old at the time of inoculation.

Mycelial discs, 6 mm in diameter, were cut with a cork borer in the margin of 10 day-old cultures of *S. lignicola* (isolate Beit Shean) grown on PDA in Petri dishes. A similar disc was removed from the bark of the stem of inoculated saplings, and an inoculum disc was placed there and tightly fastened with a flexible polyethylene grafting ribbon (10 mm), which was removed after 3 days of incubation. Canker length was measured every 1–2 days until the 8th day, when at least one replicate became wilted. Occurrence of wilt was monitored from the 8th to the 28th day after inoculation.

Effect of temperature on disease development on 'Star Ruby'

The tests were conducted in temperature-controlled chambers in a greenhouse, where three temperature regimes were maintained: 'Very Hot' (47 °C/34 °C), day/night; 'Hot' (36 °C/28 °C), day/night; and 'Moderate' (30 °C/20 °C), day/night. Length of daytime varied between 16 and 14 h according to season. The transition of temperature between day and night was gradual until the temperature was reached. Three experiments were conducted to study the effect of pre- and post-inoculation temperature on the disease. Plants were exposed to the pre-inoculation temperature regime for 7 to 8 days, and were maintained under the post-inoculation conditions until the end of the experiments 28 days after the inoculation. In each experiment, there were four to seven saplings per treatment depending on availability of plants. The results of three similar experiments were pooled so that the means in each experiment were considered as replicates in the combined computations. The results were then statistically analyzed by LSD analysis.

Susceptibility of grapefruit cultivars to S. lignicola

Saplings of 'Star Ruby', 'Marsh Seedless', 'Rio Red' and 'Flame' grapefruit were maintained under 'Very Hot' or 'Hot' regimes, as described above, throughout the experiment. Five to seven plants of each cultivar (according to availability) were held at the selected regime for 8 days before inoculation, and then canker length (mm), 3 and 5 days after inoculation, and occurrence of wilt 7 days after inoculation were monitored. The results were statistically analyzed by χ^2 analysis.

Isolation and pathogenicity of S. lignicola *from healthy groves*

Pieces of 'Star Ruby' twigs, which fell to the ground during routine management, were collected in four orchards in the Coastal Plain, where Scytalidium wilt was not found. The

presence of *S. lignicola* was assayed in the wood by placing segments of the dead twigs on PDA and incubating them at 36 °C. Fast-growing cultures were examined, and when *S. lignicola* was isolated, its pathogenicity was confirmed by inoculating 'Star Ruby' saplings in a 'Very Hot' regime.

Statistics

Differences between the treatments were determined by ANOVA and χ^2 test at $P \leq 0.05$ with JMPIN 5 software (SAS, Cary, NC).

Results

Effect of temperature on disease development on 'Star Ruby'

In all inoculation experiments wounded control plants, to which sterile agar discs were applied, did not develop any canker at any of the incubation regimes. This result is not reported in the following tables. Canker development and subsequent wilting of infected 'Star Ruby' saplings that were maintained under various temperature regimes before and after inoculation with *S. lignicola* are presented in Tables 1 and 2. Occurrence of cankers and their subsequent development were strongly dependent on the temperature regime before and after inoculation (Table 1). Among the pre-inoculation regimes, 'Very Hot' was the most conducive to infection, with no statistically significant dependence on the post-inoculation temperature, whereas the 'Hot' regime sustained canker development only when followed by a 'Very Hot' regime after the inoculation (Table 1). The 'Moderate' pre-inoculation temperature did not trigger canker development, except a statistically non-significant level of disease under the 'Very Hot' post-inoculation regime (Table 1). When wilting of the infected saplings was monitored (Table 2), only plants that were exposed to the 'Very Hot' temperature regime, either before or after inoculation, wilted. Wilt developed fastest in saplings exposed to the 'Very Hot' temperature regime both pre- and post-inoculation (Table 2). Uninoculated saplings of 'Star Ruby' exposed to the 'Very Hot' regime developed heat-stress symptoms similar to those observed on 'Star Ruby' in the Jordan Valley. These included foliar chlorosis, sometimes with laminar bleaching, necrotic lesions on young leaves, and fissures, occasionally accompanied by gum oozing in the shape of coiled protrusions. The root system of wilted plants exposed to the 'Very Hot' regime was washed and compared to that of non-inoculated ones that had been exposed to the 'Hot' regime. No differences were found, both had normal white tips, and there was no visual indication of any damage to the root system in saplings from the 'Very Hot' regime.

Table 1. Effect of three temperature regimes pre- and post-inoculation on development of cankers on 'Star Ruby' grapefruit inoculated with *Scytalidium lignicola*

Temperature regime[a]		Canker length (mm)					
Pre-inoculation[b]	Post-inoculation	Days after inoculation					
		4		6		8	
Very Hot	Very Hot	18.7	a[c]	42.3	a	42.7	a
Very Hot	Hot	7.3	ab	27.3	ab	33.0	a
Very Hot	Moderate	3.7	b	13.7	bcd	23.3	ab
Hot	Very Hot	9.7	ab	22.3	bc	32.3	a
Hot	Hot	0.0	b	0.3	d	0.7	c
Hot	Moderate	0.0	b	0.0	d	1.0	c
Moderate	Very Hot	5.3	ab	9.0	bcd	10.7	bc
Moderate	Hot	0.0	b	0.0	d	0.0	c
Moderate	Moderate	0.0	b	0.0	d	0.0	c

[a]'Very Hot' – 47 °C/34 °C, day/night; 'Hot' – 36 °C/28 °C, day/night; and 'Moderate' – 30 °C/20 °C, day/night.
[b]Seven to eight days.
[c]Numbers in each column followed by different letters differ significantly ($P \leq 0.05$) as determined by LSD test.

Table 2. Effect of three temperature regimes pre- and post-inoculation on development of wilt on 'Star Ruby' grapefruit inoculated with *Scytalidium lignicola*

Temperature regime[a]		Percent wilt					
Pre- inoculation[b]	Post- inoculation	Days after inoculation					
		8		18		28	
Very Hot	Very Hot	60.0	a[c]	86.7	a	86.7	a
Very Hot	Hot	8.3	b	65.0	ab	78.3	a
Very Hot	Moderate	0.0	b	29.0	bc	46.7	ab
Hot	Very Hot	0.0	b	43.3	abc	66.7	a
Hot	Hot	0.0	b	0.0	c	0.0	b
Hot	Moderate	0.0	b	0.0	c	0.0	b
Moderate	Very Hot	6.7	b	37.7	bc	44.3	ab
Moderate	Hot	0.0	b	0.0	c	0.0	b
Moderate	Moderate	0.0	b	0.0	c	0.0	b

[a]'Very Hot'–47 °C/34 °C, day/night; 'Hot'–36 °C/28 °C, day/night; and 'Moderate'–30 °C/20 °C, day/night.
[b]Seven to eight days.
[c]Numbers in each column followed by different letters differ significantly ($P \leq 0.05$) as determined by LSD test.

Susceptibility of grapefruit cultivars to S. lignicola

Disease development on 'Marsh Seedless', 'Rio Red' and 'Flame' grapefruit, under two constant temperature regimes, was compared with that of 'Star Ruby' (Table 3). Under the 'Very Hot' regime, both canker development and subsequent foliar wilt appeared on 'Flame', similar to symptoms on 'Star Ruby', but did not appear on 'Marsh Seedless' or 'Rio Red' (Table 3). Under the 'Hot' regime, no disease occurred on any grapefruit cultivar (Table 3).

Discussion

It is commonly accepted that plant diseases are greatly influenced by environmental conditions. Here we study the specific effect of elevated temperatures. Colhoun (1979) reviewed the predisposing effect of high temperatures on some diseases and concluded that plants can be rendered more susceptible to infection by relatively high temperatures before inoculation. Lockwood (1988) reviewed several predisposing factors, including high temperatures, and established that most of them increase disease severity in plants that are genetically somewhat resistant. He concluded that although the effects on disease are well-documented, the underlying mechanisms are not well understood. Chamberlain (1972) and Chamberlain and Gerdemann (1966) reported heat-induced susceptibility of soybean to several pathogens when hypocotyls were predisposed to 47 °C, due to suppression of phytoalexin production.

The results of the present study demonstrated that only saplings of a susceptible cultivar exposed to a 'Very Hot' regime before inoculation or during incubation are invaded by *S. lignicola*. This finding supports the hypothesis, based on field observations, that Scytalidium wilt develops on 'Star Ruby' grapefruit only as a consequence of a predisposition induced by heat stress. The extremely hot conditions that predispose the trees to infection are not common in citrus-growing areas in Israel, and therefore Scytalidium wilt does not develop, although the causal organism is present in the microflora. In Israel, events of extreme heat are accompanied by very low humidity, which exposes the trees to extreme transpiration. The foliage of 'Star Ruby' in orchards under heat stress usually turns yellow with touches of bronze hue, and the laminae curl (Oren et al., 2001). We found a similar appearance in saplings after a week's exposure to the 'Very Hot' regime. The damage to the saplings was reversible: uninfected plants regained their normal foliage three months after relief from the heat stress, as also occurred in the Jordan Valley (Oren et al., 2001).

Table 3. Development of cankers and wilt on four grapefruit cultivars maintained at two temperature regimes 8 days before and 7 days after inoculation with *Scytalidium lignicola*

Cultivar	Temperature regime[a]	Canker length (mm)				Rate (%) of wilted plants 7 days after inoculation	
		Days after inoculation					
		3		5			
Star Ruby	Very Hot	29.4	a[b]	78.2	a[b]	100.0	a[c]
Flame	Very Hot	22.2	a	64.2	a	100.0	a
Rio Red	Very Hot	0.0	b	5.2	b	0.0	b
Marsh	Very Hot	0.0	b	0.0	b	0.0	b
Star Ruby	Hot	0.0	b	0.0	b	0.0	b
Flame	Hot	0.6	b	0.6	b	0.0	b
Rio Red	Hot	0.0	b	0.0	b	0.0	b
Marsh	Hot	0.0	b	0.0	b	0.0	b

[a]'Very Hot'–47 °C/34 °C, day/night; 'Hot'–36 °C/28 °C, day/night.
[b]Numbers in column followed by a different letter differ significantly ($P \leq 0.05$) as determined by LSD test.
[c]Numbers in column followed by a different letter differ significantly ($P \leq 0.05$) as determined by nominal logistic fit (χ^2 test).

Scytalidium lignicola was readily isolated as a saprophyte from dead 'Star Ruby' twigs collected from the ground in orchards in the main citrus-growing area of Israel, where Scytalidium wilt had been sought but not found. Isolates of *S. lignicola* from those twigs were pathogenic to 'Star Ruby' after it had been exposed to extreme heat. This finding supports the view that *S. lignicola*, a common organism in the natural microflora where it is devoid of pathogenic characteristics, is an opportunistic pathogen. Two rare reports of its pathogenicity to other stressed crops have been reviewed (Oren et al., 2001). In his discussion of predisposition, Schoeneweiss (1975) suggested that stress exerts the most pronounced effect in predisposing plants toward greater susceptibility to facultative parasites, particularly weak or non-aggressive ones. He concluded that in most cases, plants tolerate or adapt to stress without permanent injury in the absence of disease-causing organisms.

Two grapefruit cultivars, 'Marsh Seedless' and 'Rio Red', which are grown in the affected region, were found to be resistant to both heat stress and Scytalidium wilt. Those cultivars did not develop any stress or disease symptoms when incubated and inoculated with *S. lignicola* in 'Very Hot' chambers. Another grapefruit cultivar, 'Flame', not grown in the affected region, was susceptible to *S. lignicola*, when inoculated after being predisposed to the 'Very Hot' regime.

Acknowledgements

We thank Miriam Kimchi for her technical assistance. This study was supported by funds from the Chief Scientist, Ministry of Agriculture and Rural Development, and from the Israeli Citrus Board. The study was conducted under the auspices of the Francis Ariowitsch Chair in Agriculture, endowed to the last author.

References

Chamberlain DW (1972) Heat-induced susceptibility to non-pathogens and cross-protection against *Phytophthora megasperma* var. *sojae* in soybean. Phytopathology 62: 645–646.

Chamberlain DW and Gerdemann JW (1966) Heat-induced susceptibility of soybeans to *Phytophthora megasperma* var. *sojae, Phytophthora cactorum*, and *Helminthosporium sativum*. Phytopathology 56: 70–73.

Colhoun J (1979) Predisposition by the environment. In: Horsfall JG and Cowling EB (eds.) Plant disease an advanced treatise (Vol. IV) (pp. 75–96) Academic Press, New York, NY.

Elshafie AE and Ba-Omar T (2001) First report of *Albizia* lebbec dieback caused by *Scytalidium dimidiatum* in Oman. Mycopathologia 154: 37–40.

Lockwood JL (1988) Evolution of concepts associated with soilborne plant pathogens. Annual Review of Phytopathology 26: 93–121.

Oren A, Sadowsky A, Gefen D, Solel Z and Kimchi M (2001) Scytalidium wilt of citrus. European Journal of Plant Pathology 107: 467–470.

Schoeneweiss DF (1975) Predisposition, stress, and plant disease. Annual Review of Phytopathology 13: 193–211.