

## Enhancement of *Sphaeropsis* Canker of Aleppo Pine by the Israeli Pine Bast Scale

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An interaction between the fungal pathogen *Sphaeropsis sapinea* (Fr.) Dyko & Sutton, causal organism of *Sphaeropsis* canker, and the Israeli pine bast scale *Matsucoccus josephi* (Homoptera: Margarodidae), causal agent of pine decline, was studied, since both were prevalent on Aleppo pine (*Pinus halepensis*) stands. *Sphaeropsis sapinea* was isolated from larvae (stage II) and molts of the Israeli pine bast scale collected in four Aleppo pine stands. When Aleppo pine saplings were infested with pine bast scale and subsequently inoculated with *S. sapinea* most plants died, whereas mortality of seedlings inoculated with *S. sapinea* alone without previous scale infestation was negligible. Scale infestation of saplings without subsequent fungal inoculation caused the death of one third of the plants. Wounding of pine shoot apices by removal of needle fascicles enhanced infection by *S. sapinea*. The results support the assumption that *M. josephi* can play an important role in transmitting the fungal inoculum to the host tree as well as enabling its penetration to the shoots through wounds in the bark.

**KEY WORDS:** *Sphaeropsis sapinea*; *Matsucoccus josephi*; *Pinus halepensis*; *P. pinea*; *P. canariensis*; infection; wounding.

### INTRODUCTION

Aleppo pine (*Pinus halepensis* Mill.) was the major species used for afforestation in Israel for many years, due to its adaptation to the edaphic and climatic conditions (17,19). During the last decade of the 20<sup>th</sup> Century *Sphaeropsis* canker disease, caused by the fungal pathogen *Sphaeropsis sapinea* (Fr.) Dyko & Sutton, became prevalent on Aleppo pine in various stands in northern counties of Israel (11). In that region the Israeli pine bast scale, *Matsucoccus josephi* (Homoptera: Margarodidae), is common on that host, and it is considered a major factor in tree decline (4,9,13). This association between the disease and the pest prompted the hypothesis of interaction between the organisms. Various interactions between insects and fungal pathogens have been reported (7,8,12,14). The insect can be a vector of the fungal spores or mycelium, or mediate in fungal host penetration.

The objective of this study was to examine the hypothesis that the Israeli pine bast scale can play an important role in the epidemiology of *Sphaeropsis* canker.

### MATERIALS AND METHODS

**Plants** The experiments were conducted with one- or 2-year-old Aleppo pine saplings, 70-80 cm tall, grown in pots. In one experiment, 2-year-old saplings of Italian stone pine (*Pinus pinea* L.) and Canary Islands pine (*Pinus canariensis* Chr. Sweet ex Spreng) were included.

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**Isolation of *S. sapinea* from pine bast scales** The study was conducted in four stands of Aleppo pine in northern Israel, where both Sphaeropsis canker and infestation with the Israeli pine bast scale were observed. Shoots of pine trees (25–35 years old) showing death of apices accompanied by needles reddening, which are characteristic symptoms of infestation by the Israeli pine bast scales, were collected at random. Twenty apices from different trees in each location were examined with a stereo microscope and molts (approx. 30) or stage II larvae (approx. 20) of *M. josephi* from each site were removed with forceps and placed on sterile potato dextrose agar (PDA) in petri plates. After incubation for 7 days, any culture that developed from an insect part, showing fluffy gray-green mycelium, a typical characteristic of *S. sapinea*, was examined for pathogenicity on Aleppo pine, to confirm its identification.

**Pathogenicity test** A small piece of bark was removed with a scalpel from the bark of the stem of one-year-old Aleppo pine saplings, and a disk of mycelium (3 mm diam) from the margin of the examined culture was placed over the wound. The plants were incubated for 4 to 7 days in a mist chamber at 24°C and then maintained on a greenhouse bench at that temperature for 3 weeks. For each of ten isolates, four saplings were inoculated. An isolate was considered pathogenic when a canker developed beyond the wounded area. Control sterile agar disks were included in the tests and never caused any canker.

**Methods of inoculating pine saplings with *S. sapinea* conidia** Inoculum was prepared by crushing pycnidia of *S. sapinea* that developed on infected branches, maintained in a humid chamber under constant light for 2 weeks. The conidial suspension was filtered through cheesecloth and adjusted to  $10^5$  conidia ml<sup>-1</sup>. The conidial suspension was sprayed with an atomizer or applied by dipping filter paper disks (3 mm diam) in the suspension and placing them on the bark of inoculated shoots. The plants were incubated for 4 days in a mist chamber at 24°C, transferred to a greenhouse bench and maintained at that temperature for 12 days.

**Combined effect of *M. josephi* and *S. sapinea* on Aleppo pine mortality** Fourteen 2-year-old saplings of Aleppo pine were infested with ovisacs (egg masses) of *M. josephi* in a screen house. One ovisac was attached over the stem at the lower part (15 cm above the soil) and the other at the upper part, close to intensive branching. Some infested saplings were subsequently inoculated with *S. sapinea* 35 days after the infestation with the scales by spraying the whole foliage with a conidial suspension. Thus, the experiment contained eight saplings treated by both *M. josephi* and *S. sapinea*, six saplings inoculated by *S. sapinea*, six saplings infested with *M. josephi*, and six untreated control plants.

**Effect of epidermal wounds on infection by *S. sapinea*** Shoot apices of 2-year-old saplings of Aleppo, Italian stone and Canary Islands pines were either wounded – by removing four needle fascicles, or left intact. For each treatment, ten shoots were inoculated either by spraying them with a conidial suspension, or by placing filter paper disks on the bark. The plants were incubated for 4 days in a mist chamber at 24°C and transferred to a greenhouse bench, maintained at that temperature.

**Statistical analyses** Data were analyzed by Fisher's Exact Probability Test.

## RESULTS

**Isolation of *S. sapinea* from pine bast scales** The pathogen was isolated from scales at all the tested locations (Table 1). The rate of insect contamination was moderate, 5.0%

to 8.7% in molts and 0 to 11.1% in larvae. All the isolates of *S. sapinea* were tested for pathogenicity and were proven pathogenic, causing typical stem canker followed by wilt.

**Effect of bast scale infestation on vulnerability to infection by *S. sapinea*** Five weeks after infesting 2-year-old saplings of Aleppo pine with eggs of *M. josephi*, plenty of larvae at stage I were observed. When mortality of saplings inoculated by *S. sapinea* was examined 32 days later (Table 2), most plants that were exposed to both organisms (87.5%) had died, whereas mortality occurred in only one third of those plants infested by *M. josephi* only. Mortality of seedlings inoculated by *S. sapinea* without scale infestation was negligible, and no mortality occurred on untreated control plants (Table 2).

TABLE 1. Isolation of *Sphaeropsis sapinea* from molts and larvae (stage II) of *Matsucoccus josephi*

Forest location	Infected molts (%) <sup>z</sup>	Infected larvae (%) <sup>y</sup>
Binyamina	6.0	11.1
Nazerat	8.7	5.0
Zikhron Ya'aqov	5.0	0.0
Zefat	7.1	-

<sup>z</sup>Number of examined molts = 23–33.

<sup>y</sup>Number of examined larvae = 15–20.

TABLE 2. Mortality of Aleppo pine saplings 32 days after infestation with the Israeli pine bast scale, inoculation with *Sphaeropsis sapinea*, or combined treatments

Treatment	Sapling mortality (%)
Fungal inoculation 5 weeks after insect infestation	87.5 a <sup>z</sup>
Insect infestation	33.3 b
Fungal inoculation	16.7 b
Control	0.0 b

<sup>z</sup>Values followed by the same letter do not differ significantly according to Fisher's Exact Probability Test at the 5% level. Number of saplings in each treatment = 6–8.

TABLE 3. Dieback rate of shoot apices of pine saplings 12 days after inoculation by *Sphaeropsis sapinea*, with or without prior removal of four needle fascicles

Pine species	Inoculation method	Needle removal	Apices dieback (%)
Aleppo pine	Spray	Yes	0.0 b <sup>z</sup>
		No	0.0 b
	Filter paper disk	Yes	33.3 a
		No	0.0 b
Italian stone pine	Spray	Yes	90.0 a
		No	0.0 b
	Filter paper disk	Yes	100 a
		No	22.2 b
Canary Islands pine	Spray	Yes	22.2 b
		No	0.0 b
	Filter paper disk	Yes	50.0 a
		No	0.0 b

<sup>z</sup>For each pine species, values followed by the same letter do not differ significantly according to Fisher's Exact Probability Test at the 5% level. Number of saplings in each treatment = 9–10.

**Effect of epidermal wounds on infection by *S. sapinea*** The hypothesis that penetration of pine shoots by *S. sapinea* was enhanced by the bast scale was tested with artificial wounding, by removal of needle fascicles. The results (Table 3) show that with spray inoculation, simulating mild inoculum pressure, no infection developed on any pine species without open epidermal wounds. With disk inoculation simulating drastic inoculum pressure, some infection (22.2% apices dieback) occurred on unwounded Italian stone pine (*P. pinea*), the most susceptible pine species. Needle removal markedly enhanced infection (Table 3). The most prominent effect was observed in the highly susceptible *P. pinea* (90–100% apices dieback). In Aleppo pine, the least susceptible pine species, only one third of the shoots became infected when wounded and inoculated drastically. In Canary Islands pine (*P. canariensis*), wounding enabled some infection with spray inoculation (22.2%) and moderate infection (50%) with disk inoculation (Table 3).

## DISCUSSION

The results of this work support the assumption that *M. josephi* may play an important role in transmitting the fungal inoculum of *S. sapinea* to the host tree as well as enabling its penetration of the shoots through wounds in the bark. Infestation with the bast scale is expected to cause wounds. This might account for the experimental results showing that inoculation of pine seedlings infested with the bast scale resulted in a markedly higher infection rate than that occurring on uninfested ones. Furthermore, in the absence of wounding *S. sapinea* barely caused disease on *P. halepensis*. The requirement of wounding – including hail damage – for development of Sphaeropsis canker is well documented (15,18,20). However, infection of some pines without wounding has been reported (6).

An association between insects and Sphaeropsis canker has been suggested. Recently, adults of the cone bug *Gastrodes grossipes* (De Geer) (Heteroptera: Lygaeidae) were shown in Italy to carry conidia of *S. sapinea* in stands of infected *Pinus nigra* (7), and the authors conjectured that the bug has a potential role in fungus dissemination. In Wisconsin (USA), an association of *S. sapinea* infection of cones and shoots of red pine (*P. resinosa*) and their being damaged by insects, especially *Dioryctria* sp., was demonstrated (8). Birch (2) in 1936 noted a correlation between dieback of *P. radiata* and infestation by pine chermes (*Pineus pini*). A species of the fungus *Cenangium* was found in Arizona in close correlation with the lesions of *Matsucoccus vexillorum*, and was suspected of being responsible for the secondary extension of the initial damage (12). It is important not to underrate the most common dissemination of spores by wind-driven raindrops splashing from affected branches and cones, where pycnidia of *S. sapinea* develop (2,15,16).

Our results suggest that both *P. pinea* and *P. canariensis* are more susceptible to *S. sapinea* than is *P. halepensis*. These pine species are common in forests in Israel, and are also found in landscape gardens. Yet, we have no records of Sphaeropsis canker on them, nor infestation with the well established Israeli pine bast. It appears that their resistance to the bast scale reduces the chance to become infected by *S. sapinea*.

Unfavorable growing conditions, especially water stress, augment the severity of Sphaeropsis canker (1,2,3,5,7,15,20). The Israeli pine bast scale causes anatomical changes in the xylem, leading to obstruction of water transport in the wood (10). Therefore it is likely that weakening the physiological activity of the pine may limit its ability to endure invasion by *S. sapinea*.

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