

Branch Dieback of Brown-Berried Juniper in Israel Caused by *Phomopsis occulta*

Z. Madar and Miriam Kimchi

Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel

[Fax: +972-3-9683543; e-mail: vpzvisol@volcani.agri.gov.il]

A new disease causing dieback of shoots and branches of brown-berried juniper (*Juniperus oxycedrus* L.) was observed in the Meron nature reserve, northern Israel, in the summer of 1996. This evergreen tree is native to the Mediterranean region and is found in Israel only over limited areas on Mt. Meron and the Mt. Hermon range (8). Many of the affected shoots and branches turned reddish brown and died. Stem cankers were sometimes accompanied by resinous exudates.

Isolations were made from the apex of affected shoots and from discolored branches by removing the outer tissue with a sterile scalpel, cutting small sections from the underlying affected tissue, and placing them on PDA. Uniform white mycelium developed after 7 days and dark pycnidia, with long hyaline cirri, were formed a few weeks later. The cirri consisted of a mass of type alpha and beta spores, typical of the genus *Phomopsis*. Alpha spores were one-celled, hyaline, fusiform, 2-guttulate with one guttule at each end. Spore dimensions (n=100) were: 3.5–8.8 (5.8) × 2.0–2.4 (2.2) μm. Beta spores were hyaline, one-celled, slightly curved, 8.8–19.3 (13.7) × 0.9–1.7 (1.0) μm. The morphology of both alpha and beta spores is similar to the description and drawing of *Phomopsis occulta* (Sacc.) Traverso given by Hahn (3). The width of alpha spores of our isolates is similar to that reported by Hahn, but the length of spores was smaller: 5.8 μm, compared with 7.2 μm. Considering the known size variability of *Phomopsis* spores (2), and the absence of yellow coloration in the medium, characteristic of *P. juniperovora* (4), we consider our pathogen to be *P. occulta*.

Two-year-old seedlings grown in plastic containers (0.5 l) were used for inoculation experiments. Inoculations with mycelia were made by removing the outer layers of the bark (5 × 3 mm) with a knife and placing a 3-mm disc of a culture of *P. occulta* over the exposed tissue. The inoculated area was covered with wet cotton and wrapped with plastic ribbon, both of which were removed after 5 days. Spores for inoculations were obtained by incubating cultures under continuous fluorescent light for 18 days. For inoculations with spores, plants were scraped slightly with a scalpel, sprayed with a spore suspension (10⁹ spores/ml water), and incubated for 3 days in a humidity chamber under controlled conditions (24±2°C). Some inoculations were made with cut branches in water, which were inoculated as above, covered with moistened plastic bags for 3 days, and maintained at room temperature (23±2°C).

With mycelium inoculum, no cankers developed in seedlings when the inoculation was not preceded by bark wounding (Table 1). Likewise, Igoe *et al.* (5) did not obtain infection symptoms with spruce inoculation without wounding. Inoculation with mycelia incited canker formation on several species of Cupressaceae (*Cupressus sempervirens*, *Juniperus horizontalis* 'Wiltonii' and *J. oxycedrus*), but not on *J. horizontalis* 'Andora Compacta', *J. chinensis* 'Obelisk', *J. virginiana* 'Grey Owl' or *J. virginiana* 'Silver Spread' (Table 1). *C. sempervirens*, which was highly susceptible to the pathogen, is a common tree in Israel, but was not found naturally infected by *P. occulta*. In Italy this fungus attacks primarily *C. sempervirens* in nurseries and adult trees in forest, parks or gardens (1). In inoculations with spores, only alpha spores germinate and cause infection. All plants which were wounded before inoculations with spores were affected, whereas nonwounded plants remained healthy, except *J. chinensis* Obelisk (Table 1). Our findings differ from those of Igoe *et al.* (5), who reported that wounding was not required for spruce infection by spores. Unwounded cut branches that developed disease symptoms following inoculation with spores could have been under physiological

¹Contribution from the Agricultural Research Organization. No. 2269-E, 1997 series. Received Sept. 7, 1997; received in final form Dec. 12, 1997; web site posting March 6, 1998.

TABLE 1. Reaction of various conifer plants to infection by *Phomopsis occulta*

Host plant	Inoculation with mycelium		Inoculation with spores	
	Wounded	Unwounded	Wounded	Unwounded
Cupressaceae				
<i>Cupressus sempervirens</i>	+	—	+	+ ^z
<i>Juniperus</i>				
<i>J. horizontalis</i> Wiltonii	+	—	+	—
<i>J. horizontalis</i> Andora Compacta	—	—	+	—
<i>J. chinensis</i> Obelisk	—	—	+	+
<i>J. phoenicea</i>				+ ^z
<i>J. oxycedrus</i>	+	—		+ ^z
<i>J. virginiana</i> Grey Owl	—	—	+	—
<i>J. virginiana</i> Silver Spread	—	—	+	—
Pinaceae				
<i>Pinus halepensis</i>	—	—	—	—

+, infection; —, no infection.

^zCut branches (see text).

stress. In reisolations from affected plants, *P. occulta* was recovered. *Pinus halepensis* was not affected by either mycelia or spore inoculum of *P. occulta* (Table 1). Some authors considered *P. occulta* to be a saprophyte (3,4), whereas recent workers reported it to be pathogenic (5,7). Our results indicate clearly that *P. occulta* is a pathogenic fungus. The disease was found only on *J. oxycedrus* in the Meron nature reserve, apparently due to the special climatic conditions there (high humidity, low temperatures) which are conducive to disease development (6).

REFERENCES

1. Ghillini, C.A. (1939) Atachi di *Phomopsis occulta* su conifere. *Riv. Forest. It.* 1(6):16-22.
2. Hahn, G.G. (1920) *Phomopsis juniperovora*, a new species causing blight of nursery cedars. *Phytopathology* 10:250-253.
3. Hahn, G.G. (1930) Life-history studies of the species of *Phomopsis* occurring on conifers. Part 1. *Trans. Br. Mycol. Soc.* 15:32-93.
4. Hahn, G.G. (1943) Taxonomy, distribution, and pathology of *Phomopsis occulta* and *Phomopsis juniperovora*. *Mycologia* 35:112-129.
5. Igoe, M.J., Peterson, N.C. and Roberts, D.L. (1995) A *Phomopsis* canker associated with branch dieback of Colorado blue spruce in Michigan. *Plant Dis.* 79:202-205.
6. Panconesi, A. (1990) Pathological disorders in the Mediterranean basin. in: Ponchet, J. [Ed.] Agrimed Research Programme Progress in EEC Research on Cypress Diseases. pp. 54-81. Commission of the European Communities, Luxembourg, Belgium.
7. Sanderson, P.G. and Worf, G.L. (1986) *Phomopsis* and *Sirococcus* shoot blights of Colorado blue spruce in Wisconsin. *Plant Dis.* 70:1159.
8. Waisel, Y. and Alon, A. (1980) [Trees of the Land of Israel.] Yad HaHamisha, Kefar Chabad, Israel (in Hebrew).