First Report of Stem Canker Caused by *Botryosphaeria obtusa* on Pistachio

Wijnand J. Swart, Senior Lecturer, and **Wilma-Marie Botes,** Graduate Student, Department of Plant Pathology, University of the Orange Free State, Bloemfontein 9300, South Africa

ABSTRACT

Swart, W. J., and Botes, W.-M. 1995. First report of stem canker caused by *Botryosphaeria obtusa* on pistachio. Plant Dis. 79:1036-1038.

Dieback of 3-year-old *Pistacia vera* L. trees, grafted onto *P. atlantica* rootstocks, was observed in trial plantings in the Prieska district of South Africa during the spring of 1992. Close examination of diseased trees revealed the presence of cankers on the lower part of the stem in the vicinity of the graft. Isolations from the margins of diseased tissue onto malt-extract agar consistently yielded black fungal colonies and pycnidia bearing mature conidia identified as belonging to the *Sphaeropsis* anamorph of *Botryosphaeria obtusa*. The fungus was shown to be highly virulent to three *Pistacia* species in pathogenicity tests in the field. Branches, measuring 5 mm in diameter, of *P. atlantica, P. vera* and *P. integerrima* developed mean cambial lesions measuring 53, 51, and 15 mm, respectively, 14 days after artificial inoculation with fungal mycelium. Wounding of branches prior to inoculation resulted in significantly larger cambial lesions. As far as we have been able to determine, there are no published reports of *B. obtusa* being associated with disease symptoms on cultivated pistachio trees.

The commercial cultivation of pistachio nuts (*Pistacia vera* L.) is a relatively recent development in South Africa. In the spring of 1992, dieback of 3-year-old trees of *P. vera* grafted onto rootstocks of *P. atlantica* was observed in trial plantings in the Prieska district of the northern Cape province. Dying trees, obvious because their foliage appeared chlorotic and wilted, were scattered throughout the plantings. Examination of these diseased trees revealed the presence of cankers on the lower part of the stem in the vicinity of the graft. The general impression gained was that more than 90% of trees seemed very healthy and vigorous. However, on closer examination the prevalence of stem infections was up to 15%. Examination of many seemingly healthy plants by scraping away a small section of bark from the stem at soil level revealed small sections of dark brown cambial tissue indicative of the early stages of infection. This study was undertaken to characterize the pathogen and examine the relative susceptibilities of three potentially important *Pistacia* spp.

MATERIALS AND METHODS

Isolation and identification. Small pieces of bark were removed from a canker on a diseased plant (Fig. 1A) and incubated in a humidity chamber at 25°C for 2 days. Conidia (Fig. 1B) that oozed from pycnidia on the surface of the bark were removed with a sterile needle and transferred to 2% malt-extract agar (MEA) (20 g of Difco malt extract, 20 g of Difco Bacto agar and 1 liter of distilled water). Plates were then incubated at 25°C under 12-h alternating cycles of dark and near-UV light to induce sporulation.

Diseased stems were rinsed in tap water and then surface sterilized with 0.5% (vol/vol) NaOCl. The diseased bark was cut away with a sterile scalpel and small sections from the margins of discolored tissue were plated on MEA and incubated at 25°C for 3 days. Fungi that grew into the agar were subsequently transferred to MEA and incubated at 25°C under 12-h alternating cycles of dark and near-UV light. The main morphological characteristics of the suspected pathogen were based on studies with diseased stems from the field and on pure cultures.

Pathogenicity studies. Inoculum of the suspected pathogen was prepared by culturing the fungus in petri dishes on MEA overlaid with sterile cotton-gauze strips (10 × 50 mm) until the gauze strips were completely colonized. In one experiment, 20 shoots measuring approximately 5 mm in diameter were chosen on young *P. integerrima, P. vera,* and *P. atlantica* trees. Ten shoots of each species were wounded by lightly scraping off a length of bark (2 × 5 mm) with a scalpel. and then inoculated by wrapping gauze colonized by the fungus around each wound. Ten unwounded shoots of each species were similarly treated with gauze strips colonized by the fungus. Another 10 shoots, of only *P. atlantica*, were treated with sterile gauze strips following wounding to serve as a control treatment. Parafilm was then wrapped around all inoculation sites to prevent desiccation of the inoculum. After 2 weeks the gauze strips and surrounding bark were removed from all treated shoots and the cambium examined for discoloration. The length of each cambial lesion was determined and Tukey's HSD procedure for comparison of means (10) was applied, in which a factorial analysis of variance showed significant variation between treatments.

In a second experiment, 20 branches of 5-year-old *P. atlantica* with a mean diameter of 13 mm were inoculated as described above after wounding 10 of the branches. Isolations were made from discolored tissue to verify the presence of the inoculated fungus. Cambial lesions were measured after 4 weeks.

RESULTS

Isolation and identification. Cankers were characterized by the presence of black, uniloculate pycnidia on the bark surface and black discoloration of tissue primarily below the scion bark of *P. vera.* Cultures derived from conidia that had formed in pycnidia on diseased bark and isolations from diseased woody tissue consistently yielded black, fast-growing fungal colonies after 3 days, with abundant formation of aerial mycelium. All morphological characteristics of the isolated fungus were identical to those of the *Sphaeropsis* anamorph of *Botryosphaeria obtusa* (Schwein.) Shoemaker (8,9). However, the perfect stage was not found on either diseased material or in culture.

Pathogenicity studies. The fungus colonized the cambial tissue of all three *Pistacia* species following artificial inoculations. In all cases in which infection had been successful, discoloration of the bark surrounding the wound and pycnidial formation on the bark surface were clearly visible. In numerous cases, girdling of the stem had resulted in dieback of the shoot above the point of inoculation. Removal of the bark revealed that the cambial tissue was severely discolored (Fig. 2A) and that discoloration had spread to the xylem in most cases (Fig. 2B). No lesions resulted from the control treatment on *P. atlantica.* There was a significant difference ($P < 0.05$) between the susceptibility of *P. integerrima* and that of the other two species (Fig. 3). Although infection had occurred through nonwounded bark in approximately 60% of cases, wounding greatly facilitated infection and resulted in cambial lesions significantly larger than those in nonwounded treatments. On the larger-diameter (13 mm) branches of *P. atlantica* that had been wounded and inoculated, the mean length of cambial lesions was 34 mm

Corresponding author: Wijnand J. Swart
E-mail: WJS@landbou.uovs.ac.za

Accepted for publication 2 May 1995.

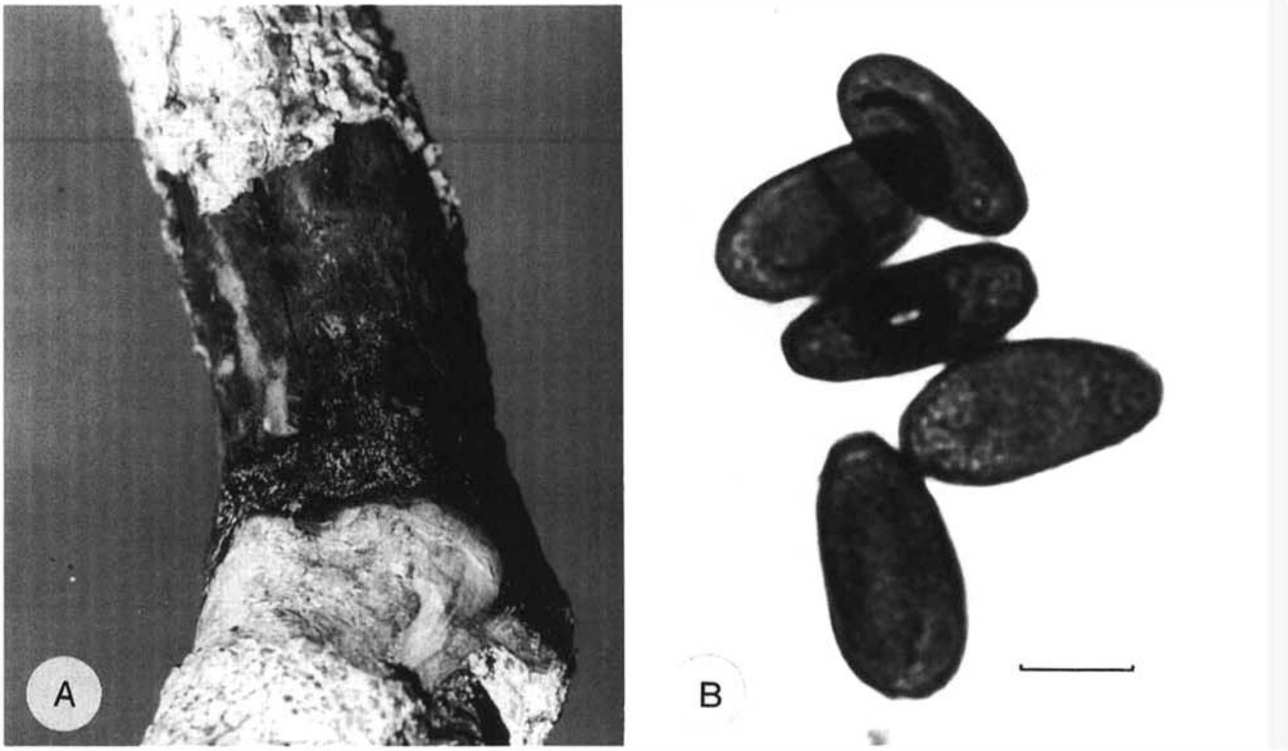

Fig 1. Canker on *Pistacia vera* scion with **(A)** black discoloration of tissue below diseased bark and **(B)** conidia extruded from uniloculate pycnidia of *Sphaeropsis* anamorph of *Botryosphaeria obtusa* on the bark surface. Bar = 10 µm.

(range 28 to 77 mm). Nonwounded branches in this trial displayed no lesion development. The pathogen was successfully reisolated from all artificially inoculated tissue.

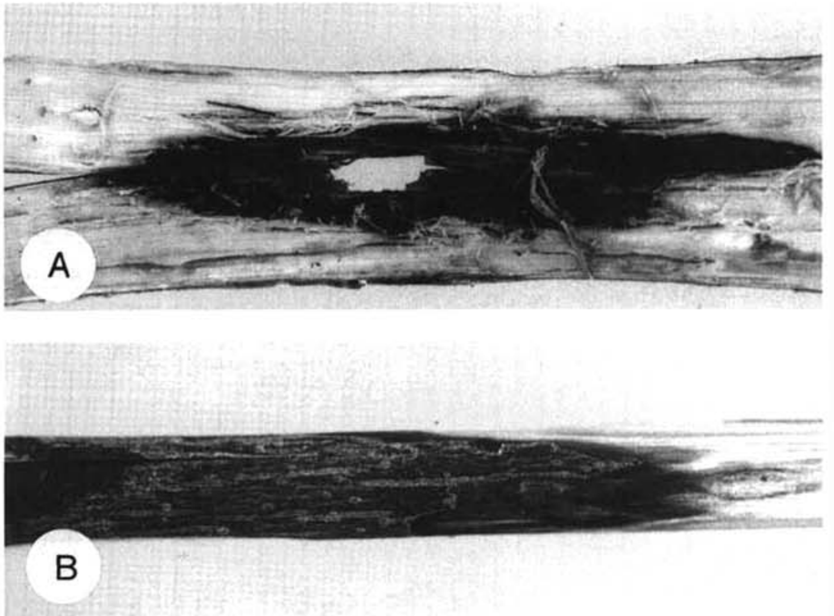

Fig. 2. Result of artificial inoculation of young *Pistacia vera* branch with *Botryosphaeria obtusa* showing **(A)** discolored cambial tissue and **(B)** discoloration of xylem.

DISCUSSION

A panicle and shoot blight disease of *P. vera* caused by the asexual stage (*Dothiorella* sp.) of *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. was observed for the first time in California during the summer of 1984 and the disease has since become a major concern to pistachio growers in Northern California (7). The latter pathogen also causes major diseases of apple (*Malus domestica* Borkh.) and peach (*Prunus persica*) in the southeastern United States, as does *B. obtusa* (1,4). The symptoms caused by these organisms are virtually indistinguishable and they often occur together in apple orchards on the same host, although *B. obtusa* is more common than *B. dothidea* (2,3). Although the *Sphaeropsis* sp. anamorph of *B. obtusa* has on some occasions been isolated from male "02-16" or "02-18" pistachio trees and from Granny Smith apples in California, it has never been found to be associated with cankers, blighting, or any other disease symptoms of *Pistacia* spp. in the U.S. (T. J. Michailides, *personal communication*). In only one published report was *Botryosphaeria obtusa* mentioned as occurring on twigs of *P. chinensis* (5). To our knowledge, *B. obtusa* has never been associated with disease symptoms on cultivated pistachio trees anywhere else in the world.

Botryosphaeria obtusa, like *B. dothidea,* is a facultative parasite and can colonize plant tissue predisposed by environmental stress or even killed by other biotic or abiotic agents. Black rot caused by *B. obtusa* has long been known to follow winter injury in apple orchards (4). In view of the steep temperature decline that occurs in the Prieska district during the months of May through July, cold damage is therefore a distinct probability that should be investigated. *Botryosphaeria dothidea* and *B. obtusa* also commonly infect plants through pruning wounds, but lenticels and growth cracks in bark are also natural avenues of entry into plants for these fungi (4,11). Our results confirm that although wounding facilitates infection of *Pistacia* spp. by *B. obtusa*, the fungus can invade through intact bark, thus emphasizing the effective parasitic status of the fungus. The reason why *B. dothidea* was not

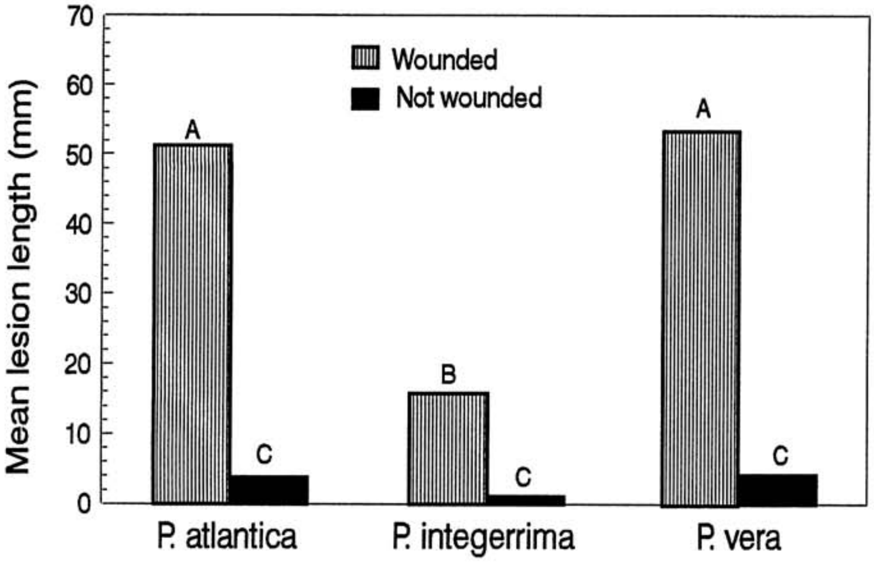

Fig. 3. Mean lengths of cambial lesions on three *Pistacia* spp. artificially inoculated with *Sphaeropsis* sp. anamorph of *Botryosphaeria obtusa*.

isolated from the diseased tissue in the present study, despite its presence in South Africa on various hosts (6), is probably related to the epidemiology of the two species. In apple orchards in the U.S., *B. dothidea* predominates in summer and fall, while *B. obtusa* predominates in winter and spring (3). Regular monitoring of pistachio orchards over all four seasons will therefore have to be conducted in order to confirm whether a similar situation exists in South Africa.

Very small differences in susceptibility to *B. obtusa* following artificial inoculation were observed between *P. atlantica* and *P. vera* in the present study. This appears to be inconsistent with field observations indicating that discoloration is restricted to *P. vera* at sites above the graft union, and is not associated with *P. atlantica*. This may be attributed to the age difference between the diseased field material and artificially inoculated material, whereby *P. atlantica* becomes more resistant to *B. obtusa* with advancing age than does *P. vera*. Another possibility is that the pathogen cannot compete with antagonistic soil organisms that are present in the soil surrounding the rootstock. If rootstocks of *P. atlantica* prove to be as susceptible to *B. obtusa* as the scion of *P. vera* under different circumstances, possibly *P. integerrima* could be used as an alternative, more resistant rootstock.

The occurrence of *B. obtusa* on pistachios in South Africa currently appears to be limited to a few scattered cases. However, its notoriety as a pathogen of apple and peach in the U.S. suggests that pistachio growers in South Africa should take its presence seriously. The current situation could be exacerbated should the presence of *B. dothidea* in pistachio orchards also become evident. Various precautionary measures will therefore be investigated to reduce the potential impact of these two important pathogens on the developing pistachio industry in South Africa.

LITERATURE CITED

1. Beisel, M., Hendrix, F. F., Jr., and Starkey, T. E. 1984. Natural inoculation of apple buds by *Botryosphaeria obtusa*. Phytopathology 74:335-338.
2. Britton, K. O., and Hendrix, F. F. 1982. Three species of *Botryosphaeria* cause peach tree gummosis in Georgia. Plant Dis. 66:1120-1121.
3. Britton, K. O., and Hendrix, F. F. 1986. Population dynamics of *Botryosphaeria* spp. in peach gummosis cankers. Plant Dis. 70:134-136.
4. Brown, E. A., II, and Britton, K. O. 1986. *Botryosphaeria* diseases of apple and peach in the southeastern United States. Plant Dis. 70:480-484.
5. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, Minn.
6. Gorter, G. J. M. A. 1977. Index of Plant Pathogens and the Diseases They Cause in Cultivated Plants in South Africa. Dept. Agric. Tech. Serv., Sci. Bull. 392. Pretoria.
7. Michailides, T. J., 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. Phytopathology 81:566-573.
8. Punithalingam, E., and Waller. J. M. 1969. *Botryosphaeria obtusa*. Descriptions of Pathogenic Fungi and Bacteria, No. 395. Commonw. Mycol. Inst. Assoc. Appl. Bot., Kew, Surrey, England.
9. Shoemaker, R. A. 1964. Conidial states of some *Botryosphaeria* species on *Vitis* and *Quercus*. Can. J. Bot. 42:1297-1301.
10. Steele, R. G., and Torrie, J. H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill, New York.
11. Weaver, D. J. 1979. Role of conidia of *Botryosphaeria dothidea* in the natural spread of peach tree gummosis. Phytopathology 69:330-334.