MORPHOLOGY AND TAXONOMY OF SPECIES OF
PHOMOPSIS ON ASPARAGUS

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ABSTRACT

Phomopsis javanica, a new species on Asparagus from Java, is described and illustrated. It differs from P. asparagi by producing paraphyses among the conidiophores and conidigenous cells, and is the first Phomopsis described with these structures. Phomopsis javanica was more virulent than P. asparagi when inoculated on asparagus foliage and stems. Phomopsis asparagicola is considered a synonym of P. asparagi. P. asparagi and P. javanica are compared in a series of photographs.

Key Words: Phomopsis javanica sp. nov., Phomopsis asparagi, Phomopsis asparagicola, paraphyses

A serious disease of asparagus (Asparagus officinalis L.) plants in Java, Indonesia was found to be caused by a Phomopsis. Comparison of this fungus with the two other Phomopsis species previously reported on asparagus revealed it to be an undescribed species. Saccardo (1878) described Phoma asparagi Sacc. on putrescent stems of A. officinalis at Padua, Italy. Although Bubak (1906) transferred this fungus to Phomopsis, it is still being reported as Phoma asparagi in current literature (Liu and Hwang, 1988; Tanaka et al., 1987).

Phomopsis asparagi (Sacc.) Bubak is well known in Europe (da Camara and da Luz, 1943; Engler and Prantl, 1900; Hruby, 1928; Lind, 1913; Roumeguere, 1889; Solla 1915), in the Middle East (Engler and Prantl, 1900), Puerto Rico (Sherf and MacNab, 1986) and eastern U.S. (Farr et al., 1989) but has not been considered an important pathogen in any of these places.

The disease caused by P. asparagi on asparagus was first described in India by Kheswalla (1936) and later by Galloway (1936), Sohi et al. (1975), and Tripathi (1985). It attacks asparagus in China (Teng, 1932; Yao et al., 1987), Japan (Tanaka et al., 1987), Korea (Nakata and Takimoto, 1928; Choi et al., 1981), Taiwan (Hsu and Sun, 1969; Wu, 1970; Yang et al., 1970), and Thailand (Anon., 1961). Reifschneider and Lopes (1982) reported it in Brazil. Few of these papers contain much information on the morphology and taxonomy of the fungus and it is possible that two or more fungi are involved.

Bausá Alcalde (1952) described a second species, Phomopsis asparagicola Bausá Alcalde on branches of Asparagus plumosus Baker. It differed from P. asparagi because the alpha conidia of P. asparagi were oblong, 7–8 × 3 µm according to Saccardo (1878) and oblong or spindle-shaped and 5.5–9 × 2–2.5 µm according to Bubak (1906) whereas they were ellipsoid or fusiform, 7–9.5 × 2–2.5 in P. asparagicola; conidiomata were subepidermal in P. asparagi according to both Saccardo and Bubak but subepidermal then erumpent in P. asparagicola; and the beta conidia of P. asparagi were reported to be shorter and thinner (18–25 × 1 µm) than those of Phlyctaena asparagi Fautrey & Roum, (30 × 2 µm), which was considered by Bausá Alcalde to be the beta form of P. asparagi.

A Phomopsis was recently collected on stems of asparagus in central Java in Indonesia. It differs from the two previously mentioned fungi in that the alpha conidia are more variable in length and are consistently broader than those of the other two, it produces paraphyses among the

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conidiogenous cells, and it is much more virulent on inoculated asparagus than is an isolate of P. asparagi.

This paper describes and illustrates the new fungus on asparagus from Java and compares it with isotypes of *Phomopsis asparagi* and other herbarium specimens and with three ATCC cultures from Taiwan. A lectotype of *P. asparagi* is designated and photographs illustrating this species for the first time are provided. Epidemiology of *P. javanica* will be discussed in a separate paper.

**MATERIALS AND METHODS**

The original collection of *Phomopsis javanica* was made by one of us (DAJ) in central Java in January, 1989. A culture (FAU-477) from this collection was grown routinely on autoclaved one-inch long pieces of stem of *Asparagus officinalis*, alfalfa (*Medicago sativa* L.), soybean (*Glycine max* (L.) Merr.), Stokes aster (*Stokesia laevis* (Hill) Greene), kiwi (*Actinidia chinensis* Planchon), and cranberry (*Vaccinium macrocarpon* Ait.), and on grains of oat (*Avena sativa* L.) on water agar (WA) plates. Specimens on each host material were fixed in formalin-acetone-alcohol (FAA), embedded in Paraplast Plus and sectioned at 7 μm. Slides were run through a standard series of xylene and ethanol to water and were stained in methylene blue-azure II (Humphrey and Pittman, 1974, omitting the basic fuchsin). Although this stain was originally applied to specimens embedded in plastic, it is also useful for paraffin sections. Used at full strength, it overstains heavily. When diluted to 25% of the original strength and applied for 5–15 min at room temperature staining was more easily controlled. Slides were then dipped in water to remove excess stain, blotted nearly dry on absorbent paper, and dehydrated and differentiated one to two h in tertiary butanol. After two 10-min baths in xylene, sections were mounted in Permount.

Isotype specimens of *Phomopsis asparagi* were rehydrated for several h on moist filter paper, fixed in FAA, and further treated as above. Conidiomata were measured on living or dried specimens using a Leitz Ultrapak incident light illuminator equipped with 4× and 11× objectives.

Study of the conidiogenous apparatus was accomplished by using a modification of the phloxine-KOH method introduced by Martin (1934). A single conidioma was allowed to expand in 3% KOH. A small portion of the conidiogenous layer was stained with 1% aqueous phloxine, the phloxine was replaced with KOH, and the specimen was flattened. All photographs of the conidiogenous apparatus were made from specimens stained with phloxine-KOH, which was then replaced with 0.2% cotton blue in 85% lactic acid (LA-CB) to extend the useful life of the preparation.

Conidia were collected on cover slips by touching the cover slip to the mucilaginous drop containing conidia at the mouth of the ostiole. A small drop of water was added and the conidia were spread in an even layer across the cover slip. In cases where conidia were not yet extruded in a drop, conidiomata were dissected in a small drop of water on a slide and the conidia were spread upon the slide. In either case, after air-drying the conidia were mounted in a small drop of LA-CB.

*Phomopsis javanica* was also grown on pieces of stem of asparagus, alfalfa, and grape (*Vitis labrusca* L.) on WA plates and the conidiogenous apparatus was observed at intervals of one to five days. Photographs were first taken on day five after inoculation, the last ones on day 40. The purpose of this series was to determine whether the conidiogenous apparatus in conidiomata on asparagus varied morphologically at any stage of development from those at other stages and from those on the other substrata.

To compare the virulence of *P. javanica* with that of *P. asparagi*, potted asparagus plants of cultivars Mary Washington and WSU-1 were inoculated with *P. javanica* and *P. asparagi* in three separate tests. The isolate of *P. asparagi* (FAU-499) was derived from naturally infected asparagus plants grown at Rutgers Research and Development Center, Bridgeton, New Jersey. Inoculum was increased on potato dextrose agar in Petri dishes placed under continuous fluorescent light at 20 to 23 C for 11–14 days. Conidiomata were scraped and washed from the agar, conidiomata were filtered through cheesecloth and inoculated onto four to seven plants per test. One drop of Tween 20 was added to 500 ml distilled water and one drop of the dilution was added to the

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inoculum. Concentration of inoculum was approximately $1.8 \times 10^6$, $2.8 \times 10^6$, and $1.5 \times 10^6$ alpha conidia, respectively, for the three tests. Inoculations were accomplished by wetting the foliage of asparagus plants with inoculum. Shoots 60–80 cm in length were laid horizontally and moved around on a 60 × 68 cm plastic sheet that contained 60–100 ml of conidial suspension. Separate plastic sheets were used for each fungus. After inoculation, plants were placed in a plastic mist chamber for 72, 48, and 64 h, respectively, for the three tests. Plants were then placed in a greenhouse with temperatures of 21–24°C during the day and 17 to 19°C at night.

**RESULTS**

**Phomopsis javanica** Uecker et D. A. Johnson, *sp. nov.*

Conidiomata brunnea, simpliciter eustromatic, immersa, plerumque dissita raro confluenda, ampulliformia vel complanata, loculo solitarii interdum convoluto, 160–430 μm longo × 85–315 alto; partes fuscus apicem versus, ad latera et inferiora undulato-pallidior, postea fuscans ubique, 10–14 μm crassus vel tot quot 25 μm prope ostiolum, textura angularis; ostiolum circulare, plerumque solitarium, 15–20 μm; conidiophora hyalina, brevia vel elongata, plus minusve decrescentia, et basi et super septata, praeter terminalem ramo laterale longo vel breve infra septum omnes cellulae conidiophori conidiogenes, 10–40 × 2–3 μm sed usque 6 μm ad basim; cellulae conidiogenae enteroblasticae, phialidicae, integrae vel discrete, hyalinae, apertura in ramo laterali apicali, canale et cello luto minutis, spissitudine periclinali crassa vel non, 10–20 × 2–4 μm; conidia acropleurogenus; conidia alpha hyalina, aspetata plerumque biguttulata, late elliptica vel elliptica vel fusiformia-elliptica, 6–13 × 3–4 μm; conidia beta hyalina, aspetata, non guttulata, recta vel curvata vel sigmoidea, 12–22 × 0.9–1.3 μm, interdum continuo inter conidia alpha et beta; paraphyses hyalinae, septatae vel aspetatae, cellula terminale conidiophori vel cellulae iisdem atque conidiophoris, usque 60 × 2–4 μm, in conidiomatabus aliquot abundantes sed in alteris sparsae.

Conidiomata (Figs. 1, 2) brown or smoky or more heavily pigmented, simply eustromatic, immersed, usually separate but sometimes confluent, ampulliform or flattened, circular or elongate in outline, papillate or not, with a single locule that is often convoluted, (160–)250–350(–430) μm long × 85–315 μm high; wall (Fig. 2) dark around the ostiole, lighter at sides and below when young but becoming darker with age, 10–14 μm thick, to 25 μm thick near the ostiole, textura angularis; ostiole usually single, circular, 15–20 μm diam; conidiophores (Figs. 3, 4) hyaline, short or elongate, more or less tapered toward apex, septate both at the base and above, 10–40 × 2–3 μm but up to 6 μm wide at the base; except for the terminal cell each cell of the conidiophore produces a short or long lateral branch just below the septum and becomes conidiogenous; conidiogenous cells 10–20 × 2–4 μm, phialidic, integrated or discrete, hyaline, aperture apical on the lateral branch, channel and collarette minute, periclinal thickenings of variable thickness; conidia acropleurogenous; alpha conidia (Fig. 5) hyaline, aspetate, usually biguttulate but sometimes with one large guttule, broadly elliptic or elliptic or fusiform-elliptic, 6–12 × 3–4 μm; beta conidia (Fig. 6) hyaline, aspetate, not guttulate, straight or curved or sigmoid, 12–22 × 0.9–1.3 μm; sometimes a continuum of sizes exists between alpha and beta conidia; paraphyses (Fig. 7) hyaline, septate or aspetate, arising from the terminal cell of the conidiophore or from the layer that gives rise to conidiophores, up to 60 × 2–4 μm, free at tips, free ends rounded or expanded, in some conidiomata abundant but in others sparse.

**HOLOTYPE**: BPI, USO 1102577, on sterilized stems of *Asparagus officinalis* on water agar. Isotypes in NY, DAOM, and IMI (abbreviations from Holmgren et al., 1981).

Other specimens examined: BPI: USO 356160, labeled *Phoma asparagi* Sacc. on *Asparagus officinalis* L., Taipeh, Taiwan, K. Sawada 15/XI/1922.

**CULTURE**: ATCC 24624, on *Asparagus*, Taiwan, S. K. Sun PA-12.

Symptoms consisted of elliptical to round lesions (less than 1 mm to over 45 mm in length) with either tan to light brown or white centers and brown to reddish brown margins. Coalescing and large lesions girdled branches and stems causing the fern and shoots to blight. Conidiomata formed in lesions and in dead tissue. In January 1989, 20 to 80% of the shoot fern in the asparagus-growing area in central Java (23 ha in five locations) had disease symptoms on 5 to 100% of the surface area of the foliage.

Lesions on inoculated plants became initially evident five to six days after inoculation with *P. javanica* and eight to ten days after inoculation with *P. asparagi*. Mean number of lesions per inoculated plant were 23, 30 and 18 after inoculation with *P. javanica* and 2, 1, and 0.5 after inoculation with *P. asparagi*, respectively, for the three tests. Differences were statistically significant (P = 0.01) using Student’s t-test.
Figs. 1–7. *Phomopsis javanica*. 1. Habit on *Asparagus* stem, ×45. 2. Section through conidioma on *Asparagus* stem, ×325. 3. Portion of conidiogenous layer showing conidiophores with septa and with conidiogenous branch emerging from below septa, ×1000. 4. Conidiophores with conidiogenous cells producing beta conidia, ×1000. 5. Alpha conidia, ×1000. 6. Alpha, beta, and intermediate conidia, ×1000. 7. Conidiophores, conidiogenous cells, and paraphyses, ×1000.
Phomopsis javanica produced conidiomata abundantly on pieces of stem of asparagus, alfalfa, soybean, and stokes aster on WA. Production of conidiomata was sparse on kiwi and cranberry stems and on oat grains. Sizes of conidiomata were similar on all these substrates. The conidiomata continued to enlarge for about two wk after inoculation and continued conidium production for about six wk. Conidiomata developed in a subepidermal position but the ostiole apex soon became erumpent through the epidermis. Young conidiomata were yellowish with a dark area surrounding the ostiole. Conidiomata became darker in color as they aged, the color spreading downward from the area near the ostiole. Conidiomata were sometimes nearly spherical but more commonly somewhat flattened. They were ostiolate and more or less papillate with great variation in the length of the papilla. The wall was thinner on the bottom and sides, thickest near the ostiole (Fig. 2).

Sporulation was profuse on asparagus, alfalfa, and soybean stems. In the few conidiomata formed on kiwi and cranberry stems and on oat grains conidium production was much less profuse.

A more or less tightly packed, palisade-like layer of conidiophores and paraphyses lined the entire cavity of the conidiomata up to the point where the ostiole begins. Each conidiophore was attached at the base to a cell on the inside of the wall. No layer of morphologically specialized cells was evident between the conidiophores and wall. As soon as conidiomata could be distinguished on stem pieces on WA, 4–5 days after inoculation, conidiogenous apparatus and conidia were already present.

Paraphyses were first observed in conidiomata on stems on WA about eight days after inoculation. Paraphyses were longer and more numerous in conidiomata 13 or more days after inoculation. After about six wk neither paraphyses nor conidiogenous apparatus were functional any longer. Paraphyses were often difficult to find in sections but were evident in nearly every squash mount.

Alpha conidia were variable in length from one crop to the next on the same host (compare Figs. 5 and 6). Beta conidia (Figs. 4, 6) have so far been seen on only one occasion, after inoculation of a living asparagus plant. Branches excised from that plant after incubation for 15 days were put into a moist chamber and beta conidia were observed after two more weeks.


= Phoma asparagi Sacc., Michelia 1: 257. 1878.

Saccardo (1878) described Phoma asparagi with conidiomata gregarious, covered by the epidermis, globose-depressed, conidia oblong, 7–8 × 3 μm, two-guttulate, hyaline, beta conidia not seen. Based on a specimen from Yugoslavia rather than on type material, Bubak (1906) transferred the name to Phomopsis asparagi (Sacc.) Bubak. He redescribed it as extremely variable, with or without stromata, stromata elongate or in a streak, subepidermal, 0.5–1 mm long and 300–400 μm wide, rupturing by a longitudinal split, black, dull, light brown within and black-brown above, pycnidia or single complete or incomplete chambers mostly lens-shaped and depressed and elongated, rarely hemispheric; conidiophores rod-shaped, 10–15 μm long, 1–1.5 μm wide, straight, hyaline; conidia elongate or spindle-shaped, 5.5–9 × 2–2.5 μm or often with Septoria-shaped conidia, elongate conidia rounded at the ends, spindle-shaped ones attenuated, appearing falsely two-celled because of two large oil drops; with a range of shapes between the spindle-shaped and Septoria-shaped ones.

Conidiomata of both the isotypes (Figs. 8, 9) and of Bubak’s specimens are black, stromatic, ampulliform or somewhat flattened, elliptic or broadly elliptic to subcircular in outline, immersed, separate or confluent, 190–390(–660) μm long × 115–240 μm wide, larger conidiomata often found in one area on a stem and smaller ones in an adjoining area on the same stem or sometimes mixed; wall of the conidioma is textura angularis (Fig. 9); conidiophores hyaline, septate, tapering toward the apex, to 40 × 5 μm; conidiogenous cells (Fig. 10) phialidic, integrated or discrete, hyaline, cylindric or tapered, to 20 × 4 μm, aperture apical on terminal cell or on lateral extensions of other conidiogenous cells, periclinal thickenings usually visible; alpha conidia 6–8 μm in Saccardo’s specimens (Fig. 11), 6–9 in Bubak’s; beta conidia were not seen in any of these specimens.
The following specimen is here designated as lectotype of *Phoma asparagi* Sacc:

**LECTOTYPE:** *Phoma (Diaphore) [sic] asparagi* Sacc.—on *Asparagus* stem, X/1876 Padua, Italy. Mycotheca Veneta No. 932, Sbarbaro Collection (undistributed), in BPI.


MICH: Ellis & Everhart 2940 (two specimens) and 2156, cited above.

CUP: 35064, *Phoma asparagi* Sacc. on *Asparagus* sp., L. Ogilvie 3/III/1926, Pomander Gate, Paget, Bermuda; CUP-A: Ellis & Everhart 2940 and 2156, cited above; CUP-F: Mycotheca Fairmai 3967, labeled

**Figs. 8-11.** *Phomopsis asparagi.* 8. Habit on *Asparagus* stem, ×43. 9. Section through rehydrated conidioma of islectotype, ×295. 10. Short conidiophores with conidiogenous cells, ×1000. 11. Alpha conidia, ×1000.

NY: labeled Phoma asparagi Berk. & Curt., on Asparagus sp., ex Herb. E.M., no date, Chester County (PA?); labeled Phoma asparagi Sacc.: de Thüemen, Mycotheca universalis 1585 on A. officinalis, H. W. Ravenel, 1877, Aiken, South Carolina; Ellis & Everhart 2940 and 2156 (two specimens), cited above; ex Herb. A. Commons 1064, on decaying stems of Phytolaema decandra L., 5/11/1889, Wilmington, Delaware; on A. officinalis, G. W. Carver 418, 12/VIII/1897, Tuskegee, Alabama; labeled Phoma asparagi f. tami: C. Roumeguer, Fungi Selecti Exsiccata 5764, on Tamus communis, Eug. Niel., no date, Ferrière pres Broglie (Eure).

MA: 12046, labeled Phoma asparagi Sacc. on Asparagus albus L., D. G. Sampaio 2/IV/1921, Faro, Portugal; 7080 labeled Phoma asparagi Sacc. on Asparagus sp., P. D. Unamuno IX/1924, Santander, Spain; 10779, labeled Phomopsis asparagi (Sacc.) Trav. & Spessa on Asparagus plumosus, Silva Teixiera XII/1932, Porto Stelae Ollisonis, Portugal.

CULTURES: ATCC 24623, S. K. Sun PA-7 on asparagus, Taiwan; ATCC 24625, S. K. Sun PA-23 on Asparagus, Taiwan.

We were unable to obtain the type of Phomopsis asparagi.ica. Types of four of the species which Bausa Alcalde described in the same publication with P. asparagi predicata are available from MA but the types of Phomopsis asparagi.ica and P. catalipica were not found (F. Pando, Curator of Cryptogamic Herbaria, Jardin Botanico, Madrid, personal communication). Comparison of the type, if found, with that of P. asparagi will probably show that both are the same species.

DISCUSSION

Sutton (1980) defined paraphyses as sterile hyphae which are free at the apex and are often produced among fertile conidiophores or conidiogenous cells. Among Phialostromateae, the suborder which includes Phomopsis, Sutton described and illustrated paraphyses in Aschersonia, Flectophomella, Titaespore, Anerosporium, Phaeocystostroma, and Massariotheca. Among Phialophydiinae, only Coleophoma and Pseudorobillarda exhibit paraphyses. The only previous indication that such structures might occur in Phomopsis is an illustration of a single paraphysis in P. theae Petch (Punithalingam and Gibson, 1972). No mention is made of this structure either in the text or legend for the figure. We consider paraphyses to be unreported previously in Phomopsis. Paraphyses have been considered worthy of description in other genera and we believe that they should also be mentioned when they occur in Phomopsis. Such a distinctive character is welcome in the study of a group noted for a dearth of such characters.

It was noted above that paraphyses in P. javanica may arise either from the cells that produce conidiophores or from various cells of the conidiophore, especially the terminal cell. Except for one paraphysis shown developing from a lower cell of the conidiophore in Aschersonia aleyrodis, all those illustrated by Sutton (1980) develop from the cell layers that give rise to the conidiophores or conidiogenous cells. Especially in the case of those arising from the terminal cell of a conidiophore, one might question why these structures are not considered simply as elongated conidiophores or conidiogenous cells that have not yet become conidiogenous. It is possible and even probable that some of them do become conidiogenous. They are sometimes septate, and have rounded ends in contrast to the acute apices of the conidiogenous cells. The paraphyses are present early in development, become more conspicuous for a time, and some are still present forty days after inoculation.

Three species of Phomopsis have been described on asparagus stems. We believe that P. asparagi and P. javanica are distinct and we consider P. asparagi.ica a synonym of P. asparagi. Comparing the isotypes of P. asparagi and Buk’s specimens with the descriptions of P. asparagi.ica indicates that there is no outstanding character to separate the two and that the conidial shape and dimensions offered for P. asparagi.ica are not unlike those for isotypes of P. asparagi. If the type of P. asparagi.ica or other authentic specimens are discovered, the question can be reevaluated.

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LITERATURE CITED


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