Observations of *Vialaea insculpta* (Amphisphaeriaceae)

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Vialaea insculpta (Fr.:Fr.) Sacc., an ascomycetous fungus with unusual characteristics, has long been known in Europe and in western North America for 50 years where it occurs in cankered branches of *Ilex* spp. The fungus was isolated from freshly collected plant material from Oregon, USA. Its characteristics in culture on several growth media and on sterile plant parts are described. Perithecia containing ascospores were consistently produced in culture. Comparisons of perithecia were made between those from freshly-collected material, herbarium specimens and cultures. Although ascospores were subjected to several treatments to stimulate germination, they were never observed to germinate on culture media. An anamorph was not observed on host material nor was one produced in culture.

Members of the Amphisphaeriaceae (sensu stricto) are ascomycetous fungi characterized by perithecia immersed in the host, perithecia with dark walls and ascal apices that are usually amyloid (Barr, 1975). Mueller & von Arx (1962) broadly defined the Amphisphaeriaceae (Sphaeriales) to include all the nonxylariaceous and nondiatrypaceous fungi that have an amyloid apical ring in their asci. However, Barr (1975) described the characteristics of ascal apices of several genera in the Amphisphaeriaceae (sensu stricto) and considered them to have a chitinoid pulvillus and an annulus which may be amyloid or nonamyloid. *Vialaea insculpta* (Fr.:Fr.) Sacc., a fungus with elongate bifusiform ascospores has most recently been placed in this family by Cannon et al. (1985). The rings within its ascal apices have been found to be amyloid (Dennis, 1978; Schrantz, 1960).

Vialaea insculpta occurs on cankered branches of *Ilex aquifolium* L., English holly (Aquifoliaceae), in Europe as well as in Oregon, Washington and British Columbia (Atkinson & Trelawny, 1962; Sacccardo, 1896; Shaw, 1973). Smith (1918) reported *Ilex angustifolia* as another host of this fungus in England. The first report of the fungus in North America was in 1939 (Milbraith, 1939). *Ilex aquifolium*, introduced into North America as an ornamental, is

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the only host for *V. insculpta* reported on this continent. The fungus was probably imported into western North America on this host.

On the host, *Vialaea insculpta* is associated with a dieback of tip of new twigs and older branches as a result of cankers and the subsequent production of a pale brown zone of necrosis with a darker brown area adjacent to healthy tissue (YOUNG & DEEP, 1952). The presence of the fungus is indicated by black dots surrounded by brown haloes on the cuticle above the immersed perithecia (DENNIS, 1978). Perithecia are produced singly or in groups and can be numerous. The bark above them opens as a longitudinal slit when perithecia are aggregated (GROVE, 1921). From the literature it is not clear whether *Vialaea insculpta* is a plant pathogen or whether it colonizes previously infected or necrotic branches. Although considered to be the causal organism of a leaf and twig disease on *Ilex* referred to as "Boydia canker" (GROVE, 1921; MILBRAITH, 1939; YOUNG & DEEP, 1952), BUDDENHAGEN & YOUNG (1957) regarded it as a "secondary organism" in cankers caused by *Phytophthora ilicis* BUDDENHAGEN & YOUNG. They found that cankers did not form on stems inoculated with *V. insculpta*. ATKINSON & TRELAWNY (1962) did not mention prior infection by *P. ilicis* and continued to refer to symptoms associated with *V. insculpta* as "Boydia canker".

MUELLER & VON ARX (1962) published the following synonymy for this fungus:

Vialaea insculpta (FR.:FR.) SACC., Bull. Soc. Myc. France 12:66 (1896)

SYN.:
Sphaeria insculpta FR., Elenchus Fung. 2:95 (1829)
Zignoëlla insculpta (FR.) SACC., Syll. Fung. 2:225 (1883)
Boydia insculpta (FR.) GROVE, J. Bot. 59:13 (1921)
Cryptospora ludwigiana KIRSCHST., Hedwigia 81:195 (1944)
Boydia remuliformis A. L. SMITH, Trans. Brit. mycol. Soc. 6:151. (1919).

CLEMENTS & SHEAR (1931, p. 75) stated that the genus *Vialaea* is characterized by "conidia in a pycnidium" but neither a pycnidial anamorph nor any other anamorph has ever been substantiated (CANNON et al., 1985; DENNIS, 1978; MUELLER & VON ARX, 1962). The observations reported here describe additional information on the biology and cultural characteristics of *V. insculpta*.

Methods

Examination of plant material and cultures

Herbarium specimens, freshly-collected material and cultures were examined with both a dissecting and compound microscope. Herbarium material was rehydrated in 2% KOH and rinsed in distilled water prior to treatment with Melzer's reagent. Waterman's

blue-black ink and Melzer's reagent were used singly and sequentially (SHOEMAKER, 1964) to stain asci. Perithecia from herbarium specimens, freshly-collected material and those produced in culture were sectioned with a freezing microtome. Sections were mounted in cotton blue in lactic acid.

Herbarium specimens (BPI). – *Vialaea insculpta:* As *Boydia insculpta* – USA: Washington, Seattle, September 1, 1938 – J. A. MILBRAITH; as *Vialaea insculpta,* J. Freedland, England; as *Vialaea insculpta,* Italy: Pollacci – Fungi Longobardiae Exsiccati #284, M. Turconi legit, Aestate, Horto botanico ticinensi. – *Vialaea minutella* PETRAK: holotype, on *Mangifera indica,* USA: Hawaii, Honolulu, December 11, 1927, coll. C. L. SHEAR & N. E. STEVENS.

Freshly-collected material. – Freshly-collected *Ilex aquifolium* stems infected with *Vialaea insculpta* were collected 8 km south of Salem, Oregon, USA, by Robert AMES, September 10, 1988 (BPI). This material was examined by the methods listed above and compared with herbarium specimens from Europe and North America.

Incubated freshly-collected material. – A recently collected twig containing perithecia of *Vialaea insculpta* was cut into 2 cm lengths, placed into a plastic petri dish containing a small amount of water and incubated in diffuse light. Dishes were examined daily.

Culture material. – A pure culture of *Vialaea insculpta* from Oregon, USA, was derived as described below from the freshly-collected material and was deposited at the American Type Culture Collection, Rockville, Maryland, USA, as ATCC 66008.

Cultural studies

Dehydrated culture media (Difco) was utilized to prepare corn meal dextrose (CMD), malt agar (MA), oatmeal agar (OA), potato dextrose agar (PDA) and 2% water agar (WA). To obtain living cultures, perithecia were removed from the freshly collected material and crushed to produce suspensions of ascospores, asci and perithecial walls with adjacent hyphae. Suspensions were placed on CMD as well as PDA and spread with the base of a flamed test tube (ROSSMAN, 1985).

Agar plugs were removed from colony margins of the isolate listed above and transferred to the center of fresh plates of culture media. Plates were incubated at 25° C in two light regimes. One group incubated in darkness was wrapped in aluminum foil to exclude light. Plates incubated in the light were placed ca. 30 cm below fluorescent lights (F15 CW + F15 BL) with a 12 h photoperiod. Observations of colony color, colony morphology and sporulation were after 13 days.

Plugs consisting of mycelium and agar were placed on WA plates adjacent to sterilized 20–25 mm lengths of dried stems of Fabaceae and Rosaceae. Leaves and stems of *Ilex opaca* AIT. were autoclaved with 2 ml of distilled water in 15 ml culture tubes and plugs were added. Plates and culture tubes were incubated in diffuse light at room temperature (20–24° C). Agar media were used to make Riddell mounts (RIDDELL, 1950).

Ascospore germination studies

Ascospores from nature and those from perithecia produced in pure culture were placed on CMD, MA, OA, PDA and WA and incubated for 60 days at room temperature. Ascospores on PDA were also subjected to temperature extremes in an attempt to stimulate germination. Some plates were incubated at 60° C for 1 h and others were stored at –5° C for 12 h. All plates were then incubated at room temperature for 14 days prior to examination for germination.

Suspensions of ascospores from nature and those produced in culture were also added to plastic petri dishes containing a thin layer of liquid 1% malt extract (Difco). Plates were subjected to the same conditions listed above prior to examination.

Results

Herbarium specimens and freshly-collected material

Dark brown spots that appeared as rings on the host cuticle indicated the presence of perithecia (Fig. 1). Perithecia were visible where the cuticle was fractured above the fruiting bodies at the limits of the spots; the cuticle was in the process of being sloughed off. In some cases, the tips of perithecial necks protruded beyond the ruptured cuticle. Sections of perithecia from nature and herbarium specimens showed a stromatic layer consisting of large, vertically-oriented, brown cells produced directly below the host cuticle (Fig. 2). The host cuticle above the sectioned perithecia was 50–55 µm thick and remained devoid of fungal pigment but was fractured by stromal development and the elongation of the tips of perithecial necks. Mycelial cells were connected to the base of the perithecia. These consisted of dark pigmented cells that were horizontally oriented and ramified throughout the area above the lignified tissue. Paraphyses were not observed. Material of *Vialaea insculpta* from Europe was similar in appearance to that from North America.

Perithecia produced in culture were surrounded by a dense layer of aerial mycelium (Fig. 3). Examination of sectioned perithecia showed that in some cases mycelium enclosed the necks. The uppermost cells of the perithecial necks and aerial hyphae enclosing the necks were non-pigmented. These cells and mycelium near the surface of the agar readily stained in cotton blue. The outer walls of perithecia were composed of two to three layers of darkly pigmented cells. The inner two to three layers were composed of thinner-walled cells. Cells of the perithecial walls tended to become darker the closer they were to the agar surface. Rudimentary perithecia were observed throughout the agar. Asci and ascospores from perithecia

produced in culture were similar to those from freshly-collected and herbarium material (Figs. 4, 5 and 6).

Examination of the ascal apex showed that the manubrium exhibited a weak chitinoid reaction and the inner ring produced a strongly amyloid reaction when these structures were singly or sequentially stained with stylographic ink or Melzer's reagent or both (Fig. 5).

The ascal apex of *Vialaea insculpta* was described in detail by CHADEFAUD (SCHRANTZ, 1960) including an "anneau amyloide" surrounding a "corpus ombilique". DENNIS (1975) referred to this amyloid region as the "inner surface of the pore". The ring was observed to be ca. 4–4.5 µm in diameter and intensely stained with Melzer's reagent (Fig. 5). The ascospores were pale brown and had no germ slits (Fig. 6).

Another *Vialaea* species, *V. minutella* PETRAK, was described on *Mangifera indica* L. (Anacardiaceae) from Hawaii (PETRAK, 1952). *Vialaea minutella* also has ascal rings that react strongly with Melzer's reagent and elongate bifusiform ascospores typical of Vialaea.

Incubated freshly-collected material

The perithecial necks produced on incubated twigs were acuminate, hyaline and gelatinous. The mean length of necks that protruded beyond the cuticle was 215 µm. Masses of ascospores were formed at the tips of necks. Ascospores were also observed on petri dish lids above perithecia. The appearance of the perithecial necks was similar to what was observed in some of the sections of herbarium specimens and freshly-collected material.

Colony characteristics

Colonies developed from perithecial fragments or hyphae adjacent to perithecia. Despite repeated attempts, ascospore germina-

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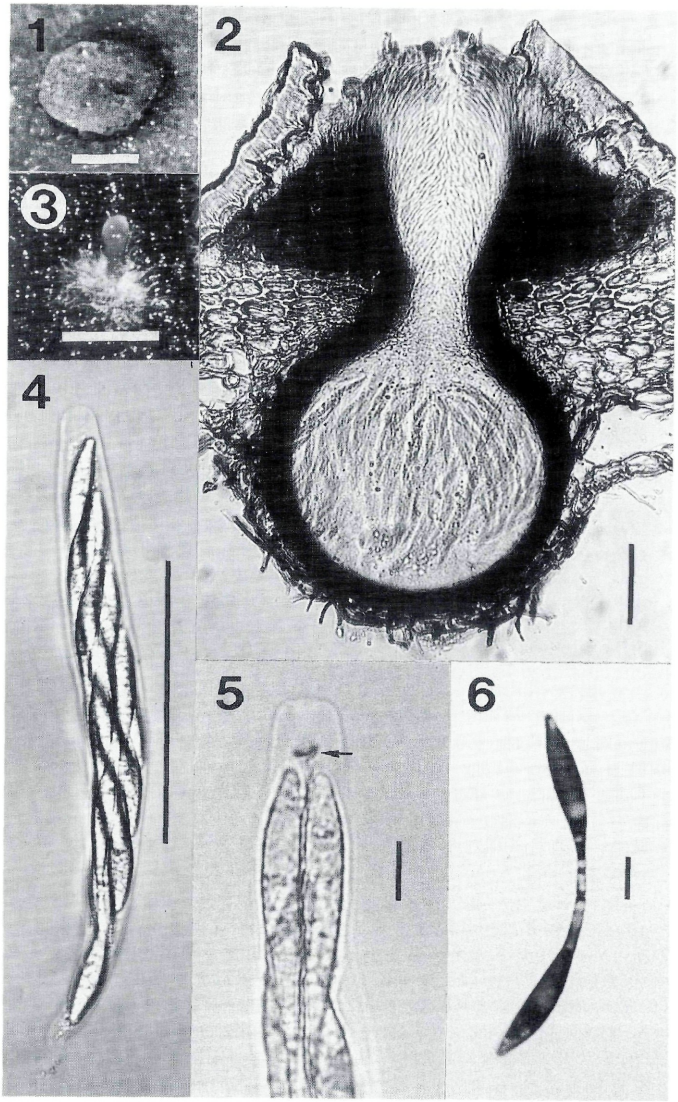
Figs. 1–6. *Vialaea insculpta*. – 1. Habit, spots produced on naturally infected twig of *Ilex aquifolium* denoting presence of immersed perithecium. Fracture at the limit of the ring produces sloughing of the encircled cuticle and exposure of ostiole and adjacent immersed stroma. Scale bar indicates 100 µm. – 2. Longitudinal section of perithecium from bark of *Ilex aquifolium* stained with cotton blue in lactic acid. Stroma surrounding the elongating neck and darkly pigmented mycelium enveloping the base of the perithecium. Scale bar indicates 100 µm. – 3. Perithecium produced in culture on malt agar after 30 days. Elongate neck with mass of ascospores oozing from the tip. Scale bar indicates 1000 µm. – 4. Short-stipitate ascus containing intertwined mature ascospores. Scale bar indicates 100 µm. – 5. Ascal apex sequentially stained with stylographic ink and Melzer's reagent. Arrow indicates amyloid ring strongly reactive to Melzer's reagent. Scale bar indicates 10 µm. – 6. Bifusiform, medially-septate ascospores. Scale bar indicates 10 µm.



tion was never observed. Diameter growth rates, colony color, colony morphology and sporulation were as follows:

On CMD in darkness: growth rate 6.1 mm/day, colonies grey, submerged mycelium, faint zonate rings on reverse side of plate and perithecia scattered, sparse and superficial.

On CMD in light: growth rate 6.0 mm/day, colonies with sparse pale grey aerial mycelium, zonate with darker grey pigment on reverse, perithecia evenly distributed throughout colony, horizontally oriented and submerged.

On MA in darkness: growth rate 5.5 mm/day, colonies black, mycelium submerged, colony reverse dark grey, perithecia numerous and submerged.

On MA in light: growth rate 6.1 mm/day, colonies pale grey, aerial mycelium sparse, colony reverse pale grey, perithecia abundant, scattered, approximately equal numbers of superficial and submerged perithecia.

On OA in darkness: growth rate 6.0 mm/day, colonies white to pale grey, mycelium tomentose covering the tops of perithecial necks, colony reverse beige with grey center, perithecia very abundant throughout colony.

On OA in light: growth rate 6.2 mm/day, colonies distinct orange-yellow, dark grey in center to middle of colonies, colony reverse beige, perithecia scattered and abundant.

On PDA in darkness: growth rate 4.2 mm/day, colonies with raised pale grey mycelium with zones containing perithecia, colonies black, outer margin of colony white on reverse of plate and perithecia clustered in a stromatic central ring.

On PDA in light: growth rate 4.5 mm/day, colonies with beige to orange margin, mycelium grey, raised, very distinct dark grey zonate on reverse, perithecia very abundant, aggregated in darker, pigmented zones.

Ascocarp formation

Perithecia with greatly-elongated necks and mature asci were produced on all culture media as well as on sterile plant parts of *Ilex opaca* within one month after mycelium was added. Perithecia were formed on culture medium within 9 days and contained ascospores at 30 days. Thickened hyphae that appeared to be rudimentary conidiophores were produced on or adjacent to perithecia. No conidia were produced from these structures.

Dense thickened hyphae often surrounded perithecial bodies and necks. Perithecia were scattered to aggregated and possessed elongate necks up to 930 µm long (Fig. 3). Small clusters of ascospores apparently violently discharged from the asci were observed on the lids of culture plates. Ascospores in pale yellow

masses also oozed from the tips of perithecial necks (Fig. 3). The walls of perithecial bodies ruptured and ascospore masses were deposited within the adjacent mycelium above the agar or extruded into the agar below the perithecia. Asci and ascospores from culture were similar to those found in nature.

After ca. 20 days perithecia were obvious on autoclaved non-host plant parts. More perithecia were produced on herbaceous stems than on woody stems. An anamorph was never observed.

Perithecia produced on agar media and on autoclaved *Ilex* leaves had elongated necks that were often obscured with dense hyphae. Perithecia produced on autoclaved parts of various plant species were similar in appearance to those produced on naturally infected holly twigs except the stroma was absent. The fungus produced fewer hyphae on autoclaved plant parts than on culture media. The outer cell layers of herbaceous legume stems were translucent which allowed the developing immersed perithecia to be more readily observed than those produced on woody stems or on holly leaves. Necks of perithecia produced on autoclaved holly leaves and stems in glass tubes were rounded when not obstructed by a physical barrier. When plant parts were adjacent to the inside glass surface necks were wide and flattened at the tip conforming to the inside of the tube.

Ascospore Germination Studies

Ascospores from crushed perithecia produced in nature or on autoclaved *Ilex* leaves or cultures did not germinate when placed on several culture media. No germination occurred after subjecting ascospores to temperature extremes. Ascospore walls disintegrated on culture media several days after treatment. The temperature extremes did not stimulate germination of ascospores in malt extract solutions. Many of the ascospores settled to the bottom of the dishes. When the plastic petri dishes were slightly tilted it was noted that at least one end of a large proportion of ascospores remained attached to the bottom surfaces. Numerous small colonies were initiated from mycelial fragments incubated at room temperature and at -5° C. Colonies grew normally when transferred onto fresh PDA plates. No colonies were formed in plates incubated at 60° C.

Discussion

The developing perithecial necks of *Vialaea insculpta* rupture the exterior of the host for ascospore liberation (Grove, 1921; Phillips & Plowright, 1885; Smith, 1918). This was observed in detail. The host cuticle was not discolored or penetrated by fungal mycelium. An apical stroma with vertically expanding cells developed and

appeared to function in the rupture of the rather thick host cuticle. This stromatic layer also observed by CHADEFAUD (SCHRANTZ, 1960), MUELLER & VON ARX (1962) and SACCARDO (1896) may exert pressure as the ostiole elongates through the mechanical barrier of the cuticle. Unobstructed development of perithecia resulted in extensive neck elongation when the fungus was grown on culture media. Conspicuously-thickened, cylindrical necks formed when necks were obstructed by a glass barrier. These observations as well as the appearance of perithecial necks in herbarium material and incubated, freshly-collected material tend to support the concept of a mechanical rupture that could be correlated to the production of a stroma within the host tissue.

ATKINSON & TRELAWNY (1962) recovered ascospores from *Ilex* cankers but did not specify if ascospores were forcefully ejected from perithecia or accumulated at the ostioles. YOUNG & DEEP (1952) observed that ascospore dispersal in nature coincided with periods of wet weather and described the production of "yellowish-white, gelatinous horns of spores" exuded from perithecia.

Incubated, naturally-infected twigs, inoculated holly leaves and pure cultures produced perithecia with elongate necks that liberated masses of yellowish-white ascospores. Similar observations were made in the field by YOUNG & DEEP (1952). Laboratory studies showed that even though ascospores accumulated at the tips of ostioles, they were also actively dispersed. Ascospore production in nature is abundant and appears to be the major source of inoculum. The adhesive nature of the ends of ascospores could function in the retention of spores on plant surfaces after deposition.

ATKINSON & TRELAWNY (1962) measured ascospore dispersal but neither their study nor any of the other literature on this fungus includes a report of ascospore germination in nature or under laboratory conditions. BUDDENHAGEN & YOUNG (1957) did not obtain infection on *Ilex* inoculated with *V. insculpta* and several other fungi implicated as the cause of Boydia canker. They did not state whether ascospore suspensions or mycelium from agar plates was used. As far as can be determined, ascospore germination has never been observed for this organism.

Phomopsis crustosa (SACC., BOMMER & ROUSSEL) TRAVERSO, the anamorph of *Diaporthe crustosa* SACC. & ROUM. (UECKER, 1988), had also been considered as a cause of the same canker disease but this was subsequently refuted (BUDDENHAGEN & YOUNG, 1957). BUDDENHAGEN & YOUNG were able to distinguish *P. crustosa* from *V. insculpta* in culture. Although *P. crustosa* has not been connected to *V. insculpta,* it is possible that CLEMENTS & SHEAR (1931) found it in close proximity to *V. insculpta* on dying or dead branches and considered it the anamorph.

BUDDENHAGEN & YOUNG (1957) isolated and identified *Vialaea insculpta* on PDA but did not describe the cultural morphology of the fungus. In the current studies, cultures incubated in the dark were generally more darkly pigmented and contained more perithecia. Colonies on malt agar produced a very dark pigment when incubated in the dark. This medium and light regime could be useful for identifying colonies of the fungus at an early stage of development. Media and incubation conditions that were unsuccessfully used to induce an anamorph were successfully and reliably utilized for the production of the teleomorph.

Perithecial development could be readily observed with the use of Riddell slide mounts. The ease of culture, the production of numerous perithecia as well as their rapid maturity make it a useful species for developmental studies of the Amphisphaeriaceae.

SAMUELS, ROGERS & NAGASAWA (1987) stated that the features that members of the Amphisphaeriaceae (sensu stricto) possess are their sessile or short stipitate asci, pale brown, septate ascospores that lack a germ slit, and the relatively broad and short paraphyses. All of these characteristics except the presence of paraphyses were observed in *V. insculpta*. *Vialaea insculpta* has been previously described as "aparaphysate" (GROVE, 1921; SACCARDO, 1896; SCHRANTZ, 1960; SMITH, 1918). KIRSCHSTEIN (1944) stated that paraphyses in this fungus were "certainly absent".

SCHRANTZ (1960) considered the ascal apices of *Vialaea* to be diatrypoid but did not place it in the Diatrypaceae because of the ascospores. He further added that it did not fit in the Clypeosphaeriaceae or Amphisphaeriaceae because of its stroma and erected the family Vialaeaceae to accommodate this genus. If *Vialaea insculpta* produced pycnidia, or if the ascus rings were non-amyloid, it might be considered to be related to the Diaporthaceae. Until an anamorph is found it seems appropriate to retain this species in the Amphisphaeriaceae.

Attempts to observe an anamorph in nature or to induce an anamorph in culture were unsuccessful. Oatmeal agar combined with incubation in reduced light intensity or in darkness has been recently used to induce the formation of anamorphs of other species within the Amphisphaeriaceae (SAMUELS, ROGERS & NAGASAWA, 1987; SAMUELS, MUELLER & PETRINI, 1987). Although anamorphs tend to be predictive of teleomorph genera, few have been connected to genera within the Amphisphaeriaceae (SAMUELS, ROGERS & NAGASAWA, 1987).

SAMUELS, MUELLER & PETRINI (1987) proposed that at least two lines of related organisms occurred in the Amphisphaeriaceae and that "conidia produced holoblastically on sympodially proliferating cells are common to both lines". *Pestalotiopsis* STEY. and similar anamorph genera represent the first group of teleomorph genera

that includes *Pestalosphaeria* Barr and allied genera. These fungi produce acervular or pycnidial conidiomata with phialidic or annellated conidiogenous cells. A second group is characterized by those that tend to form an amyloid apical ring in the ascus and have conidia produced holoblastically on sympodially proliferating conidiogenous cells. Several fungi in the Amphisphaeriaceae are not known to produce an anamorph (Samuels, Mueller & Petrini, 1987; Samuels & Rossman, 1987).

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References

Atkinson, R. G. & J. G. Trelawny (1962). A spore trap to study sporulation in tree canker diseases. – Can. Plant Dis. Survey 42:151–154.

Barr, M. E. (1975). *Pestalosphaeria*, a new genus in the Amphisphaeriaceae. – Mycologia 67:187–194.

Buddenhagen, I. W. & R. A. Young (1957). A leaf and twig disease of English holly caused by *Phytophthora ilicis* n. sp. – Phytopathology 47:95–101.

Cannon, P. F., D. L. Hawksworth & M. A. Sherwood-Pike. (1985). The British Ascomycotina, An Annotated Checklist. – CMI, 302 pp.

Clements, F. E. & C. L. Shear (1931). The Genera of Fungi. – H. W. Wilson Co., New York, 496 pp.

Dennis, R. W. G. (1978). British Ascomycetes. – J. Cramer, Vaduz, 585 pp.

Grove, W. B. (1921). Mycological Notes. – J. Bot. 59:13–17.

Kirschstein, W. (1944). *Cryptospora ludwigiana*. – Hedwigia 81:195–196.

Milbraith, J. A. (1939). Two unusual fungi on ornamental shrubs in the Pacific Northwest. – Plant Disease Reporter 23:48.

Mueller, E. & J. A. von Arx. (1962). Die Gattungen der didymosporen Pyrenomyceten. – Beitr. Kryptogamenfl. Schweiz 11(2):1–922.

Petrak, F. (1952). Ein Beitrag zur Pilzflora von Hawaii. – Sydowia 6:363–371.

Phillips, W. & C. B. Plowright. (1885). *Zignoëlla insculpta*. – Grevillea 13:77–78.

Riddell, R. W. (1950). Permanent stained mycological preparations obtained by slide culture. – Mycologia 42:265–270.

Rossman, A. Y. (1985). A single spore isolation technique. – Mycological Society of America Newsletter 36(1):59.

Saccardo, P. A. (1896). Notes Mycologiques. – Bull. Soc. Mycol. Fr. 12:64–81.

Samuels, G. J., E. Mueller & O. Petrini. (1987). Studies in the Amphisphaeriaceae (sensu lato) 3. New species of *Monographella* and *Pestalosphaeria*, and two new genera. – Mycotaxon 28:473–499.

Samuels, G. J., J. D. Rogers & E. Nagasawa. (1987). Studies in the Amphisphaeriaceae (sensu lato) 1. *Collodiscula japonica* and its anamorph, *Acanthodochium collodisculae*. – Mycotaxon 28:453–459.

Samuels, G. J. & A. Y. Rossman. (1987). Studies in the Amphisphaeriaceae (sensu lato) 2. *Leiosphaerella cocoes* and two new species of *Oxydothis* on palms. – Mycotaxon 28:461–471.

Schrantz, J. P. (1960). Recherches sur les pyrenomycètes de l'ordre des Diatrypales, sensu Chadefaud. – Bull. Soc. Mycol. Fr. 76:305–407.

SHAW, C. G. (1973). Host fungus index for the Pacific Northwest – I. Hosts. – Wash. State Agric. Exp. Sta. Bull. 765:1–121.
SHOEMAKER, R. A. (1964). Staining asci and annellophores. – Stain Tech. 39:119–121.
SMITH, A. L. (1918). New or rare microfungi. – Trans. Brit. mycol. Soc. 6:149–157.
UECKER, F. A. (1988). A world list of *Phomopsis* names with notes on nomenclature, morphology and biology. – Mycol. Mem. 13:1–231.
YOUNG, R. A. & I. W. DEEP (1952). Diseases of holly in the Pacific Northwest. – Univ. Wash. Arboretum Bull. 15(4):18,23.