Etiology and Symptomatology of Canker and Dieback Diseases on Highbush Blueberries Caused by Godronia (Fusicoccum) cassandrae and Diaporthe (Phomopsis) vaccinii

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ABSTRACT

Both *Godronia* (*Fusicoccum*) *cassandrae* and *Diaporthe* (*Phomopsis*) *vaccinii* cause severe canker and dieback diseases of highbush blueberries in Michigan. Godronia canker and dieback was epiphytotic in northern Michigan, whereas Phomopsis canker and dieback was epiphytotic in some Indiana fields and in extreme southern Michigan. Depending upon the field, either or both diseases were epiphytotic in the center of the blueberry production area. Diagnostic symptoms of Godronia canker and dieback on 1- and 2-year-old stems were red-maroon-brown elliptical lesions which were often centered about a leaf scar whereas wilting of otherwise symptomless stems was most often associated with Phomopsis canker and dieback. On stems more than 2 years old, cankers caused by *G. cassandrae* and *P. vaccinii* tended to be wide in relation to their length or long, narrow, and often covered with unbroken bark, respectively. Both fungi caused brown discoloration of stem xylem below wilt symptoms. Isolations were necessary to diagnose the cause of dieback on stems older than two years when fruiting structures were not present. Apothecia of *G. cassandrae* were common in northern Michigan fields in which Godronia canker and dieback was epiphytotic. Perithecia of *D. vaccinii* were not found.

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Additional key words: Coryneum microstictum, Fusarium sp., *Vaccinium corymbosum,* stem galls, stem blight.

During the early 1960's a canker-dieback disease was found widespread in Michigan highbush blueberry *(Vaccinium corymbosum* L.) fields. Preliminary studies indicated that *Fusicoccum putrefaciens* Shear [*Godronia cassandrae* Peck (14)] was the primary pathogen (1). Groves (11) tentatively grouped *G. cassandrae* found on different host genera into seven different forms. The form on *Vaccinium* spp. was placed in f. *vaccinii.* Because a form is not a valid taxon, in this paper *G. cassandrae* is retained; nevertheless, it is the same organism as *G. cassandrae* f. *vaccinii* sensu Groves. Further investigations, however, suggested that the problem was more complex. Symptoms, including cankers, stem galls, and dieback which were observed in Michigan blueberry fields encompassed not only those reported for Fusicoccum canker (1, 3, 12, 13, 23), but also those caused by *Diaporthe* (*Phomopsis*) *vaccinii* Shear (18, 19), *Coryneum microstictum* Berk. & Br. (22, 23), a gall-inducing *Phomopsis* distinct from *P. vaccinii* (2), and several other pathogens (6, 7, 8, 15, 20, 24).

Existing literature did not adequately describe the disease syndrome as observed in Michigan highbush blueberry fields. The objectives of this paper were: (i) to determine primary cause(s) through application of Koch's postulates, (ii) to define field symptoms, and (iii) to determine distribution and relative severity of canker and dieback diseases on highbush blueberries in Michigan.

MATERIALS AND METHODS.—Diseased tissue was collected in the field, coded according to symptom, placed in polyethylene bags and stored at 10-12 C. Stems were cut into 1- to 2-cm-long segments and surface disinfected for 1-2 minutes in 1.3% NaOCl plus 2-3 drops of Tween 20 wetting agent. Depending upon the sample, full- or half-strength Difco potato-dextrose agar (PDA and 1/2 PDA, respectively), Difco malt agar, or Difco nutrient agar were used for plating media.

Isolation plates were incubated at 10-12 C. This was

TABLE 1. Association of *Godronia cassandrae* (GC) and *Phomopsis vaccinii* (PV) with symptoms observed on blueberry stem segments in Michigan and Indiana

Symptom	Segments with GC or PV[a] (%)					
	Group A		Group B		Group C	
	GC	PV	GC	PV	GC	PV
Symptomless	20.0	38.4 (242)	22.7	71.0 (66)	96.3 (27)	38.6 (44)
Lesion-developing canker	65.0	8.1 (406)	70.9	25.8 (31)	97.0 (66)	78.6 (14)
Developed canker	32.9	27.9 (670)	40.6	57.2 (138)	89.1 (101)	73.3 (150)
Canker + stem gall	71.2	13.5 (247)	61.7	35.3 (34)	95.9 (49)	69.0 (29)
Discolored bark or epidermis	22.5	22.4 (645)	34.2	60.5 (76)	87.3 (79)	76.4 (110)
Split, flaking or exfoliating bark or epidermis	39.6	36.6 (870)	39.4	55.8 (231)	94.1 (204)	73.0 (159)

[a]Numbers in parentheses are total stem segments observed. Group A = all segments observed; Group B = segments with brown xylem taken from wilted stems; Group C = segments with brown xylem taken from wilted stems which were collected from fields in which either *G. cassandrae* or *P. vaccinii* was the only pathogen found.

important because even at optimum temperatures *G. cassandrae* (GC) grew slowly and was soon overgrown by saprophytes when plates were incubated at room temperatures. Adequate growth of GC and *P. vaccinii* (PV) at 10-12 C (60-75% and 40-60%, respectively, of growth at optimum temperatures) was maintained while sufficiently reducing that of saprophytic fungi.

Fungi growing from diseased tissue were transferred to 1/2 PDA acidified to pH 3.5 with 10% lactic acid to minimize bacterial contamination, and single spore isolates were obtained. All fungi were maintained on 1/2 PDA (pH 5.6 - 6.8) at 22-25 C and were transferred every 4-6 weeks.

Inoculations.—Forced cuttings or 3-year-old Jersey, Blueray, or Bluecrop blueberry plants were inoculated in the greenhouse. All 3-year-old plants were grown out-of-doors in field soil in 11.5-liter galvanized pails. Forced cuttings were grown in a mixture of Michigan peat and sandy loam (1:1, v/v) in 20-cm diameter clay pots in a 23-30 C greenhouse under continuous light (daylight supplemented with two 500-watt incandescent bulbs per bench) until large enough to inoculate. All plants were fertilized bimonthly with 25 ml of a solution containing 14.8 cm^3 (1 tablespoon) Plant Marvel (12% N, 31% P, and 14% K)/3.8 liters tap water. Additional inoculations were performed at 2-week intervals during the 1968 growing season on 8-year-old Jersey plants which were growing in the field.

Inocula consisted of 4-week-old cultures. After stems were swabbed with 95% ethyl alcohol, aqueous suspensions of conidia or mycelium in blocks of 1/2 PDA were placed on the stems. The inoculum and the stem tissue were pierced 1-5 times with a sterile needle, or the inoculum was inserted under 1.5-cm-long V-shaped flaps cut into the bark or epidermis. Following inoculation, the inoculation sites were covered with sterile, moist cotton and wrapped with plastic film held in place with rubber grafting strips. The number of inoculations per plant varied from 1-10 depending upon the experiment.

All greenhouse-grown plants were placed in a mist chamber (18-25 C, >90% relative humidity) for 5-10 days. Following incubation, cotton and plastic were removed and the plants were grown under continuous light at 15-20 C. In the field, cotton and plastic film were left on inoculated plants for 14 days. Controls consisted of single plants which were inoculated 1-10 times with blocks of 1/2 PDA or sterile distilled water and also single control inoculations on each inoculated plant.

Symptom progression on naturally infected plants.—Disease progression was observed by tagging naturally infected plants in one field in which both PV and GC occurred, and two in which only GC was found. Identity of fungi in diseased tissue was confirmed by isolations.

No attempt was made during the first 2 years of this research to selectively sample wilted stems. It was observed during this period that light-brown colored xylem (BX) often occurred at some point in the stem below wilt symptoms and that mycelium and various occlusions were common in zones of BX (17). In order to further define stem symptoms associated with GC and PV, random samples of over 300 wilted stems were cut into 1- to 2-cm-long segments and external symptoms noted for the entire stem and for segments with BX. Three segments from each stem, one from portions with BX, and one each from proximal and distal portions of the stem were incubated on 1/2 PDA for identification of GC and PV.

RESULTS.—*Association of fungi with symptoms, and pathogenicity tests.*—Fungi isolated from 3,130 segments of diseased blueberry tissue collected from 57 blueberry fields included GC (40.0%), PV (27.0%), *C. microstictum* (2.9%), and *Fusarium* sp. (2.0%). Inoculations made in the greenhouse showed that only GC and PV were pathogenic on actively growing 1- to 3-year-old plants. Both fungi were associated with all of the symptoms observed on blueberry stems in the field (Table 1).

Inoculation studies.—In the greenhouse lesions caused by GC on 1- to 3-year-old stems were similar to those described by others (1, 3, 12, 13, 23) in the field (Fig. 1-A to F and K). In our studies, pycnidia developed on GC lesions (Fig. 1-B) 2-3 weeks after inoculation and continued to appear for an additional 4-7 weeks. Some stems wilted within seven weeks, but <1% of the inoculated stems wilted under greenhouse growing conditions (temperature 20-30 C).

Symptoms induced by PV on succulent tissue inoculated in the greenhouse were similar to those described by Wilcox (18) (Fig. 1-G to I, and O). In

contrast to her observations, however, lesions on woody stems often expanded (Fig. 1-L and M) and killed individual stems and eventually entire plants by growing into the crown. Lesions on woody stems were similar in color, but longer than those caused by GC (Fig. 1-M). Pycnidia developed within 15 days after inoculation, and continued to develop for 2-3 months even at greenhouse temperatures >27-30 C.

In the field, symptoms associated with PV and GC on 1- and 2-year-old stems were similar to those observed in greenhouse studies. On older stems, GC and PV usually caused, respectively, wide slow-spreading cankers or long, narrow cankers usually covered with bark (Fig. 1-J and P).

None of the plants inoculated with either fungus developed stem galls. Both fungi were reisolated from inoculation sites for 18 months, even when lesions and cankers did not develop. Neither fungus was isolated from any of the control inoculations.

Many of the symptoms associated with GC and PV were similar to those previously described (1, 3, 12, 13, 16, 18, 23). Only new observations or those considered to be important in field diagnosis, pathogenesis, or epidemiology of the diseases are discussed.

Godronia canker and dieback.—First signs of infection on 1- and 2-year-old stems were observed in October as minute (<0.5-mm diameter) water-soaked lesions which turned red by December (Fig. 1-D). During the spring and summer, the lesions expanded and, depending upon the number of lesions on stems, either coalesced (causing extensive red, maroon, or brown discoloration, splitting, and flaking of epidermis) or formed typical lesions associated with GC by others (1, 3, 12, 13, 23). Most lesions were <0.5 cm-1.5 cm in length with 43.7% of all lesions and 94.4% of lesions >1.5 cm, respectively, occurring at leaf scars. Some lesions also formed on new stems during the summer. Many internodal lesions expanded during the spring, but were apparently localized during the summer.

On stems more than 2 years old, flattening, gnarling, and depressions often occurred due to infections from previous seasons (Fig. 1-J, N and S). Most cankers were 0.5 - 5.0 cm in length; however, some extended the length of the stem and were >25.0 cm long. Diagnostic GC cankers were wide in relation to their length, and xylem was often partially exposed due to disintegration of bark (Fig. 1-J and S).

In some fields, stem galls were associated with slow-growing cankers (Fig. 1-Q). It was not determined whether galls formed secondarily to cankering action of GC, or were due to other causes. However, the symptom was reproduced by mechanically girdling plants in the greenhouse.

Some stems wilted and died as buds expanded in April and May. However, most wilt started in mid-June and, depending upon the latitude, continued until August-September. Approximately 1-5% of the stems wilted due to fungus invasion from infected crowns. Often leaves of stems turned red or yellow before they wilted and turned brown. Average age of 128 wilted stems infected with GC was 2.4 years. Brown xylem (BX) always occurred at some point along wilted stems and below wilted leaves. Many stems completely girdled by GC cankers did not wilt, whereas others wilted with no visible canker or lesion.

Numerous pycnidia were produced in March-June, usually on lesions (Fig. 1-E and K), but also in bark fissures (Fig. 1-R), with conidia being visibly exuded during rainy periods. Pycnidia were not observed on lesions formed after July.

Numerous immature apothecia were first observed in April on dead pruning stubs; erect, dead stems; and on dead twigs accumulated around crowns of plants. Apothecia were observed only on dead wood, usually killed during the previous season, and occasionally were observed on dead tissues of unilaterally killed stems. Most apothecia matured by July, and were found in highly infested fields especially north of Oceana County (Fig. 2).

Phomopsis canker and dieback.—The lesion-developing canker and canker + stem gall symptoms were less frequently associated with PV than with GC, whereas wilting of otherwise symptomless stems was more common with PV infection (Table 1). Cankers caused by PV, regardless of age of stems, tended to be long and narrow, and were often covered with bark or epidermis (Fig. 1-P). As with GC infection, leaves often turned yellow-red before they wilted, and all wilted stems exhibited BX. Average age of stems killed by PV was 3.0 years. *Phomopsis vaccinii* usually occurred higher on the plant than GC, and grew downward through the stem, eventually killing major branches and often entire plants. Some infections were found in plant crowns, resulting in death of stems originating from the crown. Wilt usually first occurred during early July-August and continued until October. Pycnidia appeared during August-October mostly on dead 3- to 5-year-old stems (Fig. 1-T). No perithecia of *D. vaccinii* [ascigerous stage of PV (19)] were found.

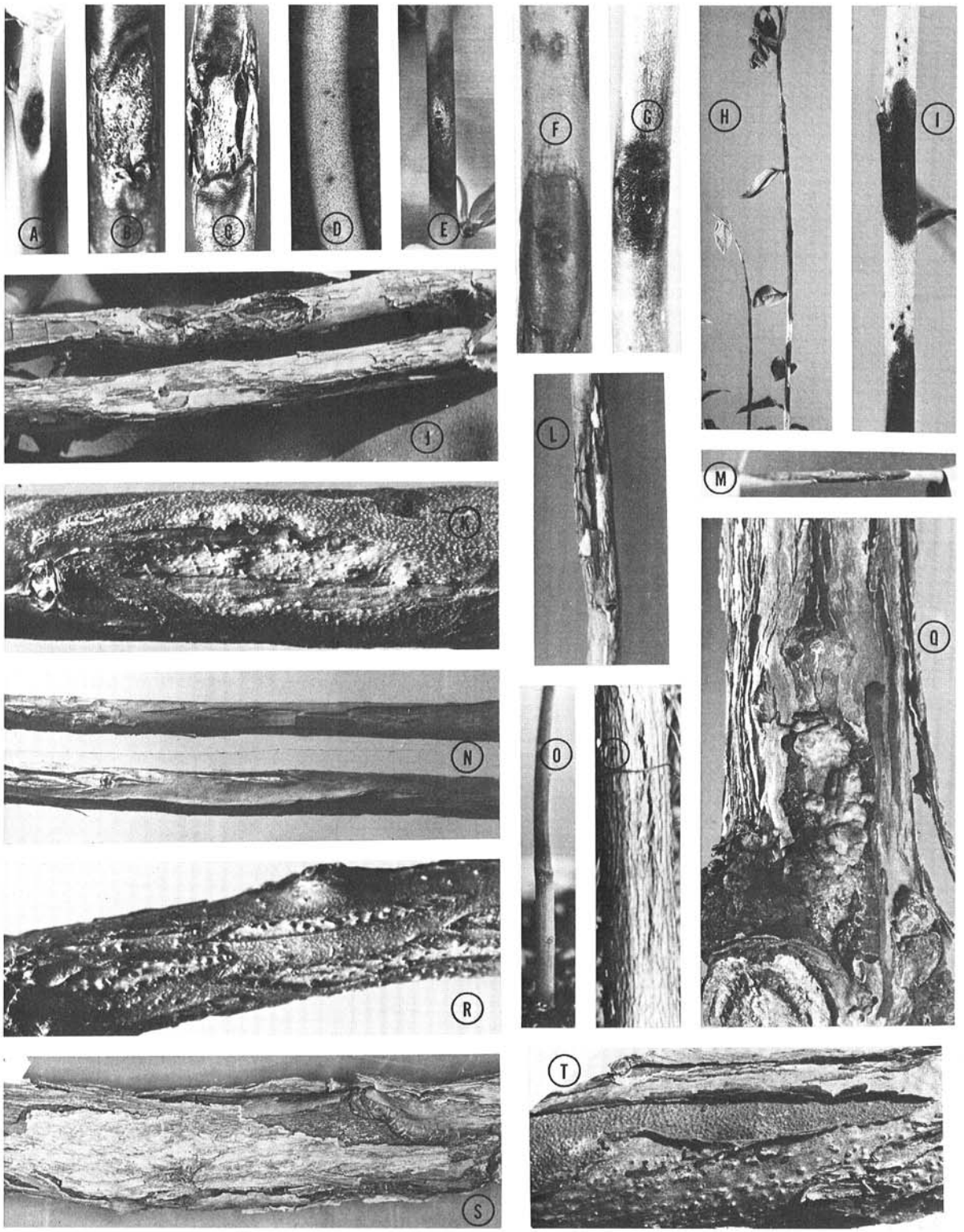
Distribution and severity of Godronia and Phomopsis canker and dieback diseases in Michigan and Indiana.—Both PV and GC were widely distributed in Michigan (Fig. 2). However, in fields north of Oceana County, GC was epiphytotic, and it was the only organism found. In the center of the blueberry production area, both fungi were common, and depending upon the field, either or both were epiphytotic. *Phomopsis vaccinii* was dominant in Indiana and in some southern Michigan counties.

Both fungi attacked all major cultivars of blueberries grown in Michigan, but the four most important cultivars (Jersey, Rubel, Bluecrop, and Earliblue) appeared to be most susceptible to GC. Jersey cultivar was the most susceptible to GC, and Rancocas (although often severely cankered) was the most resistant cultivar to wilt. In one field in which GC was epiphytotic, wilted stems were found on 6.6% of 300 Rancocas plants, whereas stems on 32.7% of 1,047 plants of other cultivars were wilted.

DISCUSSION.—This is the first report that both GC and PV cause cankers and dieback on blueberries, and the first study of these diseases on older tissue. In order to distinguish among other canker and stem blight or dieback diseases of blueberries (7, 15, 16, 20), the names Phomopsis canker and dieback, and Godronia canker and dieback are proposed for the diseases as they occur in Michigan. Phomopsis canker and dieback is used in lieu

of Phomopsis twig blight (18, 19) because the former name is more descriptive of the disease.

Godronia canker and dieback is preferred to Fusicoccum canker for several reasons. *Fusicoccum*, according to Groves (11), is an invalid name for asexual states of *Godronia* because they are morphologically



dissimilar to the type species of the genus. Use of *Fusicoccum* incorrectly implies that a taxonomic relationship exists among asexual forms of *Godronia* and the causal fungi of other diseases incited by valid *Fusicoccum* species. For example, symptomatology of Fusicoccum canker of peach is similar to that of Godronia canker and dieback of blueberry, but the disease is caused by fungus unrelated to *Godronia* (4, 5). Apothecia of GC are common in Michigan, and ascospores apparently are important in the disease cycle (17). Since few publications have used the name Fusicoccum canker (1, 3, 12, 16, 21, 23), it is suggested that the more descriptive term Godronia canker and dieback of blueberry be used.

Wilted stems (flags) are the most conspicuous symptom of both diseases in the field. Progression of wilt on noncankered stems infected with either fungus is, in most respects, indistinguishable from that of blueberry stem blight caused by *B. dothidea* in North Carolina (20).

Symptoms associated with PV and GC on 1- to 2-year-old stems in our studies were in many respects similar to those reported by others (1, 3, 12, 13, 18, 19, 21, 23). Significant differences were that both fungi caused lesions and/or dieback symptoms on 1- and 2-year-old stems and many PV lesions on woody stems expanded and caused dieback. In the field, however, local lesions caused by GC and dieback of nonlesioned stems caused by PV, respectively, were often diagnostic characters of the two diseases on 1- and 2-year-old stems.

When both diseases occurred in the same field, diagnosis on older stems was difficult because symptoms overlap. Although GC cankers tended to be short and wide, and those of PV were usually long and narrow, this distinction did not always hold, and final diagnosis was often dependent upon isolations. Isolations were always necessary for positive diagnosis of dieback on noncankered stems >2-years-old when fruiting bodies were not present.

The association of GC and to a lesser degree PV, with certain types of stem galls, and the results of girdling experiments suggest that galls at the edge of cankers may result from the slow girdling action of cankers caused by these fungi. *Phomopsis vaccinii* was not associated with galls similar to those described by Brown (2).

Distribution of PV and GC in Michigan and Indiana in part may be limited by temperature. *Godronia cassandrae,* which grows poorly at temperatures above 25 C, was not found in Indiana where warmer temperatures prevail. *Phomopsis vaccinii* which grows well at 25-30 C and caused cankers and dieback on plants artificially inoculated when temperatures were >30 C, was common in warmer areas. A similar relationship was shown in studies of dieback on cranberries, in that warm temperatures favored growth of PV over that of GC in diseased stems (10).

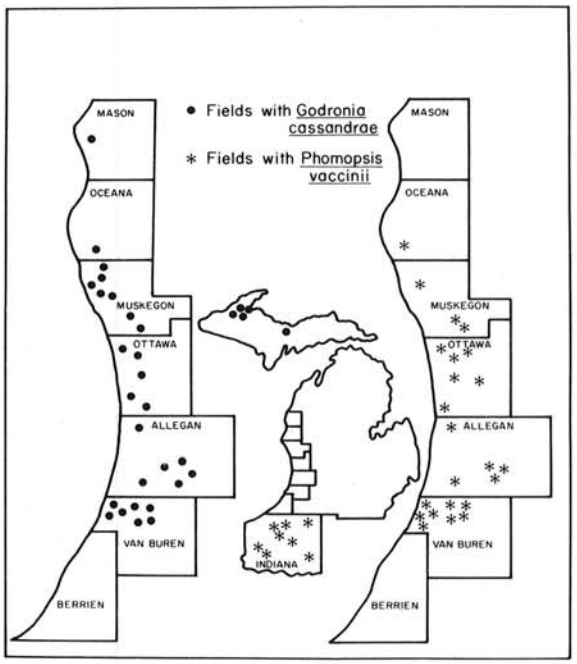


Fig. 2. Distribution of *Godronia cassandrae* and *Phomopsis vaccinii* in Michigan and Indiana blueberry fields.

During 1965-68, Godronia canker and dieback was considered to be the more important of the two diseases in the Great Lakes blueberry production area. Following a severe freeze on 11 June 1972, however, Phomopsis canker and dieback has become a major threat on most cultivars (D. Ramsdell, *personal communication*). Inoculation studies and observations that PV killed older stems than GC, suggests that PV is the more virulent pathogen. In addition to Michigan and Indiana, PV has been observed in North Carolina (18, 19), Washington (9), Massachusetts (23), and New Jersey (D. P. Weingartner, *unpublished*). All highbush blueberry cultivars in Michigan are susceptible to PV. Phomopsis canker and dieback of blueberry is therefore considered to be a potentially devasting disease on cultivated blueberries when conditions favor its spread and development.

Fig. 1-(A to T). Comparison of symptoms produced on highbush blueberry by *Godronia cassandrae* (GC) and *Phomopsis vaccinii* (PV). **A-C)** Lesions and cankers caused by GC 10 days (A), nine weeks (B), and 11 months (C), respectively, after artificial inoculations in the greenhouse. Note pycnidia on lesion in B. **D)** Incipient lesions caused by GC on naturally infected stem. **E)** Typical maroon-brown GC lesion with grey center on naturally infected stem. Note concentric rings of pycnidia around leaf scar and axillary bud. **F)** Red-brown nodal lesion and internodal lesions caused by GC. **G)** Lesion caused by PV on 6-month-old stem 10 days following artificial inoculation. **H and I)** Lesions and wilt symptoms caused by PV on succulent stems 10 days following artificial inoculations. **J)** Cankers and flattening of stems naturally infected with GC. **K)** Developing canker and pycnidia of GC on naturally infected 1-year-old stem. **L and M)** Woody stems six months and 11 weeks, respectively, following inoculation with PV. **N)** Splitting bark associated with GC infection. **O)** Die-back on 6-month-old stem artificially inoculated with PV. **P)** Long narrow canker on 5-year-old stem three months following inoculation with PV. **Q)** Callus tissue associated with GC canker on 7-year-old stem. **R)** Pycnidia of GC in bark fissures on 3-year-old stem. **S)** Wide GC canker on 5-year-old stem. **T)** Pycnidia of PV on dead 5-year-old stem.

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