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STUDIES OF CANKER AND STEM BLIGHT DISEASES OF HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) IN MICHIGAN

by

David Peter Weingartner

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ABSTRACT

STUDIES OF CANKER AND STEM BLIGHT DISEASES OF HIGHBUSH BLUEBERRY (VACCINIUM CORYMBOSUM L.) IN MICHIGAN

by David Peter Weingartner

The initial objectives of this research were to determine primary causes of cankers and stem blights of highbush blueberries in Michigan. Several fungi known to cause cankers and/or stem blight of highbush blueberries were associated with dying stems in Michigan blueberry fields. In addition, various types of overgrowths reported to be caused by other organisms were associated with some cankers. Application of Koch's Postulates showed that Godronia cassandrae Peck f. vaccinii Groves and Diaporthe (Phomopsis) vaccinii Shear caused cankers and stem blights of blueberries in Michigan. The diseases were renamed Godronia (Fusicoccum) canker and stem blight and Phomopsis canker and stem blight, respectively.

Red-brown to maroon elliptically shaped lesions, often centered by a leaf scar, were usually diagnostic for Godronia infection on 1- and 2-year-old stems. One- and 2-year-old stems which were blighted, but not cankered, were usually infected with Phomopsis. On older stems cankers caused by Godronia tended to be short and wide whereas Phomopsis cankers were often long, narrow and covered with

unbroken bark. Since both fungi caused canker and stem blight symptoms, isolations were often necessary to confirm diagnoses made in the field.

Godronia, and to a lesser degree, Phomopsis, were associated with certain types of calluses occurring along cankers on affected blueberry stems. Although calluses did not develop on plants artificially inoculated with Godronia or Phomopsis, calluses similar to those observed in the field were induced by mechanically girdling actively growing stems. Some calluses may result from the slow girdling action of cankers caused by these fungi.

Both diseases were widely distributed in Michigan, but Godronia (Fusicoccum) canker and stem blight was considered more important because, in the field, major varieties of blueberry grown in Michigan were more susceptible to this disease.

Blight symptoms caused by Godronia were more severe when artificially inoculated plants were grown under conditions inducing dormancy of the host.

The disease cycle of Godronia (Fusicoccum) canker and stem blight was studied. That isolates of G. cassandrae from Spiraea spp. and V. angustifolium were pathogenic on Jersey variety blueberries suggested that these hosts may serve as inoculum reservoirs for the disease.

Two major infection periods, 29 May - 10 July and 21 August - 9 October, were identified when different sets

of healthy 1- and 2-year-old plants were exposed to natural inoculum during the 1968 growing season. Conidia were most abundant and were observed washing down stems during April-June whereas ascospores were discharged during August-September.

Over 49% of all lesions on stems collected in April occurred at leaf scars. Isolations from attached petioles collected from the field in October showed that 45% were infected by Godronia. When plants were spray inoculated 2-3 weeks before leaf drop, 53 of 66 lesions occurred at leaf scars. Attached petioles inoculated 0, 1, 2, and 5 days after removing leaf blades resulted in 34, 31, 8, and 6% leaf scar infections, respectively. When healed leaf scars on dormant plants were inoculated, lesions did not develop until 1 year after inoculation whereas lesions developed immediately following inoculation when leaf scars were wounded before inoculation. It was concluded that leaf scars are probably infected via attached petioles before leaf drop.

Infections produced by inoculating nonwounded stem internodes and histological observations of incipient necrosis below stomates suggested that stomates also served as infection courts on 1- and 2-year-old stems.

Histology of developing cankers showed that necrosis first appeared below stomates. Godronia initially grew through longitudinal air channels in the living stem cortex.

Hyphae were observed in advance of necrosis, but chloroplasts of cortex parenchyma turned red in advance of hyphae. Hyphae grew along, but did not penetrate living cells. Vessels in discolored xylem were occluded with hyphae, various deposits and possibly tyloses suggesting that stems wilt because of vascular occlusion.

DEDICATION

To Sharon and Kris

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I want to acknowledge Dr. E. H. Barnes who started me on this research and helped in many ways until his untimely death in November, 1967; and Dr. E. J. Klos who has directed my research and has unselfishly provided assistance since Dr. Barnes' death.

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C. Michigan State University Photographic Laboratory: Figs. 1-B; 2-A; 5-B, D; 8-B; 15-A, D. Dr. P. H. Wooley: Figs. 5-E; 6-B; 12-A; and 31. Dr. J. E. Moulton: Fig. 14. Mr. J. W. Nelson: Figs. 2-B; 3-A; 4-B; 5-C. Dr. E. H. Barnes: Fig. 15-E. Canadian Department of Agriculture: Fig. 4-E.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

INTRODUCTION

Several Fungi including Coryneum microstictum Berk. and Br. (71,73) Godronia cassandrae Peck f. vaccinii Groves (16, 37), Diaporthe (Phomopsis) vaccinii Shear (66, 67), Botryosphaeria corticis (Demaree & Wilcox) Arx & Muller (25, 58), and B. dothidea (Moug. ex Fr.) Ces. & deNot. (68) were known to cause cankers and/or blights of the highbush blueberry (Vaccinium corymbosum L.). Overgrowths, tumors, or galls on blueberry were reported caused by a Phomopsis distinct from P. vaccinii (7), Agrobacterium tumefaciens Sm. & Town (21), and an unknown causal agent (69).

External symptoms observed on dying blueberry stems in Michigan included those caused by each of the above organisms. In addition, C. microstictum, G. cassandrae f. vaccinii, Phomopsis sp., Fusarium spp. and bacteria were isolated from stems with each of the symptoms reported to be associated with the several canker, blight and gall diseases. It was therefore impossible to accurately diagnose the cause of dying blueberry stems in Michigan.

Fusicoccum canker caused by G. cassandrae f. vaccinii (16, 37) was reported in Michigan in 1964 (3) and the disease was known to be widespread. Little was known, however, concerning the disease cycle and pathological histology of Fusicoccum canker.

The major objectives of this research were to: a) determine the primary causes of canker and stem blight of blueberries in Michigan and to define symptoms associated with each organism; b) develop sufficient information concerning infection periods and sites of infection by Godronia to provide a basis for fungicide evaluation experiments; c) outline pathological histology during development of cankers caused by G. cassandrae f. vaccinii.

LITERATURE REVIEW

Introduction: Modern varieties of cultivated highbush blueberries consist of over 50 interspecific hybrids developed since the early 1900's. Most varieties are derived from crosses among 7 original selections from the wild and include genes from V. australe Aiton, V. larmarkii Camp, and V. corymbosum. For consistency, all varieties will be referred to in this thesis as V. corymbosum even though the genomes of some common varieties grown in Michigan such as Jersey consist entirely of V. australe genes (38).

All blueberry literature was reviewed recently and only pertinent disease literature will be discussed (23).

Stem blight and die-back diseases of Blueberries: Stem blight or die-back diseases of the highbush blueberry are caused by P. vaccinii (66, 67), B. dothidea (68), Botrytis cinerea Deb. (61), Monilinia vaccinii-corymbosi (Reade) Honey (61), and Glomerella cingulata Spaulding & von Schrenk (61).

Botrytis causes a twig and blossom blight of blueberries which is serious only in areas where cool, rainy conditions prevail (61). Certain symptoms of Botrytis twig blight can be confused with those caused by cold injury, B. dothidea, and P. vaccinii (68). The disease is generally

confined to succulent shoots, blossoms, and fruit and occurs wherever blueberries are grown (61).

Monilinia and Glomerella can infect young stems, but twig blights caused by these fungi are not important (53, 61).

Phomopsis twig and stem blight was reported in North Carolina, New Jersey, Massachusetts (66), and Nova Scotia (36). The disease was first reported by Stevens (61) and later described by Wilcox (66). Wilcox (67) established that the blueberry pathogen was D. vaccinii which also causes a cranberry fruit rot.

Wilcox (66) reported that black lesions developed on wounded or nonwounded succulent shoots within 2-3 days after artificial inoculation at 70-76 F. Lesions expanded and the organism eventually grew down inoculated shoots to older stems which were "girdled" within a few weeks. Dead portions of stems were sharply demarcated from living sections. Such symptoms resembled cold injury (61). Localized lesions developed when older stems were inoculated directly. Leaf spots developed on leaves 2 months after spray inoculated plants were placed in a cold frame (67).

Wilcox (66) concluded that Phomopsis infects and grows down succulent shoots to older stems which are "girdled." Varney and Stretch (61) reported that older stems are frequently "girdled" when the crown is infected by the fungus. Leaves on "girdled" stems wilt and turn brown during hot weather (66).

The meaning of "girdle" was vague in the reports of Wilcox (66) and Varney and Stretch (61). They reported that blight resulted when Phomopsis "girdled" stems, but it was not clear whether this meant that stems were cankered, vascular tissues were blocked, or both. Other investigators (36, 73) however, have mentioned that cankers were associated with P. vaccinii.

According to the literature, Phomopsis twig blight is a disease of weakened bushes and is a minor disease of blueberries (20, 61, 66). Sound cultural practices and eradication by pruning are the only recommended control measures (61).

It is not known when infection occurs although symptoms on young shoots were normally observed in the spring. Pycnidia were found on leaves and overwintering twigs, but the time of year when observations were made was not mentioned (66). Pycnidia were observed in February on a plant inoculated in July. It was not indicated whether plants were grown in the greenhouse or outside or if plants were observed between July and February (66). Pycnidia occurred on dead stems during August in Nova Scotia (36).

Perithecia of D. vaccinii form on cranberry fruit (67), but are rare on any blueberry tissues. Lockhart (36) observed perithecia on stems of V. angustifolium and V. corymbosum in Nova Scotia. This is the only report of Diaporthe perithecia on blueberries.

Blueberry stem blight caused by B. dothidea was serious in North Carolina. Yellowing, wilting, and browning of leaves on affected branches were the most conspicuous symptoms of the disease. Xylem of affected stems turned pecan brown and branches with browned leaves were often in close proximity to branches with healthy leaves (68). Similar symptoms have been observed on blueberries in Michigan. Blueberry stem blight is probably confined to the south (61) although a single report of a Botryosphaeria occurring on blighted stems of blueberries collected in Ohio, Michigan, New Jersey, and Illinois was published (44).

Canker diseases of blueberries: Several canker diseases of the highbush blueberry have been serious in North Carolina (9, 25, 55), Nova Scotia (16, 35), Massachusetts (70, 73), Washington (31), British Columbia (37), and Michigan (3).

Blueberry stem or cane canker caused by B. corticis prohibits cultivation of certain blueberry varieties in North Carolina (9, 25, 55). Except for a single report of the disease in New Jersey (61), stem canker does not occur in the north.

Fusicoccum canker caused by G. cassandrae f. vaccinii (16) occurs in Quebec (11), Nova Scotia (13, 16), British Columbia (12, 37), New Brunswick (16), Washington (25, 31), Michigan (3), Maine (16), Finland (30), Holland, and England (3). The disease is considered to be the

factor limiting increased cultivation of the highbush blueberry in Nova Scotia (35) and Washington (31). The disease was first reported in 1931 (11), but few definitive studies have been published.

The asexual state of G. cassandrae f. vaccinii was isolated from rotting cranberries and described as F. putrefaciens by Shear (46). Shear and Bain (47) later showed F. putrefaciens to be the imperfect form of G. cassandrae, a Discomycete described on leatherleaf (Chamaedaphnae calyculata (L.) Moench) by Peck in 1887 (42).

The imperfect state of G. cassandrae was first isolated from cankered blueberries in 1931 (11). In 1958, Creelman (16) and McKeen (37), reporting independently, called the disease Fusicoccum canker and blueberry canker, respectively. Although both found apothecia of Godronia on diseased stems, J. W. Groves, in a communication to Creelman (16), indicated that the organism on Vaccinium was not the same as the one on Chamaedaphnae and that the former was probably undescribed. However, following a thorough study of the genus, Groves (28) indicated that the organisms inhabiting Chamaedaphnae and Vaccinium were the same species and that Godronias on Betula, Spiraea, Calluna, and Ribes were also morphologically indistinguishable from G. cassandrae. Groves therefore tentatively erected several forms of G. cassandrae based on the genera of plants on which the fungi were found (Table 1).

Table 1. *Godronia cassandrae* as interpreted by J. W. Groves (28).

Forms of *Godronia cassandrae*			Host genera
G. cassandrae	f.	*cassandrae*	*Chamaedaphnae*
"	f.	*beticola*	*Betula*
"	f.	*callunae*	*Calluna*
"	f.	*ribicola*	*Ribes*
"	f.	*spiraeicola*	*Spiraea*
"	f.	*vaccinii*	*Vaccinium*

It must be stressed that this was not a formal taxonomic treatment and according to Groves (28):

> The taxonomic status of the forms described...is somewhat doubtful.... In view of the pathogenic significance of the *Vaccinium* fungus, it was felt desirable to maintain some sort of distinction between the strains occurring on different hosts and for consistent treatment it was decided to designate them as forms.

Groves (28) indicated that *Fusicoccum* is an unacceptable name for asexual states of *Godronia* because they are morphologically dissimilar to the description of the type of the form genus *Fusicoccum*. Groves felt that *Topospora* was the earliest acceptable name for macroconidial states of *Godronia*.

In 1968, Smerlis (51) reported that isolates of *G. cassandrae* from several ericaceous shrubs (*Andromeda glaucophylla* Link, *C. calyculata*, *Kalmia angustifolia* L., *Ledum*

groenlandicum Oeder, and V. angustifolium) were pathogenic on all ericaceous shrubs tested regardless of the source of the isolate. In addition, isolates of G. cassandrae from Alnus rugosa (Du Roi) Spreng. var. american (Regal) Fern., Betula alba L., B. papyifera Marsh, B. populifolia Marsh., and Salix sp. were pathogenic on all salicaceous and corylaceous hosts tested. However, isolates from the second group were not pathogenic on ericaceous shrubs and vice versa. Smerlis therefore placed G. cassandrae found on Ericaceae (other than Vaccinium species) into form cassandrae and isolates from the other hosts into form beticola. He also noted that isolates from V. angustifolium were culturally distinct from isolates from the other ericaceous shrubs, the latter being green with abundant mycelium, whereas those from Vaccinium were black, often slimy, with sparce, grey, aerial mycelium. Smerlis, therefore, placed G. cassandrae on Vaccinium species as form vaccinii (51).

Smerlis' (51) interpretation needs further study in light of McKeen's (37) failure to infect blueberries with isolates of G. cassandrae f. vaccinii from cranberry.

Cankers on 1- and 2-year-old blueberry stems first appeared as small reddish discolorations in the epidermis (16). Such lesions were observed in the winter in British Columbia (37) and late winter to early spring in Massachusetts (73). Incipient lesions expanded rapidly in the spring (37, 73) and became dark red (73), black (37), or

brown (16) in color. Such lesions in Nova Scotia had gray centers which turned brown and died (16). Some lesions coalesced in British Columbia (48). A "bullseye" pattern of concentric zones of alternating light and dark tissue on 1- and 2-year-old stems was considered diagnostic in Massachusetts (73) and was reported in Michigan (3), but not elsewhere (16, 37).

In Nova Scotia, cankers were up to 3-5 inches long and cankers on actively growing stems caused them to become flattened or depressed. As cankered tissue died, bark sloughed and cankers usually "girdled" infected stems in 1 year (16). Other workers (3, 37, 74) did not discuss canker development on stems older than 2 years.

The most striking symptom of the disease was the "flag" or wilted stem. Infected stems began to wilt and die in May in British Columbia (37) and Massachusetts (73), but not until June in Nova Scotia (16). Wilting, which took place within a few hours on warm, dry days (16), continued throughout the growing season (16, 37, 73). In one instance, it was reported that the interval between appearance of incipient lesions and cane death was about 18 weeks in Massachusetts (73).

Most infections were centered by leaf scars in British Columbia (37) and occurred at the ground level (37) and even below the soil surface (16), but some infections also occurred on higher parts of the stem (16, 37). Creelman

(16) reported that infection progressed from cankered crowns into bases of new stems.

Xylem beneath lesions turned brown (16, 73). Creelman (16) reported, "The cortex, cambium, and to a limited extent, the xylem are invaded by the fungus." He added, "Discolored areas in the xylem do not extend deeply before death of the stem occurs." No mention was made in the literature of Godronia killing stems without causing cankers (3, 16, 35, 37, 61).

The relative roles of conidia and ascospores in the disease cycle of Fusicoccum canker and when infection occurs are not known (3, 16, 35, 37, 61). Pycnidia were present beginning in late March in British Columbia (37), from mid-March to mid-July in Massachusetts (73), and from July to September in Nova Scotia (16). In Michigan, conidia were trapped with a Hirst spore trap during July, August, and September (3).

Apothecia were abundant in Nova Scotia with successive annual crops of apothecia occurring on 3-year-old pruning stubs and diseased prunings (16), but were scarce in British Columbia (37), and were not reported in Massachusetts (73). McKeen (37) concluded that ascospore inoculum was unimportant in British Columbia because apothecia were rare. Dispersal of ascospores corresponded with the appearance of pin point lesions in Nova Scotia. It was not stated when this occurred, however apothecia were found in May (16).

Although different workers have proposed spring (16) and fall (35,37) infection periods, sound evidence in support of either is lacking. Creelman (16) and Fitzpatrick and MacSwain (14) apparently felt that infection occurred in the spring since they applied protective fungicides at that time.

McKeen (37) reported that "some, if not all" infection occurred after June in British Columbia since parts of stems which were formed during July and August were diseased. However, he did not state when or how often infections on new stems were observed. Artificial inoculations made in November and December caused cankers whereas those performed in June and July did not. Conidia were reported to be most abundant in the spring and ascospores were considered to be unimportant. McKeen concluded that infection by Godronia probably occurred during late summer and fall rains.

Lockhart (35) concluded that infection occurred in the fall because inoculations were successful during October, May, and August, but not during July "when plants were growing vigorously." He added, "The successful inoculations of August 28 and October 2 coincided with slowing down or cessation of growth." He implied that inoculations made in May which did not cause visible cankers until late August supported this conclusion.

In Massachusetts, Zuckerman (73) obtained infection on plants inoculated in a screenhouse during March and April, but did not speculate on when infection occurred in the field.

It is clear that existing data did not tell us when Godronia infects blueberries.

Several workers have reported apparent differences in field susceptibility of blueberry varieties to Fusicoccum canker. Data published on susceptibility of varieties of blueberry are summarized in Table 2.

No effective control measures have been developed, although Lockhart (38) and Nelson (39, 40) have reported limited control when compounds such as phenyl mercury acetate were applied in the fall.

Cultural characteristics of G. cassandrae varied considerably depending upon source of isolates and culture media. Shear (46) reported that isolates from cranberry varied in color from shades of yellow-green and yellow to pink and brown depending upon the culture medium. McKeen (37) indicated that isolates from cranberry and blueberry differed in color and colony texture on a range of media. He also stated that single conidial and single ascospore cultures of the isolates from blueberry differed from one another. He noted that G. cassandrae f. vaccinii varied from brown to buff-pink to grey. Cranberry isolates had more aerial mycelium and sclerotia were produced by single

Table 2. Susceptibility of varieties of blueberry to infection by Godronia cassandrae f. vaccinii.

Variety	Susceptibility[a] rating	Reference
Atlantic	-	14
Berkley	H	35
Blueray	H	35
Burlington	H	15, 35
Bluecrop	H	35
Coville	H	35
Cabot	-	73
Concord	R	35
Jersey	H[b]	3, 14, 15, 35, 48, 73
Earliblue	H	35
Johnson	H	35
Pemberton	-	3, 14, 73
Pioneer	H	73
Rubel	R-L	3, 37, 73
Rancocas	R-L	15, 35, 37
Stanley	M	3, 35

[a] H = high susceptibility; M = moderate susceptibility; L = low susceptibility; R = resistant; - = infected, but no data given on relative susceptibility.

[b] Jersey was usually considered to be most susceptible and Rancocas most resistant.

ascospore and condial isolates from blueberry (37). Smerlis (51), as mentioned earlier, also noted differences in colony texture and color when cultures of forms cassandrae and vaccinii were compared. Gremmen (27) reported that colonies were gray at first becoming gray-green to yellow-green. Groves (28) stated that cultural characters are quite variable and that even spore morphology can vary "depending upon the preparation." Groves (28) also reported that microconidia were common both on the host and in cultures of G. cassandrae.

Godronia cassandrae grew at temperatures as low as 0-4 C (16, 37). McKeen (37) reported that maximum growth occurred at 20 C on potato dextrose agar and growth was inhibited at 30 C. At 4 and 10 C, growth was 50 and 60% of the maximum, respectively (37). Stevens (52) reported that cranberry end rot caused by G. cassandrae f. vaccinii developed well at 0 C and 15-20 C, but the lower temperature was more favorable for development of rot.

Coryneum canker caused by Coryneum microstictum Berk. & Br. was reported in Massachusetts by Zuckerman (71) who later demonstrated pathogenicity of the organism on blueberries (73). Acervuli of the organism were common on sun scald areas and well developed cankers were sunken in appearance with acervuli on the surface. Cankers expanded until stems were completely "girdled" and parts of the stem above the "girdle" died. In addition to C. microstictum,

P. vaccinii was also isolated from several branches with symptoms of Coryneum canker (73).

Based on field and inoculation studies, Zuckerman (73) concluded that Coryneum affected only weakened plants. Coryneum canker has not been reported elsewhere (61).

Other organisms which are known to cause cankers of blueberries either are not important or are confined to areas of limited cultivation. Bacterial canker caused by Pseudomonas syringae Van Hall was reported in British Columbia (37), Washington and Oregon (62), but not elsewhere.

Overgrowths, tumor, and gall diseases of blueberries: Overgrowths of various types on stems of blueberries were reported caused by A. tumefaciens (21), Nocardia vaccinii Demaree and Smith (22), Pucciniastrum myrtilli (Schum.) Arth. (61), and Phomopsis sp. (7). A root gall disease was described (69), but the cause is not known.

As the name indicates, bud proliferation gall caused by N. vaccinii, is characterized by extensive bud proliferation in the crowns of infected plants (22). The disease is not economically important and is distinct from symptoms observed in Michigan.

A witches broom caused by P. myrtilli has been found in Michigan (39), but the disease is distinctive and extremely rare in the state.

In 1938, Brown (7) reported that a Phomopsis, distinct from P. vaccinii, caused galls on inoculated

blueberries. She reported the disease in Massachusetts, New Jersey, Oregon, and Michigan. The gall symptoms produced on inoculated blueberries were not striking and as Varney and Stretch (61) indicated, "...were more indicative of cankers than galls." Calluses developed on Jasmium nuidflorum and Viburnum opulum more quickly and more extensively than on blueberries (7). Brown also reported Phomopsis galls on Ulmus americana (8), Acer sp. (8), Privet (8), coral berry (8), Forsythea sp. (8), Quercus sp. (6), Viburnum (5), and Fagus sp. (6). Her results on blueberry have never been repeated (54).

Demaree and Smith (21) reported that tumors identical to the Phomopsis galls described by Brown (7) were induced by isolates of A. tumefaciens obtained from blueberries. The disease was observed in New Jersey, New York, Michigan, Washington, and British Columbia. Most galls were on branches and small twigs, but some were at bases of canes near the ground line. Galls were not found on roots. Affected buds died and galls were perrenial. Detailed studies of galls indicated that the causal organism was a strain of A. tumefaciens distinct in host range and in cultural characteristics from A. tumefaciens on apple and peach. Only A. tumefaciens isolated from blueberry was pathogenic on that host and the bacteria produced large galls on inoculated plants. Apple and peach strains, although infective on their respective hosts, did

not affect blueberry, confirming Brown's (7) results with similar isolates. Phomopsis was not associated with the tumors and none of the fungi found in galls caused callus formation on inoculated blueberries (21).

A gall disease described in 1956 by Zuckerman (69) as a new root-gall disease was caused by an unknown agent. The disease was characterized by galls on all woody portions of the plant. Zuckerman described galls as being either white and coriaceous or dark brown, woody and covered with bark. Long basal cankers were often associated with galls. According to Zuckerman (69), death of affected branches was not due to the "girdling" action of the cankers. No data supporting this conclusion were presented. He further reported that some plants had galls on the roots, but not on aerial portions. Histological sections revealed no hyphae or nematodes in gall tissue. When ground gall material was injected into Pioneer variety stems, leaves wilted within 3-5 weeks after inoculation and stems died within 3 months. Adjacent stems died during the year after inoculation. Gall and canker symptoms did not appear on inoculated plants. No pathogenic organism was isolated from affected tissue. Pioneer, Cabot, and Wareham appeared to be very susceptible, but Jersey, Rubel, and Dixi appeared resistant. Zuckerman (69) concluded that the disease was not crown gall since symptoms differed from those of A. tumefaciens affected plants. He cited, for example, that

galls did not occur on roots in Demaree and Smith's (21) study. The size and position of tumors on affected stems differed from crown gall tumors and cankers were not associated with crown gall tumors studied by Demaree and Smith (21).

In Michigan, the symptoms associated with dying stems of blueberry plants overlapped those reported for Fusicoccum canker (16, 37, 73), Coryneum canker (73), Phomopsis twig blight (61, 66, 67), Botryosphaeria stem blight (68), Phomopsis gall (7), crown gall (21), and root gall (69). Phomopsis vaccinii, G. cassandrae f. vaccinii, and C. microstictum were associated with the symptoms in the field (64).

PART I. SYMPTOMATOLOGY.

Methods and Materials.

Association of organisms with symptoms: Blueberry stems with canker or blight symptoms were collected from various locations in Michigan and Indiana over a 3-year-period. All stems were coded according to symptom, variety, date, and location of sampled fields. Preliminary investigations indicated that symptoms could be categorized according to the scheme of symptoms illustrated in Figures 1-6. All symptom data are based on these categories.

Early observations showed that growth of Godronia from diseased tissues was significantly reduced if stems dried or were exposed to temperatures exceeding 25 C for more than 24-48 hours. Stems were therefore stored at 10-12 C in tightly wrapped plastic bags after they were collected from the field.

Stems were cut into 1-2 cm long sections, surface sterilized in 1.3% sodium hypochlorite plus 2-3 drops Tween 20 surfactant for 1 minute, and placed on (see Table 3 for descriptions of all media used in this research) 15-20 ml ½ PDA (pH = 5.6-6.8) in plastic petri plates. Half strength PDA was used because preliminary investigations showed that G. cassandrae f. vaccinii sporulated more profusely on

½ DPA than on PDA or ¼ PDA. All cultures were grown in the dark at 10-12 C for 6-18 weeks. Growing cultures at 10-12 C had several advantages. Godronia grows slowly even at optimum temperatures. Maintaining isolation plates at 10-12 C allowed ample growth of Godronia, Phomopsis, and Coryneum while minimizing overgrowth of cultures by common saprophytes. Sections could be plated and held in the refrigerator for several months. Large numbers of stems were collected, sectioned, and plated during the summer and observed as time permitted. Commonly observed fungi were isolated and identified. The same procedures were used when reisolating fungi from inoculated tissue.

Table 3. Ingredients and abbreviations of media used in this research.

Medium	Abbreviation	Ingredients/ liter
Potato dextrose agar	PDA	39 g Difco potato dextrose agar
½ potato dextrose agar	½ PDA	20 g Difco PDA, 8 g agar
Malt agar	MA	45 g Difco malt agar
Nutrient agar	NA	23 g Difco nutrient agar
Dextrose, nutrient, peptone, yeast agar	DNPYA	20 g PDA, 20 g NA, 5 g Difco bacto peptone, 8 g agar, 5 g Difco bacto yeast extract.

Isolation of organisms from diseased tissue: Monoconidial isolates of fungi were obtained in one of the following ways: a) spores were harvested directly from a sporophores with a sterile needle; b) single spores were picked from the surface of agar after streaking suspensions of conidia on the agar surface; c) single colonies of fungi were harvested after spores from a suitable dilution series had germinated and grown in nutrient media.

Single ascospores of Godronia were harvested from from the surface of a thin layer of agar in plastic petri plates after spores had been ejected from inverted apothecia fastened to the lids with masking tape.

Hyphal tip isolations were necessary when fungi did not sporulate.

All isolates were transferred initially to ½ PDA acidified to pH 3.5 with 10% lactic acid in order to minimize bacterial contamination. Following initial isolation, all fungi were grown on ½ PDA (pH = 5.6-6.8) at 22-25 C in the laboratory and were transferred every 4-6 weeks.

Bacteria which grew from diseased tissue were suspended in a suitable volume of sterile distilled water and streaked on NA or DNPYA (pH = 6.8). Single colonies were then transferred and grown on the same media at 22-25 C. All bacteria were transferred weekly.

Inoculations: Depending upon the experiment, 1- or 3-year-old Jersey, Blueray, or Bluecrop variety blueberry

plants were inoculated. All 3-year-old plants were grown out-of-doors in field soil contained in 12 quart galvanized pails. One-year-old plants were grown in 1:1 (v/v) mixture of Michigan peat and sandy loam contained in 8-inch clay pots. Soil pH was not determined. One-year-old plants were transplanted from cutting beds as 7-month-old cuttings and grown at 23-30 C under continuous light (daylight supplemented with two 500 watt incandescent bulbs/bench) in the greenhouse until large enough to inoculate (Fig. 8-A). All plants were fertilized bimonthly with 25 ml of a solution containing 1 tablespoon Plant Marvel (12, 31, and 14% N, P, K, respectfully) / gallon tap water.

Inocula consisted of fungi from 4-week-old cultures or bacteria which were transferred daily for 1 week prior to inoculations. After swabbing stems with 95% ethyl alcohol, water suspensions of conidia, mycelium in blocks of ½ PDA, or a single inoculating loop of bacteria were placed on the stems. Depending upon the experiment, the inoculum and the stem tissue were pierced 1-25 times with a sterile needle. In some studies, the inoculum was inserted under 1.5 cm long V-shaped flaps cut into the bark or epidermis. Following wounding, the inoculation sites were wrapped with sterile, moist cotton and wrapped with plastic film held in place with rubber grafting strips (Fig. 7-A). The number of inoculations per plant varied from 1-10 depending upon the experiment.

All plants were placed in a mist chamber (18-25 C, 90% + relative humidity) for 5-10 days. Cotton and plastic were removed and the plants were transferred to a greenhouse and grown at 15-20 C under continuous light after the 5-10 day incubation in the mist chamber. Controls consisted of: a) separate plants inoculated 5-10 times with blocks of ½ PDA or sterile distilled water and b) single control inoculations on each inoculated plant.

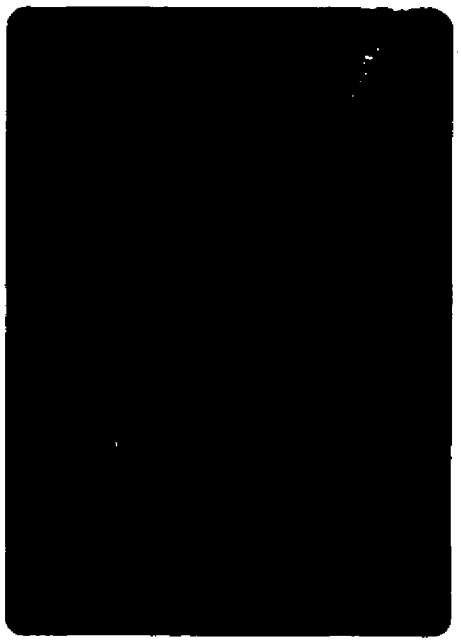
Fig. 1. Lesion-young canker infection type on 1- and 2-year-old stems. All lesions were caused by Godronia.

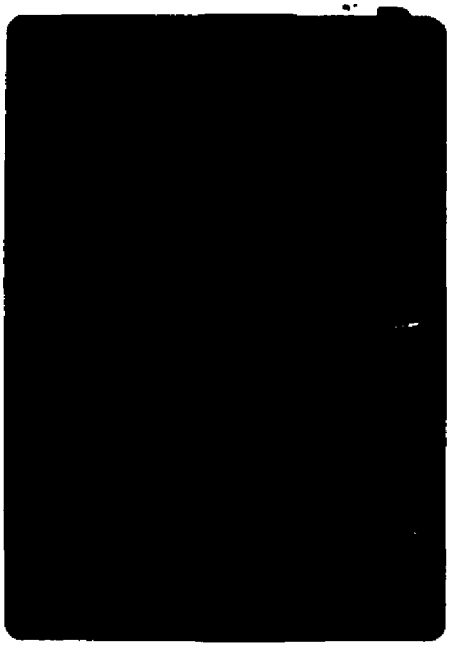
A) Incipient lesions (red spots) on 5-month-old stem collected from an upper Michigan field 3 December, 1968.

B) Lesions of various sizes on a 1-year-old stem collected from an upper Michigan field 15 June, 1967.

C) Lesion at the base of a 4- to 5-month-old stem collected from a northern Michigan field 15 October, 1965. Note the leaf scar at the center of the lesion and the absence of pycnidia.

D) Typical lesions on 1-year-old stems collected in mid-summer. Note the split epidermis and the lesion at the base of the lateral on (a) and the "bullseye" pattern of the lesions with pycnidia on (b) and (c).





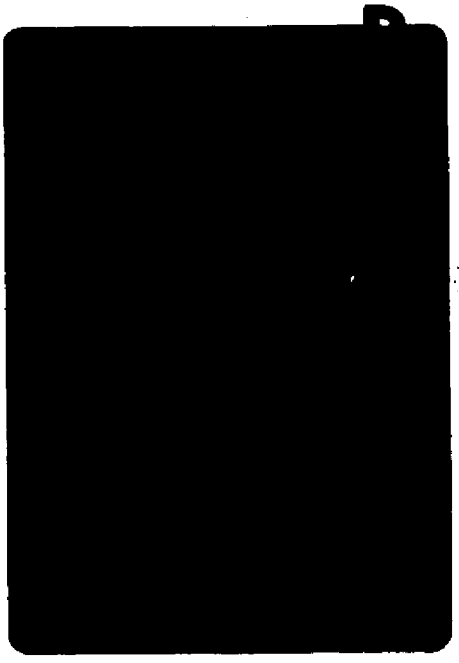


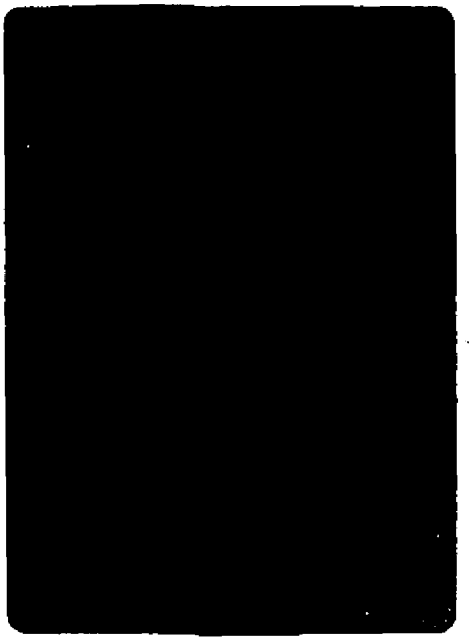
Fig. 2. Lesion-young canker infection type on 1- and 2-year-old stems. All stems infected with Godronia.

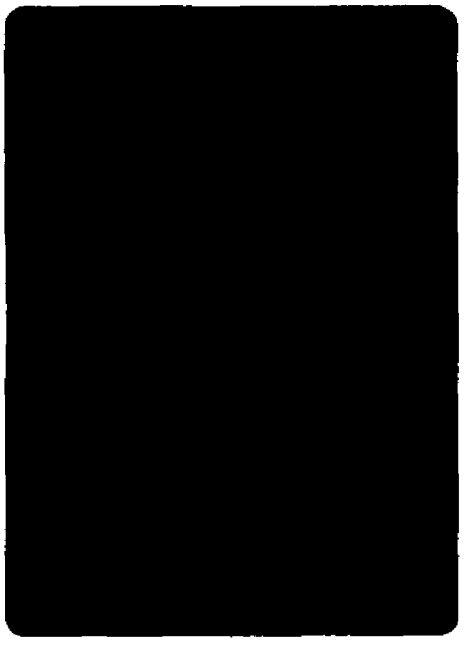
A) Lesion on 1-year-old stem collected from an upper Michigan field 15 June, 1967. Note the pycnidia. Split in the center is atypical.

B) Typical "bullseye" lesion with pycnidia on a stem collected in late May. The red-brown to maroon coloration is common.

C) Young canker on a 1-year-old stem collected in July. Note the depressed center and dead epidermis and that the canker is wide in relation to its length. A few pycnidia are visible.

D) Young canker on 2-year-old stem collected 15 October, 1965. Note the leaf scar at the center and that the canker is wide in relation to its length. Smooth, tan colored epidermis (a) was considered to be caused by sun scald.





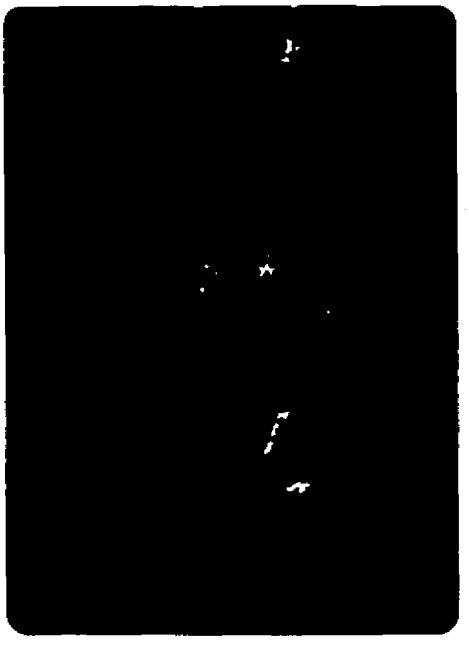


Fig. 3. Developed canker infection type on older stems. All cankers were caused by Godronia.

A) Cankers and depression on 3 to 4-year-old stems. The gnarled appearance (b) and cankers which were wide in relation to their length (a) were common on older stems infected with Godronia.

B) Developed canker on 4-year-old stem. Note that the bark is dead and flaking, the hint of callus at the edges of the canker and the leaf scar near the center.

C) Mild expression of callus type 8-E. Small internodal lesions (Fig. 1-B) observed in April were often apparently walled-off by August as shown here.

D) Callus type 8-E. This symptom was similar to calluses formed when plants were mechanically wounded. Note the smooth, rounded edges of the callus and the pycnidia at the center.

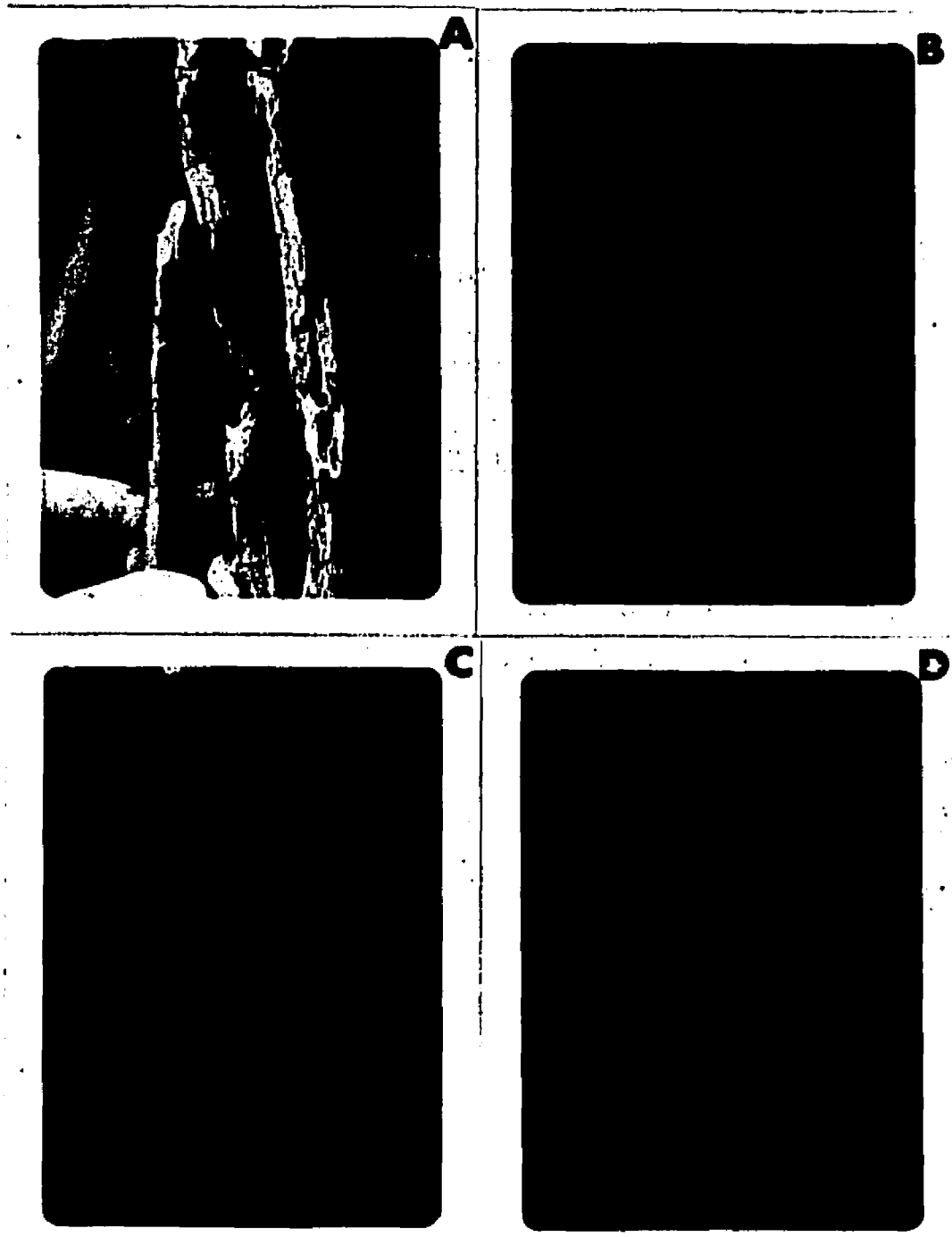

A
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Fig. 4. Callused blueberry stems.

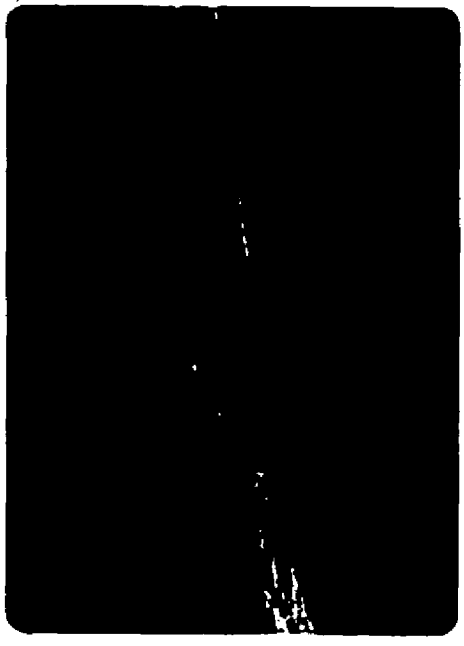
A) Callus type 8-A on 3-year-old stem of a Stanley variety plant. This type of callus was at the bases of lateral branches and there were always cankers at the base of the callus.

B) Callus type 8-A on Pemberton variety.

C) Callus type 8-B on Earliblue variety. The cause of this type of callus was not proven. Note the corky appearance and that the callus extends the length of the affected stem. Cankers (a) were usually found associated with 8-B calluses.

D) Callus type 8-B on Earliblue variety.

E) Photograph off crown gall on blueberry provided by the Canadian Department of Agriculture. Note the similarity to the 8-B calluses in C and D.



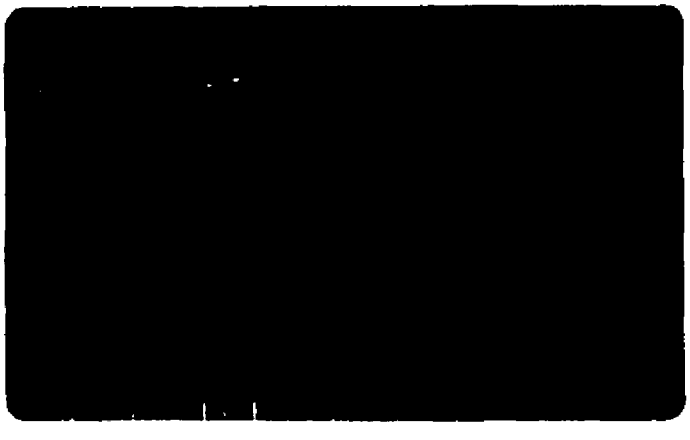


Fig. 5. Callused blueberry stems. Godronia was isolated from stems in A, B, D, and E.

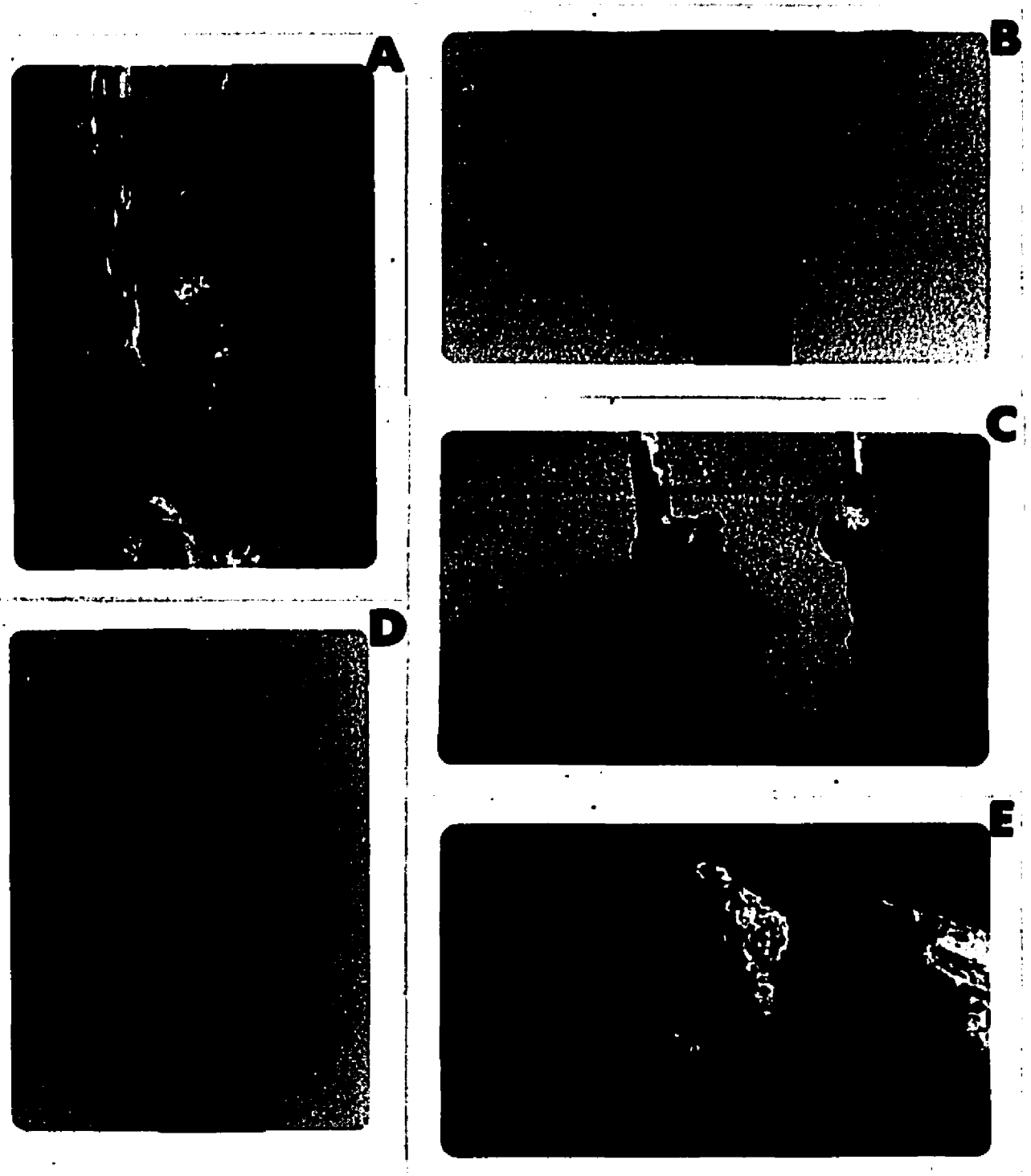
A) Callus type 8-C on Jersey variety. This type of callus was characterized by being located in crowns of affected plants. The callus was often concentrated along edges of large cankers.

B) Callus type 8-C. Note that calluses occur at the bases of each lateral originating from the cankered crown.

C) Crown gall (?) symptoms on blueberry in Michigan. Photograph supplied by J. W. Nelson, Michigan Blueberry Growers Association. As far as could be determined, diagnosis was based on symptoms only.

D) Callus type 8-D. Note that the callus occurs along edges of a canker located above the crown.

E) Callus type 8-D. Note the large size of the callus and that it occurs along the upper edges of a canker (a) located above the crown.


A
B
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E

Fig. 6. Miscellaneous symptoms associated with blight and canker diseases of blueberry in Michigan. Godronia was isolated from each of the stems.

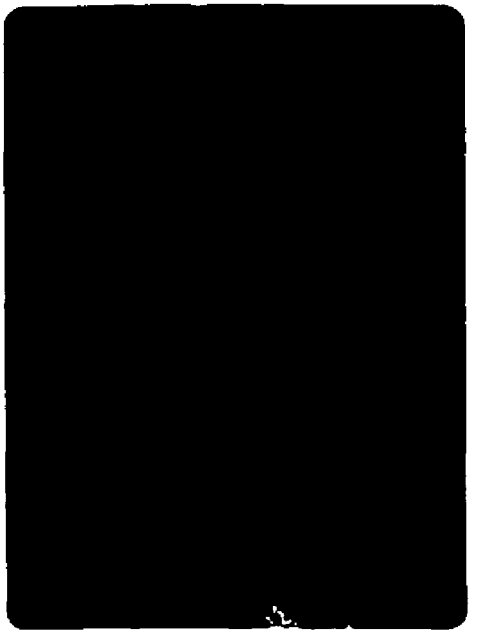
A) Discolored bark or epidermis infection type on unnamed variety photographed in May.

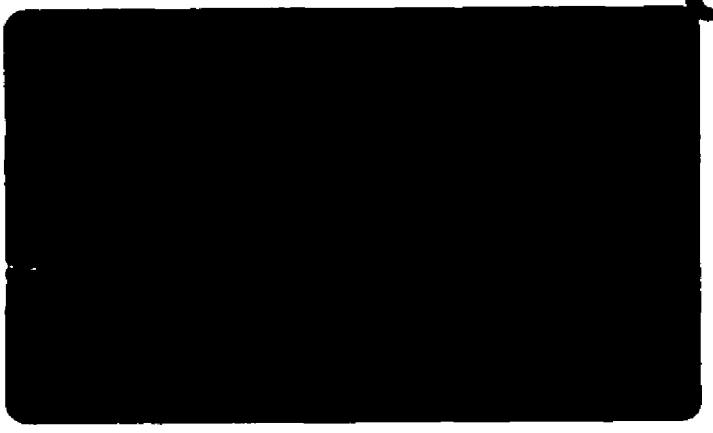
B) Split or flaking bark or epidermis infection type on 3-year-old stem photographed in late June. Note the erumpent pycnidia of Godronia concentrated in the bark fissures.

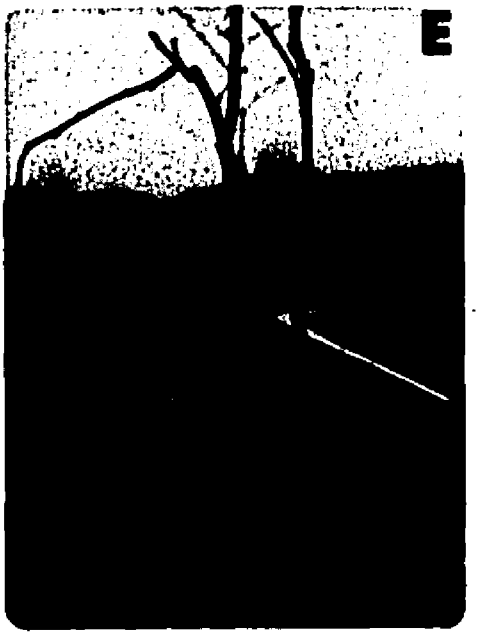
C) Split or flaking bark or epidermis infection type on 2 to 3-year-old stems. Note the depression (a) and the red-brown discoloration (b).

D) Discolored bark or epidermis infection type on 1-year-old stem of an unnamed variety photographed May 29, 1968. Note the sporulating pycnidia.

E) Discolored bark or epidermis infection type on a 2-year-old stem photographed in May.







Experimental Results

Association of organisms with symptoms: The genera of fungi identified on diseased stems are shown in Tables 4 and 5. Only those fungi which occurred on more than 2.0% of sections observed are discussed.

Alternaria spp., Epicoccum sp., B. cinerea, and Papulospora sp. were considered to be saprophytes and unimportant in canker or blight etiology. Alternaria and Epicoccum are common saprophytes on Vaccinium spp. in Massachusetts (65, 72), New Jersey (52, 65), North Carolina (65), Washington (25), and Wisconsin (26). Botrytis causes a twig and blossum blight of blueberries (61), but the association of Botrytis with diseased stems was infrequent until the cool, rainy 1968 season. Papulospora spp. are generally secondary invaders of diseased tissue and have not been reported on blueberries (58). Papulospora was generally associated with weakened tissue and especially with old, weathered calluses.

Although Fusarium spp. have not been reported on blueberries before, species such as F. solani and F. oxysporium are known to cause cankers (4, 34, 43, 48, 56, 57) and overgrowths (1) on other woody plants. Fusarium spp. isolated from cankered and callused blueberry stems did not cause disease when used to artificially inoculate healthy plants.

Table 4. Fungi isolated from stem sections taken from cankered and/or blighted blueberry plants.[a]

Fungi[b]	% sections with fungus
Godronia cassandrae f. vaccinii	40.0
Diaporthe (Phomopsis) vaccinii	27.0
Coryneum microstictum	2.9
Fusarium spp.	2.0
Botrytis cinerea	6.0
Alternaria spp.	48.4
Epicoccum sp.	27.2
Papulospora sp.	4.6
Coniothyrium sp.	<2.0
Verticillium sp.	<0.1
Phoma sp.	<2.0
Dendrophoma sp.	<2.0
Melanospora sp.	<0.1
Pullularia spp.	<2.0
Sphaeronema sp.	<0.1
+Bispora sp	<2.0
+Cephalosporium sp.	<0.1
+Cylindrocarpon sp.	<0.1
Pyrenochaeta sp.	<0.1
unidentified	<3.0

[a] Based on 3130 sections collected during 1966-68.

[b] +identification tentative.

Table 5. Sporulating fungi identified on sections of diseased blueberry stems placed in moisture chambers.[a]

Fungi	Fruiting type[b] Sexual	Asexual
Glomerella cingulata	+	+
Nectria cinnarbarina	-	+
Pestalotia sp.	-	+
Penicillium spp.	-	+
Trichoderma spp.	-	+
Sordaria spp.	+	-
Tympanis sp.	+	?
Chaetomium sp.	+	-

[a] Godronia and Phomopsis also sporulated in moisture chambers, but these data are not presented.

[b] + = observed; - = not observed; ? = not determined.

The association of Godronia, Phomopsis, and Coryneum with symptoms observed on diseased blueberry stems is shown in Table 6. Each organism was associated with all of the symptom categories, but Coryneum was relatively uncommon.

Table 6. Association of canker fungi with symptoms observed on blueberry stems.

Symptoms	Percent sections with fungi			Total sections
	Godronia	Phomopsis	Coryneum	
None	20.0	38.4	2.0	242
Lesion-young canker	65.0	8.1	0.8	406
Developed canker	32.9	27.9	1.9	670
Callused canker	71.2	13.3	3.2	247
Discolored bark or epidermis	22.5	22.4	4.7	695
Split or flak-bark or epidermis	39.6	36.6	2.6	870

Isolates of Phomopsis from diseased blueberry stems collected in Michigan and Indiana had the following characteristics. Single ∝ conidium isolates grown on ½ PDA were shite to grey in color. Colonies were velvety in texture with concentric zones of abundant and sparce aerial mycelium. All isolates attained growth at 25-30 C (Fig. 9).

Isolates obtained from blighted stems collected in New Jersey were similar except that they were black in color. Only α conidia were produced by single α conidium isolates when they were grown on ½ PDA or PDA. Alpha conidia were hyaline, oyoid, biguttulate, and measured 6-8 x 3-4 μ in lactophenol (2). Beta conidia measured 1.0 x 15-20 μ.

Pycnidia on naturally infected stems varied as to the type of conidia produced. Some pycnidia produced only α conidia or only β conidia whereas others produced both types. Production of α and β conidia was affected by light (45), culture media (67), temperature (33, 60), and host substrate (41) in studies of other species of Phomopsis. In this research single α conidium isolates from pcynidia producing both spore types produced only α conidia in culture. Production of both α and β conidia was not considered a suitable character for positive identification of Phomopsis isolated from blueberry. Therefore isolates were considered to be Phomopsis when they produced α conidia and colonies similar to those produced by single α conidium isolates from pycnidia producing both spore types. That this Phomopsis was P. vaccinii was determined by pathogenicity on blueberries, cultural characteristics and spore morphology.

Characteristics of G. cassandrae f. vaccinii isolated from blueberries will be discussed in Part II of this thesis.

Cultures of Coryneum were not studied in detail, however, single conidium isolates produced gray to brown aerial mycelium on ½ PDA. Acervuli were produced on 10-12 week old cultures. Conidia were 3-4 celled, honey colored, and measured 17-20 x 5.8-8.1 μ in lactophenol (Fig. 8-C). These observations are in accord with Zuckerman's (73) description of C. microstictum isolated from blueberries in Massachusetts.

Inoculation of plants in the greenhouse: Only Godronia and Phomopsis were pathogenic on 1-year-old Jersey, Blueray, and Bluecrop plants inoculated with Godronia, Phomopsis, and Coryneum (Table 7). There were no differences in response of the 3 varieties to a particular pathogen. Symptom development was followed on the inoculated plants for 10-24 months.

Table 7. Results of artificial inoculations of blueberry plants with isolates of Godronia, Phomopsis, and Coryneum.

Fungus	Number isolates tested	Number of inoculations	% inoculations causing disease
Godronia	8	183	82.3
Phomopsis	5	125	52.0
Coryneum	2	8	0

Both Godronia and Phomopsis caused lesions on inoculated 6 month to 1-year-old branches. Lesions caused

by Godronia were dark colored at first (Fig. 7-C) and turned red-brown to brown (Fig. 7-D, 8-B, 11-D). Bands of water-soaked and reddened tissues were often apparent at the periphery of necrotic tissues (Fig. 7-D). The centers of lesions turned gray after a few weeks due to death of cortex and epidermis (Fig. 8-B). Pycnidia developed on lesions within 2-3 weeks and continued to appear for an additional 4-7 weeks when plants were grown under continuous lights in a 15-20 C greenhouse (Fig. 7-D). Inoculated branches wilted within 7 weeks (Fig. 7-I), but fewer than 1% of branches inoculated with Godronia wilted under these conditions. The lesions attained maximum size within 4-7 weeks. Outer tissues died and sloughed off 10-15 weeks after inoculation (Fig. 7-E). As greenhouse temperatures increased during the spring, cankers appeared to become localized by the host. Such cankers did not expand when plants were transferred to a cold frame and grown outside for 1 year (Fig. 10-C).

Lesions caused by Phomopsis developed more rapidly than those caused by Godronia and by the 10th day after inoculation (Fig. 7-H) succulent shoots were wilted. Lesions on 6 month to 1-year-old stems were darker and larger than Godronia lesions of a comparable age (Fig. 7-F, G). Lesions on succulent shoots coalesced and large areas on the inoculated stems were necrotic within 10-15 days after inoculations (Fig. 7-H). Pycnidia developed in

Fig. 7. Inoculation results.

A) Wrapped inoculation site.

B) Control inoculation.

C) Response on Jersey variety 10 days after inoculating with G. cassandrae f. vaccinii. The plant was grown in the 18 C mist chamber following inoculation.

D) Response on Jersey variety 9 weeks after inoculating with G. cassandrae f. vaccinii. Note the pycnidia and the discoloration in advance of necrosis. The plant was grown in a 18 C greenhouse.

E) Canker on Jersey variety 11 months after inoculating with G. cassandrae f. vaccinii. Note the similarity to Fig. 2-C. The plant was grown in a cold frame following inoculation.

F) Response on Bluecrop variety plant 10 days after inoculating with P. vaccinii (b). Control inoculation is at (a). The plant was grown in the 18 C mist chamber.

G) Lesion on Bluecrop variety plant 10 days after inoculating with P. vaccinii.

H) Young shoots which wilted 10 days after inoculating with P. vaccinii. Plant was grown in the 18 C mist chamber.

I) Jersey variety stem wilted 6 weeks after inoculating with G. cassandrae f. vaccinii. The arrow indicates the inoculation site. The plant was grown in a 18 C greenhouse following inoculation.

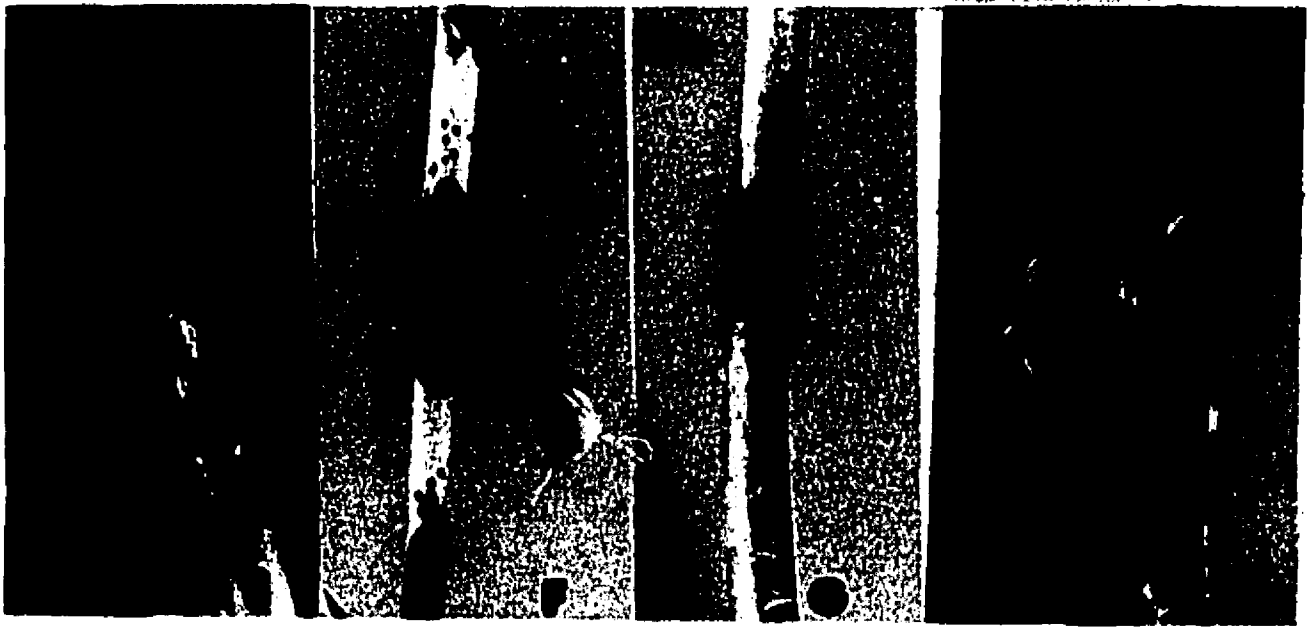
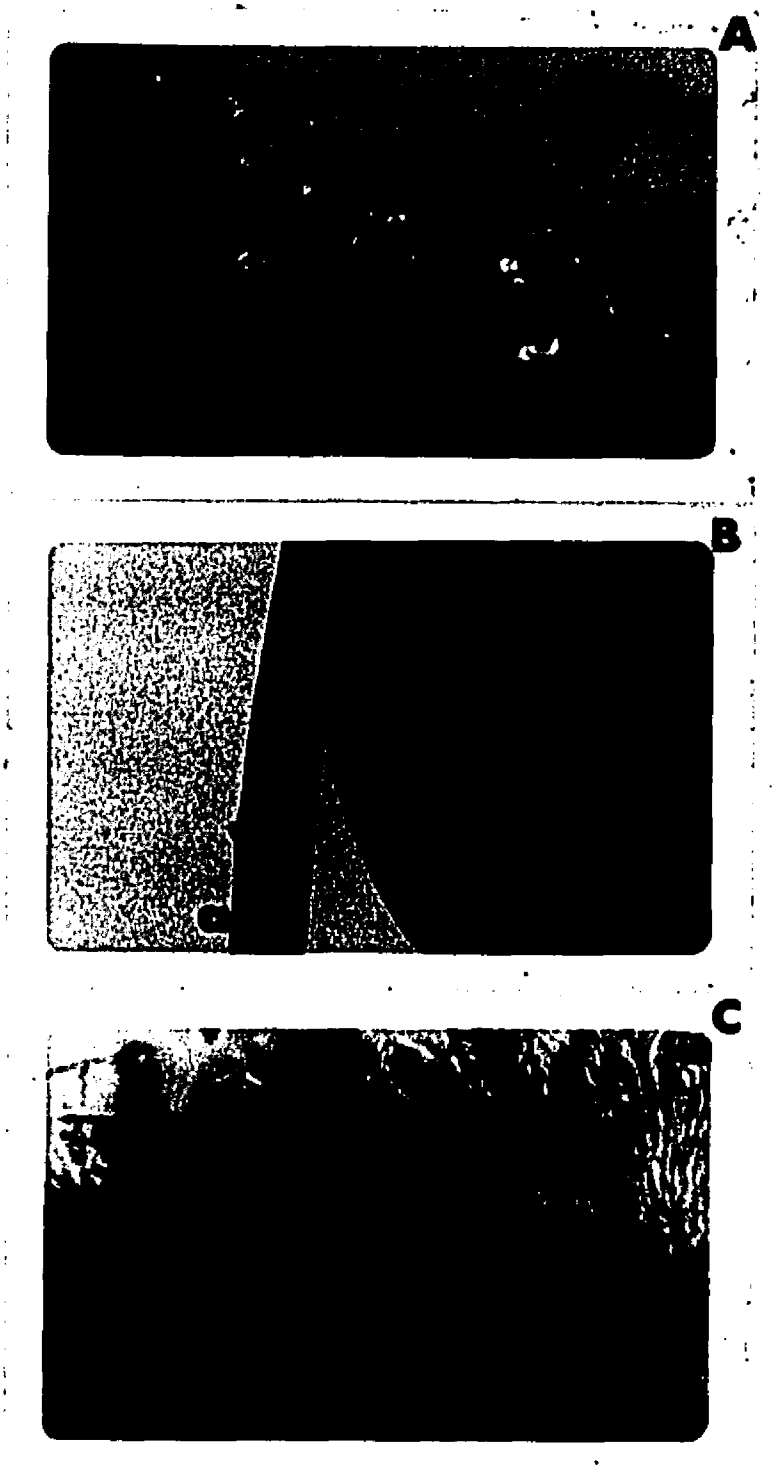


Fig. 8.

A) Typical plant inoculated in studies performed in the greenhouse.

B) Stem naturally infected with Godronia (a) and artificially inoculated stem (b). Note the gray-brown centers and maroon colored edges of the lesions.

C) Section through acervulus of C. microstictum on a blueberry stem (400 X). Section was cut with an experimental microtome (29) and mounted in water.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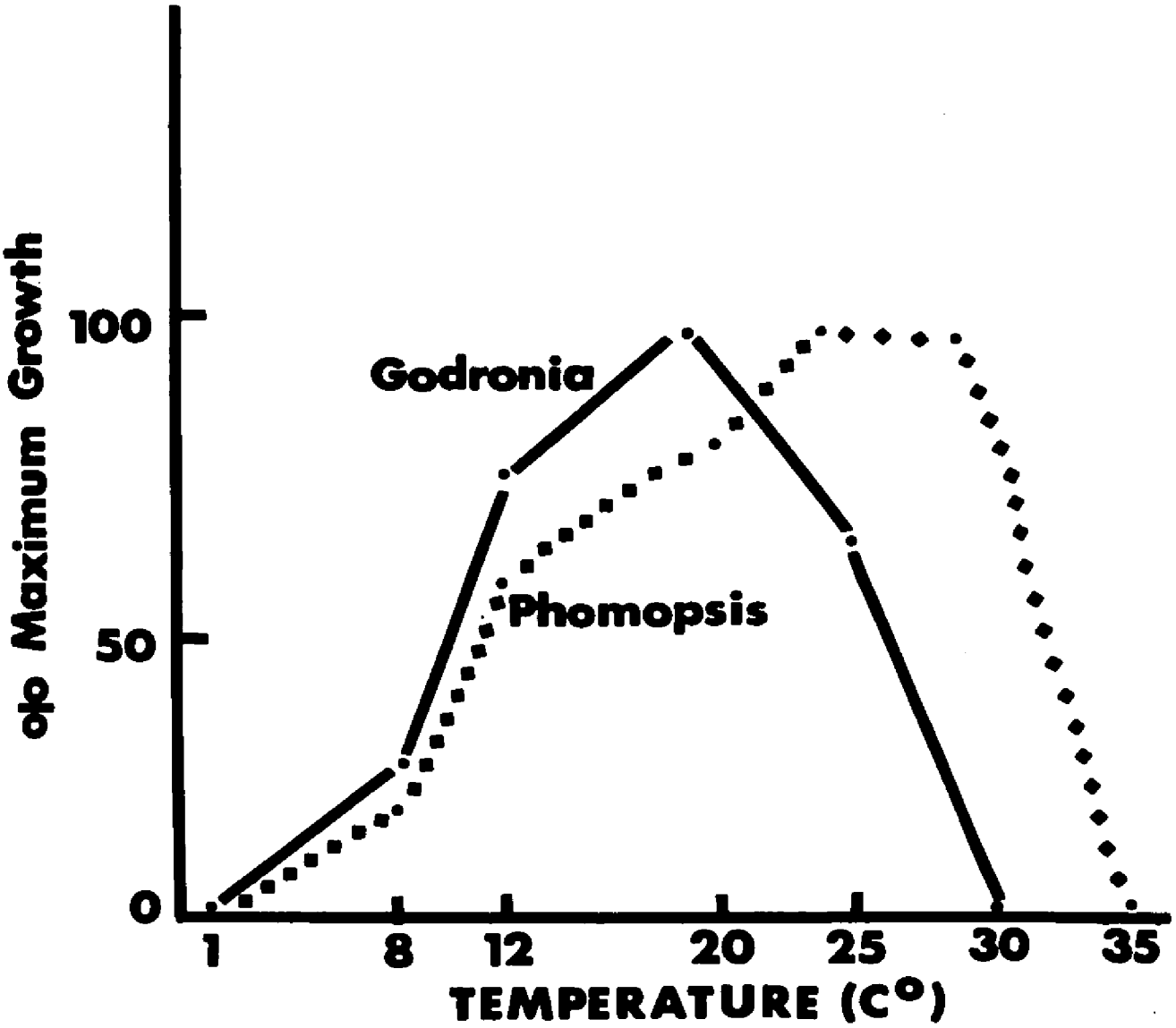

Fig. 9. Percent maximum growth of Phomopsis vaccinii and Godronia cassandrae f. vaccinii at various temperatures. Each point on the curves represents average diameters of 5 colonies of 2 separate isolates of each fungus. Measurements were taken after 14 days growth on ¼ PDA at the respective temperatures.

necrotic tissues by the 15th day on some plants and continued to appear for 2-3 months (Fig. 12-D). Extensive necrosis surrounding the inoculated branches did not develop on plants inoculated with Godronia. The disease continued to develop for several months even when greenhouse temperatures exceeded 27-30 C. Dead tissues were sharply demarcated from living portions of inoculated stems (Fig. 12-C).

All lesions did not expand on 1- and 2-year-old stems (Fig. 11-E) and only 60% caused wilt. Those lesions which expanded on older stems did so very slowly, but eventually killed most of the inoculated plant (Fig. 11-B).

Five inoculated plants on which Phomopsis lesions did not develop, were exposed to moisture stress 1 year after they were inoculated. Cankers did not develop, even though Phomopsis could still be isolated from the inoculation sites.

In another experiment, Godronia cankers which were apparently walled off, were reinoculated with Phomopsis. The reinoculated branches died within 3 weeks. Control inoculations with blocks of ½ PDA did not expand. The experiment was executed during the summer when temperatures in the greenhouse exceeded 30 C. These stems died more quickly than most stems inoculated with Phomopsis alone. The possibility of Godronia and Phomopsis being synergistic warrants further study.

Fig. 10. Naturally occurring and artificially induced symptoms on blueberry stems.

A) Callus formation from strip of cambium left protruding into mechanically girdled portion of a 1-year-old stem. Note the similarity to the naturally occurring callus along the edges of a canker caused by Godronia (B).

B) Naturally occurring callus along edges of a canker caused by Godronia.

C) Gray centered lesions and maroon colored epidermis after a plant was inoculated with Godronia and grown for the summer and following winter in a cold frame.

D) Large canker on the crown of a dead 3-year-old plant inoculated with Godronia via a chip wound. The plant was alive and uncankered prior to exposure to winter conditions in a cold frame. Note the pycnidia below the chip wound.

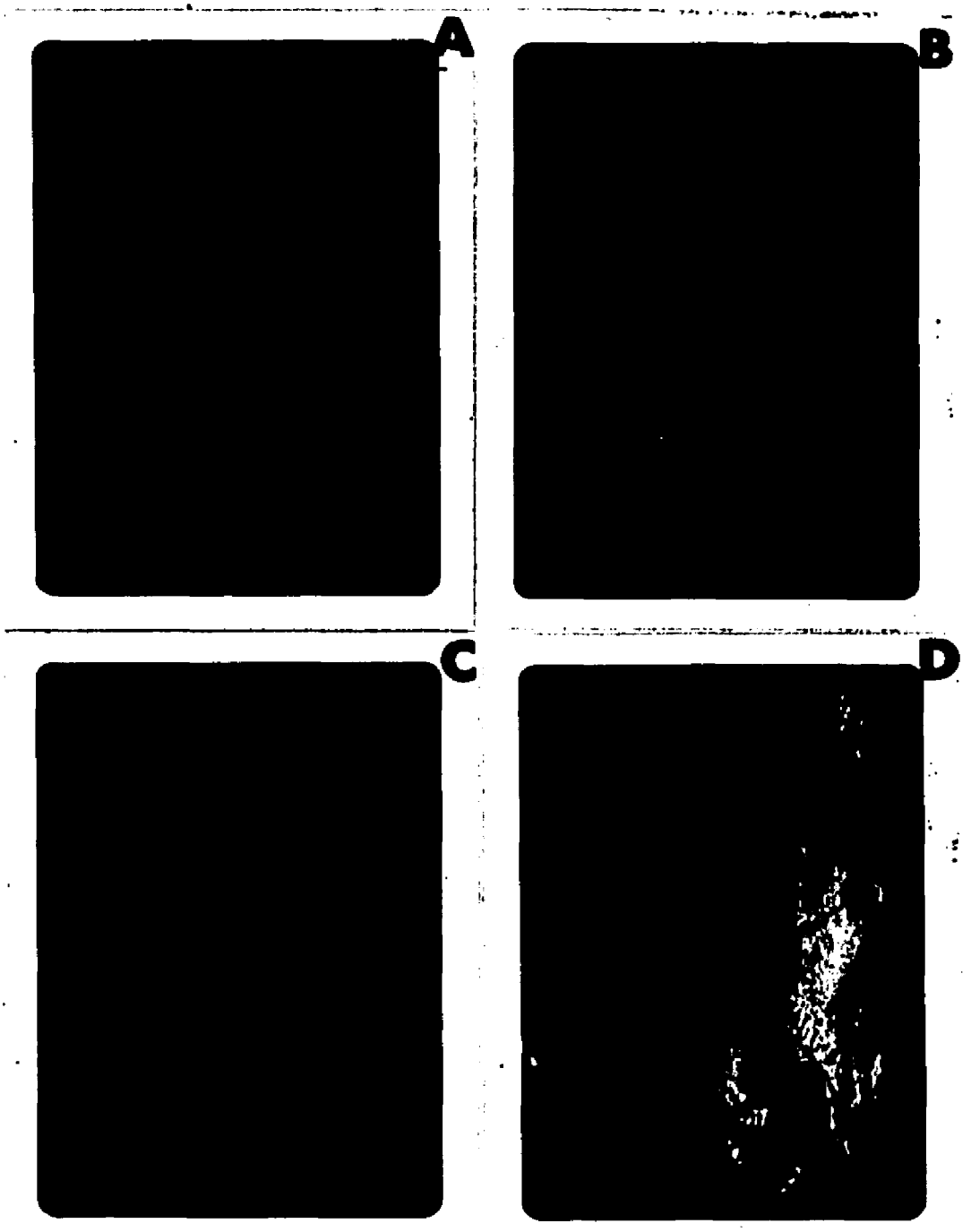
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Fig. 11. Results of artificial inoculations.

A) Lesion on Jersey variety plant 11 weeks after inoculating with G. cassandrae f. spiraeicola.

B) Control plant (a) and plant inoculated with P. vaccinii (b) several months after inoculation.

C) Canker on 2-year-old Jersey variety stem 6 months after inoculation with P. vaccinii in the field during May. Note that the canker is long and narrow.

D) Lesion on stem of Jersey variety plant 11 weeks after inoculation with G. cassandrae f. vaccinii from V. angustifolium. Note the pycnidia on the lesion.

E) Lesion on Bluecrop variety plant 6 months after inoculation with P. vaccinii in the greenhouse. Note the similarity to lesions caused by G. cassandrae f. vaccinii (D).

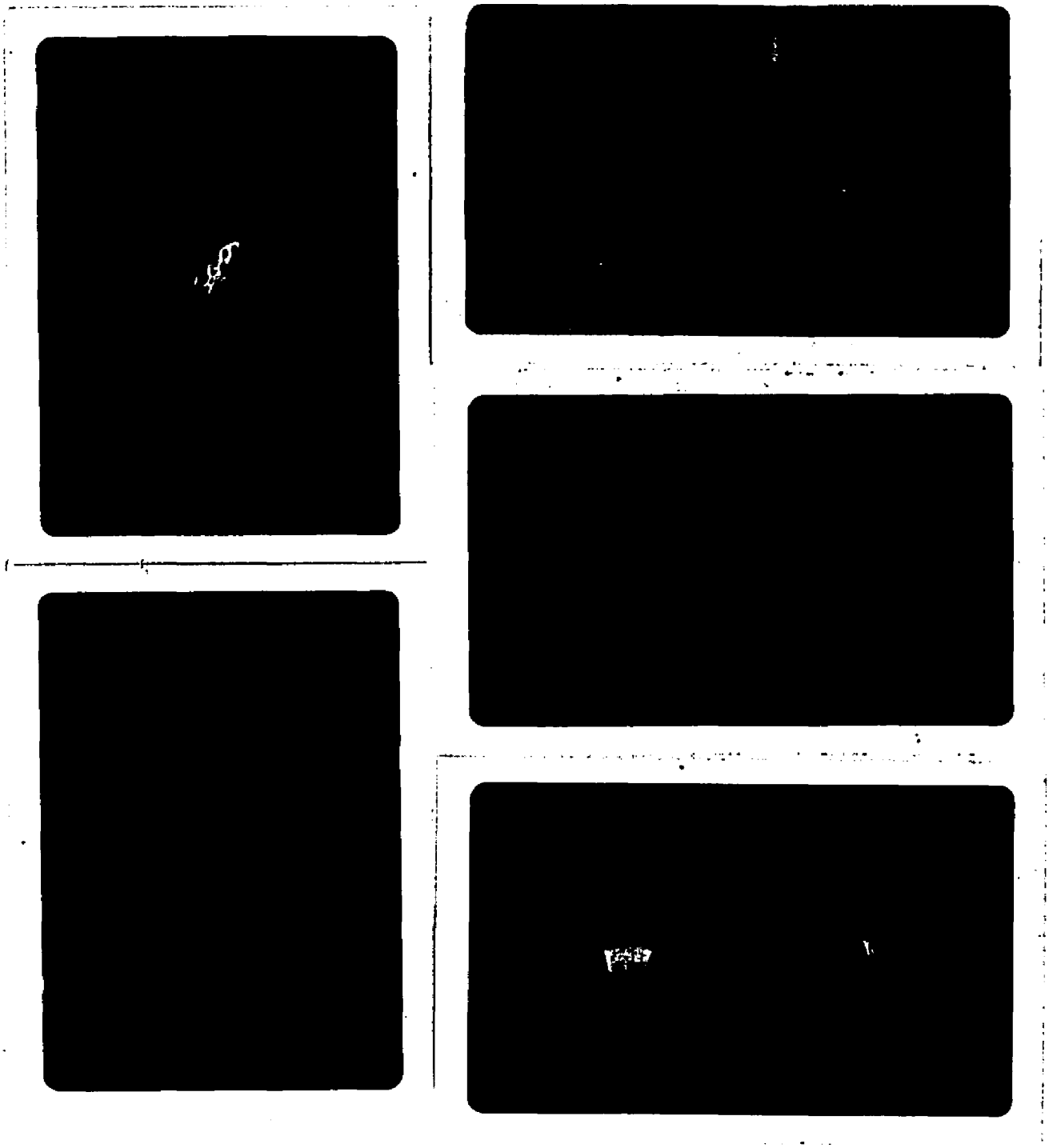


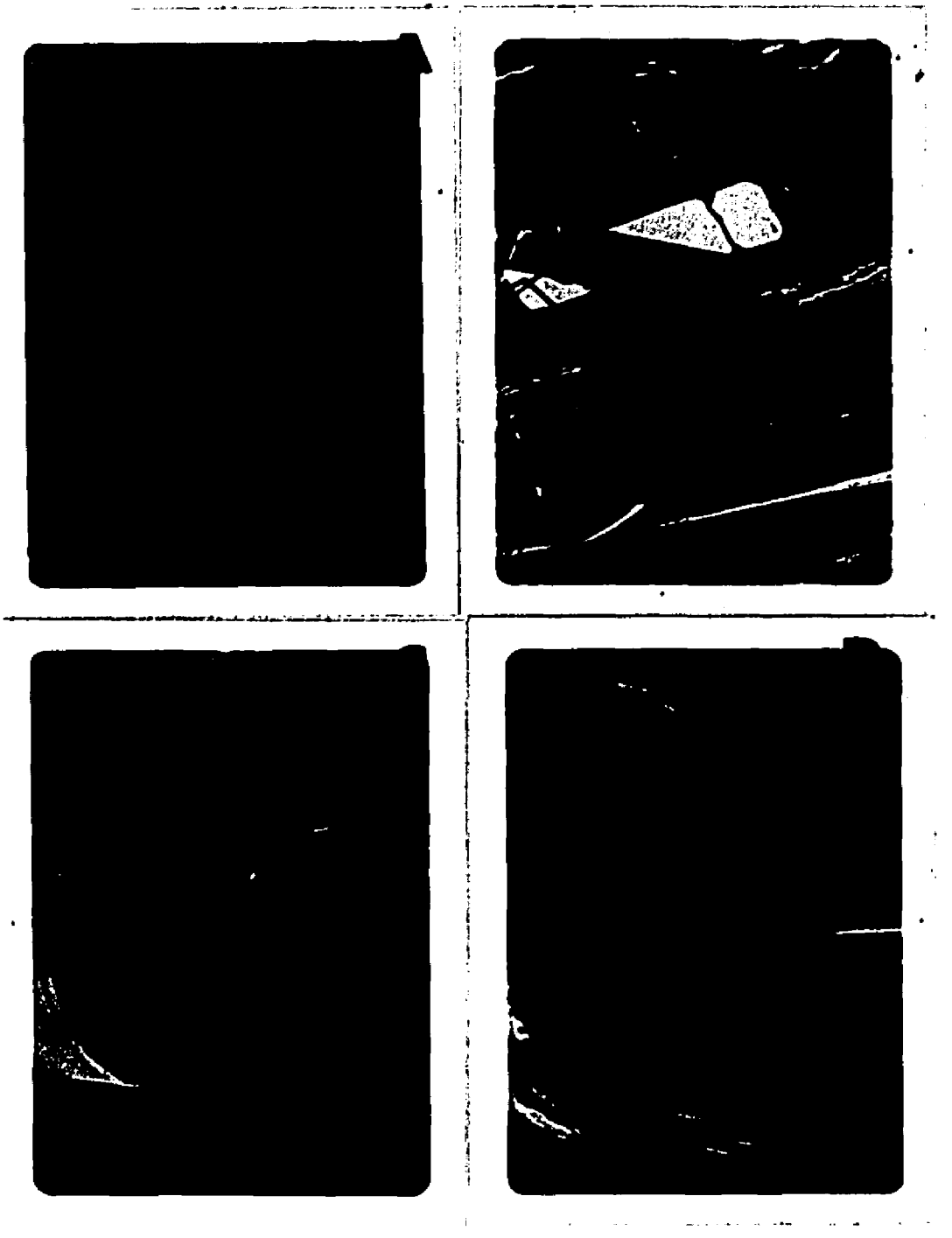
Fig. 12. Plants artificially and naturally infected with P. vaccinii.

A) Natural infection on 4-year-old stem of Weymouth variety. Note the flaked bark and the pycnidia which occur around the entire stem. The stem was dead when the photograph was taken in August.

B) Long, narrow canker on 4-year-old Jersey variety stem inoculated in the field with P. vaccinii. The stem was inoculated in May and was cankered by July.

C) Sharp demarcation between living and dead tissues on 1-year-old stem inoculated with P. vaccinii in the greenhouse.

D) Pycnidia in necrotic tissue on 1-year-old stem inoculated with P. vaccinii in the greenhouse.



Both Godronia and Phomopsis were reisolated from their respective inoculation sites for up to 1.5 years after inoculations. Both fungi could also be reisolated from inoculation sites which did not appear to be active and which did not have symptoms of canker.

None of the plants inoculated with Phomopsis or Godronia developed overgrowths or tumors.

Field studies: After it had been established that both Godronia and Phomopsis were pathogenic on blueberries in Michigan, 3 studies were designed to define further symptoms caused by each organism in the field: a) development of symptoms was followed on naturally infected plants tagged in the field; b) wilted branches were collected from fields in various areas in Michigan and Indiana and were studied in detail; c) plants in the field were inoculated with Godronia and Phomopsis.

Studies of naturally infected plants: Diseased blueberry plants were tagged in 3 fields during April, 1968. The fields and plants studied were as follows: a) a central lower Michigan (CLM) field with Earliblue variety plants infected with both Phomopsis and Godronia; b) a northern lower Michigan (NLM) field with Jersey variety plants infected by Godronia; c) an upper Michigan (UMF) field with several unnamed varieties infected by Godronia. Data confirming that Phomopsis was the only pathogen in some fields were not available until July. Therefore these fields were not included in this study.

Five randomly selected diseased plants and at least 5 branches/plant were tagged in each field during April. Additional stems were tagged as they wilted and as new shoots developed. Symptoms and occurrence of new infection were recorded every 4-6 weeks during the 1968 season. The identity of fungi causing disease was confirmed by isolating from samples of tissue collected at various times throughout the growing season.

Observations of plants tagged in the UMF and NLM fields: By the end of April, 1- and 2-year-old branches were covered with lesions of various sizes (Fig. 1, 2). When numbers of lesions on 1- and 2-year-old stems were counted on tagged branches and on a random sample of ninety-three 1- and 2-year-old stems collected from the 2 fields, an average of 20.9 lesions was found on each stem and 44.0% of all lesions were centered by a leaf scar. The percentage of nodal lesions was greater in the NLM field, with 67.9% of all lesions being at leaf scars. Most lesions were less than 1.5 cm long, but larger lesions with pycnidia were also common (Fig. 2-A, B). In the UMF field, the bases of many branches were "peppered" with numerous red spots 1 mm and less in diameter. Godronia was isolated from such incipient infection sites. The spots gradually expanded in size, coalesced, and by late August-October, the epidermis of the bases of the stems had turned red-brown, was flaking and splitting (Fig. 6-A, D, C), and as indicated

by isolations, thoroughly invaded by Godronia. Many of the larger internodal lesions expanded, but appeared to become walled-off and localized by midsummer (Fig. 3-C). It was not determined whether these cankers expanded during the winter and spring.

On stems which were more than 2-years-old, cankers were often 5-15 cm long and caused stems to appear gnarled, flattened or depressed (Fig. 3-A, B). The bark on flattened and depressed areas was usually flaked and cracked (Fig. 6-C). In wide, deep cankers, the xylem was exposed and the cankers often girdled more than 3/4 of the circumference of the stem (Fig. 3-B). Pycnidia were not found on such cankers. Xylem discoloration often occurred in parts of the stem which were not cankered externally or in areas where only small lesions were visible. Flaking and splitting of the bark was common on such stems. Godronia was isolated from flaking, splitting, or symptomless bark.

Stems on which buds were beginning to expand wilted as early as May in both NLM and UMF fields. In the UMF field, leafed-out branches began to wilt during mid-June and continued to wilt until August (Fig. 14, 15-A, C). Wilting in the NLM field started later in June and continued until September. In both fields, 1-5% of the stems wilted because Godronia grew into the bases of stems from infected crowns. Some stems wilted without visible cankers

whereas others did not wilt even when they were completely girdled by cankers exceeding 5 cm in length.

Pycnidia were produced on lesions beginning in March-April in both fields (Fig. 1-C, 2-A, B). Occassionally, pycnidia appeared in fissures in the bark and were not confined to lesioned areas (Fig. 6-B). Apothecia (Fig. 16) were abundant on pruning stubs, along the length of erect, dead stems and on dead twigs accumulated around the crowns of plants. Apothecia occurred only on dead wood, and occassionally were found in dead tissues on unilaterally-killed stems.

A few new lesions were observed in late July. Pycnidia did not appear on the lesions and lesions did not enlarge. On October 9, small darkened zones resembling water soaking were observed along internodes of stems formed during the 1968 season. Isolations showed that Godronia was present in these tissues. Small red spots (Fig. 1-A), often centered by small pockets of necrosis were observed on the stems by December 3. Isolations of Godronia from these tissues suggested that the red spots were incipient lesions caused by Godronia. Godronia was also isolated from reddened tissues surrounding leaf scars in December. Infection of stems formed during the 1968 growing season apparently had occurred before October 9, but symptoms were not apparent as lesions until December. Infection periods will be discussed further in Part II of this thesis.

Observations of plants tagged in the CLM field:

In the CLM field in which Earliblue plants were infected by both Godronia and Phomopsis, small lesions were not as common as in the other fields. Large cankers present on older (3 to 5-year-old) branches often extended into the crown. Both Godronia and Phomopsis could be isolated from such cankers. Phomopsis infections usually started on higher parts of the stem and gradually progressed down the cane, eventually killing the entire branch. Frequently, it was impossible to tell whether Phomopsis or Godronia killed the infected stem since both could be isolated from the diseased tissue. Phomopsis was also found in the crowns of plants and appeared to be actively involved in killing branches originating from the crown. It was not possible to determine whether the crown was infected directly or via infected branches leading to the crown.

Cankers from which only Phomopsis was isolated tended to be long in relation to their width and were usually covered by unbroken bark or epidermis (Fig. 11-C, E; 12-B). On these canes infected by Phomopsis but without cankers, the bark and/or epidermis were commonly flaked and splitting (Fig. 12-A).

During April and May, dead tips were noted on many stems on which buds were beginning to expand. All of the stems were growing from the crowns of established plants and had been formed late during the 1967 season. Symptoms

resembled those caused by cold injury (64), Phomopsis twig blight (68), and Botrytis twig blight (64, 70). Usually 10-30 cm of the tips were dead and living tissues were sharply demarcated from the dead tips. The pith of living portions of the stems was chambered and brown for several cm below the killed tissues. In order to determine whether pathogenic fungi were associated with these symptoms, 102 Jersey, Pemberton, and Blueray variety stems with dead tips were collected from several fields. One cm long sections were cut from the juncture of living and dead tissues and placed on ½ PDA. The fungi identified growing from such tissues are listed in Table 8. Several other fungi including Cytospora sp., Dendrophoma sp., Alternaria spp., and Epicoccum sp. were also commonly found in such tissues.

Table 8. Fungi associated with symptoms resembling cold injury, Phomopsis and Botrytis twig blights.

No. sections sampled	No. sections with fungi			
	Godronia	Phomopsis	Coryneum	Botrytis
102	8	24	3	16

One to 5-year-old stems did not wilt in the CLM field until July-August. Phomopsis pycnidia appeared during August-October and were most prevalent later in the season on older, dead stems and occurred along the length of the dead branches (Fig. 12-A). No perithecia of

Diaporthe were found in the CLM field or in any other field in Michigan and Indiana during the course of this research. Godronia pycnidia appeared during March-April as in the NLM and UMF fields. Godronia apothecia were found on pruning stubs and some dead stems, but were not as common as in the other 2 fields.

Survey of wilted branches: A detailed survey of wilting branches in Michigan and Indiana was conducted. The goals of this study were to: a) further define the symptoms caused by each organism; b) determine the relative severity and distributions of the 2 diseases in the Great Lakes blueberry region.

Random samples of wilted stems were collected and the variety, location in the state, age of sampled stem, symptoms on the stem, degree of wilt, and where the stems originated on the plant were recorded for each branch. Earlier observations indicated that xylem discoloration (XD) was common in the stems of wilting stems. Each stem was cut into 1-2 cm long sections and the presence, location, and extent of XD were recorded. At least 3 sections, taken from parts of the stems with canker symptoms or XD, were placed on ½ PDA to isolate fungi in the tissues. The number of plants infected in each field from which wilted branches were collected was recorded in order to assess the relative severity of the 2 diseases.

Fig. 13. Artificially induced Godronia lesions.

A) Lesions which developed on a 6-month-old stem which was kept in a cold room for 6 months. The stem was collected from the field in October and showed only small pockets of water soaking in the cortex and reddening of leaf petioles at that time.

B) One-year-old stem inoculated with and killed by Godronia in the field. The stem was inoculated in September, 1967, and wilted as buds expanded during late April, 1968.

C) Lesions on a 1-year-old stem inoculated with Godronia in the field during April. Photograph was taken in early June.

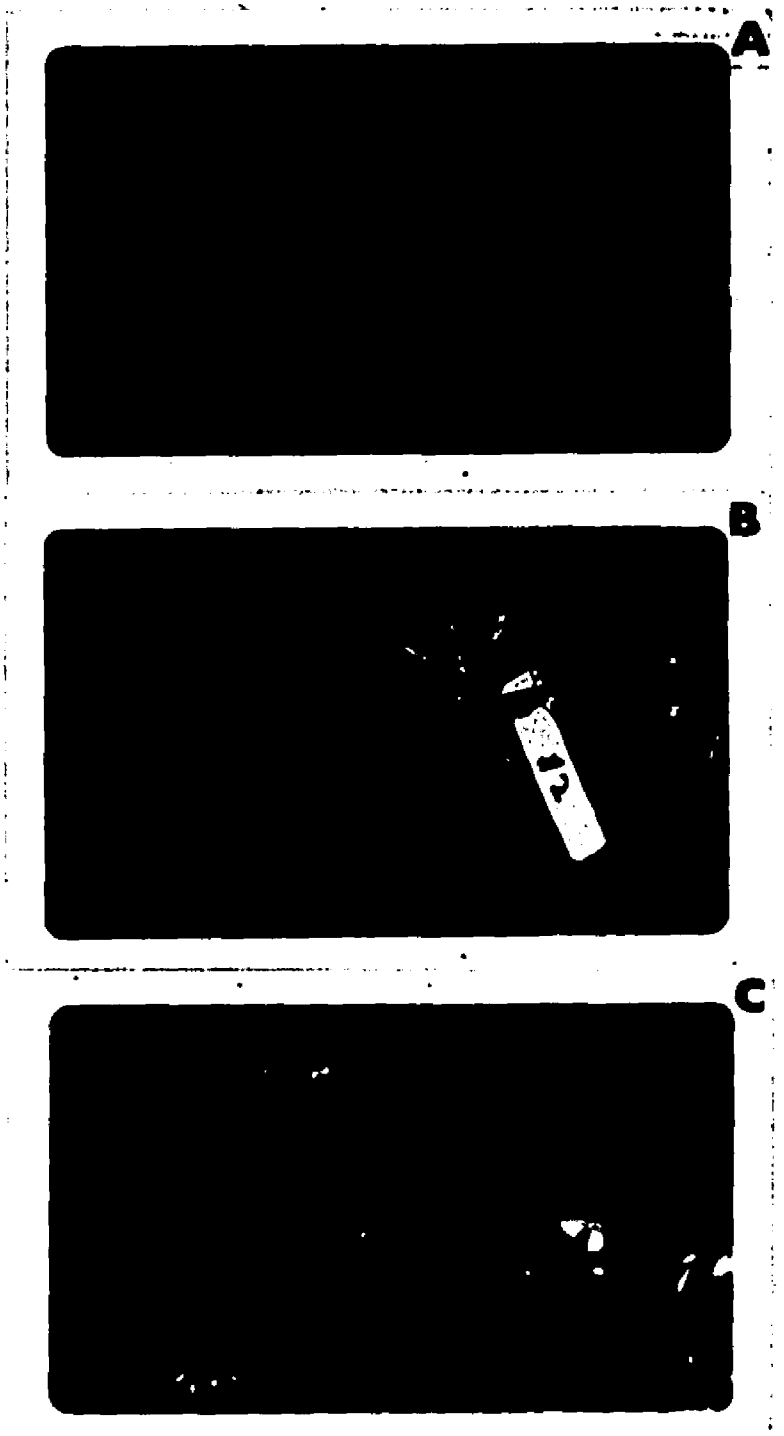

A
B
C

Fig. 14. Typical flagging seen in fields in which either Phomopsis or Godronia were epiphytotic. Godronia was epiphytotic in this upper Michigan field. The photograph was taken in July.

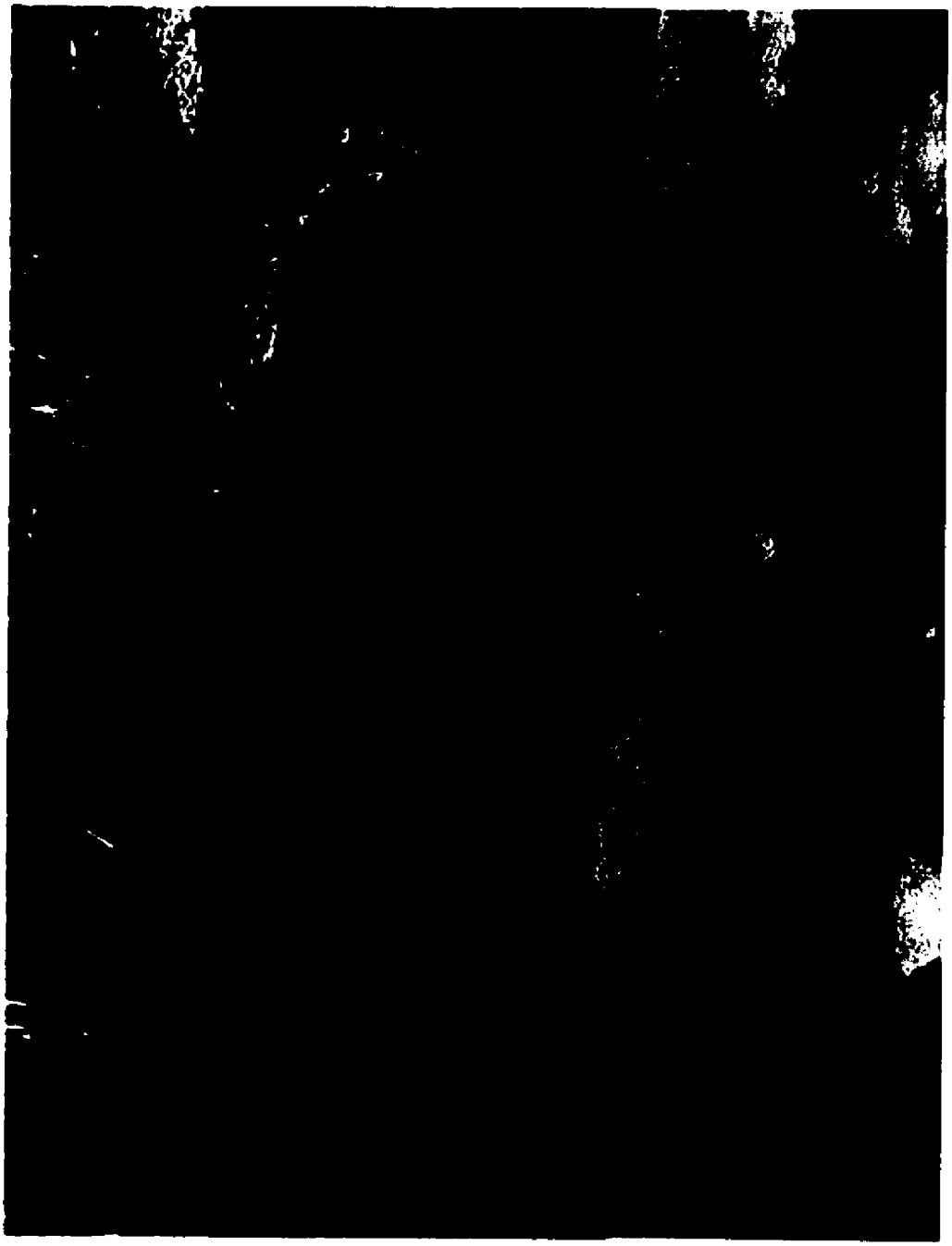


Fig. 15. Wilted branches and discolored leaves. All stems were infected with Godronia and were phtographed in July-August. Wilted branches and discolored leaves caused by Phomopsis were indistinguishable from those shown here.

A) Typical "flag" or wilted branch.

B) Discolored leaves. The first leaf at the left in each row is unaffected. The various shades of red and brown were typical.

C) Wilted leaves on a single branch.

D) Marginal browning of leaves of affected branch. The margins of leaves often showed the earliest signs of wilt. Leaf at the far left is unaffected.

E) Premature reddening of leaves on affected stem.


A

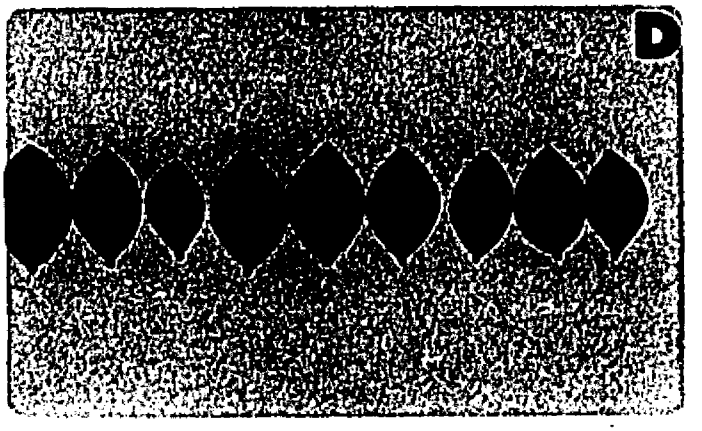
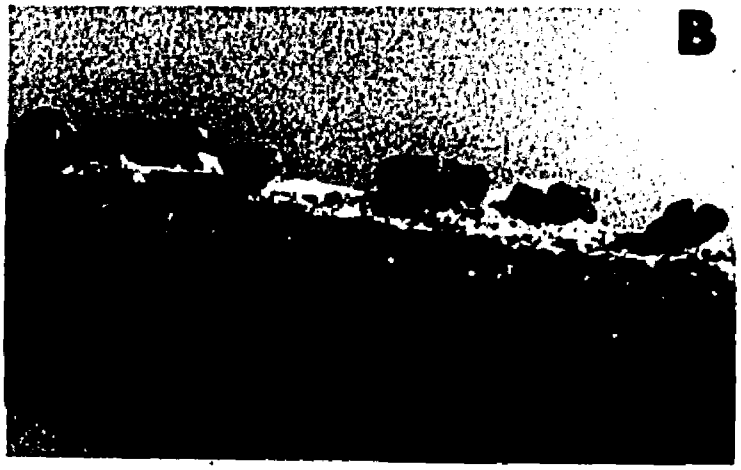

D

Fig. 16. Apothecia of G. cassandrae f. vaccinii.

A) Apothecia on dead 2 to 3-year-old stem collected in late July (3 X).

B) Lateral view of apothecia (25 X).

C) Open apothecia after being placed in a moisture chamber for 24 hours (50 X).


B


C

Leaves on stems infected with either Godronia or Phomopsis often turned red or yellow before they wilted (Table 9, Fig. 15-B, D, C). Leaves on stems infected with Phomopsis showed this characteristic more often. Margins of leaves on affected branches often darkened or wilted before the remainder or the leaf (Fig. 15-A, D). Branches killed by Phomopsis were older than those killed by Godronia (Table 10). Most stems killed by Phomopsis were 3-years-old, whereas most stems killed by Godronia were 2-years-old (Fig. 17). Both fungi were associated with each of the symptom categories observed on wilted stems (Table 11).

The distribution of symptoms (Fig. 18) on sections cut from wilted stems collected in fields in which Phomopsis was the only pathogen isolated (PH-Fields) differed from the distribution of symptoms on similar sections from fields in which Godronia was the only pathogen found (GD-Fields). The lesion - young canker and callused canker categories were more frequent in GD-Fields, whereas branches which were wilted, but otherwise normal in appearance occurred more frequently in PH-Fields. Both fungi were associated with each symptom category in the respective fields (Table 12).

Qualitative differences within symptom categories were present in these comparisons. Cankers found in PH-Fields tended to be long and narrow, and were covered with

Table 9. Characteristics of leaves on branches killed by Godronia or Phomopsis.[a]

Fungus	No. stems with discolored leaves			
	red	yellow	red-yellow	wilted, brown, or defoliated
Phomopsis	17	9	7	157
Godronia	4	4	2	137

[a] Data based on 326 wilted branches collected from 19 fields during the 1968 growing season.

Table 10. Average ages of wilted branches from which Godronia and Phomopsis were isolated.

Fungus Isolated	Average age (years)	Total number wilted branches sampled
Godronia	2.38	128
Phomopsis	2.96	184
Both	2.67	21

Table 11. Association of *Godronia* and *Phomopsis* with symptoms observed on wilted stems.[a]

Symptom category	Percent sections with fungus	
	Godronia	*Phomopsis*
None	25.6	63.1
Lesion-young canker	66.4	20.6
Developed canker	32.5	52.8
Callused canker	61.5	27.2
Discolored bark or epidermis	33.2	48.5
Split or flaking bark or epidermis	39.3	45.4

[a] Based on 1696 sections from 347 flags collected during the 1968 season.

unbroken bark or epidermis (Fig. 12-B). Cankers in GD-Fields were wide in relation to their length and often completely girdled the infected branches. Xylem was exposed due to distinegration of the bark and epidermis over the cankers (Fig. 3-B).

Xylem discoloration occurred at some point in the xylem of wilted branches regardless of the age of the stem or the fungus isolated. Zones of XD varied in length from those shorter than 2 cm to others which extended the length of the stem. Zones of XD were usually 2-4 cm long at the onset of wilt symptoms and were longest on stems with browned leaves. There was no correlation between the pathogen isolated from the xylem and the extent or position of XD. Infected stems wilted above regions of XD and leaves below remained healthy. Wilting was confined to 1 side of 3 branches in which XD was unilateral.

Both fungi were associated with all of the symptom categories occurring in areas of XD (Table 13). It is especially interesting to note that many stems infected with Godronia were not cankered or lesioned in areas of XD.

Phomopsis and Godronia were both widely distributed in Michigan (Fig. 19). However, in fields north of Mason County, Godronia was epiphytotic and Phomopsis was not found. In the center of the "blueberry belt," both fungi were common.

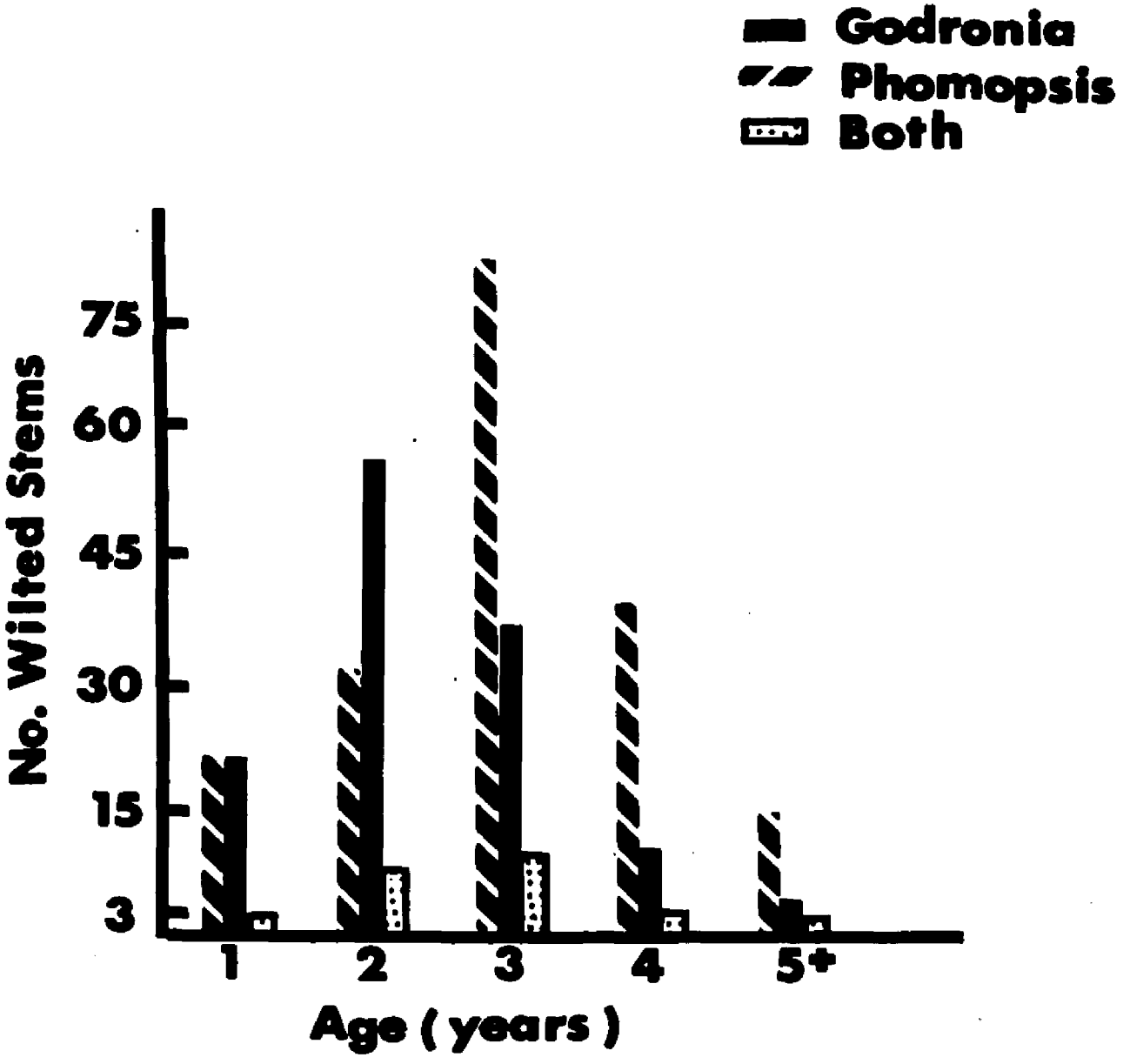


Fig. 17. Distribution of ages of wilted stems from which Godronia, Phomopsis, or both fungi were isolated. Data are based on 317 wilted stems collected from 18 fields during the 1968 season. Identification of fungi based on isolations made from discolored xylem in each stem.

Table 12. Association of *Godronia* and *Phomopsis* with symptoms on sections from stems collected in fields in which either *Godronia* (GD-Fields) or *Phomopsis* (PH-Fields) was the only pathogen isolated.[a]

Symptom	GD-Fields		PH-Fields	
	Percent sections with *Godronia*	Total sections observed	Percent sections infected with *Phomopsis*	Total sections observed
None	96.3	27	38.6	44
Lesion-young canker	97.0	66	78.6	14
Developed canker	89.1	101	73.3	150
Callused canker	95.9	49	69.0	29
Discolored bark or epidermis	87.3	79	76.4	110
Split or flaking bark or epidermis	94.1	204	73.0	159

[a] *Godronia* data based on 526 sections from 5 northern Michigan fields; *Phomopsis* data based on 496 sections from 7 Indiana and southern lower Michigan fields.

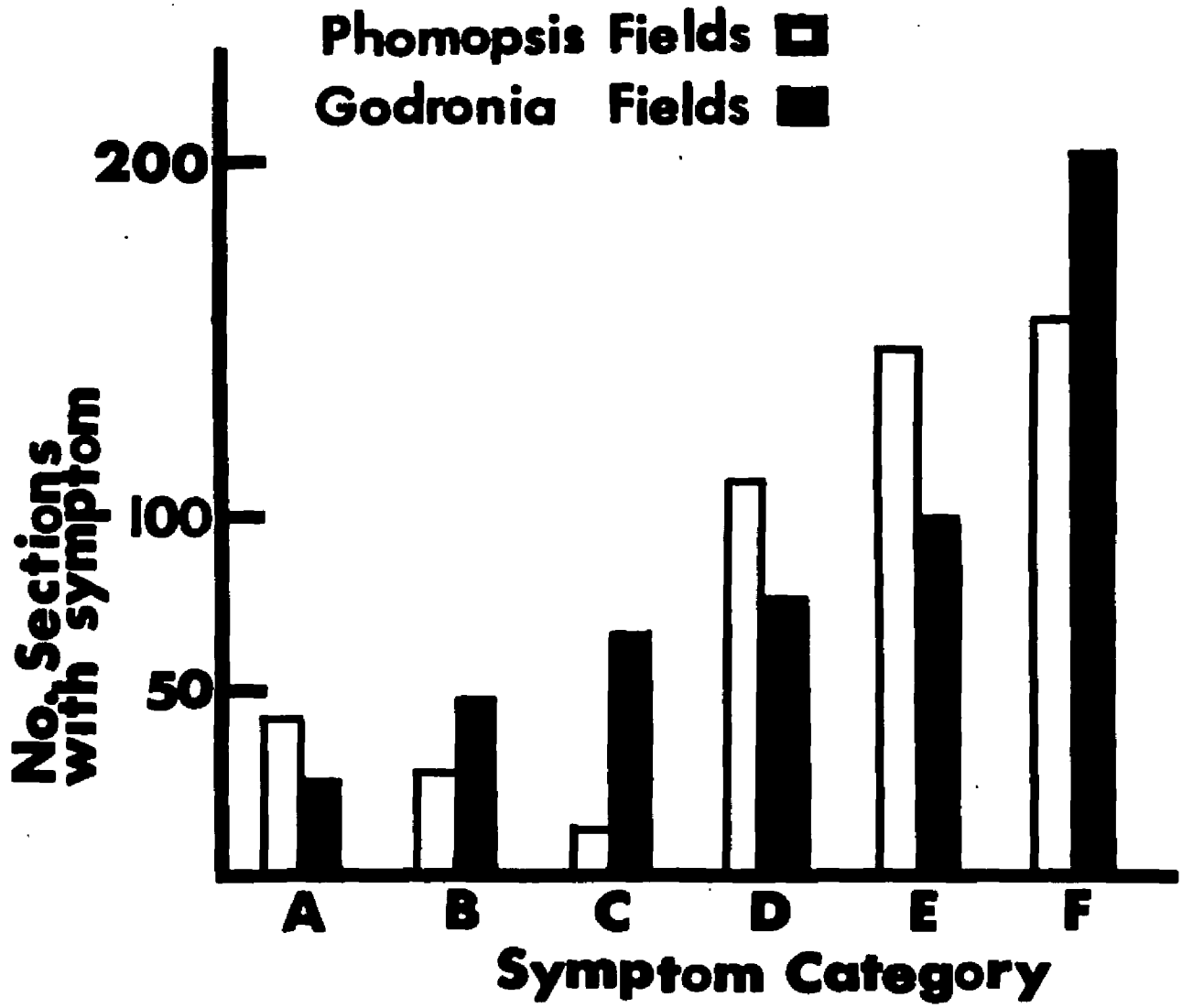


Fig. 18. Distributions of symptoms in fields in which either Godronia (Godronia Fields) or Phomopsis (Phomopsis Fields) was the only pathogen isolated. Godronia data are based on 526 sections from 5 northern Michigan fields. Phomopsis data are based on 496 sections from 7 Indiana and southern lower Michigan fields. Symptom categories are as follows: A = none; B = callused cankers; C = lesion-young cankers; D = discolored bark or epidermis; E = developed cankers, F = split or flaking bark or epidermis.

Table 13. Association of *Phomopsis* and *Godronia* with symptoms observed on sections of stems with discolored xylem[a]

Symptoms	Percent Sections with fungi		Total sections
	Godronia	*Phomopsis*	
None	22.7	71.0	66
Lesion-young canker	70.9	25.8	31
Developed canker	40.6	57.2	138
Callused canker	61.7	35.3	34
Discolored bark or epidermis	34.2	60.5	76
Split or flaking bark or epidermis	39.4	55.8	231

[a] Based on sections taken from 347 wilted stems during the 1968 growing season.

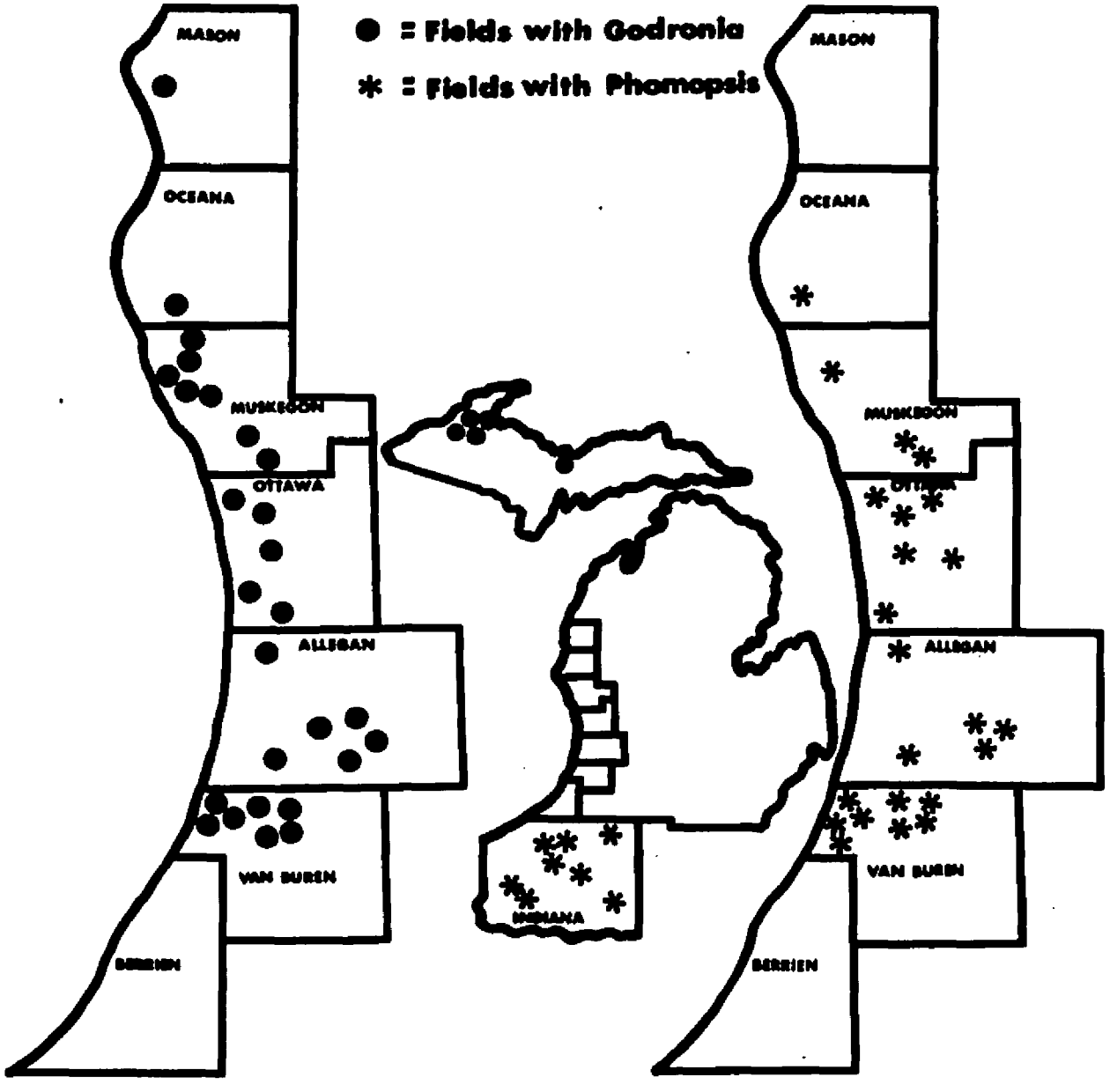


Fig. 19. Distribution of Phomopsis and Godronia in Michigan and Indiana blueberry fields. Based on samples collected from 57 fields during 1965-1968. Godronia was epiphytotic north of Oceana County and Phomopsis was epiphytotic in several Indiana and Van Buren County fields.

Both fungi attacked all major varieties of blueberries grown in Michigan (Tables 14, 15), but the 4 most important varieties appeared to be most susceptible to Godronia (Table 16). Godronia is probably the more important pathogen in Michigan. Jersey variety appeared to be most susceptible to Godronia canker and Rancocas most resistant. Earliblue was very susceptible to Phomopsis and infected plants were often non-productive.

Inoculation of plants in the field: Symptom progression was followed on plants inoculated with Godronia and Phomopsis in the field. Beginning in April, 1968, 8-year-old Jersey variety plants growing in the Michigan State University Orchard were inoculated every 2 weeks with Godronia and Phomopsis. One plant was inoculated 10 times with Godronia and another inoculated similarly with Phomopsis on each inoculation date. The same isolate of each fungus was used for all inoculations. Symptom progression was observed through the growing season.

Both Phomopsis and Godronia readily infected plants inoculated during April-June (Table 14), but the percentage of inoculations causing disease decreased during July. Further expansion of successful inoculations also stopped during this period. It was not determined whether cankers expanded during the fall and winter. During the spring and early summer, Phomopsis and Godronia lesions developed on 1 and 2-year-old stems (Fig. 13-C). A single 1-year-old

Table 14. Association of *Godronia* and *Phomopsis* with varieties of blueberries grown in Michigan and Indiana[a]

Blueberry variety	Percent 1967 acreage[b]	Total number fields		
		Sampled	*Godronia* present	*Phomopsis* present
Jersey	57.0	22	18	7
Rubel	14.30	11	5	8
Bluecrop	6.65	3	2	1
Earliblue	3.70	8	3	6
Stanley	3.06	4	2	1
Pemberton	2.75	3	1	1
Blueray	2.50	1	0	0
Berkley	2.00	1	0	1
Rancocas	1.30	1	1	0
Weymouth	0.83	6	3	4
Collins	0.60	2	1	0
Dixie	<0.92	1	0	1
Grover	<0.92	1	0	1
Unnamed	<0.92	4	4	0

[a] Based on samples collected from 57 Michigan and Indiana fields during 1965-68.

[b] Percentages based on data compiled by the Michigan Blueberry Growers Association.

Table 15. Association of Godronia and Phomopsis with varieties of blueberry grown in Michigan.

Blueberry Variety	% sections infected		No. observations	
	Godronia	Phomopsis	Sections	Fields
Jersey	47.7	9.3	869	22
Rubel	38.0	42.7	491	11
Weymouth	3.4	51.8	525	6
Unnamed	95.4	0	285	4
Earliblue	23.6	45.2	550	8
Bluecrop	66.7	4.3	253	3
Rancocas	91.3	0	46	1
Pemberton	6.2	16.6	48	3
Dixi	0	100.0	3	1
Berkley	0	62.5	8	1
Collins	7.1	0	14	2
Blueray	0	0	24	1
Stanley	3.6	2.7	217	4
Grover	0	68.9	74	1

Table 16. Relative susceptibility of major blueberry varieties to Godronia and Phomopsis.[a]

Variety	Field susceptibility[b]	
	Godronia	Phomopsis
Jersey	+++	+
Rubel	+++	++
Bluecrop	+++	+
Earliblue	+++	+++

[a] All plants were naturally infected.

[b] +++ = most severly affected; + = least severe.

branch inoculated with Phomopsis in April, died by mid-May. One cane inoculated with Godronia in September, 1967, died as buds began to expand in April (Fig. 13-B). Pycnidia developed on Godronia lesions in about 4 weeks after April, May and June inoculations, but not at all on stems inoculated later. Pycnidia developed by March on the stem inoculated in September, 1967. Few pycnidia developed on stems inoculated with Phomopsis.

Slow spreading cankers developed on 3- to 5-year-old stems when chip or pin prick wounds were inoculated with Godronia or Phomopsis. Phomopsis cankers tended to be long, narrow and covered by unbroken bark or epidermis (Fig. 11-C, 12-B). No overgrowths developed on any of the inoculated plants.

The decrease in percentage of inoculations causing disease corresponded with an increase in daily temperatures. It is interesting to note that inoculations with Phomopsis in July caused cankers, but those made with Godronia did not. That Phomopsis grows better at higher temperatures (Fig. 9) may be an explanation of this observation.

Low incidence of wilt on inoculated plants: Cankers readily developed on plants inoculated with G. cassandrae f. vaccinii in the greenhouse, but few stems wilted. Examination of wilted stems collected from the field had shown: 1) xylem discoloration occurred at some point along all wilted stems; 2) leaves were always wilted above XD and

Table 17. Results of inoculations made on Jersey variety plants in the field with Godronia and Phomopsis.[a]

Date of inoculation	% inoculations causing cankers	
	Godronia	Phomopsis
September 25, 1967[b]	100	--
April 21, 1968[c]	90	90
May 3, 1968	100	60
May 18, 1968	80	20
May 31, 1968	90	40
June 19, 1968	10	80
July 1, 1968	0	50
July 15, 1968	0	20
July 29, 1968	0	0
August 27, 1968	60	30

[a] One plant was inoculated 10 times with Godronia and another 10 times with Phomopsis on each date. None of the controls developed cankers.

[b] One stem wilted during April, 1968.

[c] One stem inoculated with Phomopsis wilted during May, 1968.

healthy below; 3) Godronia was present in XD tissues; 4) many uncankered stems wilted; 5) many severely cankered stems did not wilt. It seemed probable that wilting occurred only when the fungus was able to invade xylem tissues.

Several observations suggested that low temperatures and/or dormancy predisposed stems infected with Godronia to wilt. Stems inoculated with Godronia wilted when greenhouse temperatures were maintained lower than 18 C, whereas lesions appeared to become walled-off when greenhouse temperatures were 25-30 C. Similarly, observations of naturally infected plants indicated that many small lesions observed in April were localized during the warmer summer months. When plants were inoculated with Godronia in the field (Table 17), only spring and fall inoculations caused cankers and the only stem to wilt was inoculated in September. Similar data had been reported by others (35, 37, 73). Experiments of others (16, 37, 52) and data presented above (Fig. 9) showed that G. cassandrae grew well at 0-4 C and that the organism did not grow at temperatures above 30 C. Exposure to 5-6 C for 650 hours satisfied the cold requirement of blueberries (32). Godronia, then is able to grow well at temperatures inducing dormancy of blueberries. The following experiments were designed to determine whether low temperatures and/or dormancy predispose V. corymbosum plants infected with G. cassandrae to wilt.

Effect of dormancy on localized lesions: The objective of these experiments was to determine whether apparently localized lesions would expand when plants were dormant. Five 1-year-old plants and two 3-year-old plants were inoculated with Godronia during March, grown in a 18-30 C greenhouse, and transferred to a cold frame in June. Plants were returned to the greenhouse the following March. Controls consisted of inoculated plants grown in the greenhouse and noninoculated plants grown in the cold frame for the duration of the experiment.

Lesions had the following characteristics when plants were transferred to the cold frame in June. Centers of lesions were necrotic and stem tissues were raised along the edges of necrosis. Epidermis was raised and was red-brown to maroon colored several mm in advance of necrosis. Lesions appeared to be walled off.

Centers of lesions turned gray on plants grown in the cold frame. Maroon discoloration of epidermis was more extensive on plants grown in the cold frame (Fig. 10-C) than on plants grown in the greenhouse. None of the 1-year-old plants wilted when they were returned to the greenhouse in March.

One 3-year-old plant inoculated via a chip wound in the crown severly cankered and died before leaves were fully expanded (Fig. 10-D).

In another experiment three 1-year-old plants were inoculated and grown in the greenhouse as above and were transferred in June to a 2 C coldroom for 2.5 months. Controls consisted of inoculated plants grown in the greenhouse and noninoculated plants placed in the cold room.

Lesions did not expand and stems did not wilt on any of the plants in this experiment.

Effect of temperature on lesion development: The objective of this experiment was to determine whether low temperatures favored initiation of infection and early lesion development. Ten inoculations were made with the same isolate of G. cassandrae f. vaccinii on each of 15 plants. Each inoculation site was pierced a single time with a fine needle. Immediately following inoculation, 5 plants were placed in 5 or 15 C growth chambers (R. H. = 70-90%; light = 18 hour photoperiod, 500 fc flourescent source), or in a 18 C mist chamber (R. H. = 90%; light = normal daylight supplemented by 24 hour 200 fc incandescent source).

Lesions were initiated at each temperature. Lesions expanded most rapidly at 18 C (Table 18). Fifteen C was more favorable than 5 C for lesion expansion and pycnidia development (Table 18).

Another experiment was performed to determine whether a previous 10 day incubation period in high humidity at 18 C would increase the rate of lesion expansion at 5 and 15 C.

Two plants were inoculated and incubated for 10 days at 18 C in the mist chamber. Following incubation 1 plant was placed at 5 C and the other at 15 C.

Lesion expansion (Table 19) was similar to that (Table 18) on plants grown at 5 and 15 C without previous incubation at 18 C. No stems wilted after 27 days exposure to 5 and 15 C.

An experiment was performed to determine whether lesions on plants grown for 37 days at 5 and 15 C would continue to expand at low temperatures; and become localized at high temperatures. Plants grown at 5 and 15 C for 37 days were transferred to 2, 8, 10, 20, and 30 C growth chambers. One plant from 5 and another from the 15 C chamber was placed into each growth chamber. Plants were grown at these temperatures for 97 days and were then transferred for 60 days to a 20 C chamber.

Lesion size was inversely related to temperature (Fig. 20). Lower temperatures favored lesion development. Lesions appeared to be walled off at 20 and 30 C. Plants grown at 2 and 8 C grew rapidly when placed at 20 C indicating that they had been dormant. Stems did not wilt on any plants in this experiment.

This experiment was similar to the previous experiment except that plants were inoculated, incubated in the 18 C mist chamber for 12 days, and were placed directly into 2, 8, 10, 20, and 30 C growth chambers. Three plants,

Table 18. Expansion of lesions and formation of pycnidia on plants inoculated with Godronia grown at 5, 15, and 18 C.

Temperature (° C)	Exposure period (days)	No. lesions counted	Average length lesions (mm)	Rate lesion expansion (mm/day)	No. lesions with pycnidia
5	27	50	2.4	.09	0
5	37	50	3.0	.08	3
15	27	50	3.2	.12	14
15	37	50	4.3	.12	15
18	12	121	3.2	.27	0
18	18	50	3.7	.21	0

Table 19. Expansion of lesions on plants inoculated with Godronia and grown for 10 days in a high humidity chamber at 18 C and then for 23 days at 5 and 15 C.

Temperature (° C)	Number lesions measured	Average length lesions (mm)	Rate lesion expansion (mm/day)
5	10	2.3	.08
15	10	5.2	.16

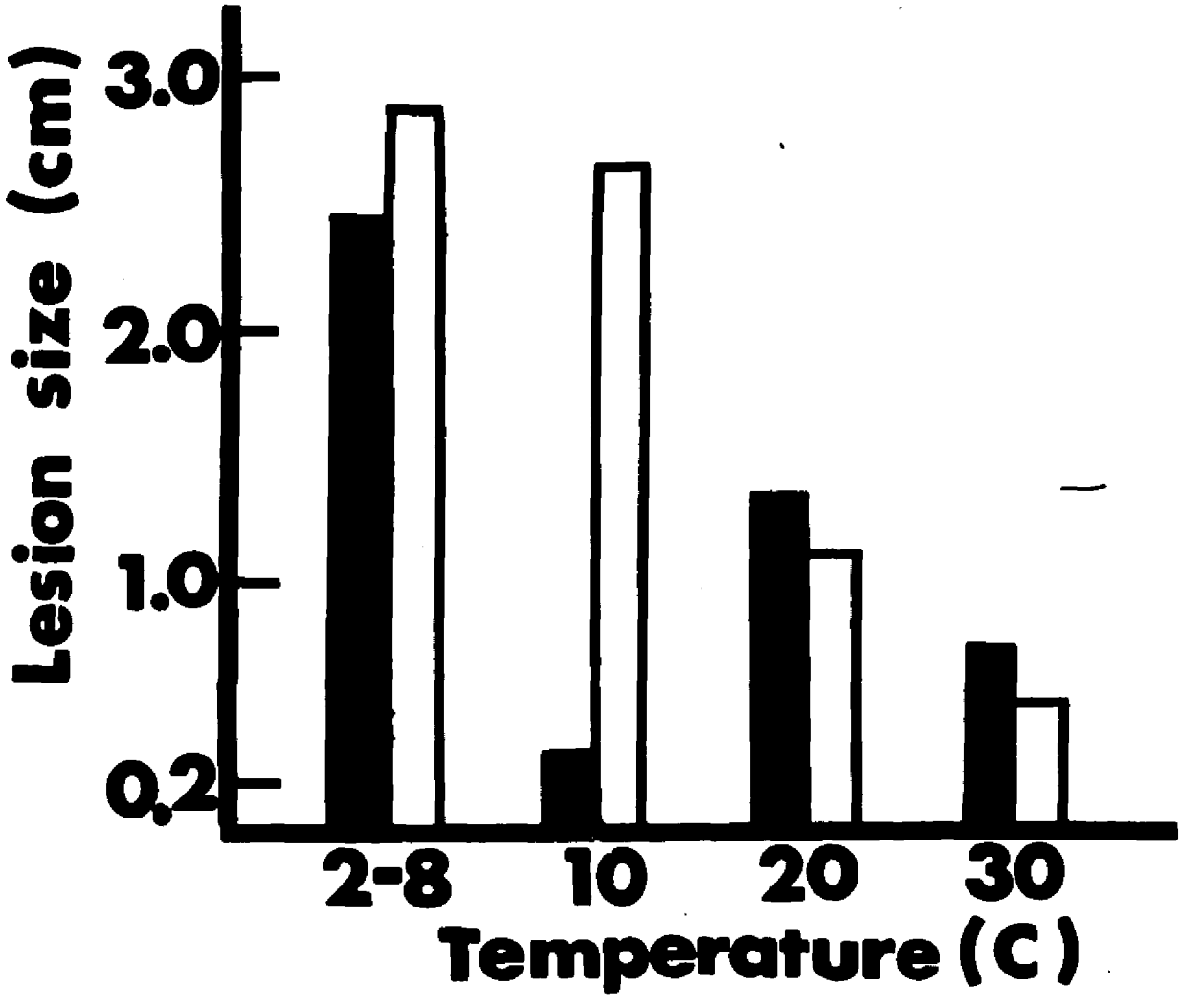


Fig. 20. Final average size of lesions caused by Godronia on inoculated 1-year-old Jersey variety plants after exposure to various temperatures. Plants were exposed to 5 (▭) or 15 (▬) C for 37 days, followed by exposure to 2-8, 10, 20, or 30 C for 97 days, and 60 days at 20 C. Data for each bar are based on average length of 10 lesions on 1 inoculated plant.

each inoculated 10 times were placed into each chamber. Plants were grown for 122 days at the respective temperatures and then grown for 63 days at 20 C.

Necrosis was most extensive (Fig. 21) at 2 and 8 C. At these temperatures, lesions were covered with pycnidia, coalesced, and extended the length of the inoculated stems. Five of 12 inoculated stems wilted when plants from 2 and 8 C were grown at 20 C. Rapid growth of these plants at 20 C indicated that they had been dormant. No differences in symptom development were noted between plants exposed to 2 and 8 C. Plants exposed to 10 C did not grow rapidly when placed in the 20 C growth chamber and stems on these plants did not wilt. Lesions appeared to be walled-off at 20 and 30 C.

Data from this series of experiments suggest that initial infection and lesion development is related to growth of Godronia at various temperatures, but that later lesion expansion is inversely related to temperature. Dormancy of host plants favored fungal invasion of xylem tissues and subsequent development of blight symptoms. The inability to control light and relative humidity detracts from the value of these data. However, further experiments under more controlled conditions are certainly warranted.

Callused cankers: Several different types of overgrowths were commonly associated with cankered blueberry stems. Detailed observations in the field and laboratory

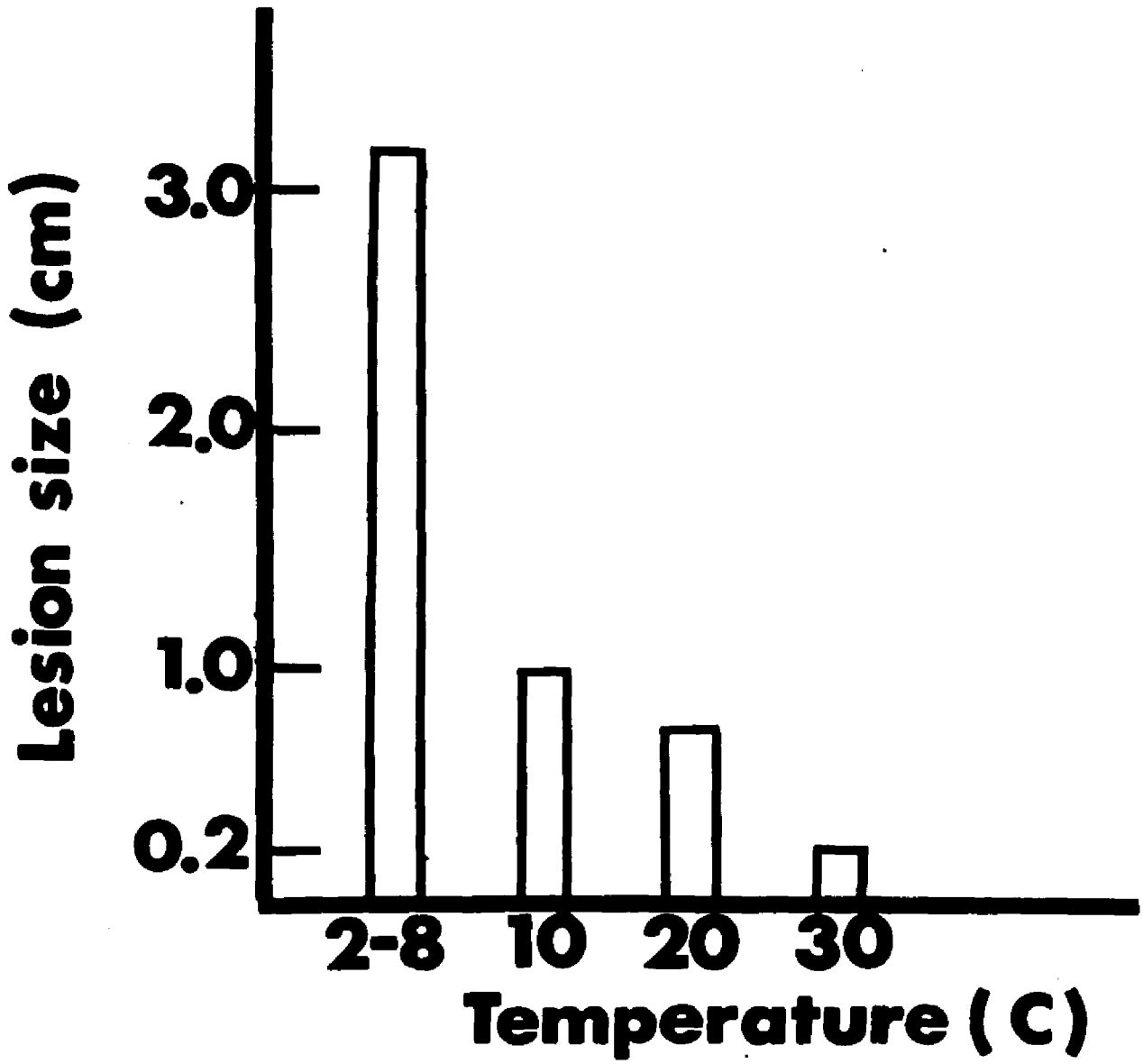


Fig. 21. Final average size of lesions caused by Godronia on inoculated 1-year-old Jersey variety plants after exposure to various temperatures. Plants were exposed to 18 C for 12 days, followed by 122 days at 2-8, 10, 20, or 30 C, and 63 days at 20 C. Data for each bar are based on average length of 30 lesions on 3 inoculated plants. Five of 12 inoculated stems wilted at 2-8 C.

showed that overgrowth symptoms could be divided into 5 categories based on the texture of the callus and the position of calluses on the affected plant. These calluses were as follows: 8-A calluses (Fig. 4-A, B) were calluses occurring at the bases of lateral branches; 8-B calluses (Fig. 4-C, D) were corky calluses extending the length of affected stems; 8-C calluses (Fig. 5-A, B) were corky and located along edges of cankers in the crowns of affected plants; 8-D calluses (Fig. 5-D, E; 10-B) were corky calluses occurring at edges of cankers located along the affected stem; 8-E calluses (Fig. 3-C, D) resembled healed wounds.

The following data are based on field and laboratory examination of over 450 callused stems collected over a period of 3 years. Regardless of the type of overgrowth, all calluses were associated with cankers. Calluses were found only on stems which were cankered. Neither boring insects such as the dogwood borer (Thamnosphecia scitula Harris), nor signs of their activity were observed in callused tissues. Nematodes were commonly found in 8-B calluses, but no attempt was made to identify or culture these organisms. No calluses were found on the excavated roots of 11 plants with callus symptoms on stems. Callus tissue was confined to the cambium, phloem, and cortex and could be easily removed from the xylem.

Stems of plants with callus symptoms often wilted and died. The xylem of dying stems was discolored at some point along the length of the branch. Since all of these stems were also cankered, it was impossible to determine whether the wilting was associated with the organism causing the canker or the agent causing the callus. Frequently, no canker fungi could be isolated from cankers on callused stems, and examination of the growth ring initially affected indicated that cankers were often initiated 3-5 years before the stem callused and wilted. The leaves of callused branches usually turned yellow or red before they wilted. Often, calluses concealed by dense foliage could be located by tracing along branches with discolored leaves.

Symptoms 8-C, 8-D and 8-E could always be found in fields in which Phomopsis and/or Godronia were common; however, these symptoms were more pronounced and more common in GD-Fields. Symptoms 8-A and 8-B were not found in all fields in which Godronia or Phomopsis were found, but Godronia was present in each of the fields in which these symptoms were found. Pruning of affected branches seemed to effectively check the spread of these symptoms in affected fields.

The association of fungi and bacteria with callused canker categories is shown in Table 20. These data are based only on those callused cankers which were sectioned and planted as indicated in Methods and Materials.

Table 20. Organisms associated with callused cankers.

Fungus	Callus type				
	8-A	8-B	8-C	8-D	8-E
	%	%	%	%	%
Godronia	21.2	51.8	57.9	82.4	34.5
Phomopsis	3.8	1.8	0	25.9	37.1
Coryneum	11.5	0	0	1.9	0.9
Fusarium	1.9	19.6	0	0.9	0.9
Alternaria	23.1	30.8	19.3	50.0	54.3
Epicoccum	36.5	1.8	10.5	28.7	44.8
Botrytis	7.7	0	5.3	5.6	4.3
Papulospora	1.9	12.5	3.5	5.6	0.9
Bacteria	2.0	40.0	3.0	5.0	5.0
Total sections	52	56	57	108	116

Godronia was commonly isolated from each type of callus. Categories 8-D and 8-E were also frequently inhabited by Phomopsis. Coryneum and Fusarium were observed most often on 8-A and 8-B, respectively. Bacteria resembling A. tumefaciens were common in 8-B calluses.

A series of experiments designed to determine the cause of callusing produced mostly negative results. Whenever actively growing plants were inoculated via chip wounds, 8-E calluses developed. Since control wounds were similar in appearance, it was concluded that 8-E calluses were a

wound response and probably develop whenever slowly developing cankers occur on rapidly growing stems.

Although 8-D calluses did not develop on plants inoculated with Godronia and Phomopsis, the symptom was induced by mechanically girdling rapidly growing stems (Fig. 10-A). These results suggest that 8-D calluses result from the girdling action of canker fungi on rapidly growing stems. Circumstantial evidence indicated that cankers caused by Godronia and Phomopsis play a role in 8-D callus formation. Both fungi were commonly associated with this symptom (Table 20). The symptom was very common in upper Michigan fields in which Godronia was the only pathogen found. Histological observations of 8-D calluses from which Godronia was isolated showed that the tissues were permeated with hyphae (Fig. 30-A). In short, when conditions are favorable, 8-D calluses probably develop in response to the slow girdling action of cankers caused by Godronia and to a lesser extent by Phomopsis which grows more rapidly in infected tissues. Whether 8-A and 8-C calluses fall into this category is unknown. Godronia, however, was commonly associated with these tissues (Table 20).

The cause of 8-B calluses was not determined. The symptoms were similar to symptoms of crown gall in British Columbia (Fig. 4-E). Attempts to transmit the symptom by using small pieces of callus tissue to inoculate blueberry plants failed. Similarly, all bacteria isolated from 8-B

calluses failed to induce symptoms on inoculated blueberries and several herbaceous indicators of crown gall. Control plants inoculated with known A. tumefaciens also failed to produce galls. Even though there was evidence that the known cultures of A. tumefaciens had lost pathogenicity, it was impossible to draw conclusions from the data.

In summary, 8-E and 8-D calluses probably result from the girdling action of cankers caused by Godronia and Phomopsis. It is possible that 8-A and 8-C calluses are similarly caused, but proof is lacking. The cause of 8-B calluses is unknown. The callus syndrome on blueberries in Michigan merits further research.

PART II. DISEASE CYCLE AND INFECTION STUDIES.

Introduction: The underlying objective of this research was to obtain sufficient information to provide a basis for designing fungicide evaluation experiments. Attempts to control "Fusicoccum" canker with fungicides had been ineffective (16, 31, 37, 73). It seemed likely that such failures were due to improper timing of fungicide applications. These studies were designed to determine when and how Godronia infects blueberries in Michigan.

Methods and Materials.

Availability of inoculum: Observations of relative numbers of apothecia and pycnidia present in blueberry fields were recorded at various times during the 1965-1968 growing seasons. In addition, fruiting bodies were collected from several different Michigan fields at various times during 1965-1968. Fruiting bodies were placed in moisture chambers at 20-25 C for 24 hours and examined microscopically for the presence of spores.

Other inoculum sources: Groves (28) reported in 1965 that G. cassandrae probably existed in several forms which were found on several genera of plants. Populations of plant species known to be hosts of forms of Godronia were surveyed in areas adjacent to blueberry fields in which

Godronia was epiphytotic. Isolates from these hosts were used to inoculate healthy Jersey variety blueberry plants. Control inoculations were made with isolates of G. cassandrae from V. corymbosum.

Infection periods: Healthy plants were exposed to natural inoculum in fields in which Godronia was known to be epiphytotic. Each month during the 1967 growing season ten 3-year-old and six 1-year-old Jersey variety plants were placed in a single field, left for 1 month, and then replaced with a similar set of plants. Control plants were kept in a nearby noninfested field for the duration of the experiment. Counts of lesions were made periodically on all plants.

Every 6 weeks during the 1968 season ten 1-year-old Jersey variety plants were placed into each of 2 fields (Field A was located in upper Michigan and Field B in northern lower Michigan) each heavily infested with Godronia. Following each 6 week exposure period, plants were returned to a cold frame in East Lansing and replaced with a similar set of plants. Controls were plants kept in the cold frame for the duration of the experiment. All plants were cut into 0.5 cm long sections at the end of the experiment. A random sample of 50 sections was taken from each plant and placed on ½ PDA to determine relative levels of infection by Godronia. Relative levels of infection were expressed as the Infection Index. Infection Index was

defined as the number of sections yielding Godronia when sections from plants were placed on ½ PDA. Thus the Infection Index would be 25 if Godronia grew from 25 of the 500 sections taken from a given set of exposed plants.

Experimental Results

Availability of inoculum: Sporulating pycnidia were most abundant during April to mid-June (Table 2.) Conidia were observed washing down stems during April and May rains, but not during rainy periods of the remainder of the growing season. Although conidia were most abundant during April to mid-June, some were present throughout the growing season. Pycnidia were found in all fields infested with Godronia.

Apothecia were first observed in late April. Ascospores, however, were not mature until early mid-July (Table 22). Evidence of ascospore discharge, as indicated by empty asci, was noted by mid-July. Most ascospores had been discharged by mid-September, but some were present in asci in mid-October. New apothecia were formed by July on a stem from which apothecia were removed in April. Apothecia were not found in all Godronia infested fields, but were common in 8 fields in which Godronia was epiphytotic.

Other inoculum sources: Apothecia and pycnidia of G. cassandrae were found on dead stems of V. angustifolium and C. calyculata adjacent to Michigan blueberry fields. Pycnidia were also found on dead stems of several species of Spiraea. Godronia was isolated from lesions and blighted stems of these hosts. Michigan isolates of G. cassandrae

Table 21. Presence of Godronia spores in fruting bodies collected at various times during the 1965-1968 growing seasons.

Sample dates	Year[a] 1965 A	1965 C	1966 A	1966 C	1967 A	1967 C	1968 A	1968 C
April 20-30	-	-	-	+++	-	-	*	+++
May	-	-	-	-	-	-	*	+++
June 1-15	-	-	*	+++	*	+++	*	+++
June 16-30	-	-	-	-	-	-	-	-
July 1-15	-	+	+++	+	+++	+	+++	+
July 16-31	-	-	+++	+	+++	+	-	-
August	-	-	-	+	++	+	++	+
September 1-15	-	-	+	+	+	+	-	-
September 16-31	0	+	-	+	-	-	-	-
October	-	+	-	-	-	-	+	+
December 1-3	-	-	-	-	-	-	0	+

[a] A = ascospores
C = conidia
+ = few spores; ++ = moderate number spores; +++ = many spores.
* = spores immature.
0 = no spores.
- = no fruiting bodies sampled.

from V. angustifolium, V. corymbosum, C. calyculata, and Spiraea spp.; and isolates from V. macrocarpon, V. angustifolium, C. calyculata, and Betula sp. obtained from other workers were studied.

Isolates from C. calyculata (Fig. 22-C, D) and V. macrocarpon were canary yellow to pale yellow in color, sporulated poorly, and produced abundant aerial mycelium on ½ PDA. Isolates from Spiraea spp. were similar except that colonies were pale yellow to tan in color (Fig. 22-C, D).

Isolates from V. corymbosum varied from canary yellow to brown or gray in color (Fig. 22-A, B, E, 23). Some isolates produced abundant aerial mycelium whereas others did not and were slimy in appearance on ½ PDA. Single ascospore isolates sectored occassionally. Cultural characteristics were maintained when mycelial transplants were made. Sclerotia did not occur.

Isolates from V. angustifolium (Fig. 22-A, B, C, D) were yellow to brown in color and sporulated well on ½ PDA. Some isolates produced abundant aerial mycelium whereas others were slimy in appearance and produced little aerial mycelium.

Form beticola (Fig. 22-C, D) produced pale yellow to tan aerial mycelium on ½ PDA and did not sporulate.

Growth of all forms of G. cassandrae (Table 22) at various temperatures was similar to growth of f. vaccinii

Table 22. Growth of forms of *Godronia cassandrae* at various temperatures.[a]

Form of *G. Cassandrae*	Host	Temperature (C)						
		0-2	8	12	20	25	30	35
		Colony diameter (cm)						
cassandrae	*Chamaedaphnae*	0.5	2.3	6.4	7.0	4.8	1.2	0
vaccinii	*V. angustifolium*	0.2	1.9	6.1	7.5	5.3	0.9	0
vaccinii	*V. corymbosum*	0.7	2.4	6.3	7.4	3.7	0.5	0
vaccinii	*V. macrocarpon*	0.5	2.3	4.4	7.2	3.6	1.0	0
beticola	*Betula sp.*	0.2	0.1	2.3	6.5	1.0	0.1	0
spiraeicola	*Spiraea spp.*	0.4	2.7	5.9	7.4	5.5	1.2	0

[a] Growth of 5 isolates grown on ½ PDA measured after 2 weeks exposure to each temperature.

Table 23. Spore morphology of forms of G. cassandrae found in Michigan.[a]

No. spores measured	Isolate Code	Host	Average size (μ)	Range of size (μ)	% with 1 septum	% spores straight	% spores curved
30	SR-7	Spiraea	9.2 x 1.3	12.7-8.1 x 2.3-1.2	100.0	76.6	23.4
50	SR-8	Spiraea	10.8 x 2.1	16.0-6.7 x 1.6-5.3	100.0	98.0	2.0
50	SR-4	Spiraea	9.8 x 2.0	12.6-6.9 x 3.2-1.3	100.0	80.0	20.0
30	CH-1	Chamaedaphnae	8.8 x 1.2	11.5-5.8 x 2.3-1.2	93.3[b]	100.0	0
50	CH-2	Chamaedaphnae	9.6 x 1.4	13.8-4.6 x 1.2-1.5	78.0[b]	90.0	10.0
50	Oct-2	V. angustifolium	9.3 x 1.7	13.8-3.5 x 2.7-0.9	86.0[2]	42.0	58.0
30	No'ed	V. corymbosum unnamed var.	11.0 x 1.7	15.0-6.9 x 1.6-1.2	96.6[b]	40.0	60.0
50	FC-11	V. australe Jersey var.	12.2 x 1.6	16.2-8.2 x 2.3-1.2	100.0	68.0	22.0
50	AS-9	V. corymbosum unnamed var.	10.0 x 1.5	17.3-7.5 x 2.1-1.3	100.0	42.0	58.0
50	AS-10	V. corymbosum unnamed var.	10.0 x 1.3	12.7-8.1 x 2.3-0.9	98.0[b]	18.0	82.0
50	AS-6	V. corymbosum unnamed var.	9.8 x 1.4	13.8-5.8 x 2.1-1.3	96.0[b]	24.0	76.0
50	FC	V. corymbosum Earliblue var.	11.2 x 1.4	15.4-7.7 x 1.0-2.6	79.1[c]	94.0	6.0

[a] All spores measured in Ammon's mounting modium (lactophenol) (2).
[b] All other spores were non-septate.
[c] Two septate 12.8%; three septate 8.1%; number septations based on count of 125 spores.

Fig. 22. Isolates of G. cassandrae grown on ½ PDA at various temperatures.

A) Single ascospore isolates from V. corymbosum (a, b), single conidium isolates from V. corymbosum (c, d), Michigan single ascospore isolate from V. angustifolium (e), Nova Scotia isolate from V. corymbosum (f) obtained from C. L. Lockhart, Quebec isolate from V. angustifolium (g) obtained from E. Smerlis. All isolates grown at 25 C.

B) Same isolates as in (A) grown at 20 C.

C) Quebec isolate of f. beticola (a) obtained from E. Smerlis, Quebec isolate of f. cassandrae (b) obtained from E. Smerlis, Michigan isolate of f. spiraeicola (c), Michigan isolate of f. cassandrae (d). All isolates grown at 25 C.

D) Same isolates as in (C) grown at 20 C.

E) Response of single ascospore isolate from V. corymbosum to temperature.

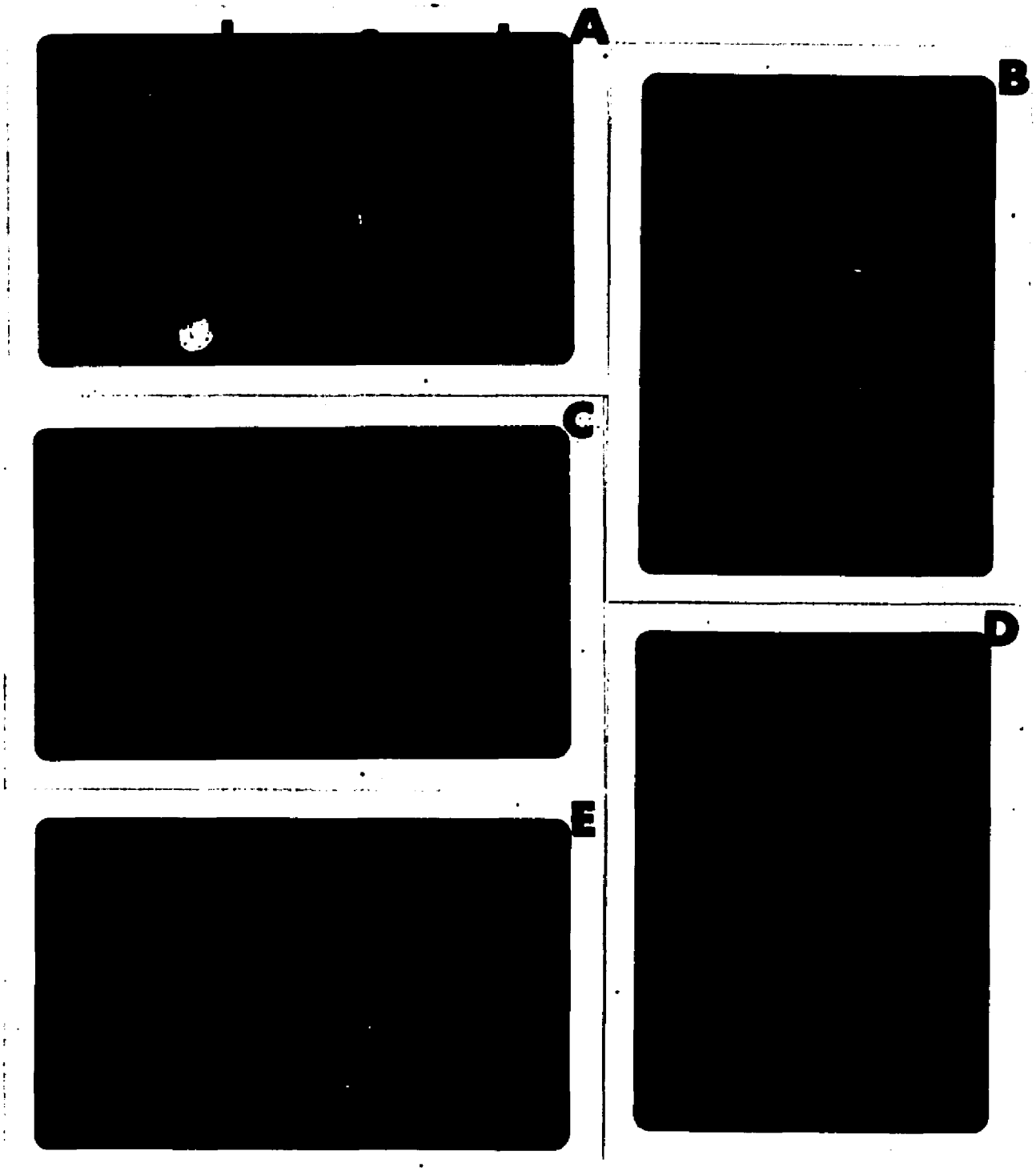

A
B
C
D
E

Fig. 23. Variations in cultural morphology of single ascospore isolates of G. cassandrae f. vaccinii from V. corymbosum. All are 4-6 week old cultures on ½ PDA.

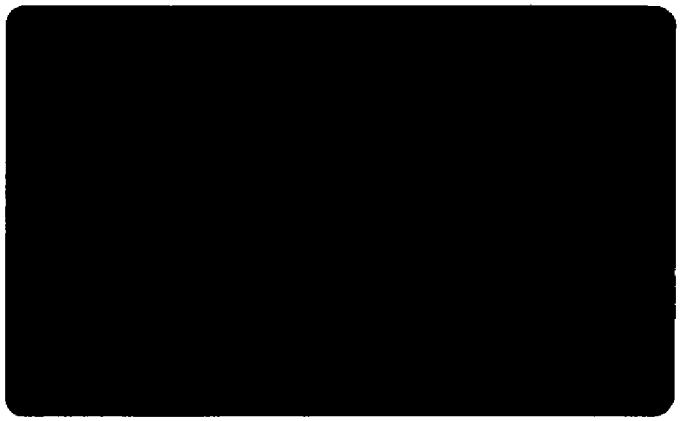


Fig. 24. Asci and spores of G. cassandrae f. vaccinii.

A) Asci mounted in lactophenol and stained with 0.1% cotton blue (800 X).

B) Single ascospore mounted in lactophenol and stained with 0.1% cotton blue (800 X).

C) Ascus mounted in water (800 X).

D) Section cut through pycnidium on an infected stem (800 X). Cut with an experimental microtome (29).

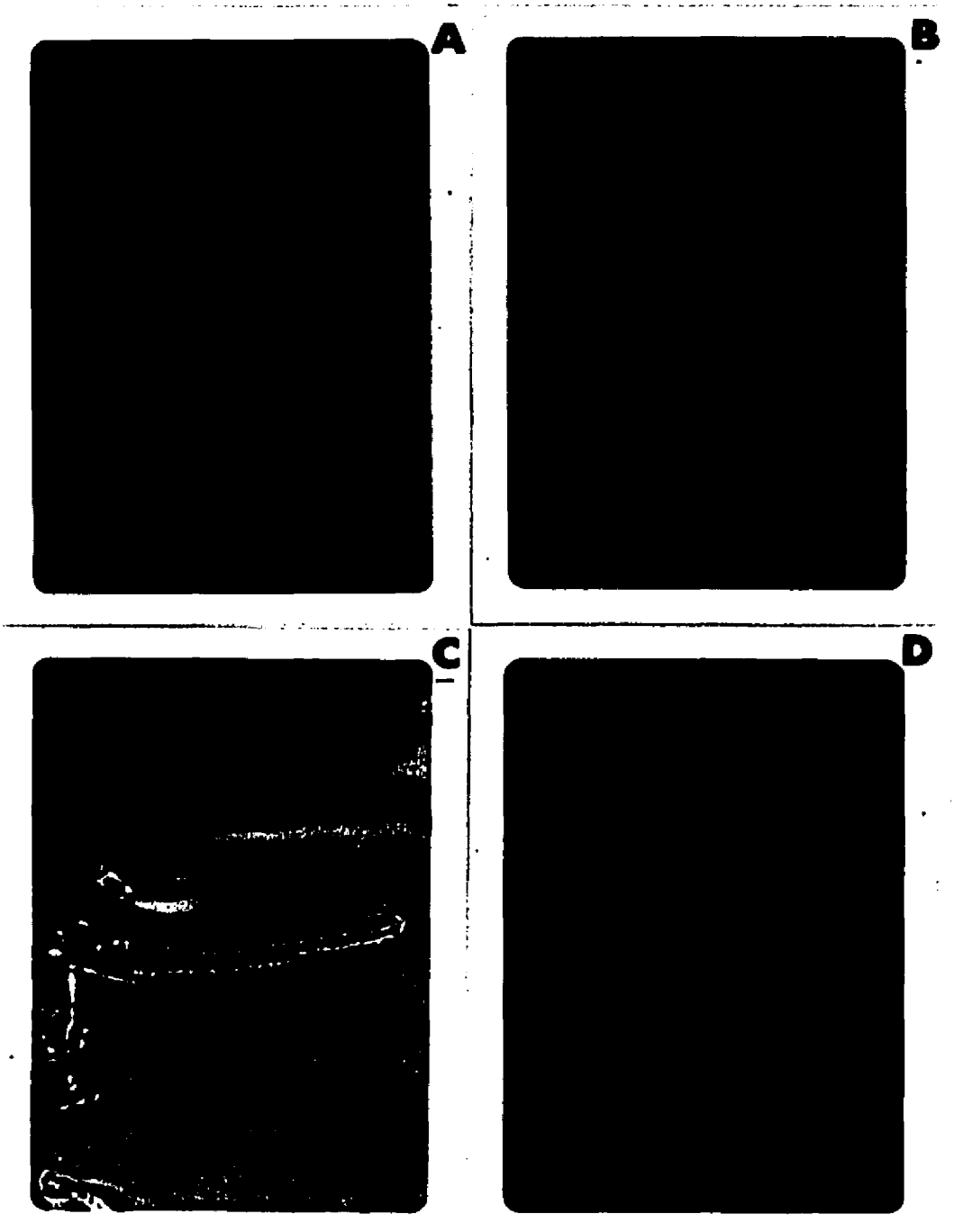

A
B
C
D

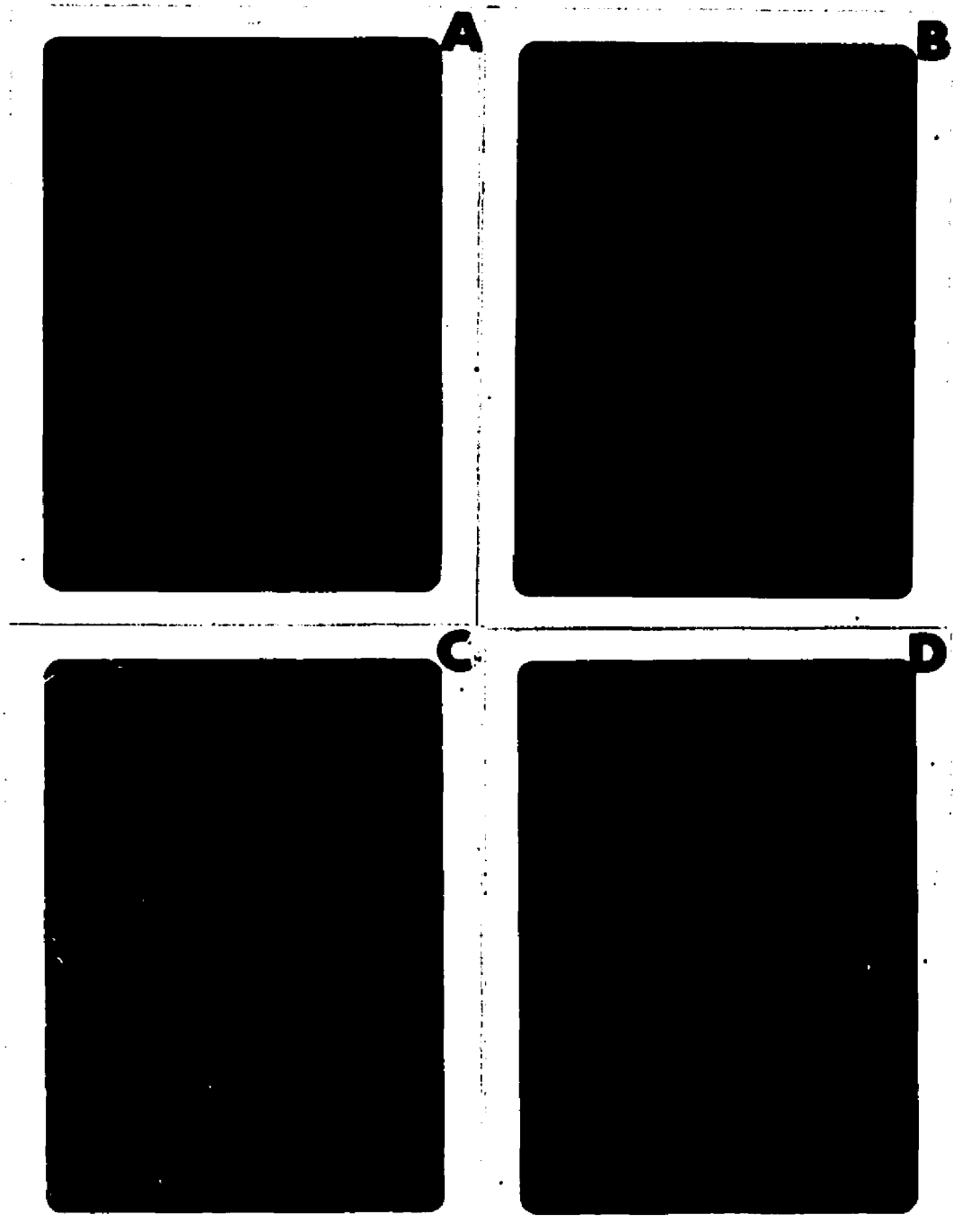
Fig. 25. Conidia of G. cassandrae (A, C, D, mounted in water and B in lactophenol and stained with 0.1% cotton blue).

A) Conidia from pycnidium on Earliblue variety (800 X). Note the spore with 3 septa.

B) Conidia from pycnidia on Earliblue variety (2000 X).

C) Conidia from pycnidium on Jersey variety (800 X).

D) Conidia of f. spiraeicola (800 X).


A
B
C
D

from V. corymbosum (Fig. 9, 22-E). Isolates from V. corymbosum and V. angustifolium which produced viscous material in culture did so only at 20-25 C (Fig. 22-A, D).

Ascospores of G. cassandrae on V. corymbosum (Fig. 24-B), V. angustifolium, and C. calyculata were 5-9 septate, straight, and measured 79.1-96.4 x 1.0-1.3 μ. Asci (Fig. 24-A, C) measured 5.3-16.3 x 82.5-106.4 μ. Microconidia were observed only in pycnidia on dead stems of V. corymbosum. Microconidia germinated and produced typical cultures of G. cassandrae on ½ PDA. Morphology of Conidia (Fig. 24-D, 25) are summarized in Table 23.

Isolates of G. cassandrae from V. angustifolium, Spiraea spp., and possibly C. calyculata were pathogenic on V. corymbosum (Table 24). Pycnidia were produced on plants inoculated with isolates from V. corymbosum and V. angustifolium, but not on cankers caused by isolates from Spiraea spp. or C. calyculata. Godronia was reisolated from lesions developing from inoculation sites. Isolates from V. macrocarpon did not cause cankers on highbush blueberries, but epidermis turned tan around inoculation sites about 1 year after inoculation. No attempt was made to isolate G. cassandrae from such tissue.

Infection periods: Plants introduced into the single field in 1967 were not infected. It was further noted that in the spring of 1968 little new infection occurred in this field. During previous years 35-40 lesions/plant were

Table 24. Pathogenicity of forms of G. cassandrae on Jersey variety blueberries.

Form of G. cassandrae	Host	Source of isolate	No. inoculations	% inoculations successful
spiraeicola	Spiraea spp.	Michigan	48	60.4
cassandrae	C. calyculata	Michigan	52	0
cassandrae	C. calyculata	Quebec[a]	20	5.0
beticola	Betula sp.	Quebec[a]	20	0
vaccinii	V. macrocarpon	Wisconsin[b]	24	0
Vaccinii	V. macrocarpon	New Jersey[c]	31	0
vaccinii	V. angustifolium	Quebec[a]	20	100.0
vaccinii	V. angustifolium	Michigan	58	79.2
vaccinii	V. corymbosum	Nova Scotia[d]	20	100.0
vaccinii	V. corymbosum	Michigan	183	82.3

[a] Isolate supplied by E. Smerlis.
[b] Isolate supplied by R. J. Friend.
[c] Isolate supplied by A. Stretch.
[d] Isolate supplied by C. L. Lockhart.

counted whereas only 2 new lesions were observed during the spring of 1968. The field was flooded by an adjacent lake during April-July, 1967 and all apothecia were destroyed. Destruction of apothecia probably explains the low level of infection in 1968.

Definite infection periods were determined during 1968 (Fig. 26). The Infection Index in Field B was greatest for plants exposed during May 29-July 10 and plants exposed during August 21-October 9. Infection Indices in Field A were similar except that plants exposed during July 10-August 21 also had a high Infection Index. Examination of precipitation records (59) from nearby weather stations showed that it rained for several days before and after August 21 in Field A, but not in Field B (Fig. 27). No sections taken from control plants were infected with Godronia.

Conidia were most abundant during April-June in both fields. Spores were observed washing down stems during rains in late May. Ascospores were not mature until mid-July. Apothecia were normally closed (Fig. 16-A) until they were placed in moisture chambers for several hours (Fig. 16-C).

Although conditions influencing ascospore discharge by Godronia have not been studied, it was recently shown that 4 hours of rainfall were necessary before ascospores of Scleroderris lagerbergii Gremmen (S. lagerbergerii has

leathery apothecia closely resembling those of Godronia) were discharged (49). Spore discharge was more related to rainfall than to relative humidity or temperature (50). It seems likely that ascospores of Godronia are also discharged during periods of rain. Assuming, then, that ascospores and conidia are dispersed during rainy periods, several conclusions regarding infection periods can be made.

The high Infection Indices on plants exposed in Field A during exposure periods 3 and 4 (Fig. 26) probably resulted because both sets of plants were exposed during the same major infection period. Ascospore infection of plants in Field A probably occurred between August 12 and September 12 since this was the only period of rainfall between 10 July and 9 October. In Field B, infection via ascospores probably occurred between August 30 and September 10.

Infection by conidia probably occurred during the spring and early summer. In Field A, infection by conidia was probably concentrated during rains between May 13 and July 2. Precipitation was not as frequent in Field B and conidia dispersal must have occurred between May 29 and July 2. It seems likely, therefore, that spring and early summer infection by conidia occurs between April and July with heaviest infection occurring in June. Ascospore infection of plants occurs between mid-August and mid-September.

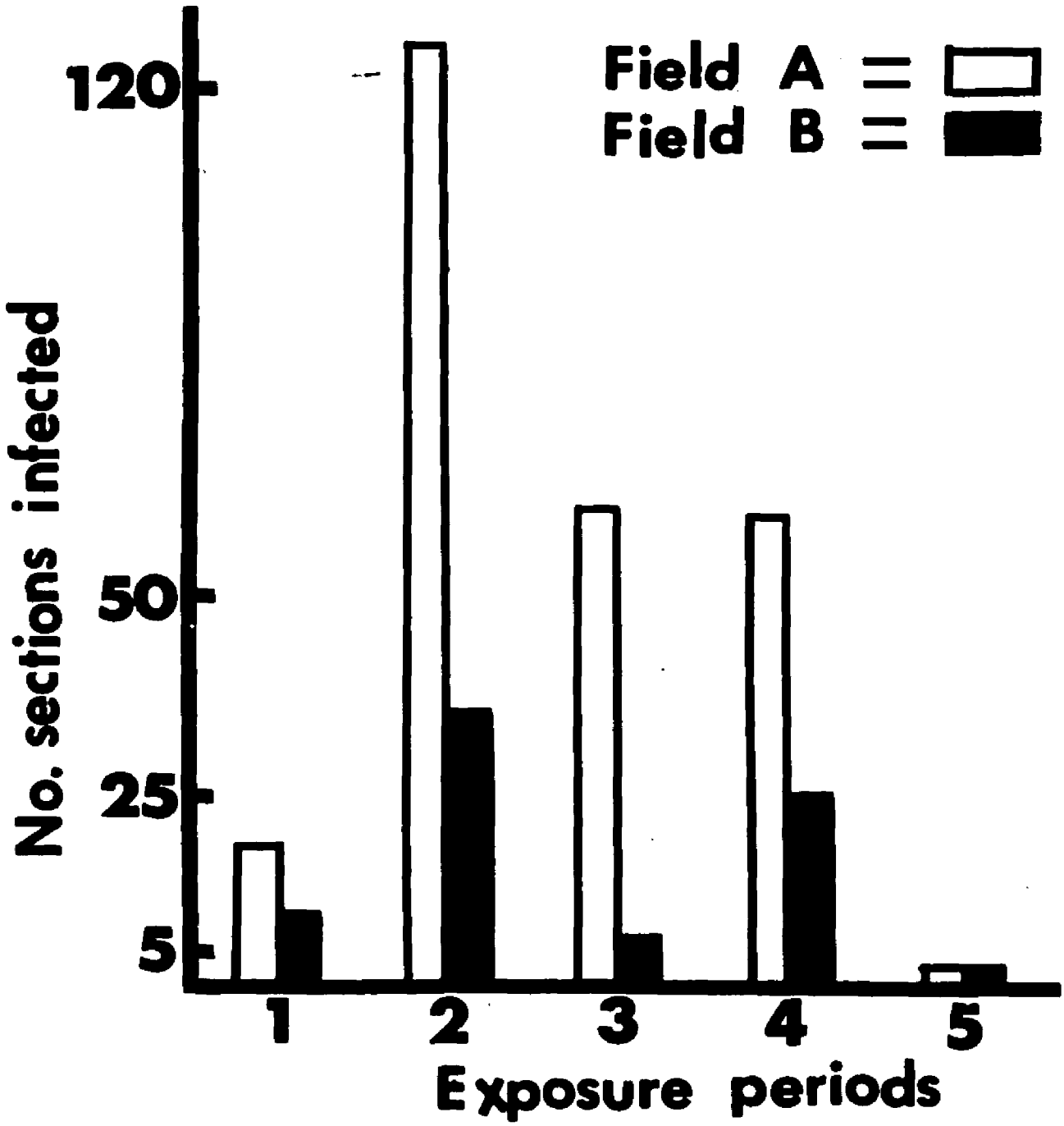


Fig. 26. Number of sections infected with Godronia (i.e., number sections yielding Godronia) when 500 sections from each group of exposed plants were placed on ½ PDA. Exposure periods were: 1 = 23 April-29 May; 2 = 29 May-10 July; 3 = 10 July-21 August; 4 = 21 August-9 October; 5 = 9 October-3 December. Field A was located in upper Michigan and Field B in northern lower Michigan.

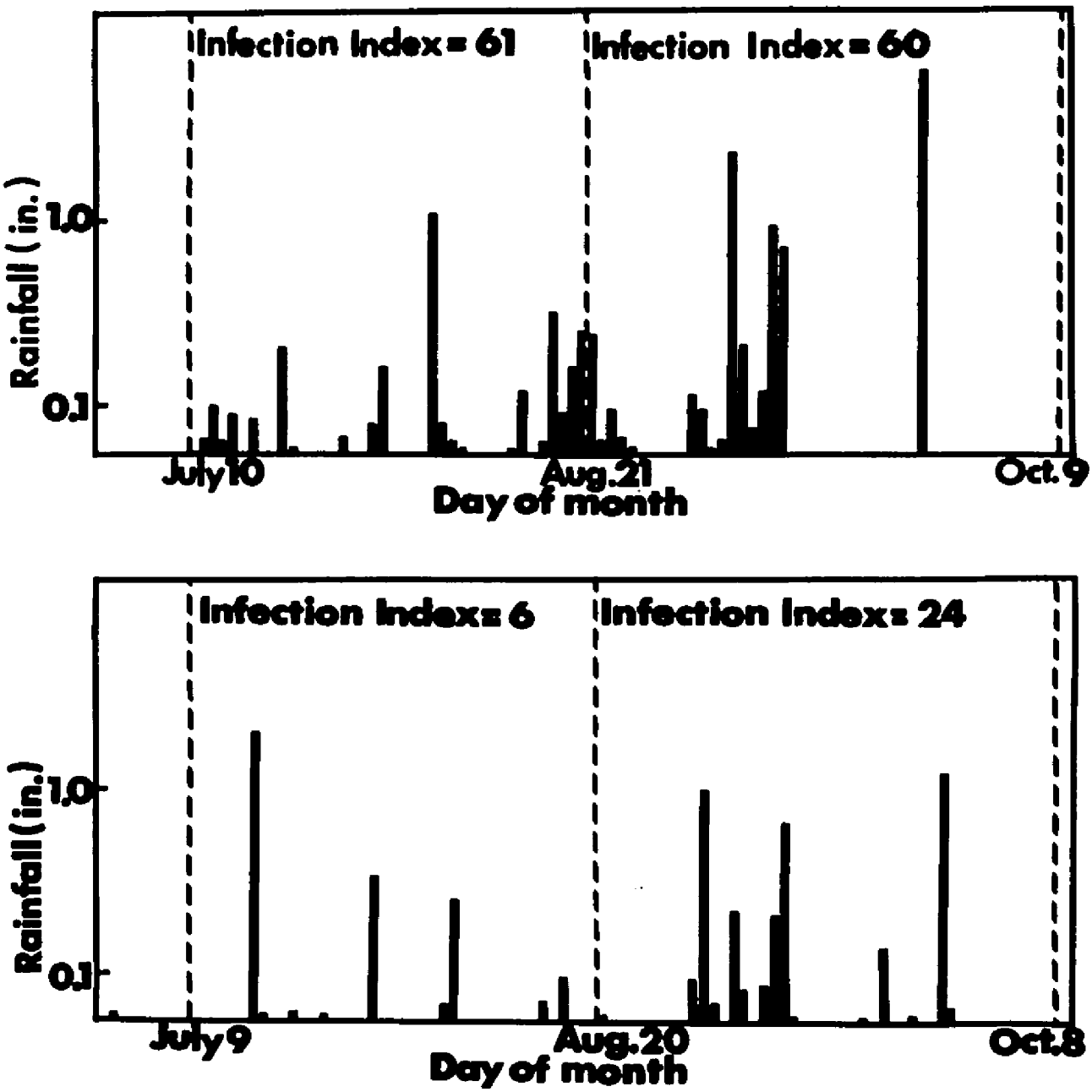


Fig. 27. Amounts of precipitation recorded at weather stations near Field A (upper) and Field B (lower) between 1 July and 9 October, 1968. Infection Index represents the number of sections yielding Godronia when 500 sections taken from each group of exposed plants were placed on ½ PDA. Vertical stippled lines indicate dates when plants were placed into and removed from the fields.

The conclusions stated above are consistent with existing data, however studies of conditions favoring spore discharge and infection would add to the value of this hypothesis.

Infection sites: Reports of others (16, 37, 73) and personal observations indicated leaf scars were important sites of infection by G. cassandrae f. vaccinii. To determine relative numbers of nodal and internodal infection sites, random samples of 1- and 2-year-old stems were collected in April, 1968. The numbers of lesions occurring at nodes and internodes were recorded. Isolations were made from lesions to verify presence of Godronia.

As shown in Table 25, 43.7% lesions observed were at leaf scars and 21.7% leaf scars were infected. Cankers at nodes were generally larger than internodal cankers. Only 28.1% lesions less than 0.5 cm long were at nodes (Table 27) whereas 94.4% cankers exceeding 1.5 cm occurred nodally (Table 27). Only 11.2% internodal infections were observed in April as developed cankers (Table 26) whereas 54.1% leaf scar infections (Table 26) were developed cankers.

Cohoon and Daines (10), Daines, et. al. (17), and Daines (18) showed that leaf scar infection sites of Fusicoccum canker of peach (F. amygdali, the causal organism of Fusicoccum canker of peach, is not related to Godronia) decreased as leaf scars healed. Several experiments were performed to determine when and how Godronia infects leaf scars on blueberries.

Table 25. *Godronia* infection sites on 1- and 2-year-old stems collected in April 1968.

Field	Number stems collected	Total number lesions	% lesions at leaf scars	% leaf scars infected
Upper Peninsula	55	1,689	40.1	29.8
Ludington	38	256	67.9	27.9
Totals	93	1,945	43.7	21.7

Table 26. Percent total infections, nodal infections, and internodal infections by *Godronia* occurring as lesions of various sizes.

	Lesion size		
	<0.5 cm	0.5-1.5 cm	>1.5 cm
% total infection sites	70.5	20.1	9.4
% total leaf scar infection sites	45.9	33.2	20.9
% total internodal infection sites	88.8	10.2	1.0

Table 27. Percent *Godronia* lesions of various sizes occurring at nodes and internodes.

	Lesion size		
	<0.5 cm	0.5-1.5 cm	>1.5 cm
% lesions occurring at nodes	28.1	71.1	94.4
% lesions occurring at internodes	71.9	28.9	5.6

Random samples of stems formed during 1968 were collected on October 10, 1968. Petioles of leaves, lateral buds, necrotic leaf tissues, and stem internodes were placed on ½ PDA to isolate Godronia. As shown in Table 28, petioles, buds, necrotic leaf tissues, and internodal tissues had been invaded by Godronia. Attempts to isolate Godronia from leaves and petioles in August had failed (Table 28).

Table 28. Infection of necrotic leaves, petioles, buds, and stem internodes by Godronia.

Date	Petioles	Buds	Leaves	Internodes
30 August				
No. sampled	30	-	30	-
% infected	0	-	0	-
10 October				
No. sampled	136	138	26	153
% infected	44.8	76.8	80.7	67.9

Petioles from which Godronia was isolated were slightly reddened, but otherwise normal in appearance. Buds showed no external signs of infection. Necrosis of leaves had been initiated from wounds. Godronia was isolated from stem internodes before lesions had developed. The only sign of infections were small zones of gray colored tissue. Observations of similar stems were made in the field December 3, 1968. At this time minute red lesions were observed

(Fig. 1-A). Godronia was isolated from most, but not all such lesions.

Several stems from the October sample were wrapped in moist paper towels, sealed with aluminum foil, and placed in a 2 C cold room. Well developed lesions with pycnidia occurring nodally and internodally were observed when stems were examined after 6 months in the cold room (Fig. 13-A).

It seemed that nodes were infected via petioles or buds. Infection of leaf scars via petioles was studied.

Infection of leaf scars prior to leaf drop: Three 4-year-old plants grown in the cold frame were sprayed in October 2-3 weeks before leaf fall with a suspension of Godronia conidia in water. The plants were covered with plastic bags and incubated for 3 weeks in the 18 C mist chamber. Plants were returned to the cold frame for the winter. Numbers of lesions occurring at nodes and internodes were recorded in April. A control plant was sprayed with water.

The number of lesions on each plant varied (Table 29). However, 83.3% of all lesions developed around leaf scars.

Infection of healing leaf scars: The ability of Godronia to infect healing leaf scars was studied in the following manner. Leaf blades were removed from petioles on actively growing plants. Petioles were inoculated with suspensions of Godronia conidia in water or mycelium in blocks of ½ PDA at 0, 1, 2, and 5 days after removing leaf

blades. Inoculation sites were wrapped and plants were placed in the 18 C mist chamber for 10 days. Following incubation plants were grown in an 18 C greenhouse. Controls were plants inoculated with water or blocks of ½ PDA.

Table 29. Infection of leaf scars by Godronia when suspensions of conidia in water were sprayed on plants 2-3 weeks before leaf drop.

Plant number	Total number lesions	Number lesions at leaf scars
1	17	12
2	10	7
3	39	36
4(H_2O control)	0	-
Totals	66	55 = 83.3%

As shown in Table 30, the percentage of leaf scar infections decreased as the length of time after removing leaf blades increased.

Table 30. Infection of leaf scars via petioles by Godronia at various times after removing leaf blades.

Days after removing leaf blade	Number petioles inoculated	% leaf scars infected
0	47	34.0
1	54	31.4
2	38	7.8
5	72	5.5

Infection of healed leaf scars: Healed leaf scars on dormant 3-year-old plants in a cold frame were inoculated in February 1968, using Godronia mycelium in blocks of ½ PDA as inoculum. Following inoculation plants were incubated for 12 days in a 18 C mist chamber and then returned to the cold frame. Leaf scars pierced a single time with a sterile needle and inoculated with Godronia; and nonwounded leaf scars inoculated with blocks of ½ PDA served as controls.

Lesions developed from wounded leaf scars inoculated with Godronia within a few days following inoculation. No lesions were discernable at healed leaf scars for 1 year. However, between February 25 and May 3, 1969, lesions developed at 30.2% of 129 inoculated nonwounded nodes. Since inoculum had been in contact with both leaf scars and axillary buds, it was not determined which served as the infection court. None of the nodes inoculated with ½ PDA were lesioned. This is the first report of latent infection by Godronia.

Infection of internodes: Two experiments were designed to demonstrate infection of nonwounded stem internodes by Godronia. Plants actively growing in the greenhouse were sprayed with suspensions of conidia in water, covered with plastic bags, and incubated for 2 weeks in a 18 C mist chamber. No infections developed on these plants when they were grown in the 18 C greenhouse.

Nonwounded stem internodes on two 4-year-old dormant plants were inoculated using the procedures outlined in previous experiments. Five of 90 inoculated internodes were infected by Godronia. Lesions did not develop on nonwounded stem internodes inoculated with blocks of ½ PDA. All internodes which were wounded and inoculated as controls were infected.

It was concluded from these experiments that leaf scars are probably infected via attached petioles or axillary buds. This conclusion is consistent with data obtained when plants were exposed to natural inoculum. That infection takes place between mid-August and mid-September and that leaf scars are invaded via attached petioles indicate that fingicides applied by other workers (16, 35, 73) were uneffective, at least in part, because they were applied long after infection had occurred.

PART III. PATHOLOGICAL HISTOLOGY.

Methods and Materials

Symptomatology of cankers caused by Godronia showed that lesions on 1- and 2-year-old stems were first visible as minute red spots on internodes and as water soaked or necrotic leaf scars. Necrosis was observed to begin in the centers of incipient internodal lesions. Bands of reddened tissue were often observed along the edges of necrotic tissues.

Development of lesions caused by Godronia was studied histologically. Lesions or cankers of various sizes on 1- to 3-year-old stems were collected. Diseased tissues were divided into categories based on the age of the lesion: a) type 1 - incipient lesions less than 2.0 mm in diameter (Fig. 1-A, B); Type 2 - 0.5-1.0 cm long, elliptically shaped lesions devoid of pycnidia (Fig. 1-B, C); Type 3 - $>$1.0 cm long, elliptically shaped lesions covered with pycnidia (Fig. 1-D; 2-A, B); Type 4 - discolored xylem from stems beginning to wilt. Callus tissue associated with cankers was also studied histologically, but no attempt was made to follow stages of callus development.

All tissues were neither fixed nor stained. Tangential and transverse 10-15 μ thick sections were cut with

an experimental microtome (29). Living sections were mounted either in water or glycerin diluted to 50% with water (v/v). Twenty to 30 sections were cut through both the centers and edges of lesions. At least 5 lesions of each Type were examined on each of 2 sampling dates. Callus tissue was collected only during June. Healthy tissue from uninfected branches of the same ages as cankered branches served as controls. Samples of lesions were placed on ½ PDA and Godronia was grown from stems with each of the symptom categories.

Observations were made with a Wild-Heebrugg M-20 compound light microscope equipped with a Kodak II 35 mm camera. Photomicrographs were taken on highspeed 35 mm Kodak Ectachrome X film.

Fig. 28. Transverse sections of healthy blueberry stem and stems infected with Godronia. All sections cut with an experimental microtome (29) and mounted in water.

A) Section of noninfected 1-year-old stem (250 X). (a) cuticle, (b) stoma, (c) substomatal chamber, (d) tightly packed cortex parenchyma, (e) air channels, (f) loosely packed cortex parenchyma, (g) pericyclic fibers, (h) phloem, (i) cambium, (j) xylem.

B) Incipient necrosis (b) below stoma (a) in center of Type 1 lesion (200 X).

C) Hypha in air channel of cortex (800 X).

D) Hyphae in air channel of cortex near reddened cortex parenchyma (400 X).

E) Hypha in air channel near reddened cortex parenchyma (800 X).

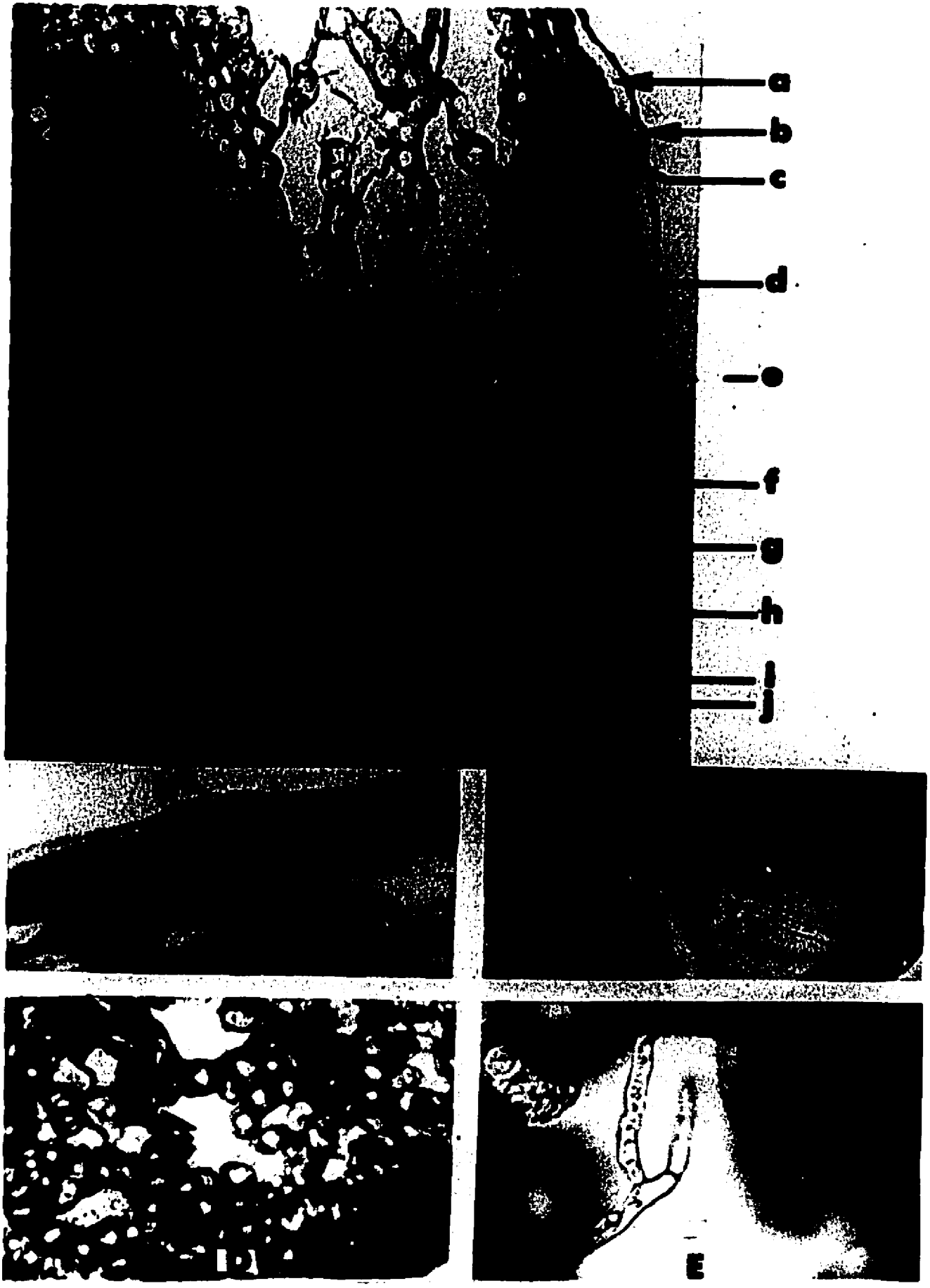

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Fig. 29. Sections of stems infected with Godronia (A-F mounted in water; G and H mounted in 50% glycerin).

A) Radial section showing hyphae in air channel of cortex (800 X).

B) Hyphae growing along surface of living cortex parenchyma cells (800 X).

C) Transverse section through Type 3 lesion showing hyphae in dead cortex parenchyma (800 X).

D) Transverse section through Type 3 lesion showing deposits in cortex parenchyma (200 X).

E) Section through center of Type 2 lesion showing general necrosis and hyphae (arrow) in air channel (200 X).

F) Longitudinal section through pycnidium of Godronia in necrotic cortex tissues (200 X).

G) Radial section showing hyphae in vessel of discolored xylem of wilted stem (800 X).

H) Transverse section through discolored xylem of wilted stem showing brown deposits (a) and hypha (b) in vessels (800 X).

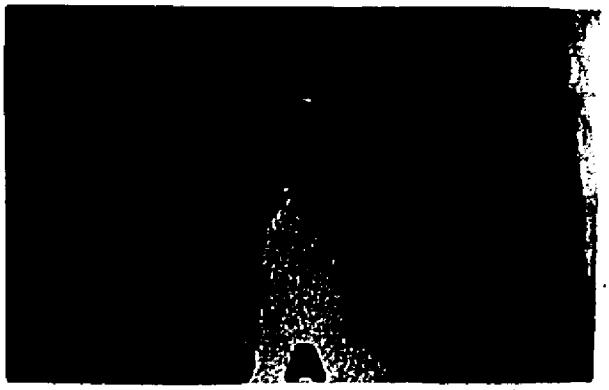


Fig. 30. Sections of diseased blueberry stems. (A, B, C, and F mounted in water; D, E, and G mounted in 50% glycerin.).

A) Hyphae in necrotic tissue of callus (800 X).

B) Hyphae and necrosis in bud tissues (200 X).

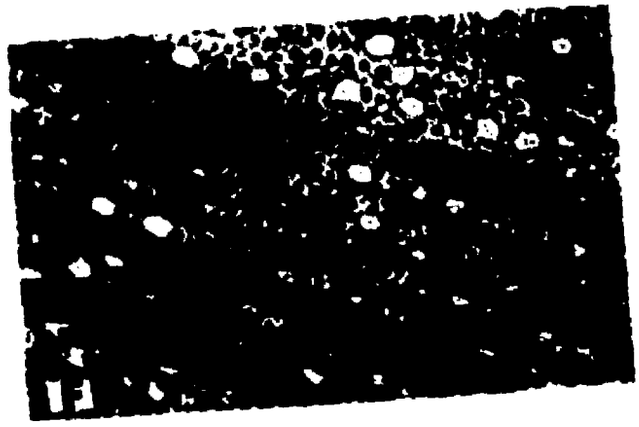
C) Disarranged vascular tissue in 8-D callus (200 X).

D) Brown deposits between 2 vessels in discolored xylem of wilted stem (800 X).

E) Hypha growing through scaliform perforation plate of vessel in discolored xylem of wilted stem (800 X).

F) Brown deposits in discolored xylem of wilted stem (200 X).

G) Possible tyloses in vessel of discolored xylem of wilted stem (800 X).



Experimental Results

The only detailed study of blueberry stem anatomy was published by Wasscher (63). The following observations (Fig. 28-A) of transverse sections of 1-year-old healthy stems of an unnamed blueberry variety agreed with Wasscher's (63) data.

The epidermis was protected by a 10-15 μ thick cuticle. The cuticle was interrupted by numerous stomata which were bordered by 2 thin-walled guard cells. The outer cortex consisted of a 5-12 cell layer of tightly packed parenchyma cells which were filled with chloroplasts. In transverse sections the remaining cortex was spongy in appearance due to presence of longitudinal air channels, which according to Eck (24), "are so oriented that 2 ducts are adjacent to one another along the radial axis." The cortex and vascular tissues were separated by a layer of thick-walled fibers called "pericyclic" fibers by Wasscher (63).

Observations of transverse and tangential sections cut through the center of Type 1 lesions showed that epidermal cells were unaffected and that parenchyma cells of the cortex immediately below stomates were the first to die (Fig. 28-B). Moribund cells were filled with dark brown deposits and hyphae could not be seen in the cells. Hyphae were, however, observed among cortex parenchyma adjacent to

pockets of necrosis (Fig. 28-C, D, E). The chloroplasts of parenchyma adjacent to necrotic cells turned red. Cells with red chloroplasts were often observed 16-20 μ in advance of growing hyphae. Hyphae were most abundant within dead cells and in longitudinal air spaces (Fig. 28-C, D, E; 29-A, B, C). Hyphae seemed to advance primarily via air channels. The fungus often grew along the surfaces of living cortex parenchyma cells (Fig. 29-B), but penetration of living cells was not observed. Hyphae were usually found in dead cells of the cortex (Fig. 29-C), but not inside of living cells. Air channels and dead cells of the cortex were filled with brown deposits and the cell walls turned brown (Fig. 29-D).

All cortex tissue beneath Type 3 lesions was necrotic and permeated with hyphae (Fig. 29-E). Pycnidia were formed in outer layers of necrotic cortex tissue (Fig. 29-F). The xylem of stems with Type 3 lesions was starting to turn brown. Hyphae, possible tyloses, and brown deposits were observed in vessels of the xylem (Fig. 29-G, H), but were confined to outermost cells.

Hyphae, tyloses, and deposits were common in the vessels of wilted stems (Fig. 29-H, 30-D, E, F, G). Thirty to 40% of all vessels in a given 10-15 μ thick transverse section were occluded (Fig. 30-F). Observations of tangential sections showed that cell walls of vessels, ray parenchyma and fibers turned brown. Hyphae grew longitudinally

through vessels and from cell to cell by growing through scaliform end plates of vessels (Fig. 30-E). The ends of vessels were often clogged with brown deposits (Fig. 30-D).

Buds associated with lesion Types 2 and 3 were necrotic and thoroughly permeated with hyphae (Fig. 30-A, B).

Callus tissue was composed to disarranged parenchyma and vascular tissue (Fig. 30-C). Hyphae were common in calluses along the edges of cankers (Fig. 30-A).

DISCUSSION

Koch's Postulates were fulfilled with P. vaccinii and G. cassandrae showing that both fungi cause cankers and stem blights of blueberries in Michigan. The diseases will be called Phomopsis canker and stem blight and Godronia (Fusicoccum) canker and stem blight, respectively.

Phomopsis canker and stem blight is used in lieu of Phomopsis twig and cane blight because the former name is more descriptive of the disease as it occurs in Michigan. Godronia (Fusicoccum) canker and stem blight is preferred to Fusicoccum canker for several reasons. Fusicoccum is an invalid name for asexual states of Godronia (28). Use of Fusicoccum canker might imply that a taxonomic relationship exists among asexual states of Godronia and the causal organisms of diseases caused by Fusicoccum spp. Such a relationship does not always exist. For example, Fusicoccum canker of peach is similar in appearance to Godronia (Fusicoccum) canker and stem blight of blueberry, but is caused by a fungus unrelated to Godronia (10, 17, 18). Apothecia of Godronia are apparently important sources of inoculum, and are easily found in severely affected fields. Therefore using the name of the sexual state of the organism appears justified. Since few publications (3, 16, 30, 39,

61, 70, 73) have used the name Fusicoccum canker, it will be less confusing in the final analysis, to use the more descriptive Godronia (Fusicoccum) canker and stem blight of blueberry.

Symptoms associated with Phomopsis and Godronia on 1- and 2-year-old stems in Michigan were in most respects identical to those reported by others (3, 16, 35, 37, 39, 61, 66, 67, 70, 73). One significant difference was that in Michigan both fungi caused lesions and stem blight symptoms on 1- and 2-year-old stems. Also many lesions caused by Phomopsis expanded and caused blight symptoms on artifically inoculated stems. This is in direct contrast to earlier observations (66) that only localized lesions followed inoculation of woody stems. In Michigan, local lesions caused by Godronia and stem blight symptoms caused by Phomopsis, respectively, are usually diagnostic characters of the 2 dieseases on 1- and 2-year-old stems.

Diagnosis in the field is more difficult on older stems. Diagnostic lesions and discoloration caused by Godronia do not occur once bark is formed. Developed cankers and stem blight are the only visible symptoms on older stems. Cankers caused by Godronia tended to be short and wide, whereas Phomopsis cankers were usually long and narrow. This distinction does not always hold, however, and isolations from infected stems are often necessary to confirm diagnoses made in the field. Blight symptoms caused by

Godronia and Phomopsis are easily differentiated if pycnidia are present on affected stems. However, isolations are necessary for positive diagnosis when fruiting bodies do not occur.

The association of Godronia, and to a lesser degree Phomopsis, with certain kinds of calluses and the results of girdling experiments suggest that calluses at the edges of cankers and wound type calluses probably result from the slow girdling action of cankers caused by these fungi. Calluses along the edges of cankers located in the crown of affected plants and calluses along cankers at the bases of lateral branches may be similarly caused. The cause of callusing along the length of affected stems remains to be determined. It is important to stress that Phomopsis was not associated with calluses similar to those described by Brown (7). Additional studies of the callus syndrome are necessary to identify positively the causal agents.

Wilted stems (flags) were the most conspicuous symptom of both diseases. Histology of discolored xylem of flagged stems infected with Godronia indicated that stems wilt as a result of vascular occlusion rather than due to girdling. In other words, the xylem is invaded by the fungus before stems are blighted. In this respect, the 2 diseases are probably similar.

Based on observations that Godronia (Fusicoccum) canker and stem blight is generally more severe and more

common in areas where cool climates prevail it has been suggested that plants are predisposed to the disease by cold injury (40, 54). Effects of cold injury on Godronia (Fusicoccum) canker and stem blight were not studied. However, increased severity of the disease at 2-8 C when inoculated plants were grown at various temperatures, indicates that low temperatures and dormancy predispose blueberry plants to this disease. These data suggest that the disease is more severe in cool climates because there are longer periods during the year when growth of the fungus is favored over growth of the host. Whether the fungus invades the xylem directly when plants are dormant or whether it must first grow to vascular tissues leading to axillary buds and leaf scars remains to be proven.

Knowledge of how Godronia invades the xylem of affected plants could have practical implications. For example, in upper Michigan, Godronia causes cankers on Rancocas variety as often as on other varieties. Cankered stems of Rancocas, however, do not wilt as often as those of other varieties. In one upper Michigan, field, for example, wilted stems were found on 6.6% of 300 Rancocas plants observed whereas 32.2% of 1,047 plants of other varieties were flagged. This observation suggests that Rancocas is infected as often as other varieties, but Godronia does not as readily invade the xylem of Rancocas plants. The reason for this phenomenon is not know. One

possibility, however, is that Godronia can not penetrate the protective cylinder of pericyclic fibers around the vascular cylinder and invades the xylem only via vascular elements leading to leaf scars and axillary buds.

Available evidence suggest that Godronia is indeed a weak parasite and can invade xylem tissues only when the plant is relatively inactive metabolically. Increased severity of the disease on plants grown under conditions favoring dormancy, for example, supports this conclusion. In addition, histological observations indicated that Godronia can not penetrate living cells and that growth of the fungus in the living cortex progresses primarily through the longitudinal air channels. Internodal lesions observed in April walled off during the summer as did lesions resulting from artificial inoculations. These observations indicate that infections occurring during the early summer and infections occurring along stem internodes may not result in development of blight symptoms. Existence of a thick layer of pericyclic fibers, fewer air channels, or few leaf scar infection sites could account for the tolerance of Rancocas. These possibilities and the way in which Godronia invades the xylem should be investigated.

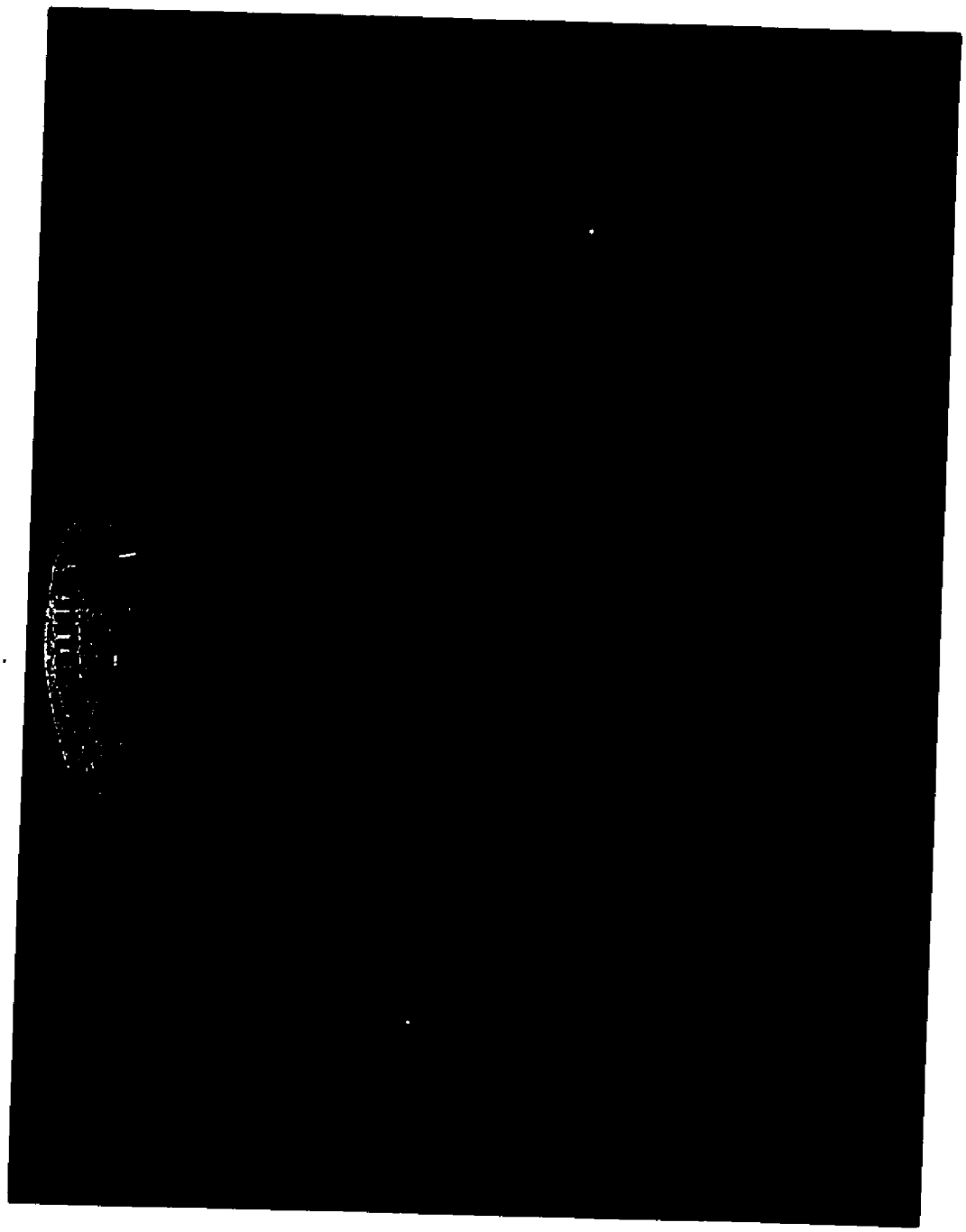
Distribution of Phomopsis and Godronia in Michigan and Indiana may be related to temperature. Godronia, for example, grows poorly at temperatures above 25 C and was not found in southern areas where warmer temperatures

prevail. Phomopsis which grows well at 25-30 C and caused cankers on plants artificially inoculated when temperatures exceeded 30 C, was common in warmer areas. That temperature plays a role in distribution of the diseases is supported by studies of a dieback disease of cranberry which suggested that warm temperatures favored growth of Phomopsis over that of Godronia in diseased cranberry stems (26). Studies of the effect of temperature on disease development, and the production, dispersal, and germination of spores of these fungi may show that temperature indeed limits the distribution and severity of these diseases in Michigan and Indiana.

Isolation of Godronia from plants exposed to natural inoculum at various times during the 1968 season provided the first experimental evidence for the occurrence of 2 infection periods during the growing season. Infection during June was probably due to conidia since conidia were observed washing down stems during this time and ascospores were immature. Disappearance of ascospores from apothecia corresponded to the mid-August to mid-September infection period. The probable disease cycle of Godronia (Fusicoccum) canker and stem blight is outlined in Fig. 31.

Data indicating that there are 2 infection periods suggest that attempts to control Godronia (Fusicoccum) canker and stem blight have failed, at least in part, because compounds were applied at the wrong time (i.e., either before or after actual infection periods). In Nova Scotia (35),

Fig. 31. Diagram of the probable disease cycle of Godronia canker and stem blight. Primary infection is defined as the first infection of new plants during the growing season.



for example, fungicides were applied during the last week in September, mid-October, and in early May. Assuming that the disease cycle is similar in Nova Scotia, each of these applications could have missed actual infection periods.

It is interesting to note that although most late infection probably occurred before September 15, 1968, lesions were not visible until December. Lesions did not develop from axillary buds and healed leaf scars until a year after inoculations. Appearance of lesions, therefore, can not be used as an accurate indication of when infection occurs.

Circumstantial evidence strongly suggests that Godronia infects leaf scars via attached petioles, but direct proof is lacking. The fungus was isolated from 45% sampled petioles following the late summer infection period which occurred 4 to 8 weeks before leaf drop. Little infection occurred on plants exposed to natural inoculum (i.e., 9 October - 3 December) during and after leaf drop. More leaf scars were infected when attached petioles were inoculated at 0 and 1 day after removing leaf blades than when petioles were inoculated at 2 and 5 days after leaf blades were removed. Healed leaf scars were not infected until a year after inoculation whereas wounded leaf scars were infected immediately following inoculation. Actual ingression of Godronia into leaf scars from artificially inoculated leaf blades was not studied. That this kind of experiment

should be executed before concluding that leaf scars are invaded via attached petioles is suggested by studies of Fusicoccum canker of peach. These studies showed that F. amygdali infected healing leaf scars (10, 17), but did not infect leaf scars when leaf blades were inoculated (10).

Evidence for infection of stem internodes via stomates is also circumstantial. Histology showed that incipient necrosis always occurred below stomates. Some infections resulted from artificial inoculations made on nonwounded stem internodes. That 56% of all lesions on 1- and 2-year-old stems occurred on stem internodes and that infected stems were not wounded suggests that Godronia either penetrates stems directly or invades stomates. Histological observations suggested that Godronia is unable to penetrate living cells. Since the stem is protected by a 10-15 μ thick cuticle interrupted only by stomates, infection of internodes of 1- and 2-year-old stems via stomates seems likely.

All major varieties of blueberries grown in Michigan are very susceptible to Godronia (Fusicoccum) canker and stem blight, whereas only Earliblue variety is severly affected by Phomopsis. Godronia (Fusicoccum) canker and stem blight is, therefore, considered to be the more important disease in Michigan. Phomopsis canker and stem blight is, however, a potentially devastating disease. For example, in one well tended field in Indiana, over 200 twelve-

year-old Earliblue variety plants were unproductive because of the disease. Losses in this field were estimated to exceed $2,000 annually. Godronia (Fusicoccum) canker and stem blight is equally as devastating in northern lower and upper Michigan where all plants were infected in several fields.

Pathogenicity of isolates of G. cassandrae from Spiraea spp. and V. angustifolium on V. corymbosum suggests that infected Spiraea spp. and V. angustifolium serve as inoculum reservoirs for Godronia canker and stem blight. These hosts are very common in Michigan blueberry growing areas and inoculum from these plants could be an important source of infection in Michigan blueberry fields.

Godronia cassandrae was divided into 6 forms according to the genera of plants inhabited by each fungus (28). Smerlis (51) used differences in ranges of pathogenicity to differentiate among forms cassandrae, beticola, and vaccinii. Forms cassandrae and vaccinii were separated by differences in cultural characteristics (51).

In my studies, 2 separate isolates of G. cassandrae from V. macrocarpon did not cause cankers on inoculated blueberry plants confirming earlier observations (37) of inoculations made with similar isolates. One of 2 isolates from C. calyculata was pathogenic on blueberry, but only 1 lesion resulted from inoculations with the pathogenic Quebec isolate. Two of 4 isolates from Spiraea spp. were pathogenic

on blueberry. All isolates obtained from V. corymbosum and V. angustifolium were pathogenic on inoculated blueberry plants. These data indicate that earlier interpretations of form vaccinii based on pathogenicity (28, 51) must be investigated further. More inoculations with single spore isolates of each form must be executed before pathogenicity can be used to differentiate among forms spiraeicola, cassandrae, and vaccinii.

Variability noted in spore morphology and cultural characteristics of G. cassandrae, especially isolates from highbush and lowbush blueberries, indicates that a large number of single spore isolates must be grown and compared under precisely controlled conditions before these characters are useful as taxonomic parameters. For example, not all isolates of G. cassandrae from V. angustifolium produced the slime and black mycelium used by Smerlis (51) to separate f. vaccinii from f. cassandrae. Some isolates, in fact, produced slime only when grown at temperatures exceeding 20 C.

Available evidence indicates that there are probably several distinct strains of G. cassandrae with different ranges of pathogenicity. However, before conclusions concerning the taxonomy of strains can be made, pathogenicity and cultural characteristics of more single spore isolates from the various hosts must be studied.

In brief summary, 2 canker and stem blight diseases of blueberry are common in Michigan. Godronia (Fusicoccum)

canker and stem blight is considered to be more important than Phomopsis canker and stem blight. Godronia cassandrae from Spiraea spp. and V. angustifolium were pathogenic on V. corymbosum. Blueberry plants were infected by G. cassandrae during June and between mid-August and mid-September. Stomates and leaf scars probably function as infection sites. Circumstantial evidence suggests that leaf scars are invaded via attached petioles. Dormancy and low temperatures favored development of blight symptoms on artificially inoculated blueberries. Histology indicated that stems wilt when the xylem is invaded by the fungus and vessels are occluded by hyphae, various deposits and possibly tyloses. Some calluses on affected blueberries were associated with cankers caused by Godronia and Phomopsis.

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