STUDIES ON THE HOST RANGES OF SOME FACULTATIVE PARASITES

BY

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ABSTRACT

Inoculation experiments were carried out to determine the relation between bark moisture level of certain host species and their susceptibility to facultative parasites. In these experiments, cuttings of 1- to 3-year old host material and the mycelial mat of the pathogens contained in an agar cylinder were used.

In the first instance, fungi that were known or found in association with bark lesions were considered: these were Cryptodiaporthe salicella (Fr.) Petrak on Salix scouleriana Barratt (Scouler willow), Dactylosporium sp. and Fusarium sp. on Acer macrophyllum Pursh. (broadleaf maple, Libertella sp. on Cornus stolonifera (Michx.) var. occidentalis (T. and G.) C. L. Hitchc. (western dogwood), Melanconis sp. on Alnus rubra Bong. (red alder).

The results demonstrated that fungi normally associated with lesions of living host material proved to be pathogenic when the relative turgidity of the host bark was lowered from the field level of above 80 per cent to the range of 69 to 77 per cent.

Secondly, an attempt was made to determine if correlation existed between bark moisture level and canker development by fungi not known, and not found to occur in association with lesions of some hosts. The following fungi and hosts were considered: C. salicella on red alder (Alnus rubra Bong), trembling aspen (Populus tremuloides Michx.), bitter cherry (Prunus emarginata Dougl.), black cottonwood (Populus trichocarpa Torrey and Gray), western dogwood (Cornus stolonifera var. occidentalis), and on broadleaf maple (Acer macrophyllum Pursh.); Fusarium sp. on red

alder, bitter cherry, western dogwood, and on Scouler willow; Libertella sp. on red alder, bitter cherry, broadleaf maple, and on Scouler willow; Melanconis sp. on bitter cherry, western dogwood, broadleaf maple and on Scouler willow.

It was shown that all of these parasites extended their host ranges to varying extent when the bark moisture level was reduced to levels within the range of 69 to 77 per cent, or in some instances to the range of 41 to 67 per cent of saturation. Cuttings with as low bark moisture levels as 41 per cent appeared to be viable as indicated by the production of roots and (or) shoots.

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CONTENTS

TABLES

FIGURES

INTRODUCTION

Two distinct approaches have been made in studies on the epidemiology of canker diseases caused by facultative parasites. In the first, the incidence of the disease has been related to climatic and soil factors. Since these factors vary considerably in different localities, there has been no basis for comparing the results of different studies of the same disease. Also, because of the interrelation of many climatic and soil factors, it has been difficult to determine the relative significance of any one factor in predisposing the trees to diseases.

In the second approach, the emphasis has been placed on host factors as influenced by changes in climate and soil. These investigators (Bier, 1959 a, b; Bloomberg, 1960) were of the opinion that although there may be a large number of environmental factors involved, their combined action was evident through the determination of an index which will express the degree of host vigor. From the results of these investigations it appeared that the field moisture content of living tissues expressed as percentage of the amount at saturation (relative turgidity) may be a useful index for the degree of host vigor.

In the present investigation the second approach was adopted. Special reference was made to the relative turgidity level of one- to three-year-old bark tissues as a possible index of the degree of host vigor in various species of hardwoods, and their vulnerability to attack by some facultative parasites.

Under field conditions in Vancouver, Cryptodiaporthe salicella (Fr.) Petrak occurred only on Salix sp., Dactylosporium sp. on broadleaf maple

(Acer macrophyllum Pursh.), Libertella sp. on western dogwood (Cornus stolonifera (Mitchx.) var. occidentalis (T. & G.) C. L. Hitchock), Melanconis sp. on red alder (Alnus rubra Bong.). Under laboratory conditions, Fusarium sp. occurred only on broadleaf maple shoots. It seemed pertinent, therefore, to determine;

1) whether any correlation existed between bark moisture content and cankering by these organisms, and
2) whether the host range of each fungus could be enlarged by changing the moisture content of the living bark tissues of a species not normally attacked.

LITERATURE REVIEW

In forest pathology, facultative parasites are often considered to be saprophytic organisms with parasitic tendencies. As parasites, the organisms occur on plants adversely affected by fire, insects and unfavourable environmental conditions (Brooks, 1928; Boyce, 1933; Day, 1948; Mooi, 1948; Gaumann, 1950; Waterman, 1955). In some instances disease development occurred during the dormant season when the physiological activities of the host were lessened, while conditions remained favourable for the development of the pathogens (Brooks, 1928; Boyce, 1948).

Undoubtedly there are many factors that are related to the establishment of the parasitic relationship. The water content of the host tissues, however, has been considered as one of the most important relationships. As early as 1909, Münch indicated that Nectria cinnabarina Fr. was pathogenic on elm when the moisture content of bark tissues was at a low level. According to Gaumann (1950), the increased water content may protect the tissues from facultative parasites by displacing the air necessary for the fungi and by increasing the vital energy of the cells.

More recently, Butin (1955) found that a low water content of poplar cuttings favoured their colonization by Cytospora chrysosperma (Pers.) Fr. because of a decreased rate in the production of secondary periderm. Gibbs (1957) suggested that short, mild periods in the winter might thaw out the younger parts of trees while leaving the roots, soil and stem still frozen. This, he suggested, would result in marked water losses from twigs, and the development of physiological stress.

Weatherley (1949) expressed the water content of the leaves of cotton plants as a percentage of that at maximum turgor. This percentage reflected

the combined effects of the amount of soil moisture and level of atmospheric humidity on the plants. Using the same method for expressing the water content of sapwood of Douglas fir and Sitka spruce, Chalk and Bigg (1956) found that the index, relative turgidity per cent, varied in trees on different sites, being higher in trees on good sites.

In a series of papers, Bier (1959 a,b,c,; 1960 a,b) demonstrated that the relative turgidity level of young bark tissues was related to the degree of host vigor. It was demonstrated that low turgor levels in the bark tissues, brought about by climatic changes, root competition and suppression, resulted in the susceptibility of the trees to canker diseases caused by facultative parasites.

According to Kramer and Kozlowski (1960), physiological processes and structure of a plant are closely related. Bloomberg (1960) argued that the lower relative turgidity of the bark of Populus trichocarpa Torrey and Gray than that of P. 'robusta' in dormancy was related to anatomical characteristics that favoured slow uptake and poor retention of water in the bark of this species.

Bier (1961) gave further evidence for the correlation of the relative turgidity of the host bark to canker resistance. He found that Populus tremuloides Michx., P. 'robusta' and P. trichocarpa shoots in the field with bark moisture content values higher than 80 per cent did not become infected with C. salicella. However, susceptibility occurred when the turgor level of the bark was lowered to levels below 80 per cent. Further, it has been demonstrated (Bier, 1961 b) that the bark tissues of P. tremuloides, P. trichocarpa, Salix sp. and Acer macrophyllum Pursh. were attacked by Hypoxylon pruinatum (Klotzsche) Cke. at higher bark moisture levels than by

Septoria musiva Pk., indicating that Hypoxylon was the more virulent pathogen on these hosts. Since these organisms did not occur on these hosts in the Vancouver area, it appeared that the host ranges of some facultative parasites might be extended if the relative turgidity per cent of the bark was reduced to levels below those normally occurring in the field.

In addition to the moisture hypothesis, the importance of the nutrition of the facultative parasite on its parasitism of the host has also been emphasized (Brown, 1936; Lilly and Barnett, 1951). These authors suggested that the chemical composition of the substrata may influence the enzyme systems of the fungi. Vasudeva (1930) demonstrated that a failure of Botrytis allii Munn. to parasitize apple may have been due to the low amount of nitrogenous substances in the host tissues. The addition of nitrogenous substances to spores stimulated the attack.

Dufrenoy (1930) placed emphasis on the hysto-chemical reactions of the host to the parasite, indicating that the host tissues produced tannin barriers preventing the advance of the parasite.

Bloomberg and Farris (1963) found a close correlation between the moisture content of the tissues and the manner and amount of deposition of tanniferous substances in the poplar bark tissues. This was considered to be a defence reaction on the part of the host against disease attack.

OBJECTIVES

In pathogenicity tests on the host ranges of C. salicella, Fusarium sp., Libertella sp., and Melanconis sp. the following hypotheses were considered:

1) The host ranges of some facultative parasites may be extended to include tree species normally not cankered by them, when the degree

of host vigor as indicated by the relative turgidity of young bark is lowered to a value below a critical level.

2) The food material in the host for the development of the pathogen may be of importance in determining the establishment of the parasitic relationship between the fungus and the host.

The objectives of this investigation were as follows:

1) To determine the water regime of the bark tissues of various host species both in dormancy and in growth.

2) To relate the water content of the dormant bark tissues of various host species to their susceptibility to cankering by C. salicella.

3) To relate the moisture content of the bark tissues of various tree species with attack by canker organisms in the field.

4) To relate the moisture content of the bark tissues of tree species to attack by fungi not known and not found in association with their bark cankers.

5) To undertake (comparative) cultural studies on the temperature requirements of test fungi, and on their possible nutritional preferences as tested on autoclaved sections of shoots.

METHODS

1) Seasonal moisture studies of tree bark.

One-year-old coppice shoots of red alder (Alnus rubra Bong.), bitter cherry (Prunus emarginata Dougl.), broadleaf maple (Acer macrophyllum Pursh.), trembling aspen (Populus tremuloides Michx.), black cottonwood (Populus trichocarpa Torrey and Gray), Scouler willow (Salix scouleriana Barratt) and three-year-old western dogwood trees (Cornus stolonifera (Mitchx.) var.

occidentalis (T. & G.) C. L. Hitchock were collected on the University of British Columbia Endowment Lands, during the winter of 1959 and the spring of 1960. All material collected had shown vigorous growth and was apparently free from disease. The excised shoots were wrapped in polyethylene bags and harvested into cuttings--ten for each species--in the laboratory. The cuttings were of uniform size, measuring 25 cm in length with a mid-diameter of 1.0 cm. The relative turgidity of the bark tissues of each cutting was then determined. Because the bark did not separate easily from the wood along the cambium, the bark samples contained small amounts of secondary xylem. The fresh weight of the samples was determined to the nearest milligram. The samples were then saturated by allowing them to float on the surface of distilled water in closed Petri dishes. After a 24-hour period samples were removed from the dishes, the excess water blotted off, and the weights determined. The samples were then oven-dried for 24 hours at 79 to 90°C and weighed again. From these three weighings the relative turgidity was determined as follows:

$$\text{Relative turgidity } (\%) = \frac{(\text{Green weight} - \text{Dry weight}) \times 100}{(\text{Saturated weight} - \text{Dry weight})}$$

Bark moisture values were also expressed on a water/dry weight basis.

2) Isolation of Fungi.

The source of C. salicella was a stock culture from the Forest Pathology Laboratory of the Department. Pure cultures of Dactylosporium sp., Fusarium sp. and Melanconis sp. were obtained by making isolations from diseased areas associated with fruiting bodies on the shoots. Cultures from single spores were also obtained for comparison and to identify the causal agency. The following techniques were used:

a) Tissue transplant: the surface contamination of the host material in the vicinity of diseased areas was eliminated by swabbing with 70 per cent ethyl alcohol. Small pieces of diseased bark tissue were removed from the advancing margin of lesions beneath the epidermis using a sterilized scalpel and transferred to malt agar plates. Pure cultures were obtained by making hyphal tip isolations from the colonies developing from the pieces of diseased bark tissues.

b) Single spore isolation: The spores of Dactylosporium sp., Fusarium sp. and Libertella sp. were large enough for isolating single spores with a pointed needle using a dissecting microscope. Spore suspensions of Melanconis sp. were poured on malt agar. After the germ tubes had become visible under the dissecting microscope but before the spores lost their identity, they were removed and transferred singly to fresh plates.

The imperfect fructifications produced on malt agar by colonies from tissue transplants and from single spores were identical for each fungus. Further, the fruiting bodies formed on the artificial medium were identical to those occurring in nature on the plants for each fungus.

The fungus isolated from the diseased shoots of western dogwood was identified as Libertella sp. by Dr. A. Funk, Mycologist, Canada Department of Forestry, Victoria Laboratory, B. C. This was the first record of the occurrence of this fungus on western dogwood in British Columbia. The pathogens on broadleaf maple shoots were identified as Dactylosporium sp. and Fusarium sp. and that on red alder shoots was identified as Melanconis sp. by the writer.

3) Inoculation experiments.

During the dormant season of 1959-60 and 1960-61, cuttings were collected from red alder, bitter cherry, western dogwood, broadleaf maple, trembling aspen, black cottonwood and Scouler willow trees. These measured from 25.0 cm to 35.0 cm in length with mid-diameters ranging from 0.5 to 2.0 cm. The red alder and bitter cherry cuttings were collected from three- and four-year-old lower branches of fifteen to twenty-year-old trees. The broadleaf maple cuttings were collected from minor and major shoots. The minor shoots were from four to six feet tall, one-year-old and pale green in colour. The major shoots were more than ten feet tall and were three-year-old with dark green colour. Cuttings of each species were harvested from the same root system in order to avoid genetic differences in the sample. The percentages for relative turgidity and water per/dry weight were then determined for each cutting.

The bark of the cuttings was surface-sterilized with 70 per cent ethyl alcohol prior to making the inoculation wounds. One inoculation and one control wound were made on each cutting. The following types of wounds were employed:

a) Burns were made using the tip of a hot iron rod three mm. in diameter.

b) Disks of bark were removed with a heat-sterilized cork borer.

c) The outer bark was incised with a heat-sterilized spear-headed needle.

An agar-plug inoculum, consisting of an agar cylinder with the fungus cut out from the advancing margins of colonies was placed on the wound and covered with Scotch tape (Figure 1). The control wound was covered with Scotch tape, but without inoculum.

Ten of the twenty cuttings inoculated were kept in a jar and under a polyethylene cover to maintain a moist atmosphere. No water was supplied to these cuttings. The remaining cuttings were identically treated except that the basal ends of the cuttings were provided with a continuous supply of distilled water (Figure 2). All jars were stored outdoors for ten days in cold frames with polyethylene covers. After this period, the polyethylene bags were loosened on the jars. The water was changed weekly and fresh cuts were made on the basal ends of the cuttings at two-week intervals in an effort to facilitate water movement into the cuttings.

Bark moisture measurements were taken as soon as there was visible evidence of canker development. Bark moisture measurements were also made on the cuttings provided with a continuous supply of water. Following this the diseased cuttings were provided with a continuous supply of distilled water and all cuttings were placed in a moist chamber in the laboratory (Figure 3). Under these favourable moisture and temperature conditions, the cuttings broke dormancy and produced roots, shoots and secondary periderm in the bark tissues at the margins of cankers. Cankered cuttings that failed to produce any of these signs of life were discarded. The bark moisture content was determined again after the canker development had ceased and the best organisms were reisolated into pure culture from the diseased areas.

4) Cultural studies.

The malt agar used in these experiments was composed of 28 gm of Difco Malt Extract and 16 gm Bacto agar per liter of distilled water. After the freshly poured plates had hardened, each was inoculated with agar-plug inoculum (4.0 mm in diameter) of the test fungi.

After inoculation the plates were wrapped in polyethylene bags to prevent rapid desiccation of the medium. Five plates of each organism were kept in constant temperature units ranging from 5°C to 35°C at five degree intervals. At two-day intervals, the average diameters of the colonies were determined by measuring the colonies along two diameters at right angles to one another and the average rate of growth was calculated for each organism.

5) Cultural studies on sterilized sections of shoots.

Fresh cuttings were collected from red alder, bitter cherry, western dogwood, broadleaf maple and Scouler willow and cut into sections 6 to 8 cm long. Each of these sections was then split into halves. A strip of the epidermis or of the periderm was removed along the length of each section to expose the inner bark. The sections were supported by glass rods in Petri plates containing 15 cc of distilled water. The plates were then autoclaved for seven minutes under 15 lbs. pressure. After sterilization the sections were inoculated with agar-plug inoculum. Eight replicates were made for each fungus on each plant species.

After inoculation the plates were wrapped in polyethylene bags and stored at 20°C for a period of eight days. At the end of this period the extent of fungous growth was determined by measuring the colonies along the length of the section. The average growth was then calculated.

RESULTS

1) Seasonal changes in the water content of the bark tissues.

The seasonal variations in the relative turgidity of bark of trembling aspen, black cottonwood, Scouler willow, red alder, bitter cherry, western

dogwood and broadleaf maple are presented in Table I.

During the dormant season of 1959 and the spring of 1960, the relative turgidity of the bark tissues of all species tested except Scouler willow was above 80 per cent. In Scouler willow the relative turgidity dropped below 80 per cent in December. Both trembling aspen and black cottonwood had higher relative turgidity values with less variation than that found in Scouler willow. Red alder, western dogwood, and broadleaf maple had relative turgidity values of 85 to 90 per cent. Relative turgidity values slightly above 80 per cent were obtained for the bark of bitter cherry. The relative turgidity of the bark of all trees studied was in excess of 90 per cent during the growing season.

On each collection date, observations were also made for the presence or absence of disease in shoots other than those sampled. During the sampling period, infection was rarely found in the field. Occasionally, in broadleaf maple and Scouler willow cankers were found that were confined to the upper parts of the shoots. On red alder, cankers occurred on living branches that were damaged. On western dogwood, small latent cankers were found in the vicinity of lenticels. No infection was found on trembling aspen, bitter cherry and black cottonwood.

TABLE I

Monthly changes in the relative turgidity of 1- to 3-yr-old bark of various species

	1959	1960					
	Dec. 10	Jan. 5	Feb. 8	Mar. 14	June 8	July 11	Aug. 8
P. tremuloides	84	83	84		95	95	94
P. trichocarpa	84	83	81	82	96	--	95
scouleriana	79	85	80	84	95	94	93
A. macrophyllum	85	84	87	85	96	97	95
A. rubra	88	89	86	---	95	97	96
C. stolonifera var. *occidentalis*	86	90	91	---	96	95	97
P emarginata	83	82	80	82	92	93	93

2) Canker development by C. salicella on inoculated cuttings of several tree species in relation to the water content of the bark tissues.

From Table II it would appear that Scouler willow, trembling aspen and black cottonwood were most susceptible to C. salicella as canker attack occurred at the highest bark moisture level among the species trees tested (Figures 4-6).

Table II also shows that the bark of red alder, bitter cherry, western dogwood and broadleaf maple became infected by C. salicella when the relative turgidity of the bark tissues ranged from 47 to 67 per cent (Figures 7-9). The susceptibility of red alder, and bitter cherry cuttings appeared to depend on the age of the host bark in addition to the bark moisture content. This was indicated by the larger number of successful inoculations obtained on cuttings of three-year-old branches than in those from one-year-old shoots. Also, the bark moisture level at which canker attack occurred was higher in cuttings from three-year-old branches than in those from one-year-old shoots.

The data obtained from initial infections of viable cuttings (Table II) shows three different bark moisture ranges at which canker attack occurred on the various tree species. These moisture ranges might indicate different degrees of host vigor thus different rates of susceptibility to the fungus. These moisture ranges were as follows:

1) In the bark moisture range of 70 to 76 per cent, trembling aspen, black cottonwood and Scouler willow cuttings were cankered.
2) In the bark moisture range of 52 to 67 per cent, cuttings of one-year-old broadleaf maple and those from three-year-old red alder, bitter cherry, and western dogwood were infected.
3) In the bark moisture range of 41 to 47 per cent, red alder and bitter cherry cuttings of one-year-old shoots were infected. (This low bark

TABLE II

Relation of the relative turgidity per cent of bark to initial infection in cuttings of various tree species inoculated with C. salicella.

Tree species and age of cutting wood	Tot. No. of cuttings			No. of cuttings initially infected	Incubation period before initial infections days	Average rel. turgidity % of host bark	
	Inoc.	Cank.	Recov.*			at the time of harvest	at the time of init. cankering
S. scoulerana 1	20	18	18	4	23	80	76
P. tremuloides 1	20	19	17	5	20	87	77
P. trichocarpa 1	20	18	12	4	25	81	70
A. macrophyllum 1	40	25	15	4	16	84	67
A. rubra 1	40	12	4	3	40	89	41
3	40	25	15	4	22	83	65
C. stolonifera var. occidentalis 3	40	16	8	4	38	93	52
P. emarginata 1	40	10	3	3	33	85	47
3	40	20	13	5	15	82	62

* Recovered cuttings were those that remained viable after infection.

moisture level appeared to approach the lowest values that may be encountered in living cuttings.)

The number of successful inoculations was found to be highest in the first bark moisture range (70-76), it was smaller in the second one (52-67) and reached the minimum number in the third one (41-47).

In general, cankers obtained on red alder, bitter cherry and western dogwood and broadleaf maple were of small size, only occasionally longer than 2.5 cm. They also expanded at a slower rate. On the other hand, cankers on cuttings of trembling aspen, black cottonwood and Scouler willow developed faster and extended to the total length of the cuttings if moisture was not supplied to them.

3) Cankers arising from latent infections on red alder, western dogwood and broadleaf maple.

Rapidly expanding cankers developed on cuttings that were harvested and left to dry gradually on the ground in the vicinity of parent trees. These cankers originated at lenticels, buds and healed or partly healed wounds. On the cuttings of red alder, broadleaf maple and western dogwood, inoculated with C. salicella, cankers of latent infections of Melanconis sp., Fusarium sp. and Libertella sp. developed before or concurrently with Cryptodiaporthe canker occasionally overgrowing the lesions caused by C. salicella. This phenomenon appeared to indicate higher susceptibility of host to the usually occurring pathogens. Reisolation experiments confirmed that Melanconis sp. was consistently associated with lesions on red alder, Libertella sp. with lesions of western dogwood, Dactylosporium sp. with lesions of broadleaf maple and C. salicella was consistently

associated with lesions of Scouler willow.

4) Relation of the bark moisture content to canker susceptibility of red alder, western dogwood, and broadleaf maple by facultative parasites that are known to occur on these hosts.

The field susceptibility of red alder, western dogwood and broadleaf maple to attack by some fungi offered an opportunity to investigate a possible correlation between the relative turgidity per cent of the bark tissues and susceptibility to canker attack. Fungi were isolated into pure culture from the lesions on each tree species. They were used in inoculation experiments on malt agar inoculum plugs. The results are given in Table III and illustrated in Figures 10, 11, 12, and 13.

Canker development caused by fungi normally associated with bark cankers of these tree species was more extensive and occurred at higher bark moisture levels than that associated with C. salicella which was not known to occur in the bark of these tree species.

5) New hosts for Melanconis sp., Fusarium sp. and Libertella sp.

The relationship between the moisture content of the bark tissues and canker susceptibility was further tested using the following test pathogens and hosts: Melanconis sp. on cuttings of bitter cherry, western dogwood, broadleaf maple and Scouler willow; Fusarium sp. on cuttings of red alder, bitter cherry, western dogwood and Scouler willow; Libertella sp. on cuttings of red alder, bitter cherry, broadleaf maple and Scouler willow.

Inoculation experiments were carried out with the same type of host material in the manner outlined by Bier (1959) and used earlier in this investigation. Malt agar plug inoculum consisting of an agar cylinder

TABLE III

Canker development in relation to the moisture content of the host bark.

Tree species and age of cutting wood	Inoculum	Tot. No. of inoculations successful inoculations*	Incubation period before init. infection days	No. of cuttings initially infected	Ave. relative turgidity (%) of host bark	
					at time of harvest	at time of init. infection.
A. macrophyllum 1-yr-old	Dactylosporium sp.	12/11	8	5	85	72
	Fusarium sp.	12/11	6	6	85	77
A. rubra 3-yr-old	Melanconis sp.	12/10	15	5	82	77
C. stolonifera var. occidentalis 3-yr-old	Libertella sp.	12/9	10	6	90	69

* Successful inoculations consisted of cuttings that remained viable after infection.

and mycelial mat of the fungus was placed on the bark wounds and covered with Scotch tape. Cuttings were then incubated and allowed to dry gradually under polyethylene covers at room temperature. Water was withheld from the cuttings until initial canker development. The results of this inoculation experiment are shown in Table IV and illustrated in Figures 14, 15, 16, 17, 18, 19 and 20.

It was found that although Melanconis sp. was associated with cankers only on red alder in the field, it readily cankered cuttings of all tree species tested if the bark moisture content was reduced to levels between 54 and 75 per cent from the field values of above 80 per cent.

Fusarium sp. also caused cankers on cuttings of tree species other than broadleaf maple from which it was isolated. It was noted (Table IV) that bitter cherry and western dogwood were cankered when the moisture content of the bark was lowered to the range of 63 to 66 per cent. Although the pathogen produced small cankers (0.5 cm. long) on red alder cuttings when the relative turgidity per cent of the bark tissues was lowered to 77 per cent, the extension of these cankers was delayed until further reduction of bark moisture content took place (Figure 17).

Libertella sp., normally pathogenic on western dogwood, produced cankers only on bitter cherry as a new host. On both hosts, however, canker development occurred only if the bark moisture content was lowered to a certain level (Table IV and Figures 18 and 19). Red alder, broadleaf maple and Scouler willow cuttings were not infected by this fungus, although hyphae and conidia-bearing sporophores were present in the inoculation wounds.

TABLE IV

Summary of inoculations and bark moisture studies on host material at room temperature (18-23°C).

Fungi	Tree Sp.	Age of cutting wood - yrs.	Tot. No. of inoc; No. of successful inoculations	Incubation period days	No. of cuttings initially infected	Ave. relative turgidity (%) of bark	
						at time of harvest	at time of initial infection
Melanconis sp.							
	Acer	1	12/10	10	5	85	70
	Cornus	3	12/10	10	6	85	71
	Prunus	1	12/12	10	6	83	64
	Salix	1	12/12	7	6	82	75
Fusarium sp.							
	Alnus	1	12/10	10	6	85	77
	Cornus	3	12/10	12	6	86	63
	Prunus	1	12/12	7	6	81	66
	Salix	1	12/00	--	-	80	--
Libertella sp.							
	Acer	1	12/00	--	-	85	--
	Alnus	1	12/00	--	-	85	--
	Prunus	1	12/12	15	8	83	60
	Salix	1	12/00	--	-	80	--

6) The growth of the fungi at different temperatures on malt agar.

The effect of temperature on the growth of the fungi was determined on malt agar at temperatures ranging from 5°C to 35°C with 5 degree intervals. The average growth of the fungi over a period of 8 days is shown in Table V.

Maximum growth of Fusarium sp. occurred between 20 and 25°C. At 30°C growth was still relatively good indicating the small retarding effect of high temperature on the mycelial growth of this fungus. At lower temperatures the size of the colonies was abruptly reduced.

Libertella sp. produced the largest growth at 25°C whereas at 5°C and at 30°C the growth was very small.

The maximum growth of C. salicella occurred at 20°C. The amount of mycelia produced at 25°C was very small, however growth at 10°C and 15°C was greater than at 25°C.

Melanconis sp. had a wide temperature range for good growth, growth being relatively good at both 5°C (0.8 cm.) and at 30°C (0.9 cm.). The greatest growth was produced between 15°C and 20°C.

7) Host preferences of the fungi.

The study of possible nutritional preferences of C. salicella, Fusarium sp., Libertella sp. and Melanconis sp. was carried out by inoculations of living cuttings from tree species other than their field host.

In addition, sections of the same shoots from which cuttings were obtained were autoclaved and then inoculated to see the extent of mycelial growth when the resistance of the host attributed to living tissues was excluded. It was realized that, besides excluding the functional resistance of hosts, changes in their chemical constitution could have taken place as a result of the sterilization process. The average lengths of the mycelial

TABLE V

The effect of temperature on the growth of fungi on Malt agar over a period of eight days at:

	5°C	10°C	15°C	20°C	25°C	30°C	35°C
	Width of mycelial mat cm.						
Cryptodiaporthe salicella	0.05	2.4	4.3	5.6	0.3	0.05	0.0
Fusarium sp.	0.05	2.5	4.3	9.0	9.0	4.2	0.0
Libertella sp.	0.05	0.65	1.2	1.6	1.8	0.2	0.0
Melanconis sp.	0.8	2.1	3.8	4.6	1.8	0.9	0.0

mats produced by the fungi on the autoclaved bark of each host was calculated at the end of an 8-day period. The extent of this mycelial growth then was compared with growth of fungi in living cuttings of each host producing or failing to produce cankers.

Fusarium sp., isolated from cuttings of broadleaf maple, produced the largest colonies on the autoclaved bark of this host (Table VI). Living cuttings of red alder, bitter cherry and western dogwood were readily cankered. Although the mycelial growth of the fungus was evident in the inoculation wounds of Scouler willow, canker development in this species did not ensue.

Libertella sp. produced the largest colonies on the autoclaved bark of its field host, western dogwood. On autoclaved bark of other hosts, the lengths of the mycelial mats were from 30-40% smaller. Cankering occurred only on bitter cherry although mycelia and conidia bearing sporophores were present in the inoculation wounds of red alder, broadleaf maple and Scouler willow.

C. salicella, occurring as a pathogen on Scouler willow in the Vancouver area, developed the largest colonies on the autoclaved bark of this host. At the same time it produced small mycelial mats on the autoclaved bark of red alder, bitter cherry, western dogwood and broadleaf maple. Canker development took place in living cuttings of all species tested. The cankers produced on the new and on its field hosts, however, differed in their growth rate. Although they grew nearly to the same length 15 days after inoculation on both the new hosts and the field host, two weeks later Cryptodiaporthe canker on its field host was about twice as long as on its new hosts (Table VII).

TABLE VI

Growth of fungi on autoclaved sections of shoots related to their ability to infect living cuttings.

Fungi	Tree sp.	Age of wood yrs.	Ave. length of 8 colonies	Cankering of living cuttings (+) successful (-) unsuccessful
C. salicella	Acer	1	0.7	+
	Alnus	3	0.3	+
	Cornus	3	0.3	+
	Prunus	3	0.8	+
	* Salix	1	2.5	+
Fusarium sp.	* Acer	1	5.0	+
	Alnus	3	3.0	+
	Cornus	3	4.0	+
	Prunus	3	4.3	+
	Salix	1	3.0	-
Libertella sp.	Acer	1	2.0	-
	Alnus	3	2.0	-
	* Cornus	3	3.2	+
	Prunus	3	2.1	+
	Salix	1	2.5	-
Melanconis sp.	Acer	1	2.5	+
	* Alnus	3	3.6	+
	Cornus	3	2.0	+
	Prunus	3	2.3	+
	Salix	1	2.5	+

* field host for the pathogen.

TABLE VII

The extension of Cryptodiaporthe canker on cuttings of its new hosts and on those of its field host.

Tree species	Age of wood yrs.	No. of cuttings	Ave. canker growth (cm) 15 days after inoc.	Ave. canker growth (cm) 30 days after inoc.	Rel. turgidity % 15 days after inoc.	Rel. turgidity % 30 days after inoc.
A. rubra *	3	6	1.1	2.0	64	42
C. stolonifera v. occidentalis *	3	4	1.0	1.8	53	40
P. emarginata *	3	5	1.0	2.3	63	41
S. scouleriana **	1	8	1.5	4.6	70	44

* new hosts for C. salicella

** field host for C. salicella

DISCUSSION

In determining the host range of certain facultative parasites the emphasis was placed on:

1) the degree of host vigour as measured by the relative turgidity per cent of the bark tissues.

2) the nutritional status of autoclaved bark tissues.

For measurements of the seasonal moisture pattern of bark tissues 10 cuttings were collected monthly for each tree species. Because of the large number of cuttings required to be collected at a time it was not possible to sample the same clone for each tree species throughout this study. It was thought, however, that the uniform size of cuttings, the healthy appearance of shoots and their similar bark texture would warrant comparison of moisture readings. In order to avoid the effect on the moisture reading the actual moisture content of the functional bark tissues was expressed as percentage of that required for their saturation.

The evidence points to the likelihood that latent infections of living host material by facultative parasites occur in the field on red alder, western dogwood, broadleaf maple and Scouler willow. The field temperatures over long periods during the dormant season, approximated the optimal temperature for the growth of these fungi in culture. In the spring, however, although temperature conditions were optimal, canker development did not occur. During this period the shoots had moisture contents of 80 to 90 per cent bark moisture content if they were dormant shoots and higher than 90 per cent if the shoots were actively growing.

Artificial inoculations of cuttings revealed that cankering by C. salicella on Scouler willow, Melanconis sp. on red alder, Libertella sp. on

western dogwood and Dactylosporium sp. on broadleaf maple consistently occurred when the field values in relative turgidity were reduced to between 69 and 77 per cent. Although these pathogens were observed to occur only on their field hosts in the Vancouver area, further inoculation studies with C. salicella, Fusarium sp., Libertella and Melanconis sp. on new hosts demonstrated that the host ranges of these parasites were extended when the moisture level of the bark tissues was lowered to values below the 80 per cent level.

Infection of new hosts by Fusarium sp., Libertella sp. and Melanconis sp. occurred in the moisture range of 60 to 77 per cent. C. salicella developed cankers on red alder, bitter cherry, western dogwood and broadleaf maple when the bark moisture ranged between 41 and 68 per cent. The 41% value appeared to approach the lowest value that could be encountered in living cuttings. The fluctuation in bark moisture under field condition never approached this low value during the course of this study. The lowest value under field condition was 79% obtained once in Scouler willow.

These results showed that the relative turgidity of the bark tissues may serve as an expression of the degree of host vigour and suggested that a high moisture content--above 80 per cent--of the bark of these tree species was a factor preventing host range expansion under field conditions.

The practical use of relative turgidity of the bark as an index of host vigour is that it might indicate periods of increased susceptibility to facultative parasites. This would be of value in nurseries and plantations.

For example, according to the experimental results, Scouler willow becomes susceptible to both C. salicella and Melanconis sp. before the

other tree species tested. Scouler willow bark showed a tendency to decrease rapidly in water content to a level where the tree would become susceptible to the pathogens. Under the same conditions with a higher bark moisture content it would be resistant to both of these pathogens. Also, it appears that C. salicella on red alder, bitter cherry, western dogwood and broadleaf maple is a minor threat because the very low bark moisture level required for predisposing the host rarely occurs under field conditions. Cankering of these trees by their usually occurring pathogens takes place long before the relative turgidity of the bark is low enough to favour C. salicella.

It was seen that Cryptodiaporthe canker on red alder, bitter cherry, western dogwood and broadleaf maple were small in size and grew slowly. The sterilized sections of these hosts supported poor growth of C. salicella whereas their usually occurring parasites (Melanconis sp. on red alder, Libertella sp. on western dogwood, Fusarium sp. on broadleaf maple) developed profusely on the sterilized host material. These factors indicated that the chemical constituents of the bark tissues of these trees proved to be relatively poor as nutrition for the growth of C. salicella.

Although Fusarium sp. grew rapidly on sterilized willow bark, living cuttings were not infected. In the inoculation wounds, however, the mycelium of the fungus was clearly visible. Similarly, Libertella sp. grew abundantly on the autoclaved bark of red alder, broadleaf maple and Scouler willow canker development did not follow, although mycelia and sporophores of the fungus were present in the inoculation wounds. It appeared that the living tissues did not provide sufficient amounts of food material for the growth of the pathogens thus cankering did not take place.

It is also possible that the saprophyte flora of the living bark (Bier and Rowat, 1962a) could have suppressed the development of the artificially introduced pathogens.

Of the two factors underlying the parasitism of facultative parasites, bark moisture content of the host is therefore considered to be most important. The chemical constituents of the bark tissues as food for the fungi are considered as a secondary factor. Obviously, because of the probable changes in the nutritional status of the bark tissues as a result of the sterilization, the significance of this latter factor could not be fully evaluated.

Recently, discoveries of Bier and Rowat (1962 a, b) have indicated that the saprophytic flora of the bark tissues has a definite role in disease resistance of trees. This aspect in general has been overlooked. Further, objections have been raised against the use of agar plug inocula (Bier 1963). In view of these latest suggestions the role of different factors in parasitism of trees by facultative parasites will have to be more intensively studied before the full implications of bark moisture content and other factors will be realized.

SUMMARY

The occurrence of facultative parasites on different hosts was investigated to determine whether any correlation existed between bark moisture content and canker susceptibility. Cuttings of red alder, western dogwood, broadleaf maple and Scouler willow were inoculated with fungi that normally occurred on them in association with bark cankers.

Each cutting with bark moisture content higher than 80 per cent of saturation was resistant to the pathogen normally encountered on that species. When bark moisture content of the cuttings was lowered within the range of 69 to 78 per cent, infection occurred in the cuttings.

Each of the pathogens studied could infect cuttings of hosts other than those on which they occurred under field conditions provided moisture contents were reduced below the level required for infection by their field hosts.

Measurements of the field level in the bark tissues of these trees indicated that it remained over 80 per cent during the dormant season and in the succeeding spring and summer.

On the basis of the inoculation studies it was suggested that the high bark moisture content of bark of trees under field conditions could have played a major part in preventing host range expansion in the field.

The cultivation of these parasites on the autoclaved bark tissues of all tree species indicated that they produced larger colonies on the sterilized bark of their field hosts than on similarly treated bark tissues of new hosts. The growth of the pathogen in living tissues was examined only in the case of C. salicella. The results showed that even if favourable

moisture conditions were provided canker development on the field host reached a larger size in the same length of time than on the cuttings of new hosts. From these results it was suggested that, aside from the bark moisture content, the chemical constitution of the bark—as food for the pathogen—may play a role in the establishment of cankers.

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Figure 1.

The method of preparing wound inoculation with agar plug inocula.

Figure 1.

Figure 2.

The method of incubation. High humidity provided for inoculated cuttings in jars covered by plastic bags.

Figure 2.

Figure 3.

Method of achieving quick recovery of cankered cuttings. Plastic cover used to maintain high humidity around cuttings with continuous water supply.

Figure 3.

Figure 4.

Cuttings of 1-year-old Scouler willow shoots inoculated with C. salicella. Column on the left indicates dates at which relative turgidity of bark was determined for each cutting.

a) water withheld from cuttings until canker formed.

b) water continuously supplied to cuttings. Note absence of cankers.

Figure 5.

Cuttings of 1-year-old trembling aspen shoots inoculated with C. salicella. Column on the left indicates dates at which relative turgidity of bark was determined for each cutting.

a) water withheld from cuttings until cankers formed.

b) water continuously supplied to cuttings. Note absence of cankers.

Figure 4a.

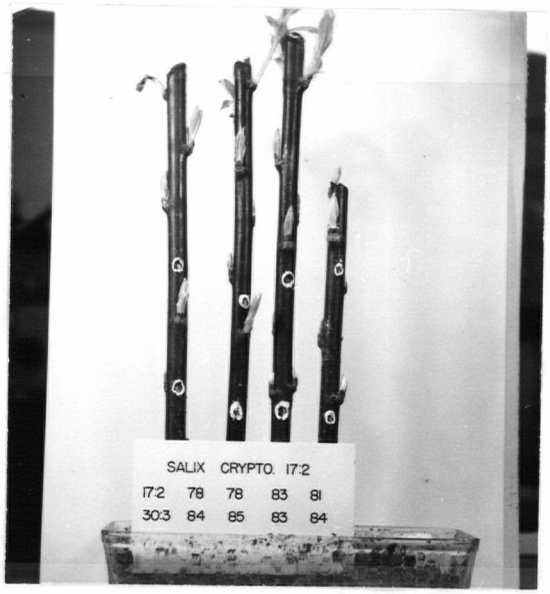


Figure 4b.

Figure 5a.

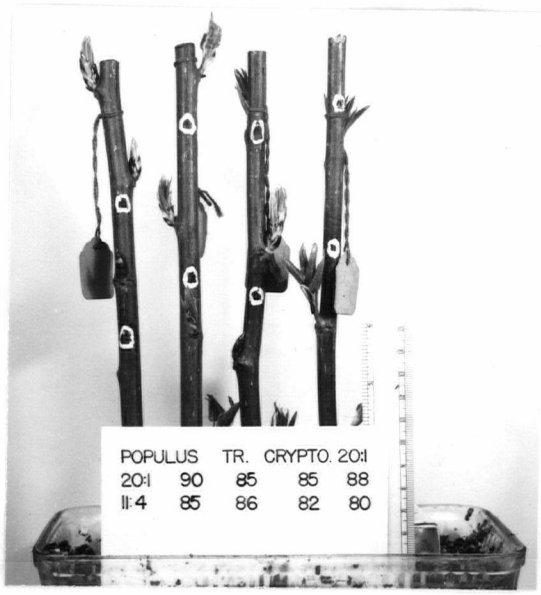


Figure 5b.

Figure 6.

Cuttings of 1-year-old black cottonwood shoots inoculated with C. salicella. Column on the left indicates dates at which relative turgidity of bark was determined for each cutting. Water was withheld from cutting until canker formed.

Figure 7.

Cuttings of 3-year-old red alder branches inoculated with C. salicella. Column on the left indicates dates at which relative turgidity of bark was determined for each cutting.

a) water withheld from cuttings until canker formed.

b) water supplied to cuttings continuously. Note absence of cankers.

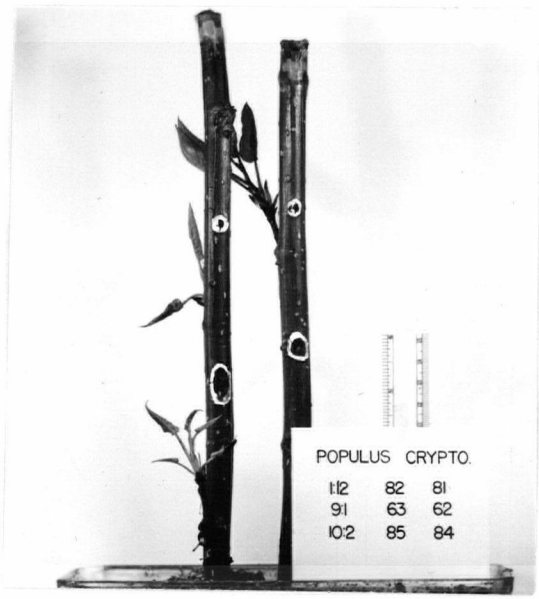


Figure 6.

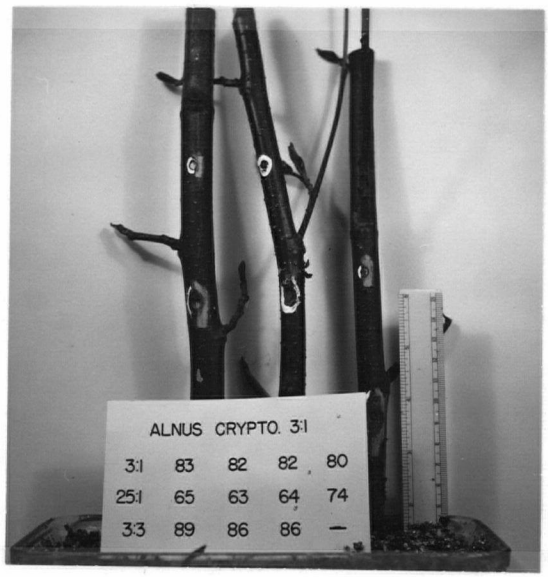


Figure 7a.

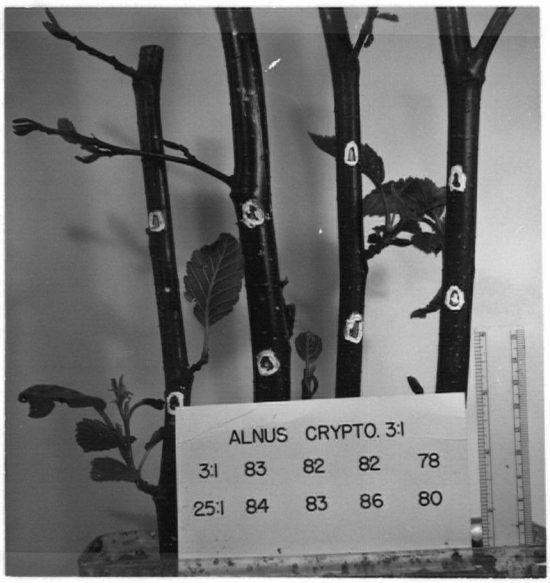


Figure 7b.

Figure 8.

Cuttings of 3-year-old bitter cherry branches cankered with C. salicella. Column on the left indicates dates at which relative turgidity of bark was determined for each cutting.

a) water withheld from cuttings until canker formed.

b) water supplied to cuttings continuously. Note absence of cankers.

Figure 9.

Cuttings of 1-year-old broadleaf maple shoots cankered with C. salicella. Column on the left indicates the dates at which relative turgidity of bark was determined for each cutting.

a) water withheld from cuttings until canker formed.

b) water continuously supplied to cuttings. Note absence of cankers.

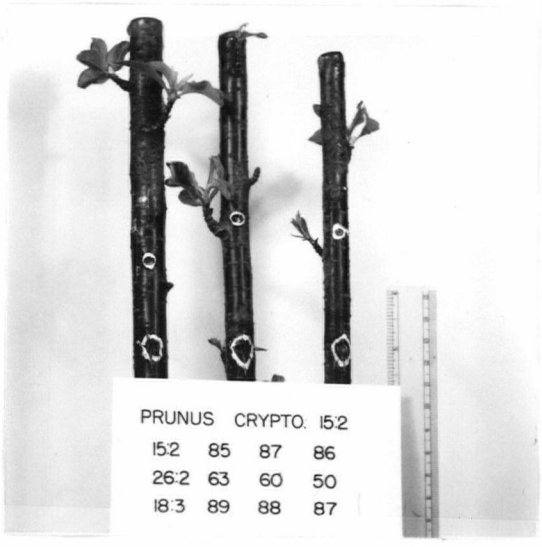


Figure 8a.

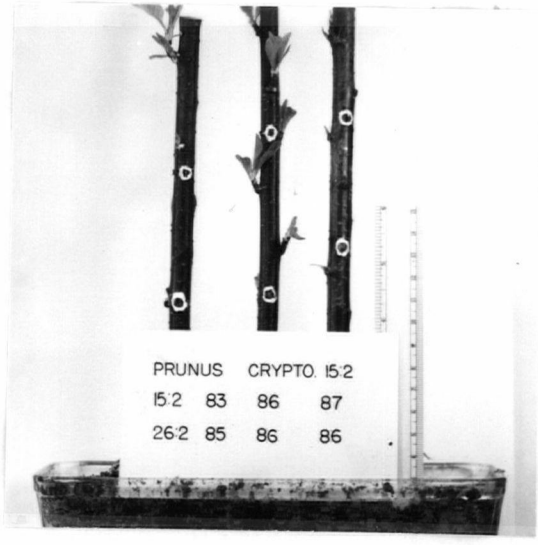


Figure 8b.

Figure 9a.

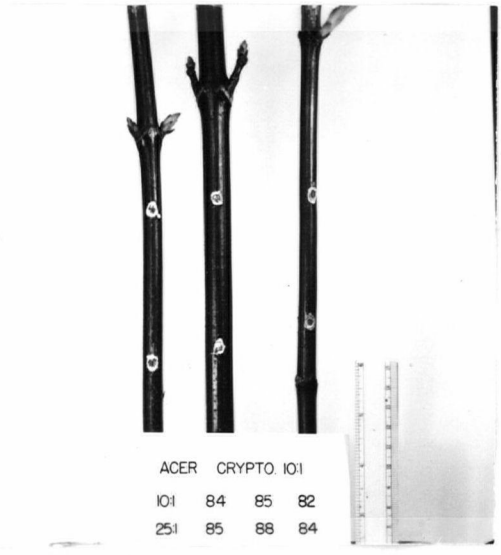
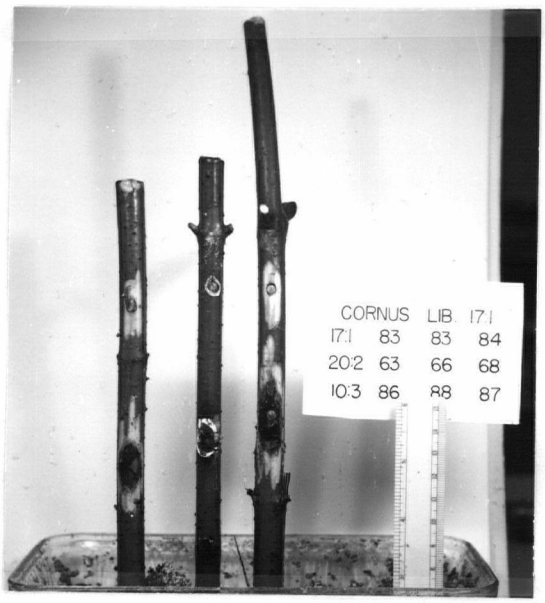


Figure 9b.

Figure 10.



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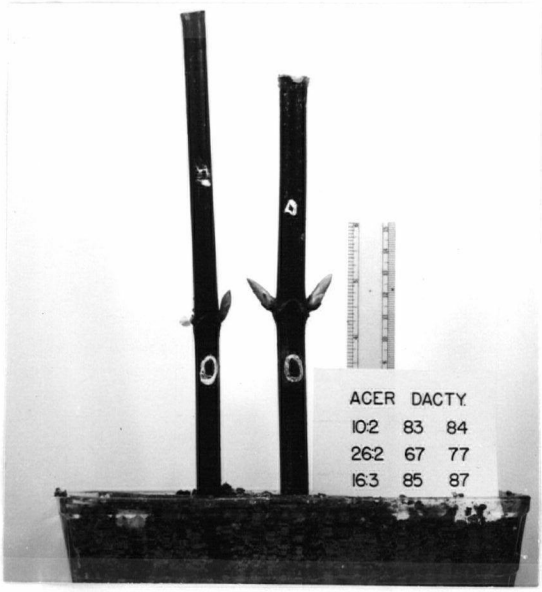

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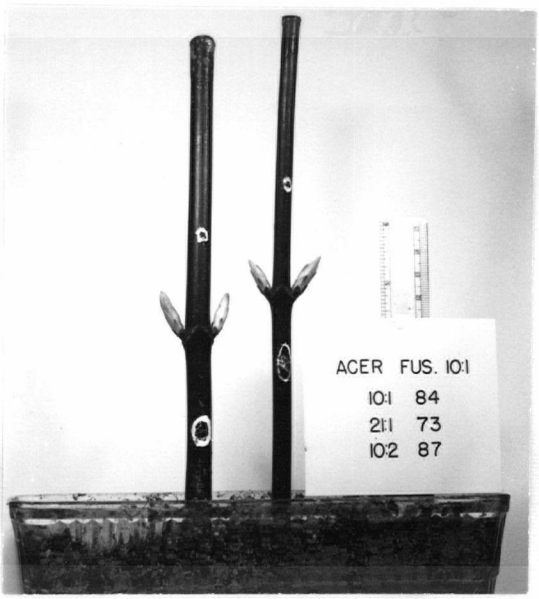

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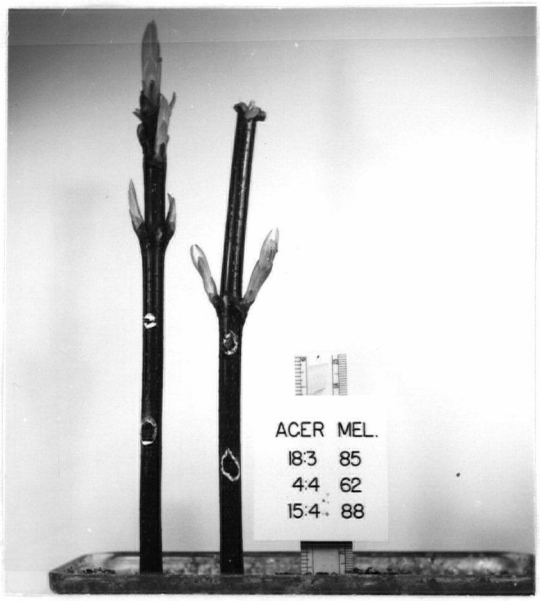

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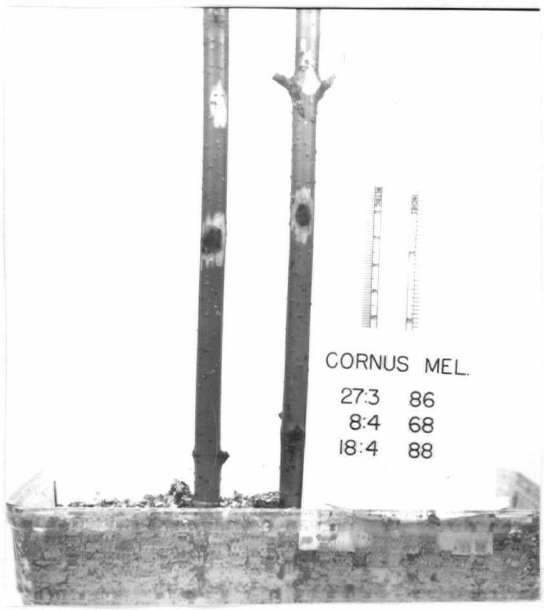
Figure 15.



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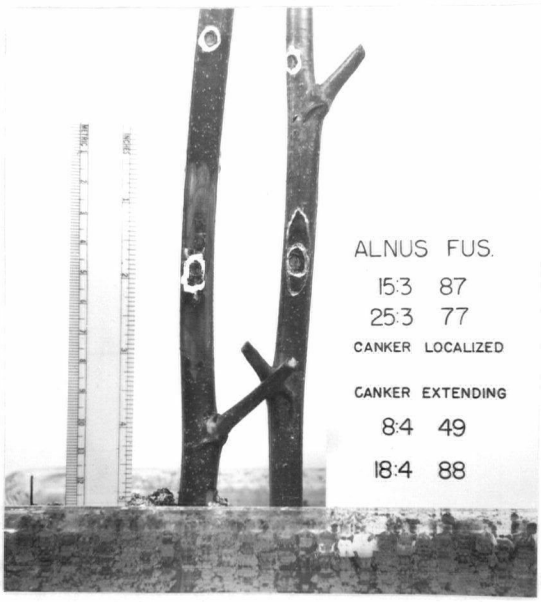

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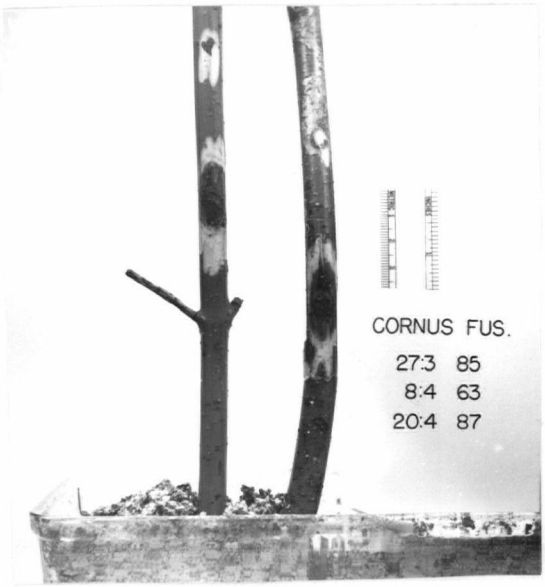

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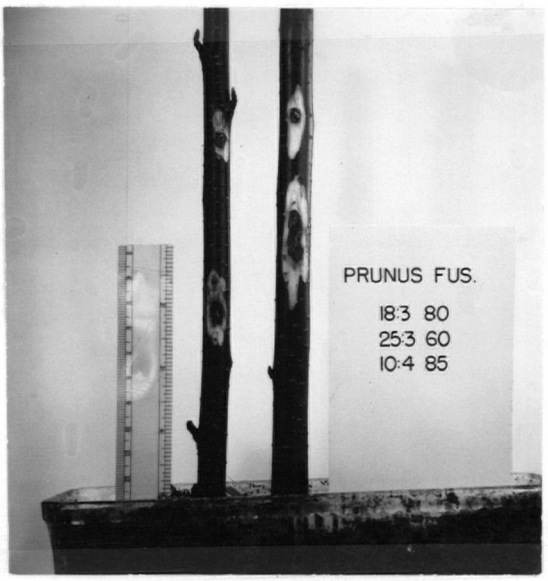

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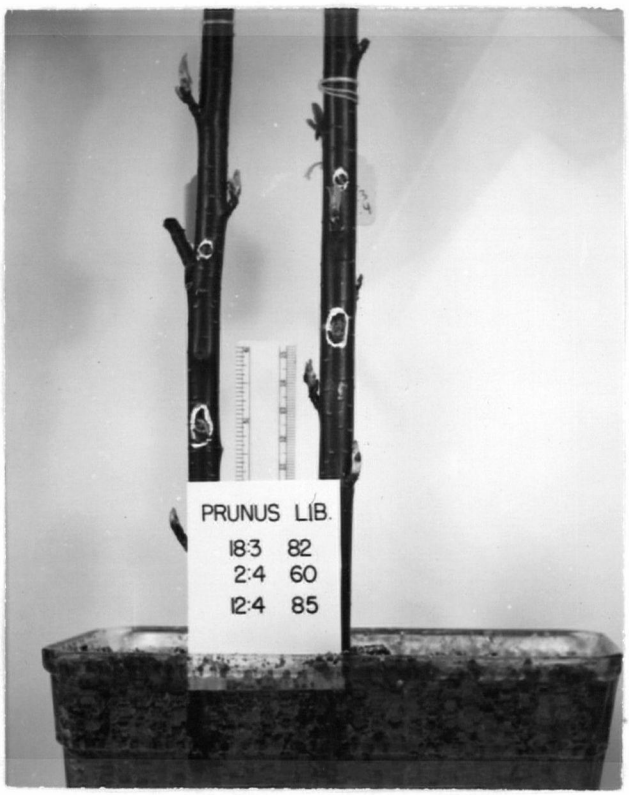

Figure 20.