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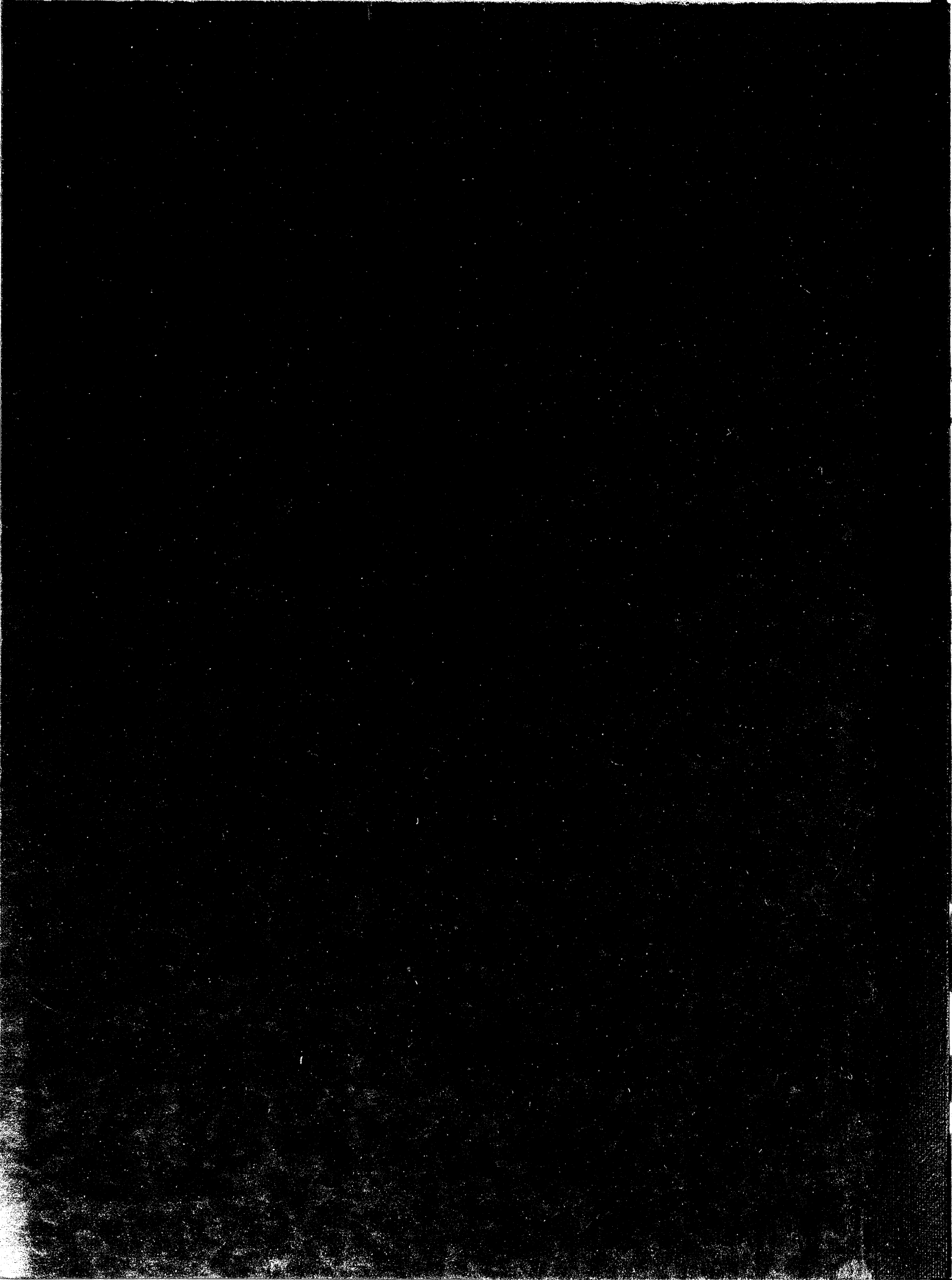

TWO STRAINS OF *VALSA* CAUSING DISEASE IN CONIFERS

by

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INTRODUCTION

Several species of the genus Valsa Fr. have become economically important as the result of the damage they cause in fruit trees and shade trees. Up until the present time most investigators in this field have confined their research to the species of Valsa attacking orchard trees or important shade tree species. In this study however an endeavour was made to discover the pathogenicity and the conditions necessary for growth and fruiting of two strains of Valsa from coniferous hosts.

PREVIOUS INVESTIGATIONS

Wehmeyer (35) working with Valsa Kunzei Fr., isolated from twigs of Thuja plicata D. Don., established the fact that two sexual strains are not necessary for the formation of perithecia in this species. He found that perithecia and ascospores are consistently produced from single spore cultures. He furthermore established the connection between the perfect stage, Valsa, and the imperfect stage, Cytospora, in this species. Perithecia were formed only in twig cultures, while pycnidia developed on agar cultures of the same isolation.

Many investigations have been made of species of Valsa that attack the poplars (Populus) particularly Valsa sordida and its imperfect stage Cytospora chrysosperma. Long (16) reports the canker disease caused by this organism to be widely distributed in the Southwestern States on several species of poplar (Populus spp.) and willow (Salix spp.). This range and host distribution is extended by Hubert (11) who found it on forest, shade, and ornamental trees in the Northwest. His host list includes twelve species and six different genera of trees. More recent papers by Povah (20), and Schreiner (24, 25) and Christensen (2) report the disease in the Eastern and Lake States.

Collections are also reported in Japan, Europe, England, and South Africa (25).

The fungus is a facultative parasite, and the severity of the injury is usually greatest on poorly growing trees or branches. On living branches entrance is gained through wounds or dead twigs. It grows in the bark of the tree causing death of the cambium (25). This injury to the living cells may, according to Schreiner, be mainly mechanical resulting from the growth of the mycelial weft--a growth probably aided to some extent by enzyme action.

Where severe damage occurs from infection by this organism some adverse site factor affecting the vigor of the tree is usually partly responsible. Such things as injury from drouth, fire, or severe pruning all make conditions favorable for spread of the disease. Damage is often serious in propagating beds where cuttings are heeled-in for storage during the winter.

In this country drupaceous and pomaceous fruit trees are susceptible to attack by Valsa leucostoma (Pers.) Fr. or sometimes placed in a separate genus as Leucostoma leucostoma (Pers.). Leonian (13) found it to be a weak wound parasite on apple trees, although severe damage from the canker has occurred when the fungus has infected trees already weakened by the woolly apple aphis or by the giant apple tree borer.

Hemmi (7, 8) described as new species, and studied the

parasitism and cultural characters of Valsa Paulowniae Miyabe et Hemmi, sp. n. on Paulowniae tomentosa and Valsa japonica Miyabe et Hemmi, sp. n. on Prunus yedoensis and P. Mume in Japan. Several comparative, pathological, and morphological studies have been made by Togashi (28, 29, 30, 31) on Vasla Mali Miyabe et Yamada, Leucostoma leucostoma (Pers.) and Valsa japonica occurring on fruit trees in Japan.

TAXONOMY

The genus Valsa Fr. belongs to a fairly well defined group of Pyrenomycetes, the stromatic Sphaeriales. The early systems of classification in this group took place before the knowledge of the spore and its morphology had developed. Therefore it was necessary to use the position of the perithecium and the configuration of the stroma in separating the group.

All of these fungi were placed in the genus Sphaeria by Fries.(5). He created in 1849 the genus Valsa for a rather indefinite group of stromatic forms (6). Nitschke (18) emended this genus in 1869 to include all stromatic forms with allantoid spores. Saccardo (23) who used spore characters for separation of genera followed this usage. Under this concept of the genus Valsa a large number of subgenera were erected, many of which were later recognized as genera.

These classifications were necessarily artificial and temporary since they were based on incomplete knowledge of the forms. In 1917 von Höhnel suggested that a natural system for the Sphaeriales should be based mainly on the character of the "nuculeus of the perithecium" or

"perithecial centrum" which includes the asci, paraphyses, and inner portion of the perithecial wall. The structure of the asci and their arrangement in the hymenial layer have been shown by von Höhnel to be important characters in the separation of large, related groups (9).

On this basis von Höhnel (9, 10) recognized that the subgenera of Valsa represented two distinct types of "perithecial centrum." Most of the subgenera of Valsa were relegated to his family Allantosphaeriaceae. The subgenus Euvalsa which retained the generic name Valsa and the subgenus Leucostoma were placed in a subfamily, Valseen or Valsaceae of the above family. Wehmeyer (36, p. 587) shows that Valsaceae should be a subfamily of von Höhnel's family Diaportheen or Diaporthaceae instead of the Allantosphaeriaceae.

That leaves us with but one point in the classification to clear up. Leucostoma was raised to generic rank by von Höhnel (9) mainly on the basis of the characters of its stroma. The stroma in Valsa, as limited in this system, is poorly developed and hardly distinguishable from the bark tissue, without any dark marginal line or zone beneath. The disk is seldom pure white. In Leucostoma (Nitschke) von Höhnel the stroma is well developed and limited by a distinct, dark marginal line, and the disk is often pure white. Wehmeyer (36) also recognizes the generic rank of Leucostoma on the basis of stromatic development.

The imperfect stage has been used to bring out

relationships in the Sphaeriales. An important step in the development of an adequate basis for classification in the Ascomycetes was made by L. R. Tulasne in 1851 when he established the occurrence of two or more spore forms in the life history of one fungus (32). The imperfect stage of Valsa is Cytospora. The genus Cytospora was founded by Ehrenberg (4); and recognized by Fries (6, p. 410) as a pycnidial stage of Valsa.

Classification by means of the entire life histories of the fungi is now developing. In this system it is necessary to have authentic connections between perfect and imperfect stages to determine the relationship of genera and species.

MATERIAL

The two strains of *Valsa* used in this study were isolated from coniferous hosts. *Valsa Kunzei* Fr. (3821) on *Abies balsamea* Mill. came from Victoria Park, Truro, Nova Scotia; and *Valsa superficialis* Nit. (3540) on *Pinus excelsa* Wall. came from Saginaw Forest, Ann Arbor, Michigan. It is difficult to distinguish between these two species on a morphological basis, and they may be only host varieties. Collections of the same morphologic type are known on the following hosts: *Pseudotsuga taxifolia* Britt., *Larix laricina* K. Koch., *Pinus strobus* L., *Pinus sylvestris*, Linn., *Tsuga canadensis* Carr., *Pinus Banksiana* Lamb., and *Thuja plicata*. In the von Höhnel system of classification these two organisms would be placed in the genus *Leucostoma* because they have a dark marginal line limiting the stroma below.

The cultures of these two organisms were supplied from Professor L. E. Wehmeyer's collection in the Cryptogamic Botany Laboratory at the University of Michigan. They originated from single ascus spore isolations.

The culture media used are as follows:

Malt agar

Malt extract 20 gr.

Agar agar 25 gr.

Distilled water 1000 cc.

Leonian agar (21)

KH_2PO_4 1.2 gr.

$MgSO_4$ 0.6 gr.

Peptone 0.6 gr.

Maltose 6.0 gr.

Malt extract 6.0 gr.

Distilled water 1000 cc.

Agar 15 gr.

Corn meal agar (21)

Corn meal 20 gr.

Agar 15 gr.

Distilled water 1000 cc.

Oat meal agar (21)

Rolled oats 50 gr.

Agar 17 gr.

Distilled water 1000 cc.

Leonian agar for C/N experiment

No. 1 No peptone	No. 2 0.6 gr. peptone
No. 3 1.2 gr. peptone	No. 4 1.8 gr. peptone

EXPERIMENTS

THE EFFECT OF TEMPERATURE ON GROWTH AND FRUITING

A comparison of growth and formation of pycnidia of the two test fungi was made on three different agar media and at five temperatures. The cultures were grown in each agar in constant temperature ovens at 8°, 20°, 25°, 30°, and 35° C. The ovens were so constructed that practically no light was admitted to the cultures except at time of observation.

Growth

Valsa Kunzei on corn meal and malt agar attained the greatest rate of growth at 20° C, and on Leonian agar at 30° C (see Fig. 2). The growth rate comparatively speaking, was greatest on malt agar, somewhat lower on corn meal, and at the minimum on Leonian agar.

V. superficialis reached the peak of its growth curve at 30° C. on corn meal and malt agars. On Leonian agar the maximum rate of growth was at 25° C., but the growth at 30° was nearly the same (see Fig. 1). Malt agar produced the most rapid growth response of the three media, Leonian agar somewhat less, and corn meal the least.

Plates of the two fungi were started at 25°, 30°, and 35° C. on oatmeal agar, but the rest of the series was not completed when it became evident that growth measurements would be impossible because of cloudiness of the agar.

Both of the test fungi survived the two extremes of temperatures used in this experiment although growth at 35° C. on some agars was nearly lacking. Plates taken from the 35° C. oven and placed at room temperature responded with rapid growth. V. Kunzei, however, produced no growth in the 35° C. oven within ten days. Some growth did appear on corn meal agar after 30 days, but it consisted of only a few scattered hyphae. V. superficialis, on the other hand, grew at least three mm. in eight days on all three media in the 35° C. oven. This growth continued, but was very scattered and never reached the edge of the plates.

A comparison of the growth graphs (Figs. 1 to 4) will show both that V. superficialis is a faster growing strain on the media used than is V. Kunzei; and that it responds favorably for growth at higher temperatures than does V. Kunzei.

The growth measurements used in making these graphs (Figs. 1 to 4) were taken on V. Kunzei at the end of six days. This period seemed to give the best representation of the growth at the temperatures used. V. superficialis,

Fig. 1.--Valsa superficialis

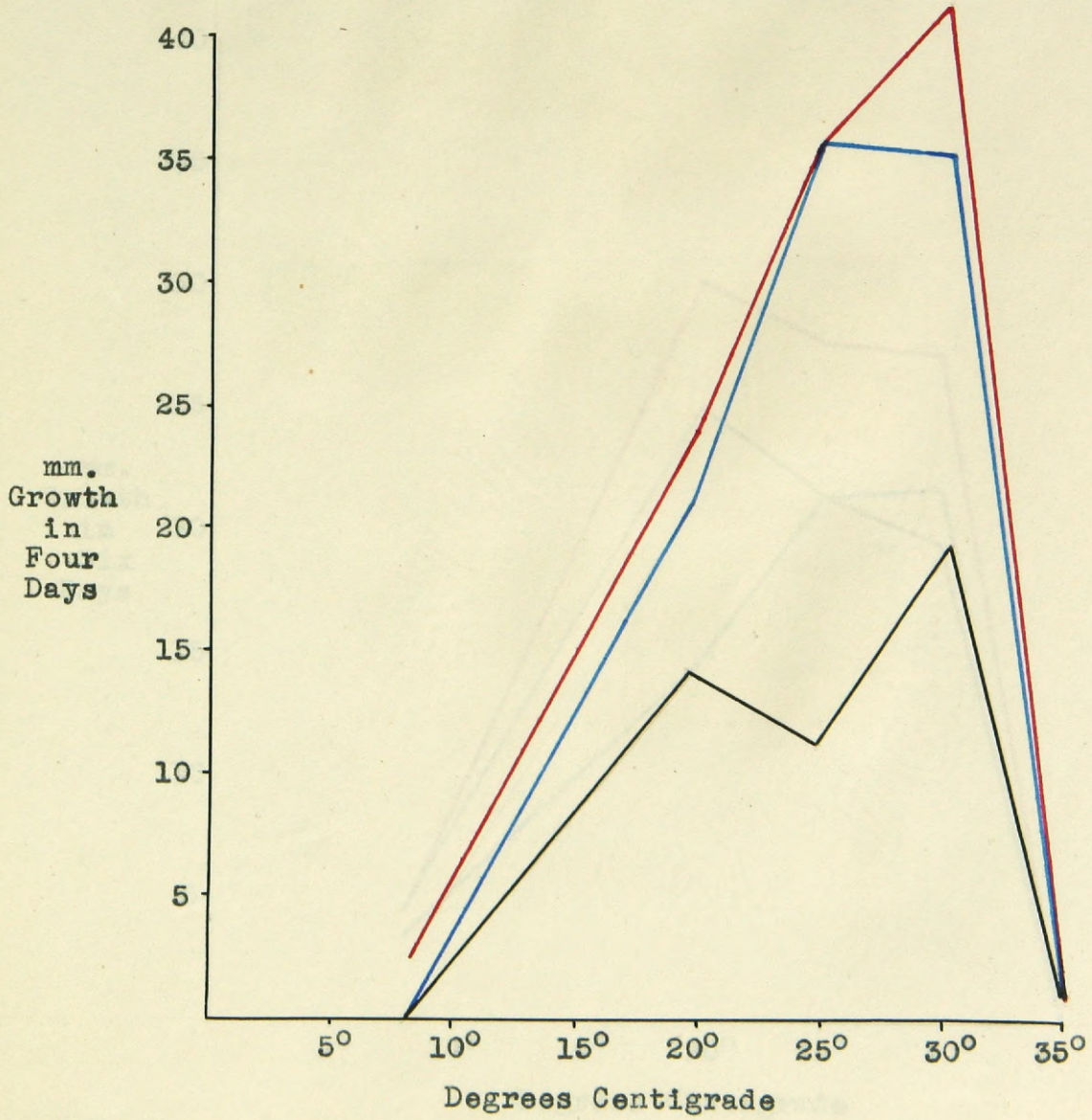


Fig. 2.--Valsa Kunzei

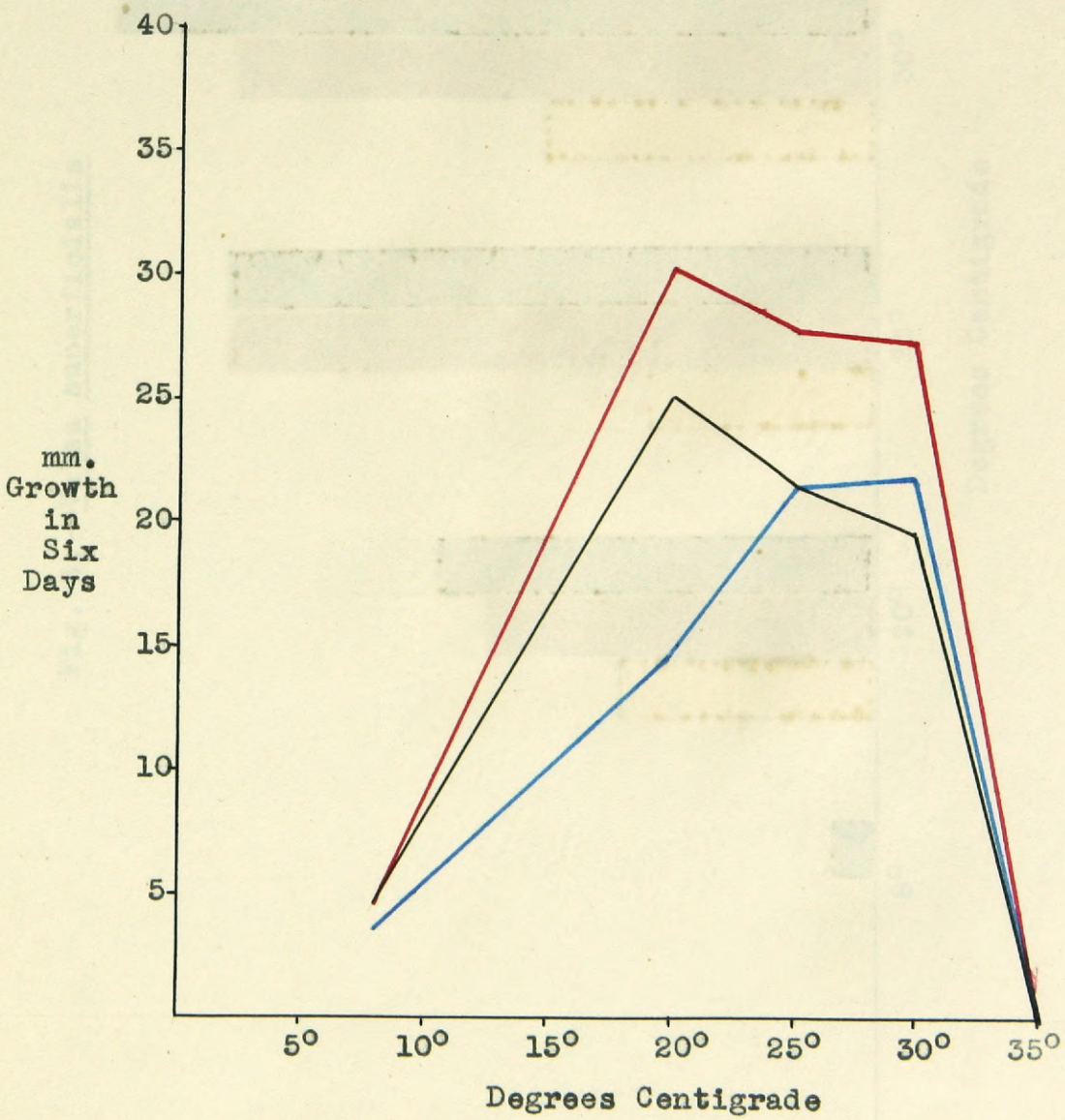


Fig. 3.--Valsa superficialis

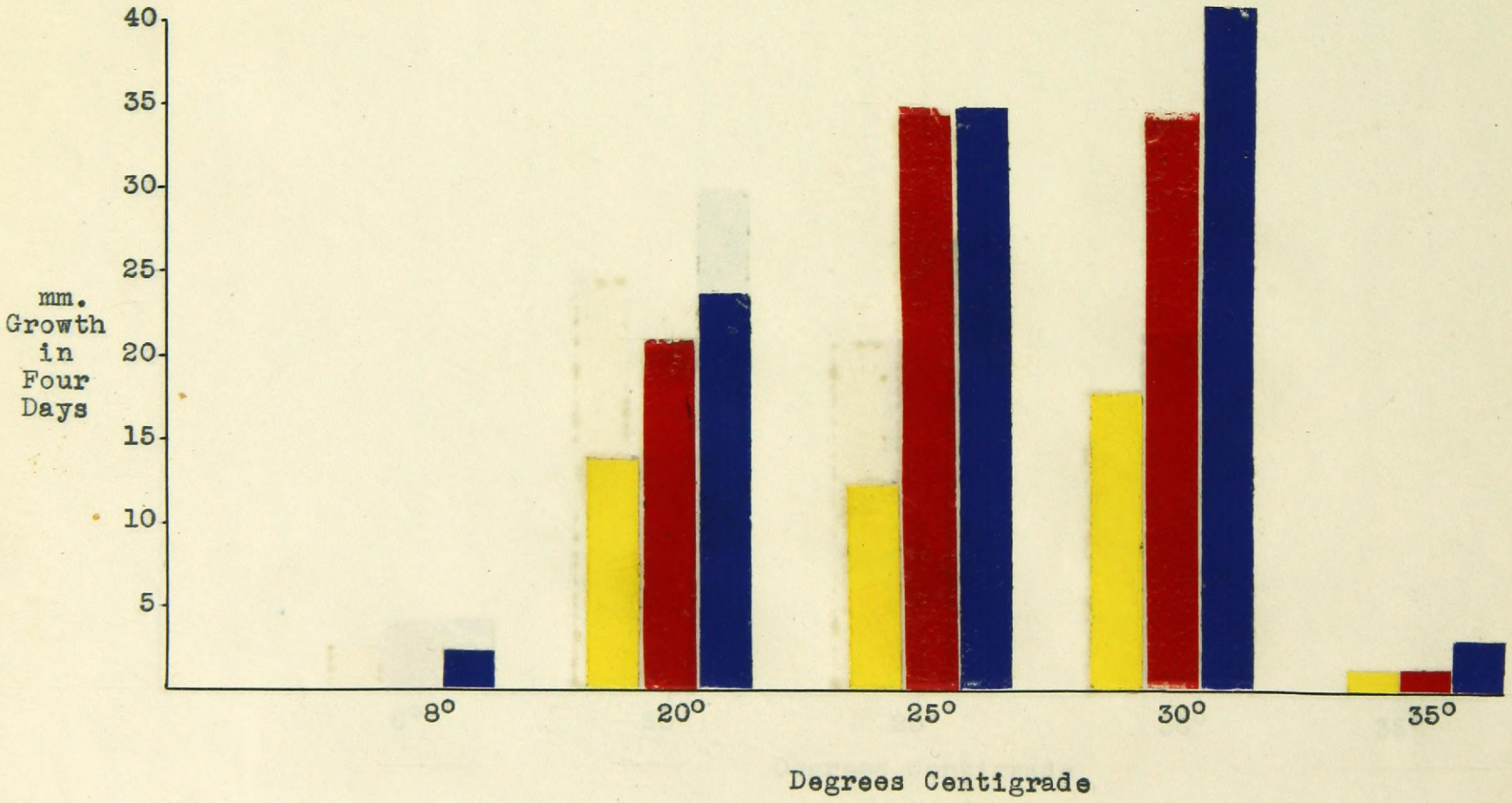
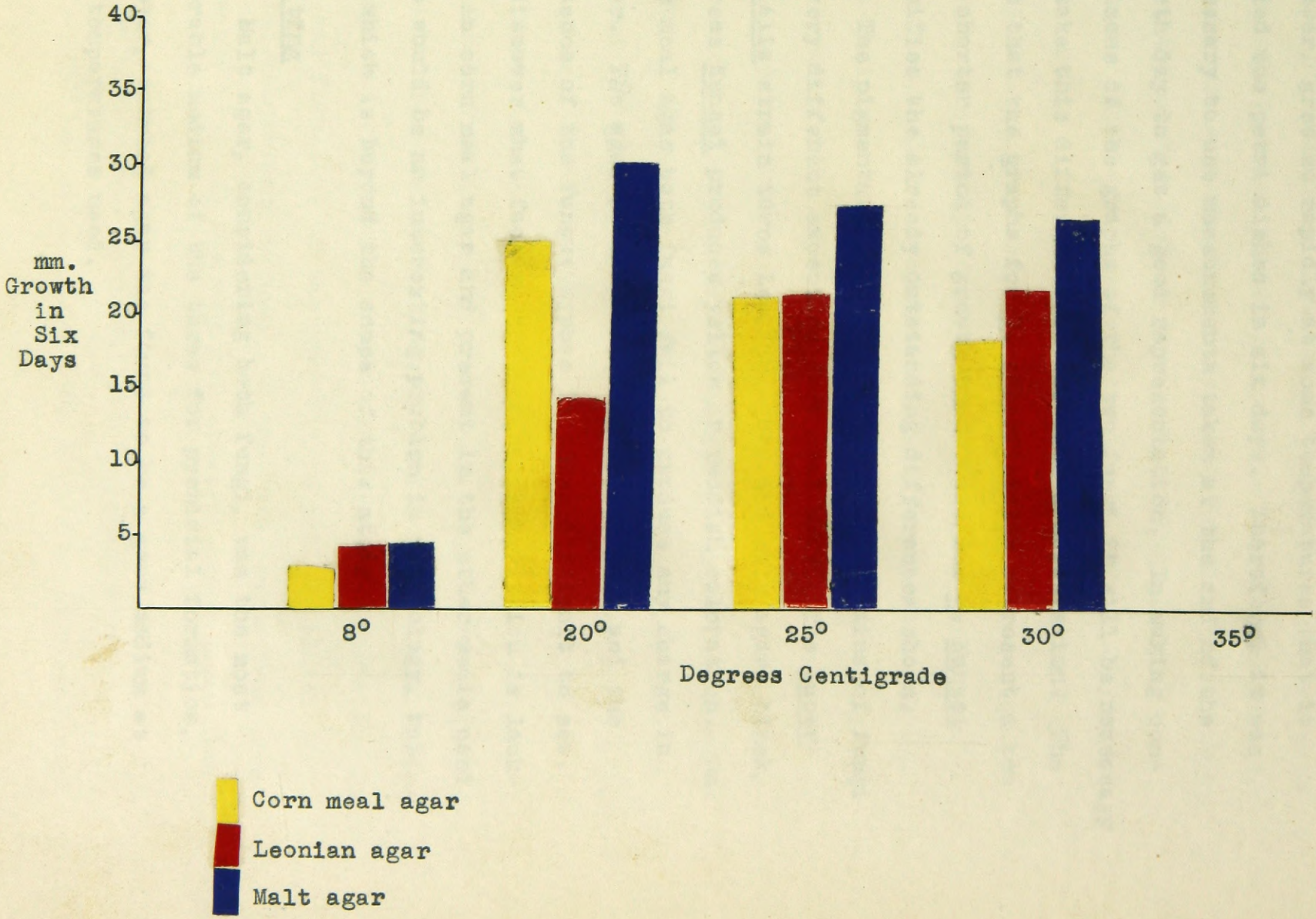


Fig. 4.--Valsa Kunzei



however, grew so rapidly at some temperatures that it filled the petri dishes in six days. Therefore, it was necessary to use measurements taken at the end of the fourth day to get a good representation. In making comparisons of the graphs of the two fungi it will be necessary to take this difference of time into consideration. The fact that the graphs for V. superficialis represent a two day shorter period of growth than those for V. Kunzei magnifies the already outstanding differences shown.

The pigmentation produced by the two strains of fungi is very different except on corn meal agar. The superficialis strain turns Leonian, oat, and malt agars black, whereas Kunzei produces yellow or reddish coloration. On corn meal agar both fungi fail to produce any change in color. The agar remains clear and colorless, and the presence of the fungus hyphae is often difficult to see. To discover what factor influencing pigmentation is lacking in corn meal agar and present in the other media used here would be an interesting problem in physiology, but one which is beyond the scope of this study.

Fruiting

Malt agar, considering both fungi, was the most favorable medium of the three for pycnidial formation, however, oatmeal agar was found to be a good medium at the temperatures used.

The temperature at which V. Kunzei produced, on malt agar, the greatest number of pycnidia was 25° C. At this temperature a profusion of stromatic fruiting bodies began to appear within fifteen days. They developed from a darkened layer of tissue on the surface of the agar and were more or less spherical in shape being 0.5 to 4 mm. in diameter. These stromata were covered with a light mouse gray (Ridgway) weft of hyphae. The pycniospores were born within labyrinthiform locules that had one or more outlets. The spores were exuded from these openings in a creamy white mass.

At 30° and 20° C. the pycnidia appeared within the same time, but they were smaller and fewer in number than at 25° C. The exudation of spores was also much less at 30° and 20° C. than at 25° C. No pycnidia were found within thirty days at 8° or 35° C.

It is impossible to evaluate in exact figures the favorability of the different temperatures for pycnidial formation because of the wide variation between the plates of any one temperature set and because of the variation in size and fertility of the pycnidia on any one plate. A careful comparison, however, of the five plates from each set makes possible the relative classification given here.

On Leonian and corn meal agar stromata developed only at 20° and 25° C. There was little or no significant

difference between reactions at these two temperatures. Corn meal agar produced only small submerged pycnidia 1 mm. or less in diameter. They were black and arranged more or less in concentric rings around the inoculum. Both aerial and submerged fruiting bodies appeared on Leonian agar. The submerged pycnidia were arranged in concentric rings on this medium also.

V. superficialis did not fruit at all on corn meal agar. On malt and Leonian agars, however, a few pycnidia appeared at 8° and 20° C. The most favorable temperature is probably somewhere between these two limits. The fruiting bodies on these two media were small as compared to those of V. Kunzei. They were 1 mm. or less in diameter and often sterile with no locules formed. The stromata that did contain locules had no openings and thus the spores were not exuded from them.

THE EFFECT OF LIGHT ON GROWTH AND FRUITING

Leonian agar was used as the growth medium in this experiment. Five dishes were inoculated for each of the test fungi, V. Kunzei and V. superficialis, and placed in a glass jar on the laboratory table in full light of the west windows. A duplicate set was wrapped in heavy opaque paper and placed on the same table. No light was admitted to these cultures except for a few minutes every two days

when observations and measurements were made. The room temperature remained fairly constant during the experiment, not varying more than 2° C above or below 25° C.

Growth

Valsa superficialis in the light grew on an average of 37.6 mm. in eight days and in the dark 38.8 mm. in the same period. V. Kunzei averaged 28.6 mm. growth in the light and 26.4 mm. in the dark for the period of eight days. The difference in rate of growth between light and dark is not significant for either strain. However, V. superficialis grew considerably faster in both light and dark than did V. Kunzei. This difference in rate of growth of the two fungi shows up in the other experiments also.

Fruiting

The significance of light in the production of pycnidia is brought out very clearly in the results of this experiment. On the plates of V. superficialis that developed in the light superficial pycnidia began to appear after eight days of growth. On the fifteenth day there were many pycnidia formed in a zone near the center of each dish. On the plates that developed in the dark there were no signs of pycnidial formation after eight days; at fifteen days there was one pycnidum on one of the

dishes; and after twenty days one dish had many, one dish a few, and three dishes no pycnidia.

V. Kunzei reacted in much the same way. In the light superficial pycnidia began to appear after eight days. In fifteen days these pycnidia were profuse on all parts of the culture surface. On the five plates in the dark there were no signs of pycnidia at eight days; a few on three plates at fifteen days; and a profusion on one plate at twenty days, a few on two plates and none on two other plates.

Around the outer edge of the mycelial mat of V. Kunzei grown in the light, minute, submerged pycnidia developed at the rate of about 10 per square centimeter. On the plates grown in the dark, submerged pycnidia as large as 2 mm. in diameter appeared. They were larger and fewer in number than the ones that developed on the plates in the light. There were no submerged pycnidia on the plates of V. superficialis grown in the dark and only a few on two of the plates in the light.

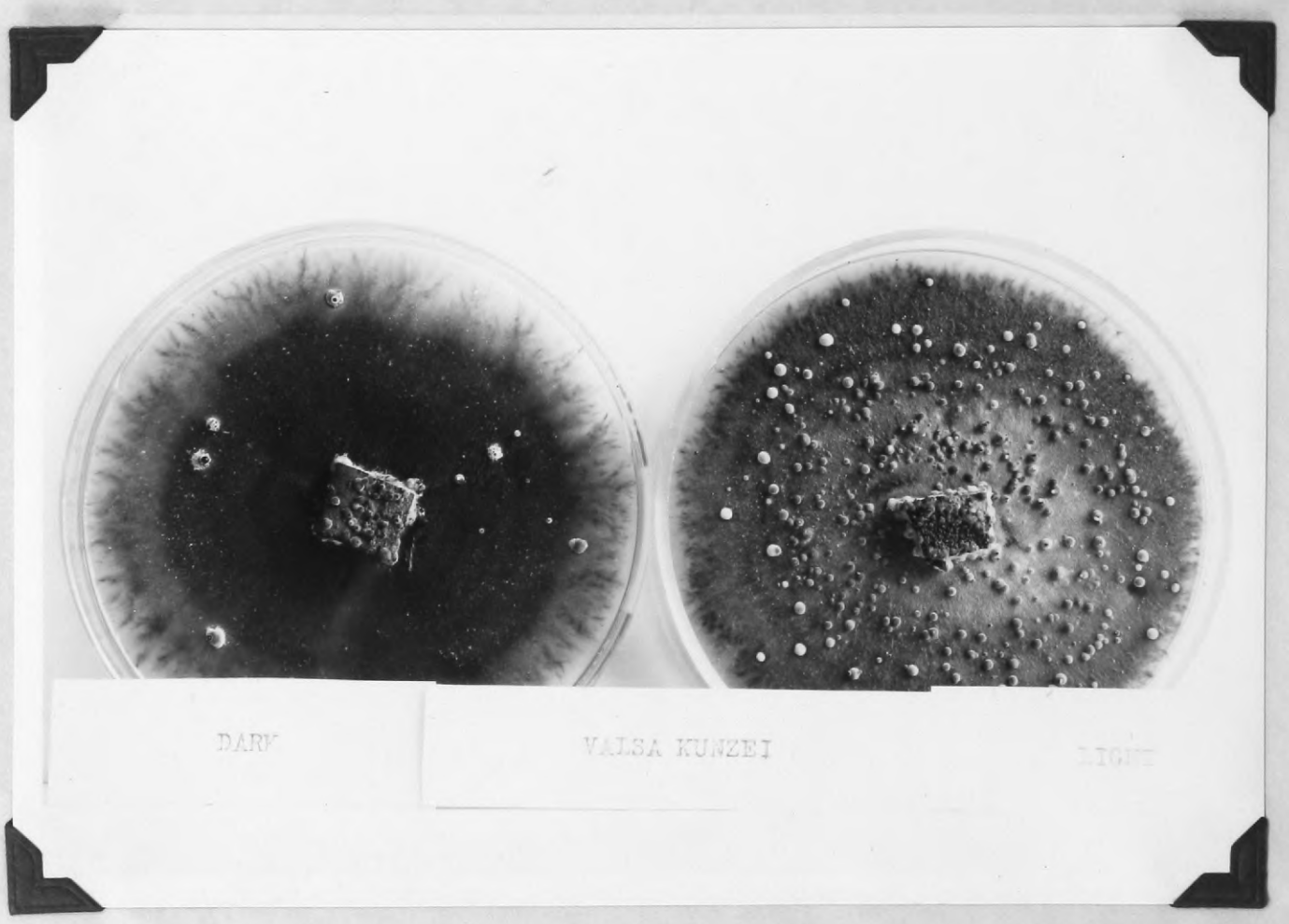
V. superficialis grew faster in both light and dark than did V. Kunzei, but it produced fewer pycnidia. It is important to note that the formation of fruiting bodies did not start on any of the plates until the mycelium had nearly filled the available space and lateral growth had stopped.

TABLE I

COMPARISON OF FINAL RESULTS OF THE EFFECT PRODUCED BY LIGHT ON PYCNIDIAL FORMATION

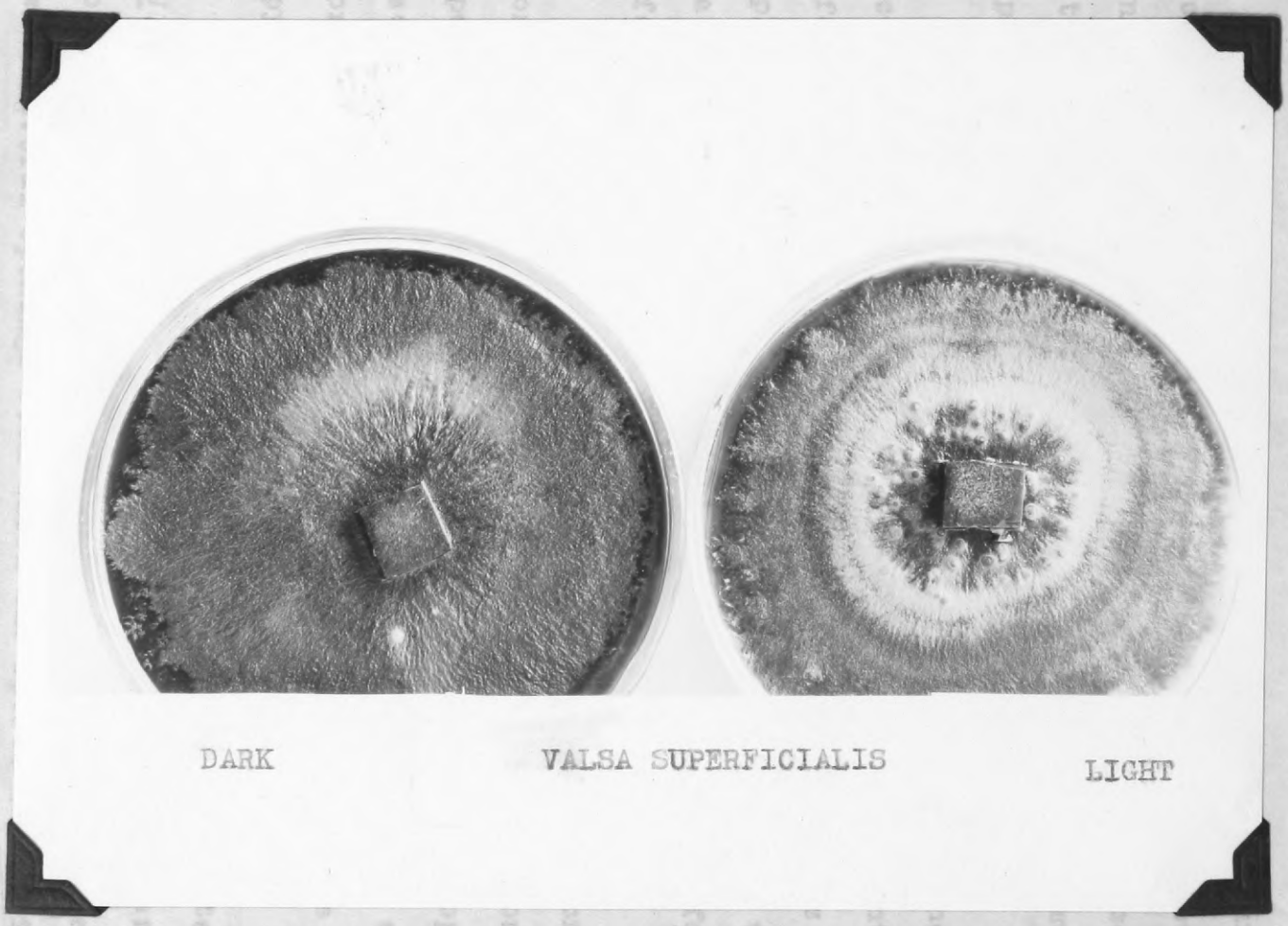
Light		Dark	
V. superficialis	*V. Kunzei*	*V. superficialis*	*V. Kunzei*
Average Number of Superficial Pycnidia Per Plate at Thirty Days		Average Number of Superficial Pycnidia Per Plate at Thirty Days	
50	226	25	55

PLATE I



A COMPARISON OF PYCNIDIAL FORMATION ON
LEONIAN AGAR IN LIGHT AND DARK

PLATE II



A COMPARISON OF PYCNIDIAL FORMATION ON
LEONIAN AGAR IN LIGHT AND DARK

INFLUENCE OF CARBOHYDRATE NITROGEN RATIO

The medium for this experiment was Leonian agar. The carbohydrate content was held constant, and the amount of nitrogen varied, with four degrees of nitrogen concentration used. The agar was made according to the formula given previously, with the variation in peptone.

After inoculation the plates were placed in the 25° C. constant temperature oven. There was no significant difference in the rate of growth between the four sets of plates. A comparison of the growth rate on plates within a set showed more variation than did the averages for all four sets of a species.

V. superficialis produced very few fertile pycnidia on any of the plates regardless of the concentration of peptone. A few rudiments of fruiting bodies developed on some plates in each set, but they remained sterile except on the plates with 1.2 concentration peptone, where one pycnidia was found to be fertile.

Pycnidial stromata were formed on all the plates of V. Kunzei. Without any peptone the fungus formed large, often compound fruiting bodies 2 to 3 mm. in diameter, with several openings from which the spores exuded in a brown mass. The color of the agar was dark brown, and became lighter as the concentration of peptone was increased. The number of stromata became greater with the

increase in peptone content, but they were much smaller. On the dishes with 1.8 concentration of peptone the pycnidia were from 0.2 to 1 mm. in diameter, and so closely crowded in some places that it was impossible to estimate the number accurately.

Even though it was not possible to estimate the relative productivity of the fungi on the four different concentrations of peptone, the experiment showed very clearly that V. Kunzei produces more pycnidia at all concentrations of peptone used than does V. superficialis.

The results of this experiment can be considered as only a preliminary indication of the probable reaction of the fungi tested. The cultural methods used do not give exact enough results for definite conclusions. Liquid culture solutions made up on the basis of their molar concentrations will give the most satisfactory standard for comparison.

Robinson (22) working with Pyronema confluens Tul. in liquid cultures found that "the ratio of nitrogen to carbohydrate available in the medium is not the primary factor in determining when the reporductive structures shall arise, but that the dominant factors are absolute amounts of nitrogen and carbohydrate available. Reproductive structures begin to appear in a given culture when the supply of nitrogen is becoming exhausted, if at the same time carbohydrate available for mycelium is not excessive in

amount." The most suitable ratio for fruiting was found to be about 4 to 1 by molecular proportions of maltose to ammonium nitrate. Coons (3) working with Planodomus fuscomaculans arrived at a 5 to 1 ratio of maltose and asparagin as the most favorable combination although others were found suitable.

These results may be applicable to the fungi now under consideration, within limits, since, as Coons (3) points out, fungi behave alike in their physiological reaction to the substrata in the vast majority of cases.

DEVELOPMENT IN TWIG CULTURES

Two sets of cultures were made from branches of the following tree species: Pinus resinosa, Pinus strobus, Abies concolor, Thuja occidentalis, Pseudotsuga taxifolia, and Tsuga canadensis. Each branch was placed in a large tube with about 50 cc. of malt agar and sterilized at fifteen pounds pressure for fifteen minutes on two successive days. One set of tubes was inoculated with V. Kunzei, the other with V. superficialis. Both sets were placed on the laboratory table in full light of the west windows.

At the end of sixty days the cultures were opened and examined. V. superficialis produced perithecia on balsam and Douglas fir branches. On balsam the fruiting bodies developed just below the periderm and were thickly scattered

over the surface of the branch. An abnormal amount of superficial buff mycelial growth developed on these perithecia formed in twig cultures, probably because of the high moisture content of the air in the tubes. On the upper and drier portion of the twig the stromata appeared as erumpent tufts 0.5 to 1 mm. in diameter through which the black ostioles of the perithecia protruded slightly. Large stromatic cushions 2 mm. in diameter were formed by the stroma that developed on the more moist portions of the branch.

The stroma around the perithecia consisted of a mixture of interwoven, hyline hyphae and dark colored bark cells that developed into a stromatic plug above through which the black perithecial necks led to the surface. The dark, thick walled perithecia were 150 to 200 μ in diameter and grouped 6 to 10 in a stroma. A distinct black marginal line limited the stroma below. All of the perithecia examined were sterile probably because of the adverse growing conditions in the cultures.

The perithecial stromata on Douglas fir were not as large and fully developed as on balsam, and there were fewer perithecia within a stroma. Pycnidia also formed in this culture. They grew both superficially and within the bark tissue, and coiled yellow spore horns exuded from them.

On red pine only superficial pycnidia were formed. They were up to 3 mm. in diameter, but had no openings.

These large stromatic cushions contained a single lybrinthiform locule where the pycnidia were produced. The surface of the branch was covered with brown mycelium. In the culture of white cedar the brownish mycelium was profuse on the surface of the twig and several brown pycnidia 1 to 2 mm. in diameter developed from the superficial mycelium, but no spore horns were present.

In the white pine culture the mycelial growth was profuse, but no mature fruiting bodies appeared in the sixty days. The growth on hemlock was very thin and pure white. It did not cover the entire twig, but some mycelium appeared at the upper end where the branch had been cut. No fruiting bodies appeared within the sixty day period.

V. Kunzei produced no perithecia in any of the cultures within the time allowed. The most profuse formation of pycnidia was on the twig of balsam. They developed beneath the periderm and were errumpent singly over the entire surface of the twig. An abnormal mycelial growth formed spherical cushions as large as 2 mm. in diameter where the condensed moisture on the glass was near the branch. Coiled spore horns exuded from many of the pycnidia.

On white pine the mycelial growth was within the bark tissue as it was on balsam. A canker-like area about 2 cm. in diameter developed near the upper end of the branch. The limits of this area were very distinct because of the

contrast between the black color of the canker and the light brown of the rest of the branch. There was a narrow raised ridge around the outer edge of the black portion. Minute gray ectostromatic disks protruded through the epidermis in this raised portion. These appeared to be the beginnings of perithecia, but they were immature and it was impossible to distinguish them for certain from the pycnidia that were errumpent through the pustulate periderm of the remainder of the canker surface. Scattered over the twig a few pycnidia developed within the bark, but formed sperical masses of olive gray hyphae on the surface after the periderm had been broken. No spore horns were visible on any of the pycnidia.

The growth on hemlock, cedar, red pine, and Douglas fir twigs was much the same in appearance. There was a thin mat of superficial mycelium on all these twigs. No pycnidia formed on hemlock; but a few superficial, gray fruiting bodies 1 mm. or less in diameter appeared on the other branches, and on Douglas fir some were produced within the bark also. Coiled yellow spore horns exuded from these pycnidia.

TREE INOCULATIONS

In order to determine, to some extent, the degree of parasitism of the two organisms four species of conifers were inoculated with the test fungi. The inoculations were made by wounding the tree and placing the fungus mycelium in contact with the wound.

The area chosen for inoculation was first sterilized with alcohol; then several parallel cuts 1 mm. or less apart and two or three cm. long were made with a sterile scalpel. A mat of mycelium and the nutrient agar on which fungus grew was placed on this wounded surface. To hold the inoculum in place and to prevent contaminations the whole area was covered with adhesive tape.

The seedlings varied in height from two feet to ten feet. They were all in pots and kept in the University of Michigan's botanical garden green house. The inoculations were made in November when the trees were dormant; but temperature conditions were favorable for fungus growth in the green house.

Thuja occidentalis, Pinus strobus, Abies balsamea, and Pseudotsuga taxifolia were the species chosen for inoculation. By May 20th, six months after inoculation, no sign of disease was outwardly apparent on any of the trees. The failure to get infection was probably due to two causes:

1. the vigor of the seedlings, 2. the method of innoculation.

Steven (26) using the burning method of inoculation on species of Sorbus found that Valsa cincta Curr. failed to spread in tissues of healthy plants and infected only plants weakened by other causes. Hemmi (8) working with Valsa japonica experienced difficulty in producing the disease artificially. He attributes the failure of some of his inoculations to the "powerful resisting power of seedlings." His attempts were most successful when he burned a small portion of the bark in addition to making a cut wound. Leonian (13) and Hubert (11) also found that healthy plants were difficult to infect with Valsa.

The inoculations in this experiment may have been more successful if the area had been burned before the cut wound was made; so that the fungus would have had dead cells in which to get a start. If further work is done this method will be tried.

DISCUSSION

Klebs, as a result of his investigations, established the following basic principles regarding the reaction of lower organisms (12): The limits of reproduction with regard to temperature, oxygen, light etc. are narrower than those of growth. Growth and reproduction are processes that are opposed to each other, and conditions that favor growth are unfavorable for reproduction.

In these experiments on Valsa the formation of fruiting bodies did not begin to appear in the petri dish cultures until the mycelium had covered the medium and lateral growth had stopped. While the growth processes were going on no reproductive activities were evident.

According to the investigations of Coons (3) the lower organisms are greatly overfed by the ordinary laboratory media, such as those used in this study. Fruiting cannot be expected then unless some other factor checks growth. Growth is the process first inaugurated, and it continues as long as the food supply is abundant and outer conditions permit. It is an energy storing process, whereas reproduction is energy consuming.

Ordinary metabolism of growth uses the oxygen supply and thus prevents reproduction; but when growth is checked

respiration is reduced. Then if oxidation is increased by light or some other factor, after growth is checked, the energy released is used in reshaping the reserve stuffs into complex protein bodies--the spores (3).

Wakefield (33) in working with two Hymenomycetes, Schizophylum commune, and Stereum purpureum, found it possible to induce the formation of fruiting bodies by reducing or entirely removing the nutritiment of the mycelium. In the study of Melanospora destruens made by Asthana and Hawker (1) high food concentrations reduced and delayed perithecial formation and, further more well nourished mycelium growing into a less concentrated medium was stimulated to form perithecia.

The influence of light, in the production of fruiting bodies under the conditions tested in this study, is very significant. Reference to Table I and Plates I and II will show clearly the difference in pycnidial formation on cultures exposed to light and those not exposed.

Leonian (15) in studying pycnidial formation of the Sphaeropsidales found that in some instances a higher temperature not only replaces the effect of light, but can become a more efficient agent for promoting the formation of fruiting bodies. This is not true, however, with the fungi under consideration here. V. superficialis produced no pycnidia at 25°, 30°, and 35° C. in the dark, but pycnidia were produced at 25° C. in the light. V. Kunzei

produced fewer pycnidia at 30° C. than at 25° C. in the dark, and fewer at 25° in the dark than at 25° C. in the light.

Eight of the test organisms used by Leonian failed to produce any pycnidia in the dark at 8° C. However, when transferred to light at 8° C. pycnidia were formed. Under some conditions, then, light was the determining factor in fruitication.

Light was also found to have a direct effect on fruiting in Wakefield's (33) experiments. Coons (3) states that oxidation of oily reserve materials in the protoplasm of well nourished mycelium by light or other oxidizing agent serves to stimulate fruit-body formation. The action of oxidation is to release energy. It is believed that the influence of light is catalytic in the activation of oxidase enzymes along with the inauguration of a reaction favorable to their continued action. The few minutes of exposure to light every two days while growth measurements were being taken, may have caused enough of a stimulus to account for the pycnidial formation that occurred on the plates that developed in the dark in the second experiment. (See Table I)

The factors that influence the formation of perithecia are of interest in this study. No perithecia were formed on any of the agar cultures, but they developed in two of the twig cultures of V. superficialis. Wehmeyer (35)

working with some 30 stromatic forms in both twig and agar cultures found that conditions are seldom favorable for perithecial formation for this group in culture. Four of his forms produced the perfect stage in twig cultures, but none of them did on agar. Nearly all of the organisms, however, developed the imperfect stage in both twig and agar cultures.

The factors controling perithecial formation in this group are probably mainly physiological. The necessary nutrient substances for this type of reproduction are probably present in both agar and twig cultures. The moisture conditions within the substrata, and the exchange of moisture between the substrata and the air appear to be important factors. In cultures the humidity is kept high by evaporation from the media, but the exchange does not go the other way so readily. It is difficult to get moisture into the twig or agar after sterilization. This high atmospheric humidity and lack of circulation may account for the abnormal amount of superficial mycelial growth that occurs in cultures. Under these conditions the food supply is apparently used for vegitative growth and imperfect fruiting.

According to Wehmeyer (35) perithecial formation seems to depend somewhat on a proper balance of moisture within the substratum, and evaporation from its surface. The movement of moisture within the substrata may also be

important since the fruiting bodies are formed within the bark. In agar cultures a free interchange of gasses and moisture is not possible beneath the surface as is possible in cellular tissue. Hence the mycelial growth tends to be superficial.

Leonian (14) found in working with Valsa leucostoma that ordinarily the life cycle of this fungus goes through a mycelium-pycnidia-perithecia rotaion even in agar cultures regardless of the quantity of food, provided the quality is favorable. However, the range of pycnidial formation is much wider than that for perithecia. Both Leonian (14) and Schreiner (25) indicate that single ascospore cultures may give rise to different types of mycelia, and possibly to different strains of the same fungus that may differ in fruiting ability, specificity as to host, and in pathogenicity. All of these factors indicate, as Wehmeyer points out, that the conditions controling fruiting and especially perithecial formation are both specific and complex.

The situations under which the two strains of Valsa studied here cause disease and canker formation in conifers were not brought out in the limited scope of the inoculation experiment. More extensive inoculations in the field should be made on trees in several age classes, on vigorous and weak trees; and the inoculations should be made at different seasons of the year. Work of this kind will

be necessary to determine the pathogenicity of the organisms studied. Most of the species in the genus Valsa are merely saprofitic fungi, but a few have been shown to be facultative parasites. A comprehensive study of the group as a whole may show some correlation between stromatic development and the degree of parasitism.

SUMMARY

V. superficialis attains a greater rate of growth on all the media used than does V. Kunzei. It also responds favorably for growth at higher temperatures than V. Kunzei does. A growth curve for either fungus reaches its peak at different temperatures on different agars showing that both the medium and the temperature influence the rate of growth.

Malt agar was the most favorable medium of those used for pycnidial formation. V. Kunzei produced the greatest number of pycnidia at 25° C. on this medium. For V. superficialis it was 20° C. or lower. Under the conditions of light and temperature tested and on the media used V. Kunzei is much more prolific in pycnidial fruiting than is V. superficialis. The twig cultures, however, indicate that V. superficialis produced perithecia more readily than does V. Kunzei.

Light had a direct effect on the amount of pycnidial fruiting that occurred in the agar cultures. It noticably increased the number of pycnidia formed.

V. superficialis produced perithecial stromata in balsam and Douglas fir twig cultures in 60 days, but V. Kunzei produced only pycnidia.

The two strains of Valsa used here did not prove parasitic enough to infect healthy coniferous seedlings.

The principles of Klebs are substantiated. The experiments show very clearly that the limits of reproduction are narrower than the limits of growth for these species. The formation of fruiting bodies only after lateral growth had been stopped by the limits of the dishes indicates that conditions favoring growth are unfavorable to fruiting.

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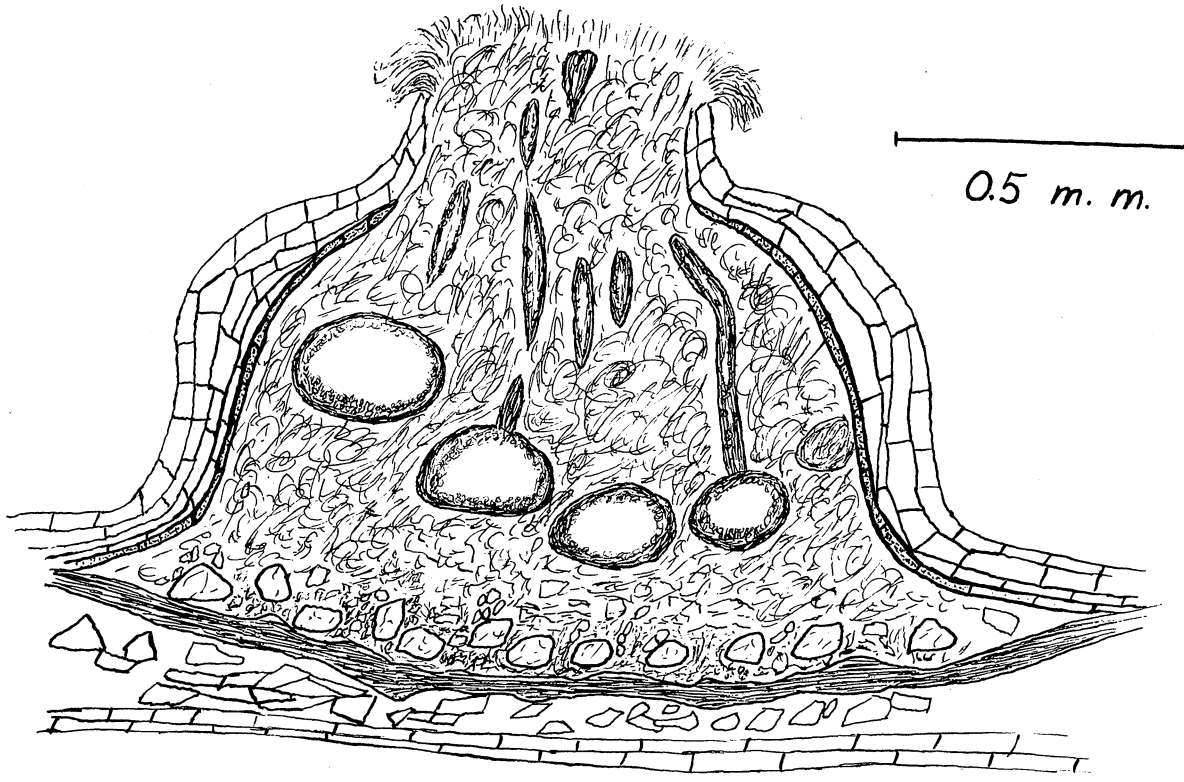
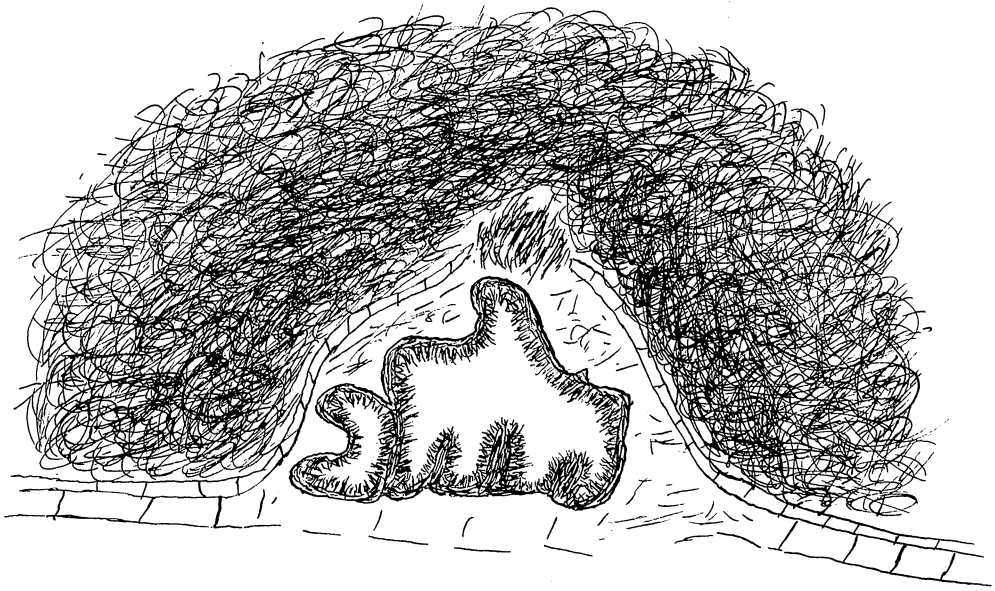
PLATE III

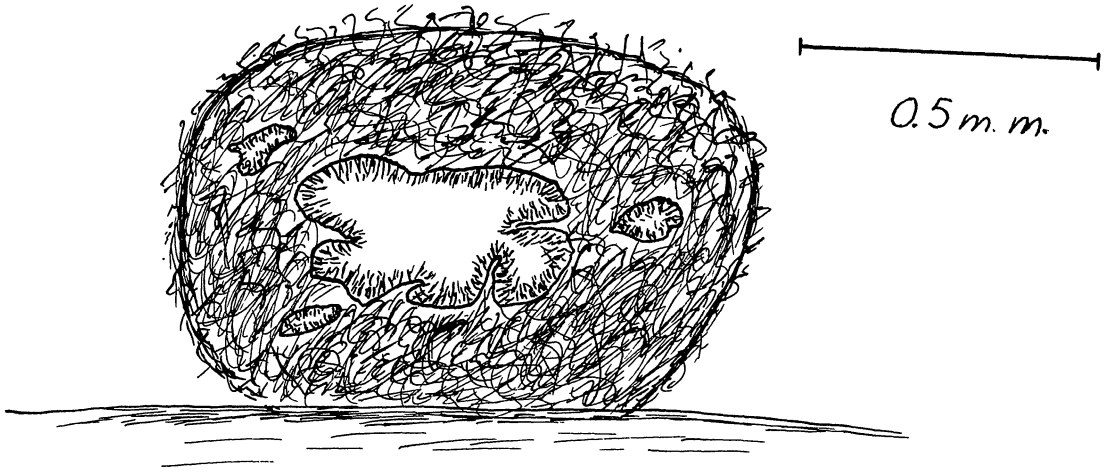


Perithecia of V. superficialis in balsam twig culture.



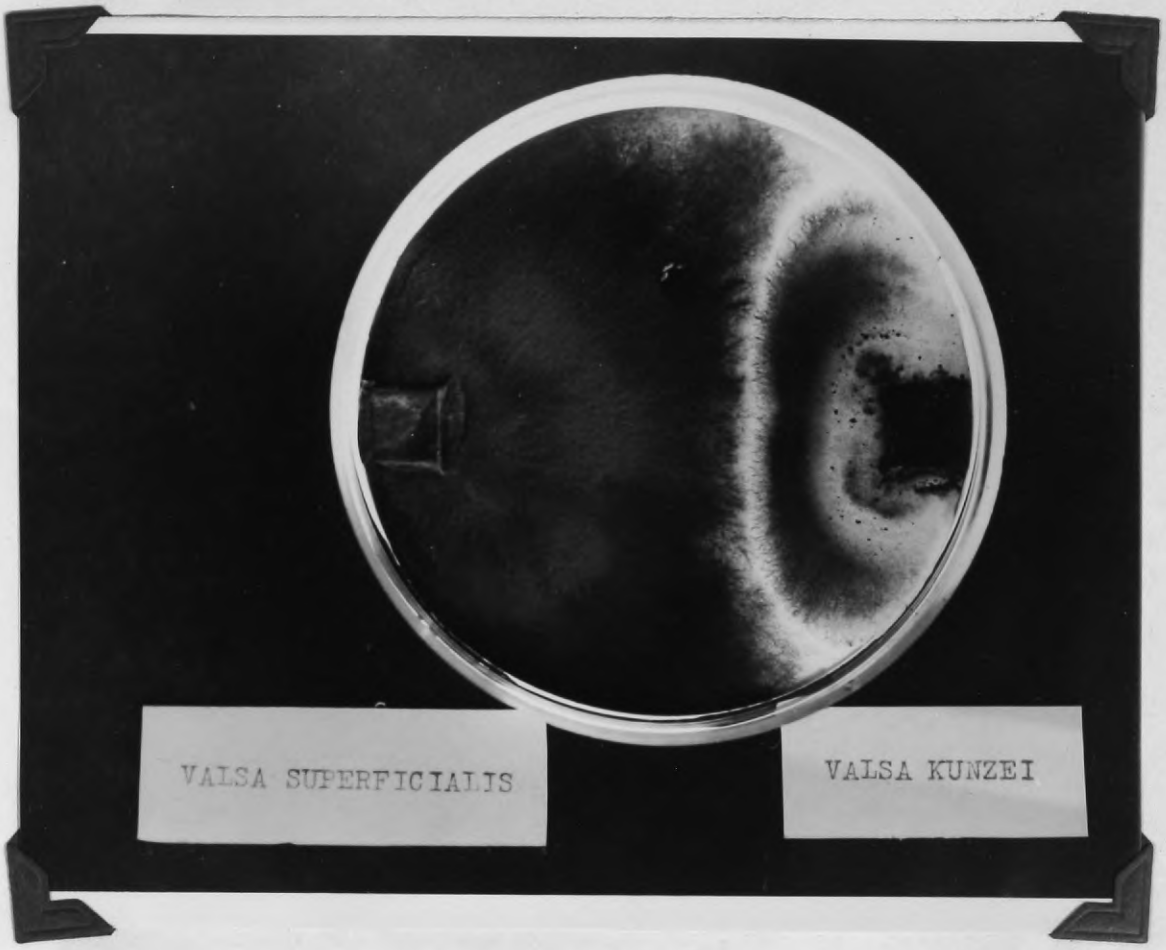
Pycnidia of V. superficialis in red pine twig culture.

PLATE IV



Pycnidia of V. Kunzei on Leonian agar.

PLATE V



Culture on Leonian agar showing antagonism between the two fungi and the difference in rate of growth

PLATE VI

Twig cultures of V. superficialis and V. Kunzei on balsam branches.

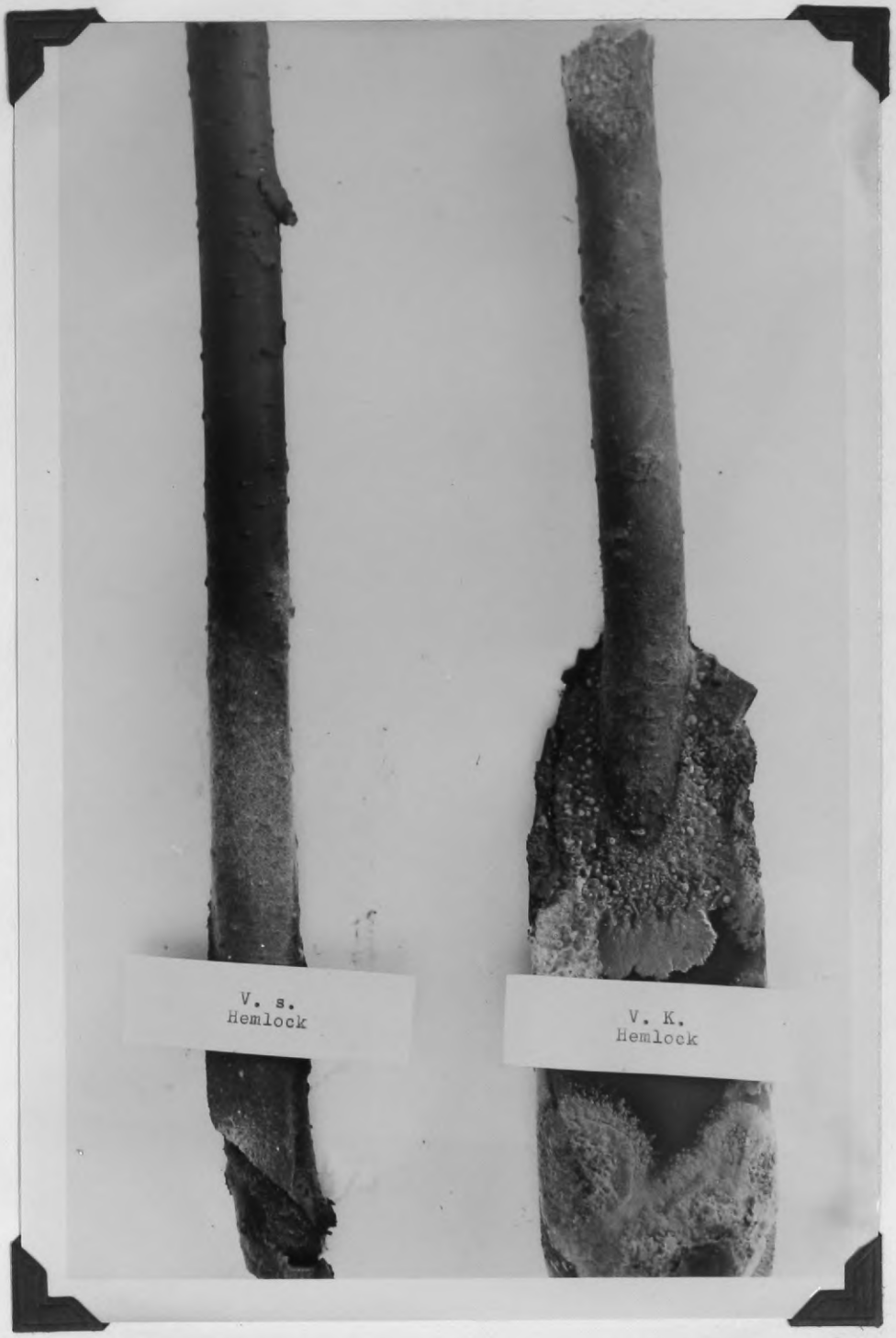
PLATE VII

Twig cultures on Douglas fir.

PLATE VIII

Twig cultures on northern white cedar.

PLATE IX



Twig cultures on hemlock.

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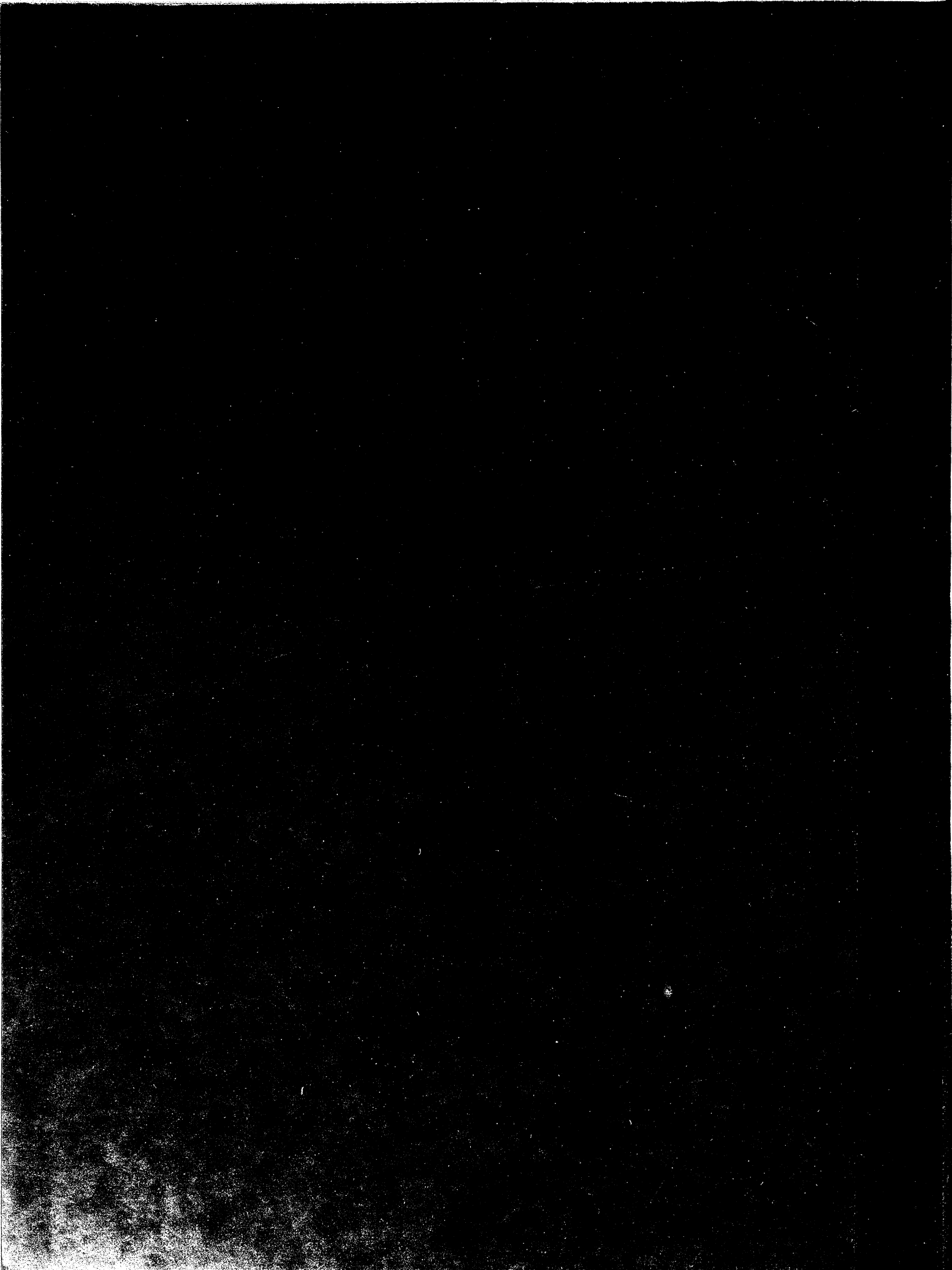
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