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Title: FACTORS AFFECTING DEVELOPMENT OF IRIS RHIZOME ROT CAUSED BY BOTRYTIS CONVOLUTA WHETZEL AND DRAYTON

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Abundant conidial and sclerotial production occurs on iris plants infected with Botrytis convoluta Whetzel and Drayton during the cool moist months of the year. Experiments were designed to study the survival and inoculum potential of conidia and sclerotia. Basic nutritional requirements of the fungus in culture were also studied.

Results of field and laboratory studies indicated a large percentage of iris plants apparently free of B. convoluta carried latent infections. The evidence indicates that these latent infections originate from contact of healthy tissue with senescent or dead leaf and/or rhizome tissues. Progression into healthy tissues is halted by increasingly higher soil and air temperatures and periderm formation which walls off the infections. Active rot development occurs when conditions favorable for pathogenesis return. These latent

infections which are undetectable visually would be a very important means of dissemination of the disease since growers believe they are shipping sound rhizomes. Chemical control would be difficult because the infections are inaccessible to non-systemic fungicides.

Field inoculation of rhizomes with conidia, sclerotia and rolled-oat cultures of B. convoluta resulted in significantly increased infection of iris plants inoculated with conidia. Sclerotia placed onto wounded areas of rhizomes also caused significantly increased infection incidence. Differences in field resistance to the pathogen were also noted. Iris plants of the variety Cotlet were found generally more prone to infection and more extensively rotted than Apricot Glory and Sunset Blaze varieties.

Laboratory results indicated that conidia and sclerotia were able to cause infection of intact iris rhizome offsets. Conidia applied to offsets colonized senescent leaves and, using this as a food base, invaded healthy juvenile leaves in contact with them. Germ tubes were observed lying parallel to and over longitudinal leaf epidermal cell wall junctures and in several instances, they appeared to be between cells. Terminal appressorium-like swellings of germ tubes were observed on leaf surfaces; however, no direct penetration of leaf tissue was observed.

Sclerotia remained viable for one year at 5 to 25 °C under dry storage conditions. Survival of conidia stored in situ on colonies for

257 days decreased with increasingly higher temperatures, dropping to less than five percent at 25°C after 210 days. Survival of sclerotia or conidia was not markedly affected by storage at -70°C for 90 days, and conidia remained at least 70 percent viable at -70°C after 257 days.

Sclerotia survived in moist soil for 60 days at temperatures of 15°C or below. No sclerotia were found viable after 60 days when soil temperatures were above 20°C. Non-viable sclerotia were found to be colonized by other fungi; mainly species of Trichoderma, Fusarium, Mucor, and Sepedonium as well as by bacteria.

Nutritional studies in vitro showed B. convoluta to utilize the carbohydrates maltose, glucose, sucrose, starch, galactose, and fructose, from good to poor carbon sources respectively. Lactose and sorbose were poor carbon sources. Nitrogen sources, from good to poor, were casein hydrolysate, asparagine, ammonium tartrate, ammonium sulfate, and glutamate. Potassium nitrate, glycine, and urea were poor nitrogen sources. Growth was most rapid when the pH of the media was pH 3-4. B. convoluta required light to be stimulated into sporulation. Sporulation was stimulated by exposure to white or to near-ultraviolet light. Optimum temperatures for linear growth was near 23°C and optimum for sclerotium formation was 10-15°C. Germination of conidia incubated in distilled water was generally very low (less than 10%) and germination was invariably much higher (85-100%) when an exogenous carbon source was supplied.

Factors Affecting Development of Iris Rhizome Rot Caused by *Botrytis convoluta* Whetzel and Drayton

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

FACTORS AFFECTING DEVELOPMENT OF IRIS RHIZOME ROT CAUSED BY *BOTRYTIS CONVOLUTA* WHETZEL AND DRAYTON

INTRODUCTION

Botrytis convoluta Whetzel and Drayton causes a rhizome rot of bearded irises in North America and Europe (Figure 1). The disease is economically important to commercial iris growers and an unknown amount of loss occurs in ornamental plantings.

The disease apparently originated in Europe on native bearded irises and was brought to North America on infected rhizomes prior to the mid-1920's when it was first reported from Canada. In the Pacific Northwest, the disease was reported to have become serious in Washington by 1931.

Taxonomically, the causal organism is separated from other species of *Botrytis* by the possession of a perfect stage, placing it in the Sclerotiniaceae, a family of inoperculate, discomycetous, sclerotium-forming Ascomycetes. The perfect stage of *Botrytis convoluta* has been produced in culture, but has not been observed in nature. *B. convoluta* differs from other *Botrytis* spp. in the type of decay produced. *B. convoluta* produces a characteristic firm, brown, odorless decay, quite unlike other *Botrytis* spp. which cause soft, watery rots. *B. convoluta* is restricted to rhizomatous irises and the disease is generally confined to the rhizomes.

Figure 1. Iris rhizome infected with Botrytis convoluta showing conidia (C) and typically convoluted sclerotia (S). Rhizome inoculated by placing sclerotia on freshly cut wound.

Although leaf bases and buds are often attacked, the organism is not considered a foliar, floral, or fruit pathogen as are most other species of Botrytis.

Since Whetzel and Drayton (1932) described Botrytis rhizome rot of iris, little information has been contributed to the disease cycle. Whetzel and Drayton indicated that B. convoluta required wounds for infection; however, Dosdell (1944) observed that rhizomes planted in fields heavily infested with B. convoluta became heavily infected and that rhizomes apparently free of the disease planted in soil with no previous history of iris cultivation often developed disease symptoms. Rhizome offsets were suspected by Dosdall to harbor latent infections. Jackson (1966) found that sclerotia required certain minimum exposures to light before germination would occur.

B. convoluta survives the cold months of the year in infected rhizomes, alleviating the need for resistant resting bodies to carry it over this period (Whetzel and Drayton, 1932). However, summer survival presents a quite different situation. Iris rhizomes escaping destruction by B. convoluta begin active growth in mid-spring and flower in late spring or early summer (May to June in Oregon). Not only are infected areas of rhizomes walled off by cork formation, but higher temperatures prohibit further growth of the fungus (MacWithey and Metcalf, 1962). No work has been done to the present to indicate how B. convoluta survives hot dry summer

months, until iris growth ceases in fall and dormancy begins. MacWithey (1967a) has shown that B. convoluta could colonize rhizome tissue at 5 to 15° C, but not at 20° C and above. It might be assumed then that summer survival would be dependent upon sclerotia or latent infections in living rhizomes. Survival of B. convoluta sclerotia in soil has not been established according to the literature. Powelson (1960) has shown that mycelium of Botrytis cinerea existed in senescent floral parts of ripening strawberry fruits as latent infections from which hyphae invaded fruit tissue when it became susceptible. Results of other investigators have shown that colonization of senescent tissue prior to infection of healthy unwounded tissue is a common occurrence for certain Botrytis spp.

The purpose of this study was to pursue areas of research on B. convoluta that have been neglected by previous investigators, such as (1) basic nutritional studies on the fungus in culture, (2) relative importance of the three types of inocula produced by B. convoluta (conidia, sclerotia, and colonized plant material), (3) to study the survival of conidia and sclerotia under laboratory conditions, and (4) to determine if host predisposition is necessary for successful infection and disease progression.

REVIEW OF LITERATURE

Botrytis convoluta Whetzel and Drayton causes a serious disease of bearded irises known as Botrytis rhizome rot, which appears to be widespread in North America. In Canada, it was first reported in Ontario in 1928 (Drayton, 1928) and later from British Columbia (Conners, 1939). In the United States the disease was intercepted by U. S. D. A. quarantine inspectors on iris rhizomes imported from France, Germany, England and Holland (Whetzel and Drayton, 1932). Additional reports have shown the disease to occur in New York, Washington (Whetzel and Drayton, 1932); Minnesota (Dosdall, 1935); New Jersey (Wood and Nance, 1938); Idaho (Dosdall, 1944); Missouri (Maneval, 1940); West Virginia (Leach and Elliott, 1965); California (Bald et al., 1959); and in Oregon (Oregon State University Plant Clinic records, 1958). Although it was first observed in North America on irises imported from Europe, the first report of its presence in Europe (Germany) was not until 1961 (Gerlach, 1961). Earlier reports of a serious Botrytis disease of iris in England was evidently not caused by B. convoluta because the descriptions of the disease and sclerotia produced by the fungus are more typical of Botrytis cinerea than of B. convoluta (Green and Wilson, 1938). The host range of B. convoluta is limited to rhizomatous species of the bearded irises. It has been reported on Iris germanica L.,

I. pallida Lam., I. variegata and closely related species as well as horticultural varieties which have been derived from the germanica group. B. convoluta has not been reported from bulbous iris species or from genera closely related to Iris (U. S. D. A. Agr. Handbook No. 165).

The degree to which commercial and non-commercial iris culture may be threatened by this disease is sometimes considerable. Dosdall reported that in 1934 Botrytis rhizome rot was so destructive in Minnesota iris plantings that continuous iris cultivation in at least three nurseries was threatened. In Oregon some nurseries have been severely limited in commercial export of rhizome stock to other states because of high incidence of rhizome rot (Schreiner, personal communication, 1965).

According to one estimate, approximately 80 acres are under iris cultivation in Oregon, with an annual crop value of $100,000-125,000. Most of these irises are exported to other states and abroad (Schreiner, personal communication, 1967); and according to Schreiner, the market for Oregon irises would presumably be much higher if it were not for Botrytis rhizome rot.

The symptoms of Botrytis rhizome rot are unique enough to be easily distinguished from those of other iris diseases. The following description is from Whetzel and Drayton (1932, p. 470), who first described Botrytis rhizome rot of iris as a new disease:

> Affected plants either fail to develop new leaves in the spring or a few shoots may appear which later turn yellow and finally die by midsummer. On the exposed portions of the rhizomes and at the bases of the leaf sheaths of the previous year's growth, in fact often involving the entire shoot, a dense short felt of dark grey to purplish-brown conidiophores and conidia of the Botrytis develop very early in the spring. On the surface of the rhizomes or breaking through the epidermis among the conidiophores and in the soil among the dead roots are agglomerations of characteristically convolute, shiny black sclerotia. The plant is easily removed from the soil because of the death and decay of the roots. The rhizomes are shrivelled and partially or completely decayed; the diseased flesh is grey-brown in color, essentially dry and pithy in texture, with distinct rifts in the disintegrating tissues. In partially affected rhizomes, distinct zones of decay may be noted, with a darker colored band sharply delimiting the diseased from the healthy tissue. No disagreeable odor accompanies this decay.

The disease is active in early spring, but may also occur in late fall. It is generally favored by cold, wet weather. Warm, dry weather appears to slow rot development and is favorable for rapid periderm formation around the lesion (Dosdall, 1944; MacWithey and Metcalf, 1962; Whetzel and Drayton, 1932). Appearance of the disease tends to be sporadic in fields and varies in severity from year to year, even seeming to disappear for one, two or several years (Dosdall, 1944). Loss due to this disease may be subtle. Often irises are said to be lost to so-called "winter kill", which is the failure of plants to emerge in spring, leaving no above-ground evidence of a fungal disease. In many cases "winter kill" or "winter injury" is thought to be a result of Botrytis rhizome rot (Dosdall,

1944; Guterman, 1935).

Whetzel and Drayton (1932) established that a new species of Botrytis, Botrytis convoluta, was the causal agent of the disease. Later Drayton (1937) related Botrytis convoluta to its perfect state Sclerotinia convoluta by inducing production of typical Sclerotinoid fruiting bodies, or apothecia, in culture from sclerotia of Botrytis convoluta. The final name change occurred when Whetzel (1945) established a new family of stromatic inoperculate Discomycetes in which he also erected the genus Botryotinia, based on Botryotinia convoluta. Some mycologists have reservations on this concept. Dennis (1956), for example, would like to treat Botryotinia as a subgenus of Sclerotinia on the basis that sclerotial differences used by Whetzel to separate the genera were not sufficiently distinct to warrant erection of Botryotinia.

Botryotinia as of 1963 (Hennebert and Groves) was comprised of 13 species, all of which have Botrytis imperfect states. Seven of these species have Botrytis of the cinerea type imperfect states; B. convoluta (Drayton) Whetzel; B. fuckeliana (DeBary) Whetzel (Botrytis cinerea imperfect state); B. pelargonii Rǿed; B. calthae Hennebert and Groves; B. ficariarum Hennebert and Groves; and B. ranunculi Hennebert and Groves. The other species are B. ricini (Godfrey) Whetzel, B. narcissicola (Gregory) Buchwald; B. globosa Buchwald (Botrytis globosa Raabe conidial state); B. squamosa

Viennot-Bourgin (Botrytis squamosa Walker imperfect state); B. allii Swada Yamamoto (Botrytis byssoidea Walker conidial state); and B. arachidis (Swada) Yamamoto.

Drayton (1937) induced the ascigerous stage to develop from sclerotial masses under carefully controlled cultural conditions, using microconidia for spermatization. The most consistent results were obtained by allowing the fungus to grow on autoclaved whole wheat at 14° C in the dark for 45 days. At the end of this time the sclerotia were placed in moist quartz sand at 0° C for three to four months, then stored at 5° C. When the apothecial initials were 2-3 mm long, the cultures were moved to a greenhouse and placed under cheese cloth, the temperature was maintained at 7° C during the night and below 15° C during the day. Apothecia usually matured within four weeks. Drayton (1937), Dosdall (1944), Moore (1949) and Jackson (1966), all of whom have worked extensively with this organism, and myself have not seen the apothecial state in nature. The importance of the apothecial stage to the fungus is obscure, and possibly vestigial.

In culture B. convoluta was found to grow readily, producing conidia, sclerotia and microconidia on such media as PDA, oatmeal agar, Czapek's agar, in liquid nutrient medium (sporulation only), on bean plugs, steamed whole wheat, and on steamed stems of various succulent plants (Whetzel and Drayton, 1932). Appressoria

were seen to develop on all solid media where hyphal tips came into contact with hard surfaces; they were said to be typical of Botrytis and Sclerotinia (Whetzel and Drayton, 1932). The ability of germ tubes from B. convoluta conidia to form appressoria on a surface has not been found mentioned in the literature.

Appressorial formation in B. convoluta has not been studied beyond this point, but in other species of Botrytis appressorium formation may be stimulated by physical contact and independently of the host (Brown and Harvey, 1927). Penetration peg formation was seen to follow and may have been the result of the same stimulus, but actual penetration was controlled by hardness of the substrate. The substrate used was a series of gelatin membranes of graded hardness. In like manner the thickness or hardness of leaf cuticle may prevent infection peg penetration, as illustrated by the resistance of certain plum varieties to Sclerotinia (Curtis, 1928; Valleau, 1915). Entry of Sclerotinia cineria was through stomata and lenticles rather than by direct penetration (Valleau, 1915). Other species of Botrytis differ in their ability to form appressoria. Hopkins (1921) noted that germ tubes of Botrytis tulipae penetrated directly through the tulip leaf epidermis without forming appressoria. Conidia of Botryotinia ricini (Sclerotinia ricini), on the other hand, produce appressoria before penetrating castor bean leaf cuticle (Godfrey, 1923). Conidia of Botrytis cinerea and Botrytis allii were shown by

Segall and Newhall (1960) to germinate on onion leaf surfaces, causing typical onion blast symptoms. They were unable, however, to find any histological indication that hyphae of either species actually penetrated the host cuticularly or stomatally, and apparently appressoria were not formed. Symptom development was ascribed to enzymes or toxins which were secreted from the germ tubes and diffused through the cuticle and killed underlying cells. Infection of host tissue by Sclerotinia trifoliorum took place by the formation of a pressure cushion which with enzymatic action ruptured the cuticle and allowed growth into epidermal cells (Prior and Owen, 1964). Alfalfa and clover are infected by growth of hyphae through stomata as well.

Some species of Botrytis are more or less obligate wound parasites and are commonly associated with diseases of dormant storage organs or mature plant material held in storage or in transit, such as flowers, fruit and vegetables. Infection courts are often provided by abrasion or wounding of plant surfaces. Quite often Botrytis may become established saprophytically on dead or senescent plant parts which serve as a food base for infection of healthy tissue. Powelson (1959) found hyphae of Botrytis cinerea to lie dormant in senescent petals, stamens and calyces of ripening strawberry fruit until conditions were favorable for infection of healthy fruit. Botrytis rot of the globe artichoke (Link et al., 1924),

Botrytis fruit rots of apple, cranberry, eggplant, pear, strawberry, Botrytis storage rots of squash and carrots (Heald and Dana, 1924) are but a few of the innumerable examples of Botrytis rots of stored food materials. Botrytis spp. also cause destructive diseases of many standing crops such as peony, onion, lettuce, gooseberry, rose, tulip (Heald and Dana, 1924); pear (Zeller, 1926); castor bean (Godfrey, 1923); strawberries (Stevens, 1916); begonia (Thompkins, 1950). The U.S.D.A. Plant Disease Handbook (1960) lists over 180 diseases caused by Botrytis cinerea alone in the United States.

Spore Production and Germination

According to Whetzel and Drayton's (1932) original description, pale-brown conidiophores arose in fascicles from groups of melanized thickwalled hyphae near the surface of the substratum or from sclerotia. Conidiophores varied in height but averaged approximately 1 mm tall. The main axis gave rise to short, lateral, divaricate branches, which in turn were usually dichotomously branched. The ultimate conidiophore tips were enlarged to form ampullae upon which numerous spiny sterigmata developed. The tips of the sterigmata swelled to form young conidia. Sterigmata were usually no longer in evidence after the conidia matured and fell away. Conidia were smooth and generally one-celled and usually

germinated by a single germ tube. Conidia of B. globosa may germinate by one to four germ tubes (Buchwald, 1953) while those of Botrytis gladiolorum may germinate by one and sometimes two germ tubes. The conidiophores with conidia of B. convoluta were characteristically of the Botrytis cinerea type. Size of conidia varied with culture media, averaging 9-11 × 7-9μ, those produced on diseased rhizomes being slightly larger than those produced on artificial media (Whetzel and Drayton, 1932). Microconidia of B. convoluta were produced on densely branched fascicles of conidiophores arising from hyphal cells or sclerotia. They have been shown to function as spermatia, to fertilize receptive ascogonial initials in sclerotia prior to ascocarp formation (Drayton, 1937). Optimum temperature for conidiophore and conidium development in culture was 20° C, and these structures varied in abundance with light and humidity (Drayton, 1937). Optimum temperature for conidial development of Botryotinia pelargonii was 10-12°C (Rǿed, 1949).

Drayton (1937) mentioned that light stimulated sporulation of B. convoluta in culture, even when the fungus was returned to dark incubation. Induction of cultures into a reproductive state by light may be due to inhibitory effects of light on vegetative growth. Page (1956) showed that specific intensities of light inhibited mycelial growth of Botryotinia squamosa in culture and that alternating light

and dark periods produced zonate colonies. Ridging and sporulation of Botrytis gladiolorum cultures were stimulated by blue and shorter wavelengths of light (Bjornsson, 1956), and sporulation of Botrytis gladiolorum increased as light intensity increased after seven hours exposure to white florescent light. Peiris (1947) noted that illumination of high intensity was required for sporulation of Botrytis gladiolorum in culture. No spores were produced in dark-grown cultures. Walker (1926) showed that humidity was a determining factor for sporulation of Botrytis allii, Botrytis byssoidea, and Botrytis squamosa. Botrytis allii appeared to sporulate readily at low relative humidites over the temperature range of 4 to 25°C in culture and on onion tissue. Botrytis byssoidea sporulated readily on onion tissue, but not in culture. Optimum relative humidites were high and optimum temperatures were between 13° and 27°C. Botrytis squamosa sporulated only under high relative humidities in the temperature range of 3° to 18°C in culture and on onion tissue.

There are numerous reports on factors which affect conidial germination of Botrytis species, some of which are apparently contradictory. For example, some have stated that conidia of Botrytis cinerea were able to germinate in pure water (Link et al., 1924) while others found that a stimulant such as an exogenous carbon source was required (Kosuge and Hewitt, 1964). Walker

(1926) observed that conidia of Botrytis allii, Botrytis byssoidea, and Botrytis squamosa were all able to germinate in "tap water" on glass slides within eight hours when incubated at 20-27° C for the first two species, and within four hours at 13.5 to 25.5° C for the latter species. Germination took place throughout the temperature range tested (3.5 to 27° C) within 24 hours for all three species. Brown (1922b) noted that a great deal of variation existed among Botrytis cinerea spores with regard to ability to germinate in pure water. He proposed that this was due to the age of the spore, the youngest being most able to germinate in water. Orellana and Thomas (1965) found that Botryotinia ricini conidia were unable to germinate in pure water, and speculated that this was due to the young age of the spores. They achieved 100 percent germination with a two percent sucrose solution. Orellana and Thomas also found that gallic acid present on pericarp tissue of susceptible castor bean varieities stimulated germination of B. ricini conidia. Kosuge and Hewitt (1964) also observed confusing results in their germination experiments with Botrytis cinerea, but could find no correlation between age and germinability with conidia from five days to six weeks old. Germination was stimulated by exudates from grapes, which contained mostly sugars. Amino acids did not appear to affect germination. Gilles (1959) observed that conidia from an isolate of Botrytis cinerea from strawberry

germinated 90-100 percent in sterile water at 15 and 20° C. Conidia in strawberry juice, however, germinated much quicker and over a broader temperature range than in sterile water. He found a difference in spore germinability and length of time required for germination due to spore age and spore density. In sterile water and in strawberry juice, 12-day old spores germinated more rapidly than six or three-day old spores.

Brown (1922b) and Gilles (1959) observed that Botrytis cinerea spores were better able to germinate in pure water at low spore densities than at high densities. However, in strawberry juice, spores at even the highest densities germinated 100 percent within seven and one-half hours (Gilles, 1959). Last (1960) observed a sharp decrease in ability of Botrytis fabae spores to germinate after they were over 40 days old. Addition of carbon sources appeared to increase germinability of old spores. Ability of spores to germinate and penetrate broad bean leaves also fell rapidly with sporeage, from 100 percent penetration at ten days old to less than ten percent at 25 days old. Infectivity increased rapidly with old spores when a carbon source was added to the inoculum suspensions.

The effect of increased carbon dioxide concentration on fungi has been well documented. Brown (1922a) observed that germination of Botrytis spores in water fell off rapidly as CO_2 concentration of the incubation environment was increased from atmospheric to ten

percent and 20 percent CO_2. No germination occurred at 30 percent CO_2 or higher. However, 50 percent CO_2 was required to prevent germination in a nutrient solution. The germination process was much more tolerant of high O_2 than of high CO_2 partial pressures. It is possible that the effect of high CO_2 concentrations was due to increasing the acidity of the incubation medium. Webb (1921) showed that Botrytis cinerea spores could germinate 40-60 percent and grow in media ranging in pH from 2.5 to 6.7. The natural buffering action of Brown's nutrient medium could account for the higher CO_2 concentration required to inhibit germination in the nutrient medium than in water.

Survival of Botrytis spp. spores has been somewhat neglected in the literature. Walker (1926) indicated that conidia of the Botrytis neck rot of onion organism (Botryotinia allii) were able to remain viable after being exposed to Madison, Wisconsin winter weather while in situ on onions. Spores in pure cultures subjected to the same conditions also remained viable. Walker suggested that B. allii may exist saprophytically on organic matter between growing seasons and that spores arising from saprophytically colonized debris constitute the primary inoculum for the following year. He did not state specifically if spores, mycelium or sclerotia actually over-wintered in the onion fields. Powelson (1959) found that Botrytis cinerea sporulated on mummified strawberries and on

other plant debris throught Pacific Northwest winter months, producing abundant inoculum. It seems possible that conidia of Botryotinia convoluta produced on decaying iris plant parts may be a source of primary inoculum for the following growing season.

Conidial germination studies have not been carried out with Botryotinia convoluta previous to this study. No indications have been given in the literature as to the ability of B. convoluta conidia to germinate on living or dead plant parts. As indicated by Whetzel and Drayton (1932) and others who have observed this disease in the field, copious conidia are produced on above ground portions of iris plants. No reports were found to indicate that conidia could infect a healthy plant in the field. Previous experimentors used such undefined inocula as "naturally infested soil" (Dosdall, 1944); wheat-grown cultures for laboratory studies (Whetzel and Drayton, 1932) and field inoculations (MacWithey, 1967a); and agar-grown inoculum for field inoculations (MacWithey, 1967b).

Niethammer and Baessler (1954) have shown that spores of Botrytis spp. may remain viable for 12 to 14 months on carrot slices. Their germinability probably suffered, however, as indicated by the work of Last (1960). He found that germinability of Botrytis fabae conidia decreased with age, from 90 percent germination at 40 days old to less than 20 percent at 65 days. As one might anticipate, Botrytis cinerea spores were unable to survive five years

storage in dry soil (Atkinson, 1954).

Temperature and relative humidity may have a great effect on survival of conidia in storage. Bagga (1967) found that Botrytis cinerea survived better at refrigerator temperatures under low relative humidities (0 to 25 percent) than at high (50 to 75 percent) relative humidities. The effect of temperature on variability of Botrytis cinerea spores has been studied by Smith (1923) who found that thermal inactivation of conidia over a period of time resulted in a non-linear response. This departure from a constant death rate presumably arose from the multi-nuclear nature of the spore, in that a time lag is required to kill enough nuclei to inactivate the entire spore. In this respect, Botrytis cinerea spores may be more comparable to multicellular propagules than to single-celled uni-nucleate spores. The multinucleate nature of Botrytis cinerea spores was shown by Barnes (1930) and Hansen (1938) to be a source of a great amount of variation between single spored colonies which arose originally from the same monosporous culture. The "dual phenomenon" is not the result of genetic mutation, although often expressed by cultural variation, but is a reflection of the hetero-karyotic nature of the fungus and of the existence of natural strains of the fungus. The effects of this phenomenon should be considered before using only single spore isolates of Botrytis spp. in pathological studies.

Sclerotia

Sclerotia are resistant bodies which generally function to carry a fungus through periods of unfavorable environment which may be limiting factors for the survival of these fungi. The interior cells of a sclerotium are pseudoparenchymatous and filled with stored food. The outer cells are thick-walled, almost sclerenchymatous, and usually dark in color. The production of sclerotia of plant pathogenic fungi at least, seems to be induced by nutrient or environmental conditions suboptimal for growth. Townsend (1957), for example, found that although sclerotium initiation depended upon sufficiently good nutrition, maturation occurred only after mycelial growth ceased, due to either nutrient exhaustion or from other causes such as antagonism from other microbes (Townsend, 1957; Sanford, 1956).

Sclerotia of Botrytis convoluta are produced abundantly on infected and decaying iris rhizomes in late fall and early spring (Drayton and Whetzel, 1932; Dosdall, 1944). As far as this writer knows, no field studies have been done to determine the ability of these sclerotia to overwinter naturally or to produce primary inoculum for infection of healthy plants. B. convoluta sclerotia apparently do not produce the sexual or apothecial stage under natural conditions. B. convoluta sclerotia commonly germinate by

producing tufts of conidiophores bearing copious conidia. Microconidia are also produced from sclerotia in culture and these have been shown to be necessary for spermatization of receptive hyphae in the sclerotia, but do not germinate and form hyphae themselves (Drayton, 1937). Germination of sclerotia by apothecium formation in nature has been noted for some species of Botryotinia other than Botryotinia convoluta. Buchwald (1953) observed the perfect stage of Botrytis globosa among decayed remnants of the host plant, Allium ursinum, in Denmark. Naturally produced ascospores were able to infect the host to complete the disease cycle. Apparently this species is homothallic and self-fertile (Buchwald, 1953). B. convoluta is heterothallic and self-infertile (Drayton, 1937) and this may explain in part why its perfect stage is rare in nature.

According to Garrett (1960) sclerotia of flower- and foliage-infecting fungi are generally larger than those of root-infecting fungi. He suggested that the major function of the latter type is to germinate by producing new infective vegetative mycelium, whereas the sclerotia of the former generally germinate to produce apothecia whose ascospores initiate new infections. It is likely that Garrett would have also categorized sclerotia which germinate by production of conidiophores in the foliar- and flower-infecting group since he cited species of Botrytis and Sclerotinia as examples of this group. Accordingly, B. convoluta might be expected to fall in the foliar- and

flower-infecting group of sclerotial fungi.

Park (1965) concurred with Garrett's (1960) theory on the relationship of size and function of sclerotia produced by air-borne and soil-borne groups of plant pathogenic fungi; however, he added that "such bulky structures might be expected to have survival value in soil." Few investigators have attempted to demonstrate quantitatively the importance of Botrytis spp. sclerotia as resistant bodies which may carry the fungus through periods of unfavorable environmental conditions. Pethybridge et al. (1921) felt certain that sclerotia produced on flax stems infected with Botrytis sp. overwintered in soil and produced inoculum for the following year. Green and Wilson (1938) stated that sclerotia of the Botrytis sp. causing a "grey mould basal rot" of iris overwinter in the soil and infect young plants the following growing season. The Botrytis involved was probably Botrytis cinerea rather than B. convoluta, judging from the description of the sclerotia. Similar reports have been made for sclerotia of Botryotinia porri and Botrytis allii, both onion pathogens, and of Botrytis cinerea, which causes gray mold diseases of various vegetable crops (Chupp and Sherf, 1960) in which the sclerotia of these fungi may overwinter in fields and produce inoculum the following year but not in the form of ascospores.

Quantitative and qualitative studies by Jackson (1966) on the

germination and survival of sclerotia of B. convoluta indicate that sclerotia required certain minimum light exposures in order to germinate consistently and profusely. Optimum conditions for light activation were at 5° C. The sclerotia had to be in a moist state in order to be activated. While only four days of light treatments were necessary to achieve 100 percent germination, the quality of germination, i.e.: number of conidiophores produced, was poor. Conidiophore production was most profuse after 14 days of light treatment. Jackson's data indicated that the prime functions of the sclerotia would be to carry the fungus through periods of adverse conditions and to produce primary air-borne inoculum for spring and fall infections, rather than to act as primary infective units themselves. His thesis is strengthened by the low germinability of sclerotia which had not been exposed to light; sclerotia apparently did not germinate when in contact with iris roots; and the sclerotia could regerminate up to four times. Apparently, then, the sclerotia have to be exposed at the soil surface during periods of cool, wet weather before they may be light activated. After the sclerotia are light activated, they may germinate by conidiophore production.

In vitro production of sclerotia by Botrytis spp. seems to be influenced by nutrition as much as by any other factor. According to Peiris (1947), sclerotial production in culture by Botrytis cinerea is favored by a relatively high carbon/nitrogen ration, such as a

relatively high initial concentration of glucose and low concentration of peptone. Townsend (1957), however, differentiating between initiation and maturation, observed that initiation of sclerotia depends upon sufficient carbon and nitrogen sources, but maturation depends upon an accumulation of factors which cause cessation of mycelial growth. She found generally that sclerotia were produced in most abundance on media which promoted maximum mycelial growth. Sclerotium production by Botrytis cinerea and Botrytis allii was inhibited on media with extremely high or low carbon/nitrogen ratios. The best carbon sources for mycelial growth and sclerotium produciton of Botrytis cinerea, Botrytis allii, and Sclerotinia gladioli in a basal synthetic medium were glucose, fructose, sucrose, maltose, lactose and starch (1 percent carbon basis). Nitrogen sources producing abundant growth and sclerotia were peptone, asparagine and potassium nitrate.

Sclerotium production seems to be favored by certain temperature and light conditions also. Optimum temperature for sclerotium production by Botrytis cinerea and Botrytis allii was near 20° C, but production was inhibited at 25° C. Sclerotinia gladioli produced sclerotia best at 25° C. Røed (1949) found that sclerotium production by Botryotinia pelargonii on PDA and malt agar media was most profuse at 10-12° C, less so at 20° C, and none at 26° C. Light may also affect sclerotium production. According to Bjornsson (1956),

Botrytis gladiolorum sclerotia were stimulated to form in cultures receiving four to seven days of darkness prior to an exposure of less than 30 minutes of white florescent light. Red light apparently favored sclerotium production, whereas blue and shorter wavelengths did not. It is difficult to perceive how light could directly affect the production of sclerotia by Botryotinia convoluta when they are usually produced well beneath the soil surface.

Varietal Differences of Resistance and Susceptibility of Irises

As stated previously, Botrytis rhizome rot of iris is restricted to bearded rhizomatous irises, especially those varieties originating from Iris pallida, I. variegata, and I. germanica. There is some variation in field resistance and susceptibility to this disease among these iris varieties (Dosdall, 1944; MacWithey and Metcalf, 1962). According to MacWithey and Metcalf (1962) the varieties El Mohr, Violet Mohr, And Thou, Orchid Mist, Amigo, Celestar, and Fair Elaine were among the most susceptible of 46 varieties tested, while Timber Line, Crystal Cap, Sea Lark, Blue Valley, Mary Vernon, Moonlight Midnight, and Tom Thumb were among the more resistant varieties. Evaluation of the degree of resistance or susceptibility, in this case, was not based on field inoculation studies, but on dormant rhizomes inoculated and stored at 15°C. Dosdall

(1944) observed in her field studies that field resistance in the varieties Miss Maggie, Monsignor, Shakespeare, Edith, and Columbia was from low to high, respectively. Her work was primarily concerned with fungicidal control of the disease, however. Evidently no work has been done on the possibility of breeding for resistance to control Botrytis rhizome rot of iris.

Infection and Colonization

According to MacWithey and Metcalf (1962) and MacWithey (1967a), colonization of wounded rhizome tissue in steamed soil, raw soil, or vermiculite occurred between 5° and 15° C. Colonization did not take place above this range. Dormant rhizomes, however, were infected at temperatures from 5° to 25° C, with the optimum at 20° C. It was found that actively growing rhizomes could quickly wall off wounds by suberization at 15° to 25° C, while 30 days was required for complete suberization at 10° C. In steamed and unsteamed soils infested with B. convoluta grown on autoclaved wheat kernels, MacWithey (1967a) found that colonization of rhizome slices by B. convoluta was maximum (48 percent) at 5° C, and decreasing at higher temperatures to eight percent at 15° C. Using a soil immersion tube technique, recovery of B. convoluta was highest from the steamed soil. Percentage recovery increased gradually from 68 percent at 5° C to 88 percent at 20° C, but fell abruptly to less than

25 percent at 25°C. Recovery in unsteamed soil was greatest at 5°C (48 percent) then decreased to 16 percent at 15°C and zero at 20°C. In cases where recovery of B. convoluta from infested soil was high, recovery of other microbes was relatively low and vice versa. Other organisms consistently picked up by the soil immersion tube technique were bacteria, mucoraceous fungi, and a species of Fusarium. The mucoraceous fungi were very numerous in steamed soil and rare in unsteamed soil, while Fusarium was most prevalent in unsteamed soil and rare in steamed soil. MacWithey suggested that the low competitive saprophytic ability of B. convoluta in soil is important in limiting successful colonization of rhizome wound tissue. He also felt that efforts to stimulate the antagonistic microflora might be made by adding crop residue or other organic amendments to B. convoluta infested soil in order to reduce infection of irises by the fungus (Mac Withey, 1967a and personal communication).

Another form of antagonism is in the ability of certain fungi to parasitize sclerotia. Sclerotia of Sclerotinia sclerotiorum, S. trifoliorum, S. borealis, and Botrytis cinerea were found to be parasitized by the following species: Acrostalagmus roseus, Gliocladium roseum, Trichoderma viride, Fusarium sp., Verticillium sp., Fusarium avenaceum, Hormodendrum sp., Mucor hiemalis, Mucor spinosus; Penicillium frequentans, P. lilacinum, Penicillium sp., and Trichothecium roseum (Karhuvaars,

1960; Makkonen and Pohjakallio, 1960). Exactly how important parasitism of sclerotia is to the overall survival of the fungi is not known at this time. Other organisms take their toll of sclerotia; according to R. S. Jackson (1966) mites and nematodes were found to be common predators of B. convoluta sclerotia.

As suggested by MacWithey (1967a), organic soil amendments may stimulate fungi and other organisms which would be antagonistic to B. convoluta, inhibiting saprophytic colonization of plant debris and infection of iris wound tissue. Certain organic soil amendments could also stimulate fungi which parasitize sclerotia of Botrytis, Sclerotinia, and Botryotinia, thus affecting adversely the survival of these sclerotium-producing fungi in the soil.

Control

Measures prescribed for controlling the Botrytis rhizome rot have been chiefly chemical. However, other means of control, while not currently in practice, are at least theoretically possible; these are breeding for varietal resistance, crop rotation and organic amendment of the soil. MacWithey and Metcalf (1962) and Dosdall (1944) have noted that varietal resistance does exist and this gene pool could potentially be the foundation for commercially suitable resistant lines. Demand for certain varieties, however, is often limited and even the most popular varieties may lose their popularity

after 10 to 15 years; therefore, it is likely that efforts to combine resistance with beauty would be more restrictive than beneficial to commercial iris breeders. Also, losses due to B. convoluta are usually low enough that control of this disease by breeding resistant varieties is somewhat impractical.

Crop rotation may provide a means of disease control to commercial iris growers, but would not be adaptable to home garden and landscape conditions. Effective disease control through crop rotation depends on the ability of the pathogen to survive as a saprophyte on the organic material and on the longevity of resistant resting bodies of the fungus. According to MacWithey (1967a), B. convoluta is poorly adapted for competitive saprophytic survival in soil. If certain groups of soil microflora inhibit colonization of iris wound tissue as MacWithey suggests, it may be possible that crop rotation and concomitant organic amendment of the soil would decrease the severity of the disease under certain conditions although this has not been tested in field studies. Long-term survival in absence of host plants (iris) would necessitate that the sclerotia be long lived in the soil. Data has not been found in the literature concerning the longevity of B. convoluta in soil, except MacWithey (in Jackson, 1966, pg. 5) stated the sclerotia could persist in soil for as long as eight years. No qualifications on this accomplishment attended the statement, however, so it is possible that this survival period

existed under controlled experimental conditions rather than under field conditions.

Effective chemical control methods for this disease are lacking at present. Dosdall (1944) found that certain mercurials showed promise when used as preplant rhizome dip treatments. However, when treated rhizomes were planted in heavily infested soil, the effectiveness of these treatments was nullified. Later, MacWithey (1967b) found that the mercurials Ceresan (ethylmercury chloride) and Semesan (2-chloro-4-(hydroxymercuri)phenol) significantly reduced losses from the rhizome rot in infested soil. The chemicals prevented infection of wound tissue on rhizomes, however, they were not effective in eradicating existing infections in rhizomes. It is also possible the fungus may be able to pass from parent rhizome to new offsets in a perennial fashion without new infections occurring. Obviously, a fungicide whose action is limited to the rhizome surface would be incapable of complete control of the disease. In this respect, some of the new systemic fungicides currently being developed may prove effective in limiting the advance of the fungus in infected rhizomes. At present, then, the best control measure available is to plant healthy rhizomes in soil free from B. convoluta, which is not practical for most commercial iris growers. In other cases, certain mercurial rhizome dips balanced with crop rotation may give limited control (Dosdall, 1944).

METHODS AND MATERIALS

Origin of Botrytis convoluta Isolates

Isolates were obtained from iris rhizomes infected with Botrytis convoluta from Schreiner's Iris Nursery, Salem, Oregon in February 1965. An additional isolate was obtained from infected irises grown at Brown's Sunnyhill Gardens, Walla Walla, Washington. Unless otherwise stated, the Salem, Oregon isolate was used throughout this thesis. B. convoluta was readily isolated from portions of diseased rhizomes, plated onto and maintained on potato-dextrose-agar (PDA) in pertri plates. B. convoluta was kept in continuous culture during this study by periodic mass transfer of mycelium and spores to fresh PDA media.

Media

Media most generally used in this study were PDA and Streptomycin-PDA (100 ppm Streptomycin sulfate). PDA was formulated from 20g. dextrose plus 17g. agar added to an effusion from 200g. peeled potatoes and enough distilled water to bring the volume to one liter. The media were reautoclaved and streptomycin sulfate added after sterilization. A basal synthetic medium (BSM) was employed, which was used in both liquid and solid forms (Lilly and

Barnett, pg. 20, 1951). BSM had the advantage of a defined medium in which the carbon and nitrogen sources could be substituted for comparative purposes. The BSM was sterilized by filtration through a sterile Millipore filter apparatus containing a filter with a pore size of 0.45μ and delivered automatically into sterile culture vessels. A rolled-oat medium was used for growing mass inoculum. It consisted of two parts distilled water to one part rolled-oats. The mixture was autoclaved in petri plates and in metal containers for 45 minutes, allowed to cool, and resterilized.

Nutritional Studies

In order to determine basic requirements for growth and sporulation of B. convoluta in culture, various carbon sources, nitrogen sources, initial pH, light, and temperature factors were examined.

Liquid media for culture studies were contained in 125 ml. Erlenmeyer flasks stoppered with cotton plugs. Shake cultures were incubated on a platform shaker adjusted to slow agitation. Illumination was supplied by four 40 watt Ken-Rad cool-white fluorescent lights suspended 60 cm. over cultures in liquid or on solid media. In one experiment, cultures were illuminated by short exposures to low intensity red light (15 w. photographic safe-light) when certain linear growth measurements were taken. Near ultraviolet (NUV) fluorescent lighting was also used, supplied by four General Electric 40w.

"Black Light" lamps (320-420mμ, approximately) suspended 60 cm. over the cultures. Cultures were grown at room temperature (24 ± 2°C. Cultures grown on liquid media were harvested by filtration of the culture through preweighed Whatman glass filter disks (GF/A, 7cm. diam.). The filtered cultures were oven dried at 100°C for 24 hours, cooled in a desiccator and weighed to obtain dry weight increments of growth. Culture filtrates were saved for pH readings which were made on a Beckman Zeromatic pH meter. Linear growth measurements of two colony diameters at right angles were taken on the underside of the colonies in petri plates, and averaging these, radial growth increments were computed.

Mass inoculum, rather than single spore inoculum, was generally used. Three millimeter diameter plugs were cut with a sterile cork-borer from peripheries of young non-sporulating (dark-grown) colonies. Three or four replications were made for each harvest of cultures in liquid media, and three to five replications of agar-grown cultures. Five to eight harvests were made, from zero time to peak growth and generally one during autolysis.

Carbon Sources

Various carbon sources used were c.p. grades of glucose, sorbose, fructose, galactose, sucrose, lactose, maltose, and soluble starch supplied in liquid and solid BSM on an equal weight

basis. A malt extract-yeast extract medium (Difco Bacto agar media) and a no-carbon medium were used as controls.

Nitrogen Sources

Nitrogen sources used were c.p. grades of glycine, ammonium tartrate, urea, ammonium sulfate, potassium nitrate, asparagine, glutamine and casein hydrolysate in BSM incorporated into silica gel. Glucose was supplied as the carbon source. Nitrogen sources were supplied on an equivalent nitrogen basis. Linear growth measurements were taken after seven days growth.

Hydrogen Ion Concentration

Effect of initial pH growth of B. convoluta in glucose-asparagine BSM was examined. Initial pH values were adjusted to 3, 4, 6 and 8 with 0.1N HCL and 0.1N NaOH.

Temperature

PDA petri plate cultures were placed in controlled temperature boxes held at six temperatures described previously. These experiments were confined to determinations of rate of growth on solid media.

Light

Effects of light on growth and sporulation were studied by growing B. convoluta cultures under continuous white and NUV light, 12-hour periods of white and NUV florescent regimes, short exposures to red-light and continuous darkness. Cultures were grown on malt extract-yeast extract and PDA media in petri plates.

Inoculum Preparation

Three types of inoculum were used in this study: conidia, sclerotia, and colonized rolled oats. Conidia were produced by growing B. convoluta cultures on PDA for five to seven days at 24° C in the dark and then exposing them to continuous white florescent light for five to six days or until the colony surface was covered with a dense felt of conidiophores bearing conidia. The conidia were washed off the mycelial mat with sterile distilled water plus Tween 20 (one drop per liter of water) and filtered through several layers of cheese cloth. The conidial suspension was diluted with distilled water to give approximately 50,000 spores/ml for most inoculations in the laboratory and 350,000/ml for field inoculations. Field inoculations with conidia were made by dipping either the leaves only or the entire plant into the conidial suspension. Excess water was allowed to drain before the plants were planted. In

the laboratory, iris leaves were inoculated by spraying them with a conidial suspension.

B. convoluta grown on rolled oats in petri plates at 24 °C in the dark for one to one and one-half months produced abundant sclerotia. Petri plates were emptied into a large container where the rolled oats and mycelium conglomerate was broken into smaller pieces by hand. Sclerotia were removed from the substrate and sorted to size by passing through a series of soil screens. Sclerotia which passed through eight mesh/inch but not 14 mesh/inch were used in experiments on longevity and for inoculation studies in the field and the laboratory. Sclerotia were stored in plastic capped test tubes at various temperatures (-70, -15, 5, 10, 15, 20, 25, and 30 °C) and sampled periodically for viability. When sampled, 20 to 80 sclerotia were surface sterilized in 20 percent clorox + detergent for two minutes with agitation. Sclerotia were then transferred aseptically to PDA plates and incubated at 20 °C for two to three days until sufficient visible hyphae had grown to determine viability.

Large quantities of sclerotia-free B. convoluta mycelium for field inoculations were raised on rolled oats. The rolled oats was mixed 1:2 with water in metal quart cans and sterilized in autoclave. After cooling, the oat mixture was aseptically transferred to eight sterile plastic crisper boxes (9 × 12 × 4 inches). Inoculation of the oat medium was done by breaking up and distributing one rolled oat

petri plate culture of B. convoluta into each box. The box cultures were incubated at 22-24° C for only seven days so that sclerotia would not form.

Germination of Conidia

Initial germination studies were carried out to determine whether B. convoluta conidia required an exogenous carbon source for germination. Spores were collected by placing plugs taken from sporulating agar cultures into 15 cm test tubes containing sterile distilled water plus Tween 20 and agitated for 30 seconds on a Vortex Jr. mixer and diluted to approximately 50,000 spores/ml final concentration. Tween 20 (Carolina Biological Supply House, Burlington, N.C.) was added to the distilled water to aid dispersal of spores in solution. Spore suspensions were placed in 250 ml flasks for incubation at 20° C for 12, 24 and 48 hours. After incubation, spore suspensions were agitated and small samples pipetted onto slides and stained with lactophenol plus Aniline blue for microscopical examination. Spores were considered germinated when their germ tubes were at least as long as the width of the spore. Carbon sources tested were sucrose, lactose, dextrose, ethanol. Germination was also determined on PDA, SPDA and water agar in petri plates.

Cultures producing conidia on PDA plates under white florescent light were placed in sealed plastic bags and stored in

controlled temperature boxes to test the longevity of conidia in culture at various temperatures from -70° C to 30° C. These cultures were sampled periodically by removing a 10-12 mm plug from each colony with a cork borer, washing the spores off with sterile distilled water plus Tween 20 and plating onto PDA plates which were incubated at 20° C for 12 to 24 hours. The staining solution was deposited directly onto spores on the PDA plates for direct microscopical examination for germination. This assay was carried out on PDA plates because in previous experiments conidia germinated as well or better on PDA than on any other medium tested.

Conidial suspensions were sprayed with an atomizer onto detached iris leaves without addition of exogenous nutrients. Inoculated leaves were placed in moist chambers at 20° C for 24 hours incubation. The material was prepared for microscopical examination by stripping the cuticle and epidermal cells off the leaf and placing the strips in a dilute lacto-phenol plus Aniline blue stain on a microscope slide for observation of germination and possible penetration.

Laboratory Inoculation Experiments

In order to evaluate the effects of wounding and wound healing, various inoculation procedures were carried out with rhizome tissue and whole offsets in the laboratory.

Fresh rhizome disks (Sunset Blaze) 11 mm in diameter and 0.2 to 0.5 mm thick were sprayed with spore suspensions and incubated in moist chambers at 5, 10, 15, 20, 25 and 30° C for 12 to 24 hours to test the ability of spores to germinate on freshly cut rhizome tissue.

Rhizome offsets (Cotlet) were wounded by spot freezing a portion of the knee region with dry ice (-56.6° C) for 1-2 minutes and allowing that portion to thaw. Freezing in this manner killed a hemispherical mass of tissue about two millimeters deep. Offsets were inoculated with conidial suspensions and planted in plastic crisper boxes filled with field soil moistened to 50 percent FMHC. All offsets were surface sterilized in 15 percent clorox for five minutes prior to use. Soil was screened through window screening before moistening. Spore suspensions were pipetted onto wounded and unwounded offsets.

Inoculated rhizomes were incubated at several temperatures, viz., 1° C, 3° C, room temperature (21-24° C) and in a garage (referred to here as "outside") which varied from a mean daily maximum of 9.3° C to a mean daily minimum of 5.8° C and an average daily temperature of 7.5° C between October 25, 1967 and January 10, 1968.

For the sake of convenience, and lack of a better nomenclatorial system, I gave descriptive names to various distinct

morphological features of a normal rhizome, Figure 2. In brief, these are: Apical = apical bud or flowering stalk initial; top = dorsal surface; knee = area between top and toe; toe = portion where the offset was connected to the mother; sole = ventral surface where roots emerge; and heel = portion between sole and apical.

Rhizomes wounded by cutting a small portion of the knee area were placed at 25 °C for 54 and 100 hours to initiate and promote wound healing prior to inoculation. These rhizomes were inoculated with either sclerotia or mycelial plugs removed from a young PDA colony with a 3 mm diameter cork borer. One plug was placed on the wounded area of each rhizome. Two to three sclerotia were fixed into position onto rhizomes with cellophane tape. The tape evidently had no effect on germination of sclerotia or infection of rhizomes. The inoculated offsets were planted in soil moistened to 50 percent FMHC and incubated at 3 °C in plastic crisper boxes covered with polyethylene bags which were punctured to improve aeration.

Field Plot Procedure

Iris plants used in this study were donated by Schreiner's Nursery, Salem, Oregon. Three varieties, Sunset Blaze, Cotlet, and Apricot Glory were used in the various inoculation experiments in the field. All field experiments were conducted at the Oregon

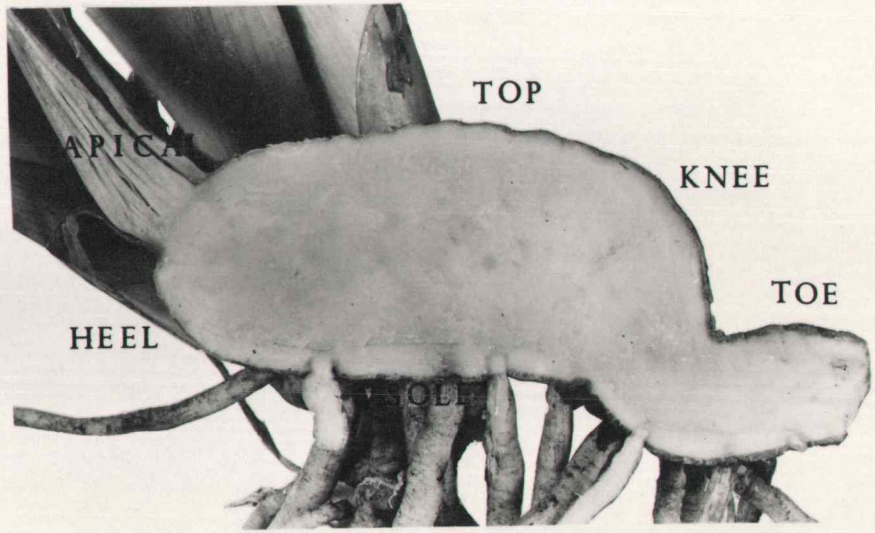

Figure 2. Longitudinal section of healthy iris rhizome labeled to illustrate external morphological features.

State University Botany and Plant Pathology Field Laboratory east of Corvallis. The soil in the area is a Chehalis silty clay loam with a "field moisture holding capacity" (FMHC) of 30 percent as determined from a repacked soil column. FMHC is defined as the amount of water a soil will hold after all gravatational water has drained away following saturation (Truog and Engelbert, 1956).

Rhizomes were surface sterilized in 15 percent clorox for five minutes before using. One series of iris plants was inoculated with conidia by either dipping entire plants or only the leaves into a conidial suspension (Ca. 350,000 spores/ml), allowing excess water to drain before planting. Another series of plants was inoculated by placing ten sclerotia (8-14 mesh size) onto partially buried wounded and unwounded rhizomes before covering completely with soil. Rhizomes were wounded by cutting off thin sections near the knee area with a potato peeler. A third series of plants was inoculated with colonized rolled oats (approximately one tablespoon per rhizome) placed against wounded and unwounded rhizomes before covering with soil. Care was taken to apply the sclerotial and colonized rolled-oat inocula to the upper surfaces of the rhizomes to avoid confusion of latent infections in the toe and sole regions with those caused by inoculations.

Control plots of uninoculated, unwounded and wounded rhizomes of each variety were included. Wounds were of two types, each of

which is commonly the result of removing offsets from old mother rhizomes for propagation purposes. Toe ends were cut off cleanly with a knife or were broken off ~~or were broken off~~ leaving a rough torn surface.

Plantings were made between September 6 and October 13, 1966. Rhizomes were lifted after flowering for examination between May 31 and July 24, 1967. Severity of necrosis was assessed by cutting rhizomes vertically lengthwise and estimating percentage necrosis attributable to B. convoluta using a modified Horsfall and Barratt (1945) rating system. Isolations were made from necrotic lesions of dubious origin. Location of decay was noted to distinguish between possible latent infections and those caused by inoculation.

RESULTS

Nutritional Studies

Carbon Sources

In order to ascertain nutritional factors which may have an effect on the growth of B. convoluta and the etiology of Botrytis rhizome rot of iris, several experiments were conducted to determine the ability of the fungus to utilize various carbon and nitrogen sources, and the effect of initial pH of the culture medium on growth. In liquid BSM containing different carbon sources, the greatest dry weight in shake-grown cultures was produced in the maltose medium, with growth on starch, galactose, glucose, sucrose, fructose, lactose, sorbose and the no-carbon control following in order from good to poor carbon sources (Table 1). Linear growth and sporulation were assessed on solid media. B. convoluta grew most rapidly on agar media with glucose as sole carbon source, followed by maltose and sucrose. Starch, fructose, and galactose were intermediate in their effect on linear growth, while lactose gave practically no increase in growth over the no-carbon control. Sorbose inhibited growth. Differences in growth response to carbon sources between liquid and solid media cultures were apparent, but these represent different methods of growth measurement and comparisons between

Table 1. Growth and sporulation of B. convoluta in liquid and agar media containing the different carbon sources.

Added Carbon Source[a]	Growth in liquid media (mg. dry weight)		Radial growth on agar media		Degree of sporulation on agar media
	mg	%	mm	%	
Maltose	72[c]	100[b]	36.5[d]	90.8	+++[e]
Starch	60	83.4	29.0	72.2	++
Galactose	58	80.5	28.5	71.0	+++
Glucose	55	76.3	40.2	100	++++
Sucrose	50	69.5	35.0	87.0	++++
Fructose	23	32.0	29.0	72.2	++++
Lactose	17	23.6	12.8	31.7	+
Sorbose	11	15.3	0	0	0
No carbon	10	13.9	10.5	26.3	0

[a] Cultures grown on Argninine-BSM. Carbon supplied at 10 gms saccharide/1.

[b] Comparative growth on a percentage basis; the medium which produced the most growth equals 100%.

[c] Average of six replications, cultures 11 days old.

[d] Average of three replications, cultures five days old.

[e] Based on visual evaluation: Profuse (++++); None (0).

the two should be done cautiously. Extent of linear growth does not necessarily reflect the same growth responses as gain in dry weight would (Cochrane, 1963).

Sporulation occurred most profusely on solid media which stimulated maximum mycelial growth. Sporulation was most profuse on glucose, sucrose, and fructose media, less so on maltose, galactose, and starch media, and sparce to none on lactose and sorbose respectively. No sporulation occurred in sorbose and no-carbon control cultures.

Nitrogen Sources

On silica gel BSM with different nitrogen sources, linear growth of B. convoluta was greatest with casein hydrolysate (Table 2). BSM containing equivalent amounts of nitrogen from asparagine and ammonium tartrate promoted less growth. Urea and glycine supported less growth than the no-nitrogen control. Sporulation was most profuse with casein hydrolysate, asparagine, ammonium tartrate, and ammonium sulfate, less profuse with glutamate and potassium nitrate, and absent with glycine, urea and no nitrogen.

pH

In several experiments B. convoluta grew fastest in liquid media with low initial pH, pH 3 to 6. Data in Table 3 illustrate the

Table 2. Growth and sporulation of B. convoluta on silica gel media with different nitrogen sources.

Nitrogen source[a] added	Linear growth		Degree of sporulation
	mm	%	
Casein hydrolysate	41.7[b]	100[c]	++++[d]
Asparagine	38.2	92	+++
Ammonium tartrate	35.6	85.5	+++
Ammonium sulfate	30.3	73	+++
Glutamate	29.5	71	++
Potassium nitrate	23.8	57	++
Glycine	6.1	42	0
Urea	4.0	14.6	0
No nitrogen	19.1	9.6	0

[a] Cultures grown on glucose-silica gel-BSM for seven days. Nitrogen supplied at the rate of 430 mg. N/l of medium.

[b] Average of three replications.

[c] Comparative growth on a percentage basis; the medium producing the most growth equals 100%.

[d] Based on visual observation. Profuse (++++); None (0).

Table 3. Growth of B. convoluta and pH change in sucrose + potassium nitrate and glucose + asparagine media.

Medium	Time of harvest	Growth (mg) and pH at harvest							
	(day)	mg	pH	mg	pH	mg	pH	mg	pH
Sucrose + KNO_3[a]	0	0[b]	3	0	4	0	6	0	8
	6	15.0	5.7	34.7	3.9	18.3	5.2	7.5	7.3
	8	49.7	6.3	37.5	6.3	53.9	5.5	27.5	5.7
	15	45.3	6.3	29.9	6.1	51.3	6.1	46.9	5.3
Glucose + Asparagine	0	0	3	0	4	0	6	0	8
	6	78.3	3.3	67.1	6.5	66.2	6.5	42.3	5.9
	8	52.7	7.3	47.9	7.4	48.9	6.9	55.1	6.1
	15	45.5	7.4	44.3	7.3	42.8	7.2	58.4	6.0

[a] 15 ml BSM per 125 ml flask.

[b] Average of three replications per harvest.

facility of B. convoluta to alter initial hydrogen ion concentration during growth. It was observed that growth on media with pH 8 was slow initially until the medium became acidified and then rapid growth occurred. The fungus utilized and depleted available nutrients rapidly and began autolysis at pH values below pH 6 in less time than it took to reach maximum growth in the pH 8 medium. Similar data are presented in Figures 3 and 4. In these experiments, changes in growth and culture filtrate pH were followed in asparagine + glucose BSM. It was found, whether the fungus was grown in light or dark, that the pH of the medium became lower as fungal growth increased most rapidly and pH was lowest when growth was at its peak. After peak growth was reached, autolysis began, and a concomittant rise in pH started. In media lacking a carbon source, no peak growth was reached, but the filtrate pH increased steadily. No attempt was made to determine the cause of the pH change as B. convoluta grew, but according to Gentile (1954), similar pH changes and growth response in Botrytis cinerea were correlated to production of oxalic acid which appeared to be a metabolic end-product of glucose metabolism. Oxalic acid could not be used as a respiratory substrate or as a carbon source by Botrytis cinerea.

Figure 3. Growth of B. convoluta cultured in liquid glucose-BSM under continuous light and dark regimes, and pH of culture filtrates at each harvest.

Figure 4. Growth of B. convoluta cultures in liquid maltose-BSM and pH of culture filtrates at each harvest.

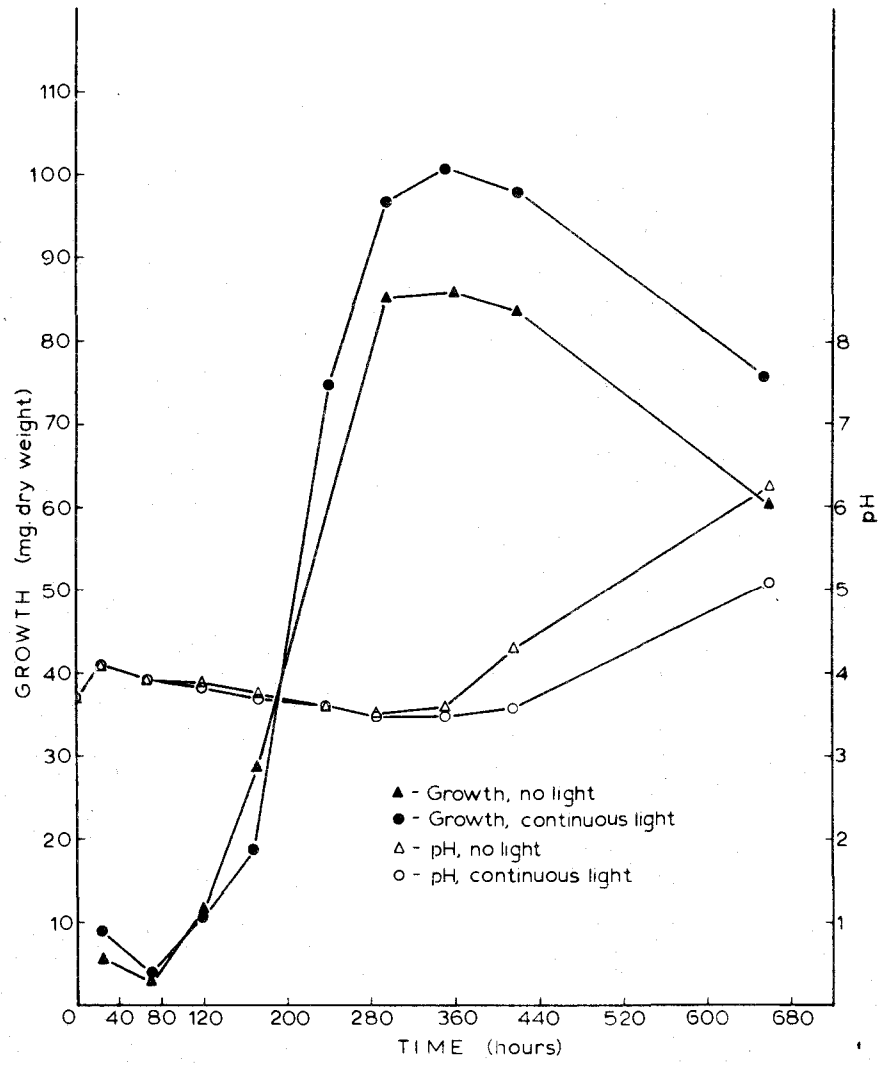

Figure 3.

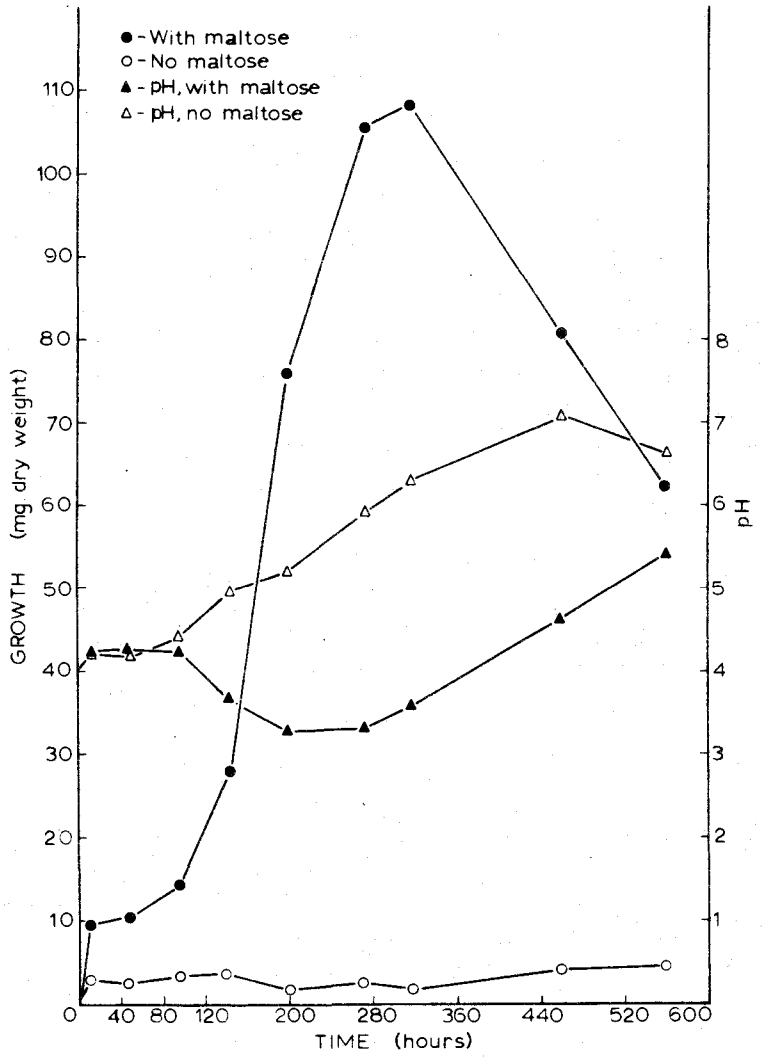

Figure 4.

Effect of Light on Growth and Reproduction

Many fungi require light for initiation or maturation of reproductive structures (Leach, 1967). Light of the visible range (400mμ to 760 mμ, approximately) often has the effect of increasing the number of reproductive structures formed. This effect is even more pronounced by near ultra-violet light (NUV = 320-420 mμ, approximately) in certain Ascomycetes and imperfect fungi. The occurrence of light-induced zonation of cultures on solid media is a common phenomenon. Generally, it occurs in fungi which sporulate poorly in the dark but may be stimulated into sporulation by a brief light exposure.

B. convoluta cultures required light for initiation of sporulation. PDA-cultures grown in complete darkness at temperatures ranging from 5-26°C failed to sporulate after 43 days. Cultures grown under 24 hours of continuous light or 12 hours of white fluorescent light alternating with 12 hours dark regimes were induced to sporulate. Zonation of sporulation was noted in cultures exposed to 12 hours of light alternating with 12 hours of darkness. Sporulation was profuse in cultures exposed continuously to white light and moderate in cultures exposed to alternating 12 hour light and 12 hour dark periods. Cultures exposed to continuous NUV sporulated profusely, and cultures exposed to alternating 12 hour

periods of NUV and darkness developed definite zonate patterns (Figure 6).

Vegetative linear growth rates of B. convoluta in agar (Figure 5 and Table 4) and dry weight increase in liquid (Figure 3) cultures was unaffected by light. Two different agar media (Table 4) were used to determine if the constituents may modify the response of the fungus to light treatments. B. convoluta was grown on PDA and exposed to continuous white and continuous NUV fluorescent light and in total dark. In order to observe the effect of light on growth, linear growth measurements were taken periodically and the resulting data plotted on a logarithmetic scale (Figure 5). Linear growth rates under the three light conditions were nearly identical. B. convoluta was also grown in liquid glucose + arginine BSM. The results (Figure 3) did not indicate that light affected the growth rate of B. convoluta in liquid culture. Length of lag periods and slopes of the logarithmetic phase of growth were very similar although peak growth of light-grown cultures was near 101 mg and dark-grown colonies near 87 mg. This difference was thought to have been due to small differences in incubation temperatures between the two treatments rather than due to light, since dark-grown cultures were incubated at 20-21 °C while cultures exposed to light were incubated at near 26 °C.

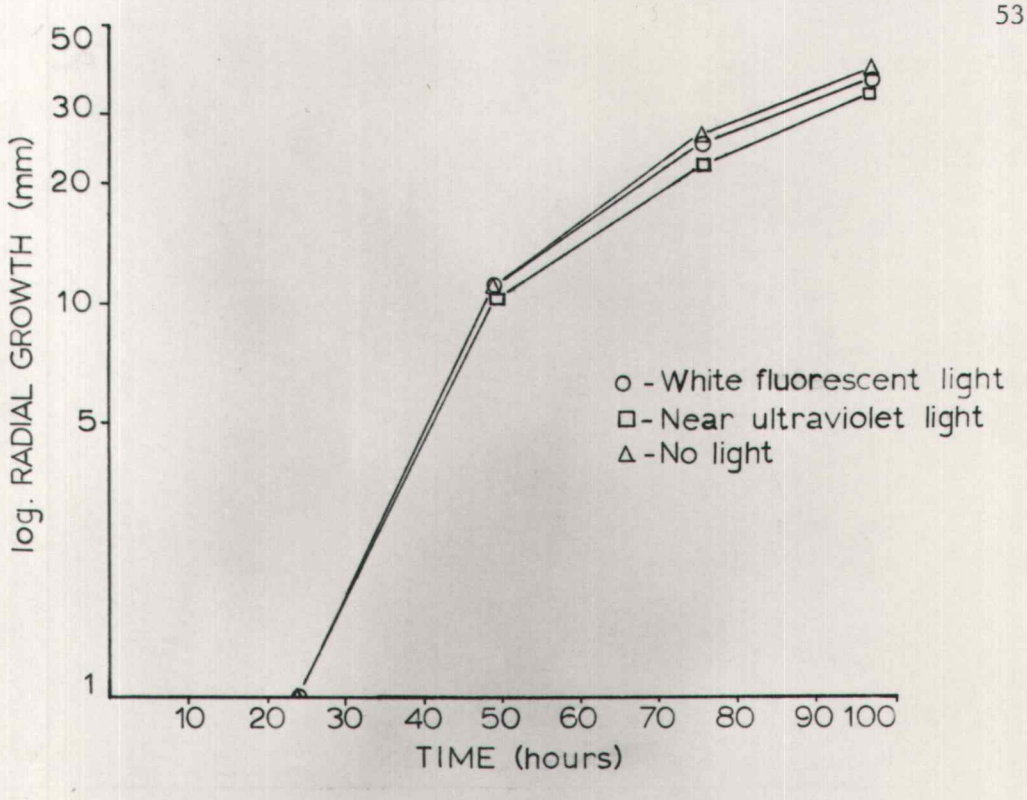

Figure 5. Comparative growth rates of B. convoluta on PDA media under three light regimes.

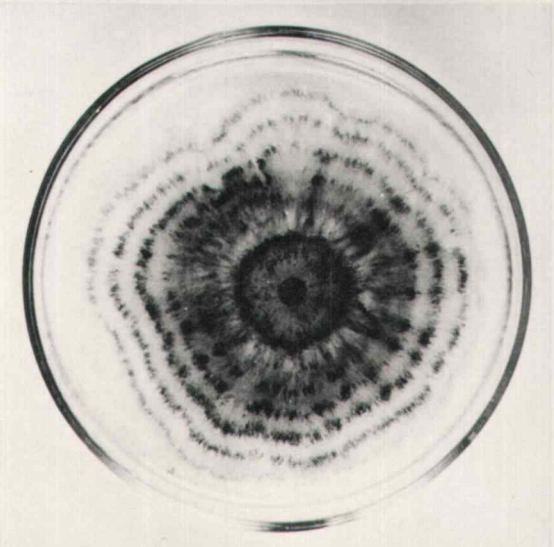

Figure 6. Growth of B. convoluta on PDA under alternating exposures to 12 hours near-ultraviolet light and 12 hours darkness for 10 days. Culture 10 days old, incubated at room temperature (23±2°C).

Table 4. Effect of light on growth of B. convoluta on two solid media.

Medium	Treatment	Linear growth at time (hours) of measurement						
	hours:	44	80	92	101	116	126	140
		mm	mm	mm	mm	mm	mm	mm
m + y[a]	24 hr. light	5.5[b]	17.3	20.5	--	29.2	--	37.1
	12 hr. light	5.0	--	19.1	--	28.6	--	37.9
	Dark + short red exposures[c]	--	18.0	--	26.5	--	37.6	--
	Continuous dark	--	--	--	--	--	36.0	--
s + a	24 hr. light	3.3	11.7	15.0	--	22.9	--	30.4
	12 hr. light	3.8	--	17.6	--	25.6	--	33.4
	Dark + short red exposures	--	12.9	--	21.2	--	30.6	--
	Continuous dark	--	--	--	--	--	29.1	--

[a] m + y = malt extract + yeast extract Difco Agar. s + a = sucrose + arginine BSM.

[b] Average of three replications.

[c] Red photographic safe-light, 15w, used to take colony measurements.

Temperature and Growth

According to Lilly (1963) fungi may show quite different growth patterns on different media, which may be attributable to the carbon or nitrogen sources used. Differences in growth response of B. convoluta due to carbon and nitrogen sources have been dealt with above; however, it was thought necessary to compare the growth of B. convoluta on PDA, the medium most generally used in this study, with several others including two composed of infusions of iris leaf and rhizome tissue. Infusions of iris leaves (50g dry iris leaves/liter) and iris rhizomes (190g air dried and peeled rhizomes/liter) were substituted for the potato infusion ingredient of PDA. Corn meal agar (Difco Bacto Agar No. B386) and SPDA (100ppm streptomycin sulfate) were also used. In addition to the Salem isolate, the growth curve on PDA for an isolate of B. convoluta from Walla Walla, Washington was determined. Results (Figure 7) showed that on all media used, the optimum temperature for linear growth was near 23.5°C. No growth at all occurred at 31°C. These data represent results of three experiments; however, assuming that the colonies remain at a nearly constant growth rate at each respective temperature until restricted physically, a comparison of hourly growth rates of B. convoluta in each experiment may be made. These growth rates were calculated by dividing

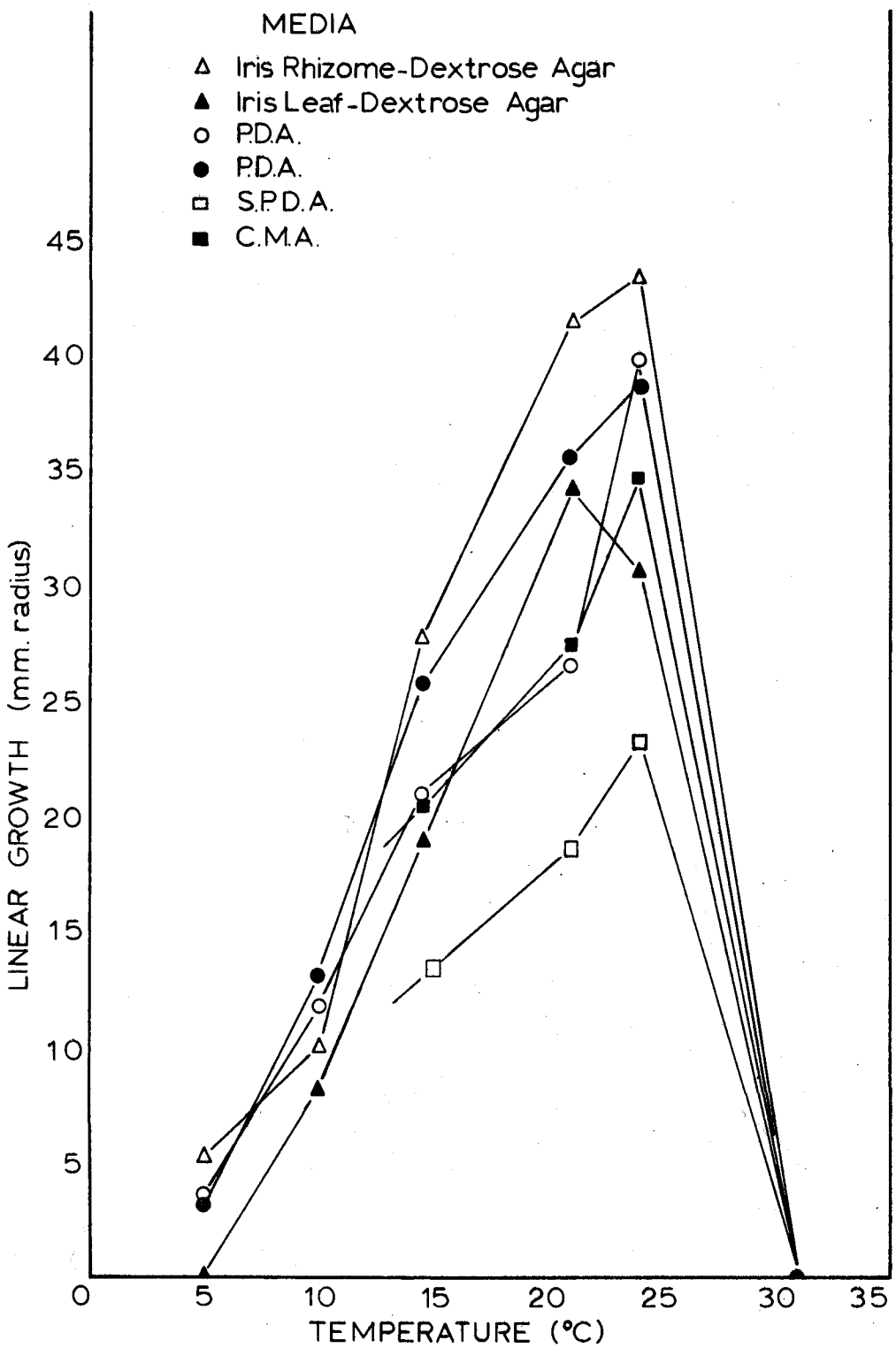

Figure 7. Growth of two isolates of B. convoluta on different media. (0) = Walla Walla, Wash. isolate. Remainder = Salem, Ore. isolate.

maximum linear growth (Figure 7) in a series by the total hours elapsed from inoculation of plates to that reading (Table 5). These data seem to indicate that PDA was the best medium of this group for mycelial growth. The addition of 100 ppm streptomycin to PDA was inhibitory to B. convoluta. Inhibition of growth by 100 ppm streptomycin was unexpected. Vörös (1963) reported that species of fungi with chitinous cell walls (as B. convoluta would be expected to have) were unaffected by 400 ppm streptomycin.

Germination and Survival of Conidia and Sclerotia

Sclerotia

Sclerotia of B. convoluta are generally produced on below-ground portions of iris rhizomes, although occasionally produced on leaf bases and above-ground portions of rhizomes. Development of rot in infected rhizomes in the field occurs during late fall and early spring. In the Willamette Valley development of rot could be expected to continue through the mild winter months. Jackson (1966) speculated that the sclerotia function to carry B. convoluta over the hot and dry summer season, during which the mycelium is relatively inactive and the host is growing rapidly, walling off infected areas. I thought it necessary to determine the optimum temperature for sclerotium formation and survival.

Table 5. Comparison of maximum growth rates of B. convoluta on different media at 21-24°C.

Isolate	Medium	Max. linear growth (mm)	Total time (hours)	Growth rate (mm/hr)
		mm	hr	mm/hr.
Walla Walla, Wash.	PDA[a]	39.9[b]	93	0.429
Salem, Oregon	PDA	38.7	93	0.416
Salem, Oregon	ILDA	34.3	120	0.286
Salem, Oregon	IRDA	43.5	168	0.259
Salem, Oregon	CMA	34.8	144	0.242
Salem, Oregon	SPDA	23.4	144	0.163

[a]Media: PDA = Potato Dextrose Agar; ILDA = Iris Leaf Dextrose Agar; IRDA = Iris Rhizome Dextrose Agar; CMA = Corn Meal Agar; SPDA = PDA with 100 ppm streptomycin sulfate.

[b]Ave. four replications

The optimum temperature range for sclerotium formation and maturation (characterized by intense melanization) was found to be 10-15°C, as indicated by the number of sclerotia and dry weight of sclerotia per PDA plate (Figure 8). Values are averages of three replications and the experiment was carried out twice. Dry weights of sclerotia were found to average 90.2, 125.0, 109.6, and 68.1 mg respectively at 5, 10, 15 and 20°C. Numbers of sclerotia produced for the same temperature range averaged 41, 66, 52, and 24 sclerotia per plate respectively. The optimum temperature for sclerotium production was lower than for linear growth which was 23-25°C. The average weight per sclerotium remained somewhat constant from 5 to 21°C, varying from 1.89 mg at 10°C to 2.84 mg at 20°C, indicating a balance between mass and numbers of sclerotia produced over the temperature range.

In some instances sclerotium formation by other fungi may be stimulated by mechanical injury to the mycelium. Henis et al. (1965) found that Sclerotium rolfsii could be stimulated to form sclerotia by tearing or cutting the mycelium in culture. An attempt was made to determine if this were true of B. convoluta. The mycelia of several PDA cultures of B. convoluta were injured mechanically by taking irregular plugs from the colonies and slashing the colonies. It was found that sclerotia were formed at sites where the mycelia were wounded. However, sclerotia which were formed on the

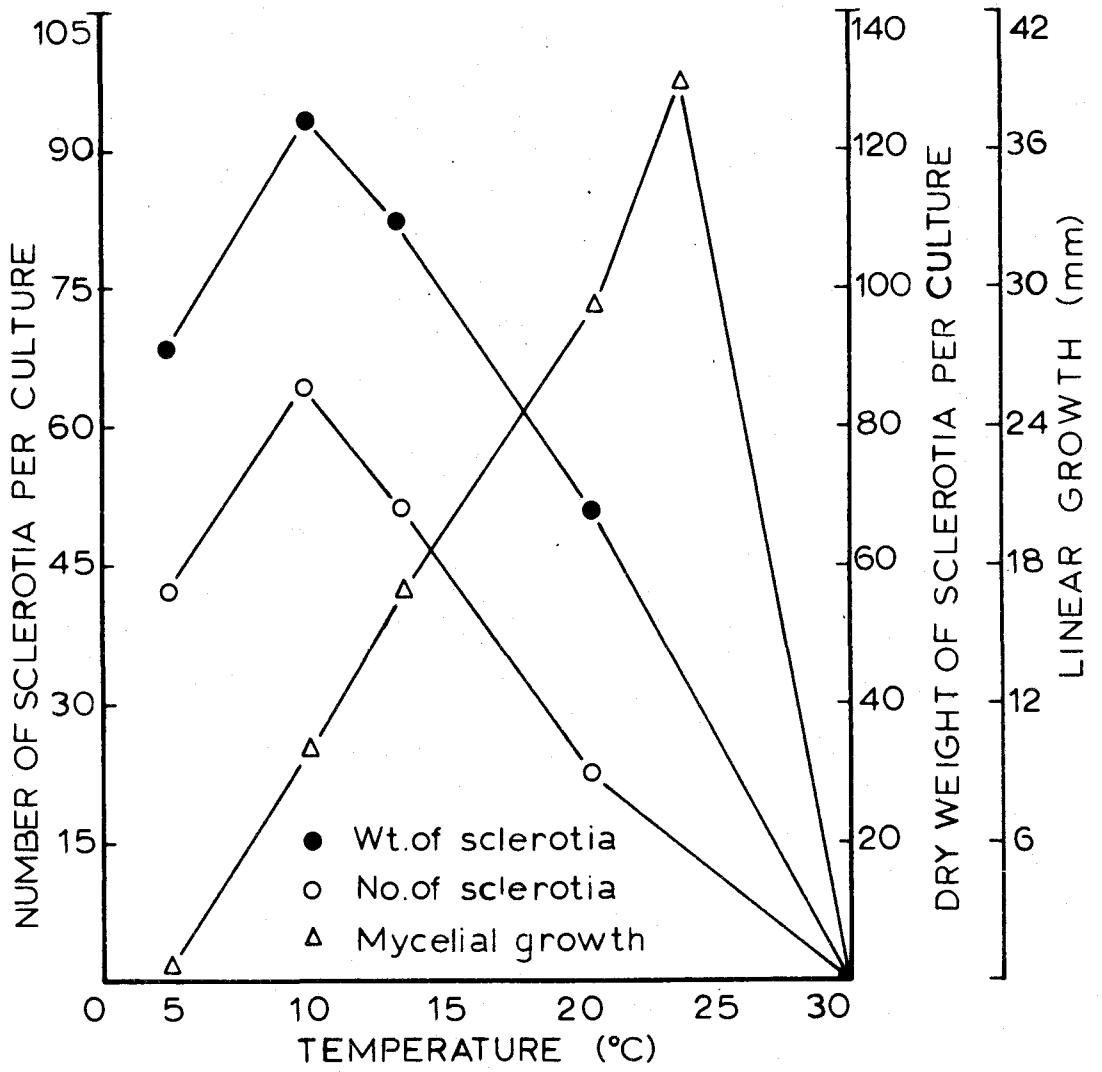

Figure 8. Influence of temperature on production of sclerotia and mycelial growth by B. convoluta in culture.

unwounded colonies and the majority formed on wounded colonies were located near the sides of the petri plates, which suggests sclerotium initiation may be due more to a combination of nutrient depletion and physical restriction of mycelial growth than to wounding of the mycelium.

Preliminary experiments showed sclerotia could germinate at temperatures from 5 to 25° C, the optimum temperature for germination on agar plates and on freshly cut rhizome disks was near 20° C. Germination occurred at 25° C on both substrates, but it was only half that at 20° C. No sclerotia germinated at 31° C. Germination occurred at 15, 10, and 5° C, but was decreasingly slower at the lower temperatures.

Several experiments were designed to determine the survival value of B. convoluta sclerotia. Sclerotia produced on rolled oat cultures were not handled aseptically and so were surface sterilized with clorox before plating them onto PDA to test their germinability. Tests showed sclerotia could remain 100 percent viable after immersion to 20 percent clorox for ten minutes. Subsequently, surface sterilization in 20 percent clorox plus a small amount of detergent for two minutes was found adequate.

Sclerotia produced an autoclaved rolled oats, which passed through a soil screen of eight meshes per inch but not 14 meshes per inch were placed into test tubes which were stoppered with

plastic caps, and stored at various temperatures from -70° C to 30° C and sampled periodically for viability. Initially, germination was 94.5 to 100 percent. After four days, frozen sclerotia were removed and thawed slowly at -15° C for $3\frac{1}{2}$ hours, and then placed at 5° C for $7\frac{1}{2}$ hours before plating. Germination of sclerotia held at -70 and -15° C was 98 and 100 percent, respectively. After removing samples, test tubes were replaced in their respective temperature storage units. In subsequent sampling of frozen sclerotia, they were thawed rapidly by placing them in a 24-30° C water bath. After 85 days the -70° C sclerotia germinated 100 percent, but the stock was unfortunately lost after this sampling, so further readings were not made. Germination of sclerotia held at -15° C dropped to 73 percent after 355 days. Germinability of sclerotia (Figure 9) held at 5 to 25° C remained high for 85 to 96 days, but declined rapidly at the higher temperatures. Since relative humidities of the incubation chambers were not held at a uniform level, dessication could have been involved in loss of viability of these sclerotia. Sclerotia were able to survive 366 days at 5 to 25° C under dry storage conditions.

Similar long term survival of B. convoluta sclerotia in soil was not found. Sclerotia contained in nylon mesh bags were buried in soil (50% FMHC) in jars and held at 5, 10, 15, 20, 25 and 30° C and "outside". When the bags were removed from the soil after 62

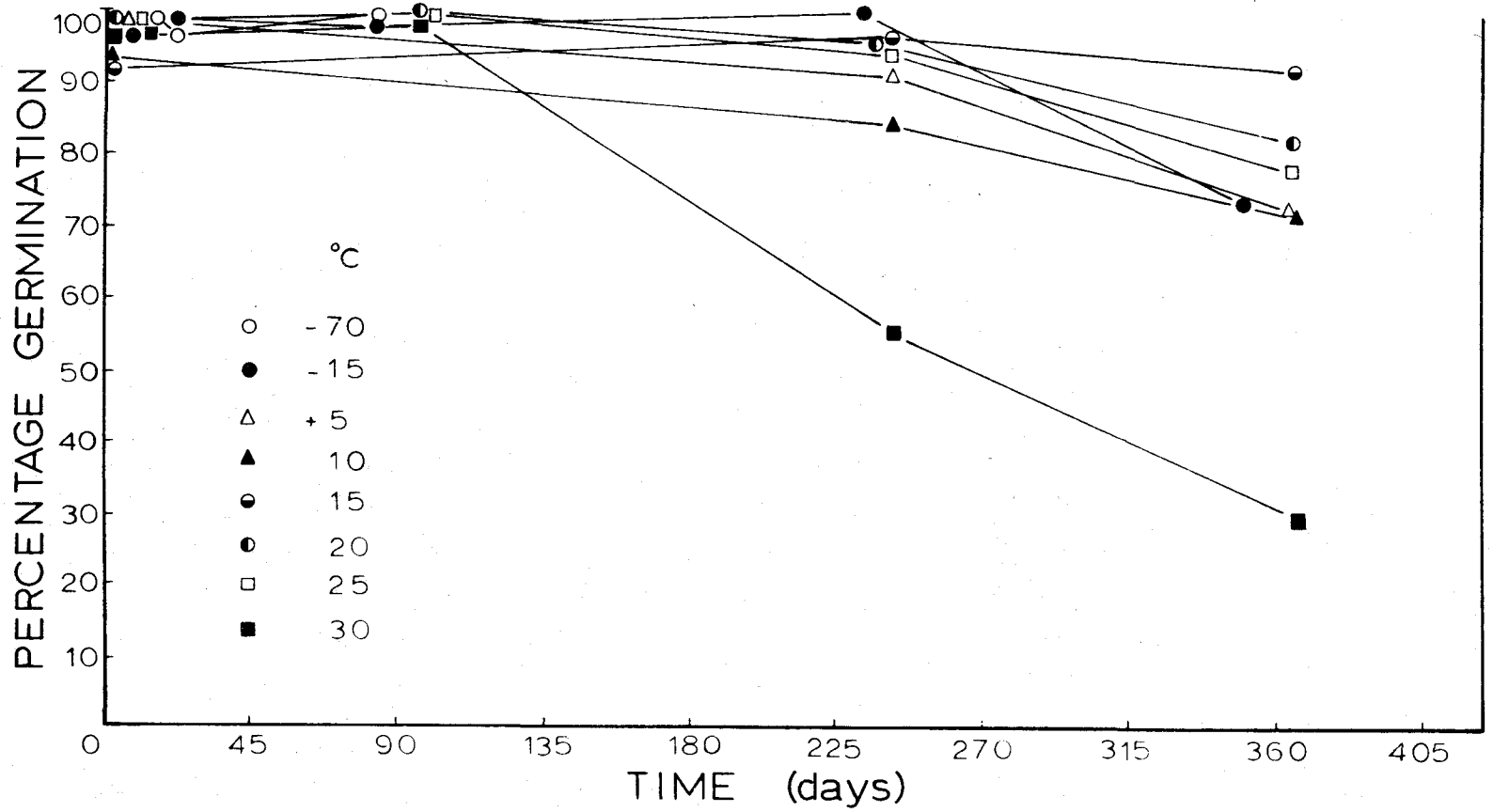

Figure 9. Influence of storage temperature on viability of B. convoluta sclerotia.

days burial, profuse sporulation was observed on sclerotia kept at 5 and 10° C, and "outside". According to Jackson's work (1966), B. convoluta sclerotia would not be expected to germinate while buried in soil. Sclerotia were removed from their soil-bags after 60 days burial, surface sterilized and plated on PDA. The cultures were incubated at 20° C for five days, then removed to room temperatures and exposed to white florescent light to promote sporulation. Ten days after removal from the soil, characteristic B. convoluta conidiophores developed on colonies originating from viable sclerotia. Survival was 42 percent at 5° C and only nine percent at 15° C. No sclerotia remained viable at 20 to 30° C (Table 6).

Examination of fungi which grew from non-viable sclerotia revealed a preponderance of Trichoderma spp., Fusarium sp., and phythiaceous fungi. Other fungi found growing from these sclerotia were a Mucor sp., a Sepedonium (?) sp., a basidiomycete, probably Trechispora sp., a Cephalosporium sp. and a Fusarium sp. (F. roseum ?). Several sclerotia were also colonized by bacteria.

Germination and Survival of Conidia

Iris plants infected with B. convoluta develop brown felty patches of conidiophores bearing copious conidia on above-ground portions of rhizomes and lower portions of leaves during cool, wet fall and spring weather. No real effort has been made by other investigators to determine the role of conidia in the disease cycle

Table 6. Effect of temperature on survival of B. convoluta sclerotia in soil.[a]

Temperature of storage (°C)	Total sclerotia	B. convoluta colonies	Percentage survival[b]
°C	No.	No.	%
5	100	42	42.0
"Outside"[c]	60	24	40.0
10	55	9	16.4
15	44	4	9.1
20	83	0	0
25	47	0	0
30	110	0	0

[a] Chehalis silty-loam soil at approximately 50% FMHC.

[b] Sixty days after burial.

[c] Temperature from late November, 1967 to early January, 1968, Corvallis, Oregon. Mean daily max.: 9.3°C. Mean daily min.: 5.8°C. Mean: 7.5°C.

of Botrytis rhizome rot of iris. In order to demonstrate their possible function, it was necessary to examine their germination and survival.

Age of spores and incubation conditions had a pronounced effect on spore germination. For example, spores seeded on PDA in petri plates germinated 95-100 percent within 12-18 hours, but in liquid media containing a limited amount of carbon source, they required up to 48 hours to attain a comparable germination rate (Table 7). Germination of spores incubated in distilled water was less than 5-11 percent. When an exogenous carbon source (sucrose) was supplied, over 80 percent germination occurred.

Sporulating cultures of B. convoluta were stored at temperatures from -70 to 30° C and sampled periodically for conidial viability (Figure 10). Germination of spores stored at 30° C decreased from 65 percent after seven days to one percent after 140 days, while germination of spores kept at 25° C decreased from 86 percent to 43.5 percent in the same time intervals. Viability of spores kept at temperatures from -70 to 20° C remained high (80-99%) after 140 days, except spores held at -15° C. Germination of spores kept at -15° C was 89 percent after seven days; but after 140 days, they were only 6.5 percent viable. The reason for this sharp decline in viability seemed to be that this group of plates was inadvertently thawed slowly and refrozen sometime before the 140 day harvest.

Table 7. Effect of exogenous nutrients on germination of B. convoluta conidia.[a]

Incubation media	Time of incubation (hrs.)	No. spores germinated	Total spores	Percent germination
	hrs.	No.	No.	%
Water only	24	23[b]	582	4.9
Water only	48	49	643	7.4
Water + Tween 20[c]	24	58	613	9.5
Water + Tween 20	48	65	595	11.1
2% Sucrose	24	220	647	34.3
2% Sucrose	48	513	582	88.1
4% Sucrose	24	226	629	35.5
4% Sucrose	48	437	514	83.2
PDA Plates	12	116	154	75.5
PDA Plates	24	106	111	95.7

[a]Spores approximately 14 days old; spore concentration approximately 50,000/ml.

[b]Average of four replications.

[c]Tween 20 (wetting agent) from Carolina Biological Supply House.

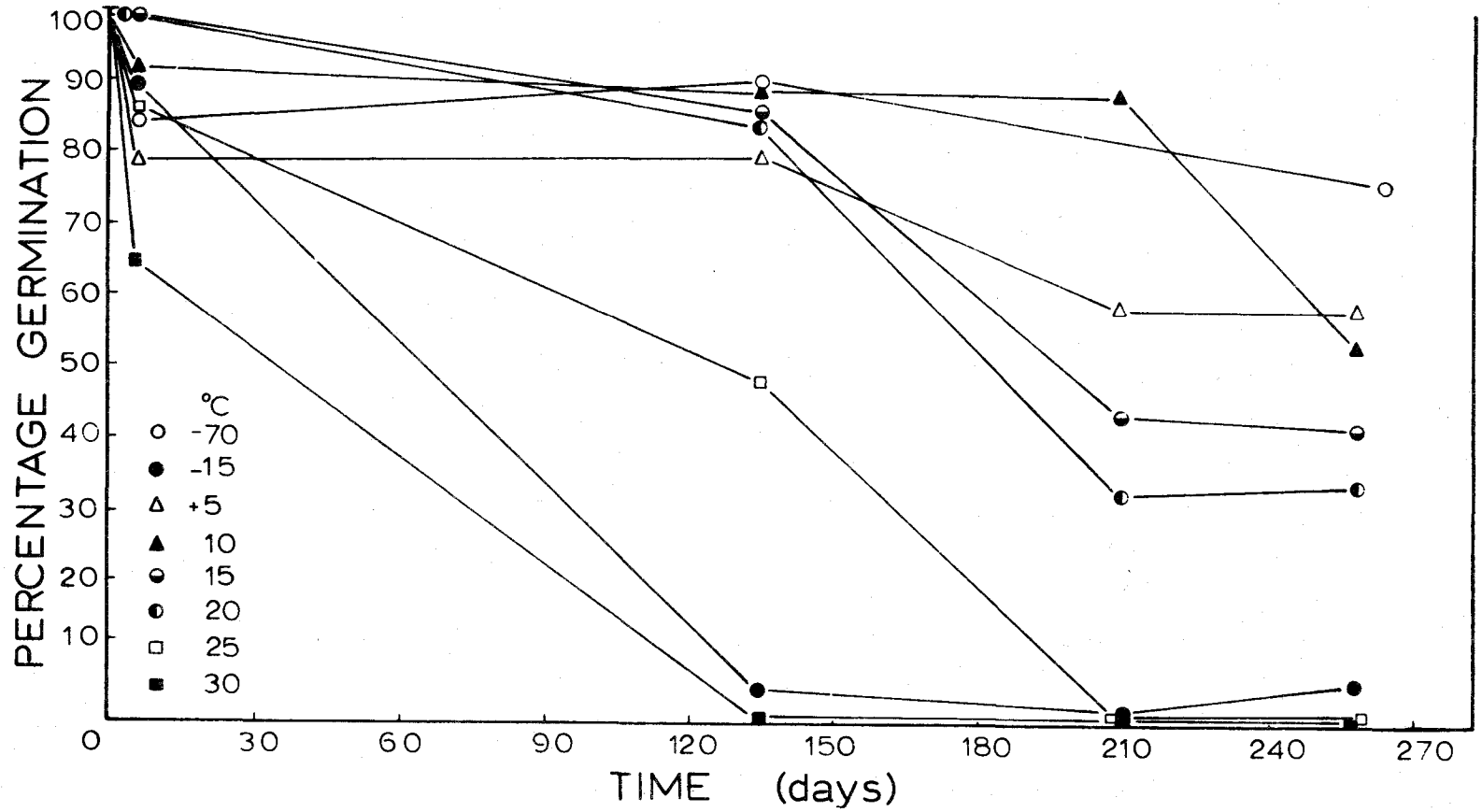

Figure 10. Influence of storage temperature on viability of B. convoluta conidia.

After 210 days, time and temperature appeared to take their toll, and after 257 days viability of spores stored at 20, 15, and 10° C was reduced to 32 percent, 41.3 percent, and 53.6 percent, respectively. Spores kept at 5° C remained 58.5 percent viable, while those frozen at -70° C were found to be 70 percent viable. These data suggest B. convoluta conidia lose their viability quickly under high temperature conditions similar to those encountered during late spring to early fall months.

Inoculation Studies

Preliminary experiments with conidia and sclerotia showed both were capable of causing infection of iris rhizome tissue under certain conditions. Sclerotia placed onto unwounded rhizomes with cellophane tape were able to germinate and infect the underlying tissue. Data (Table 8) suggest that spore germination and infection may be limited by high incubation temperatures. Although germination was nearly as high on rhizome disks as on PDA at 20° C, average germ tube lengths were considerably less on disks than on PDA. However, this did not prevent infection of the rhizome tissue.

Conidial suspensions placed on iris leaf tissue and incubated at 20° C for 12-24 hours germinated readily, producing long branched germ tubes over the leaf surface (Figure 11a). Terminal hyphal thickenings were observed which appeared to be appressoria (Figure

Table 8. Effect of temperature on germination of B. convoluta conidia on iris rhizome tissue disks.[a]

Temperature	Substrate	No. spores germinated	Total spores	Percent germination	Ave. length of germ tubes (μ)
°C		No.	No.	%	μ
30	Rhizome disks	0[b]	100	0	---
25	Rhizome disks	0	100	0	---
20	Rhizome disks	113	131	86.5	24.5
20	PDA	198	200	99	180
5	Rhizome disks	2	142	1.4	8

[a]Disks (var. Sunset Blaze) 11mm diam × 0.2-0.5 mm. 18 hrs incubation.

[b]Average of four disks.

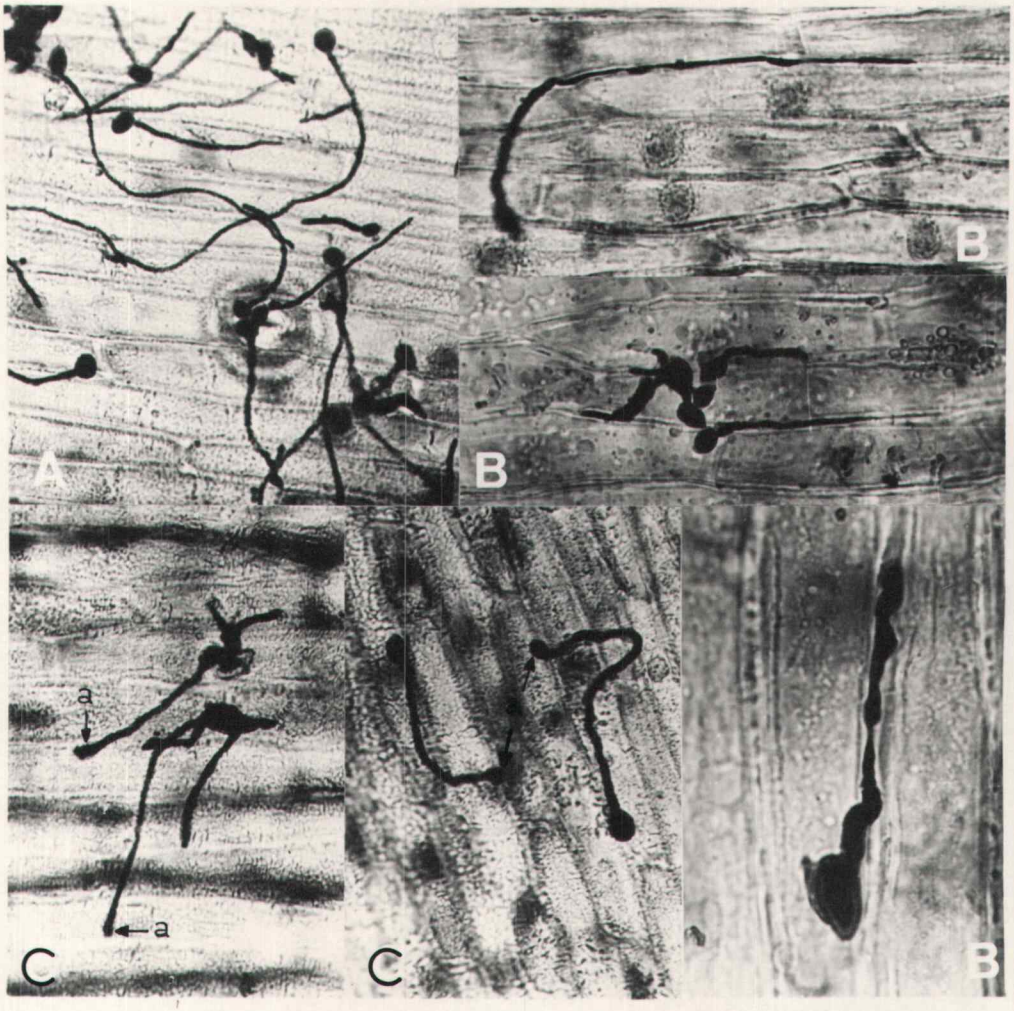

Figure 11. Germinated conidia of B. convoluta on iris leaf tissue. Fig. 11A; germ tubes showing no attraction to stoma. Fig. 11B; germ tubes lying parallel to and between longitudinal epidermal cell walls. Fig. 11C; germ tubes with appressorium-like terminal swellings.

11c); however, no penetration hyphae could be detected even after 48 hours. Stomatal invasion was not observed and there seemed to be no attraction of germ tubes towards stomata. Germ tubes produced by conidia directly adjacent to stomata often grew away from or over them, but not into the stomata (Figure 11a). Germ tubes and hyphae were commonly observed lying directly parallel to and over underlying epidermal cell walls. In some instances, hyphae appeared to be growing between epidermal cells (Figure 11b).

Young offsets were planted in plastic crisper boxes with 50 percent FMHC soil and inoculated with conidia. Infection of juvenile leaves occurred when they were in contact with colonized senescent or dead leaf tissue. In such cases, brown water-soaked lesions developed which were ramified with hyphae (Figure 12a, b).

It was observed in a preliminary experiment that wounded and unwounded iris rhizomes inoculated with sclerotia became infected within 21 days when incubated at 2.2°C. Plants similarly treated but incubated at -1.7°C remained healthy, suggesting that sclerotia are unable to infect iris rhizomes held slightly below zero degrees centigrade, but infection could occur at slightly above zero.

In a second experiment, sections of rhizomes were cut away and the wound allowed to heal at 25°C for 54 or 100 hours before being inoculated with sclerotia or mycelial plugs and buried in soil. Another series of rhizomes was wounded by spot freezing with dry

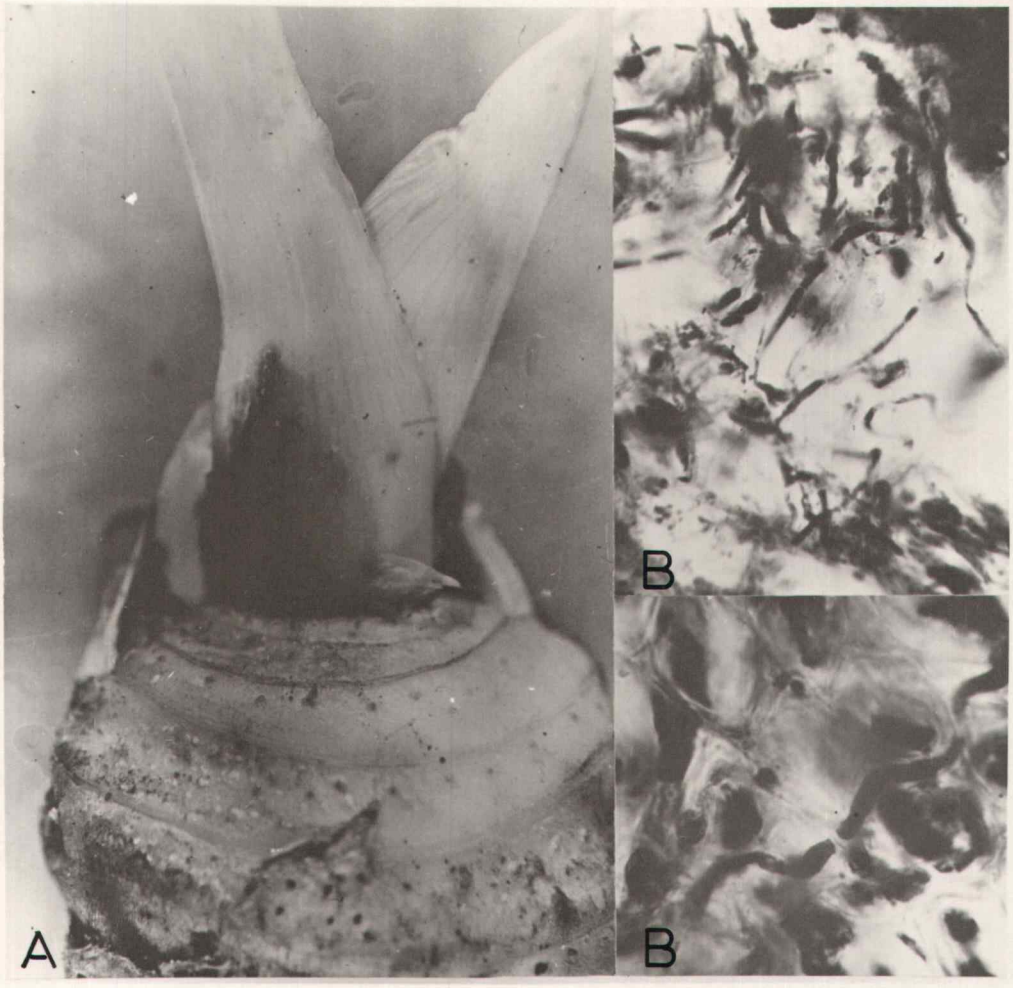

Figure 12. Fig. 12A; Lesion on juvenile iris leaf base resulting from contact with senescent leaves colonized by B. convoluta. Fig. 12B; Hyphae of B. convoluta ramifying iris leaf tissue.

ice and inoculated with conidia. Rhizomes were planted in plastic crisper boxes with 50 percent FMHC soil and incubated at various temperatures [1°C, 3°C, "outside", and room temperature (Table 9)] for 75 days.

Uninoculated control rhizomes developed a high incidence of rot in the toe, heel, or sole areas. The origin of rot in these regions would indicate that the infection originated from diseased mother rhizomes before separation of offsets. The infection must have been latent since offsets showing visible infections were discarded. The importance of latent infections will be presented in the discussion.

Offsets inoculated with conidia and incubated at 3°C showed a high degree of infection, but less was observed on rhizomes incubated at the other temperatures. Unlike the control series, rhizomes inoculated with conidia developed major lesions in areas associated with the point of inoculation. Of 100 plants in the conidial inoculation series, 35 were found to have lesions; of these, 18 were necrosed primarily in the toe region, 12 plants had lesions associated primarily with the apical region and juvenile leaves (Figure 12), and the remaining five had lesions primarily in the knee region. This distribution of infection loci was found only with plants inoculated with conidia, not in plants inoculated with sclerotia or mycelial plugs. Conidia applied to the offset rhizome may have germinated and colonized dead and senescent iris tissues. Using this as a food

Table 9. Effect of wounding and wound healing on infection of iris offsets inoculated with B. convoluta sclerotia, conidia, and mycelial plugs.

Treatment[a]	Incubation temperature	Offsets infected	Percent infection	Percentage infected offsets with major necrosis areas located at:		
				Toe, heel or sole	Apical region	Top or knee
	°C	No.	%	%	%	%
Uninoculated controls	1	3[b]	12	12	0	0
Uninoculated controls	"outside"[c]	8	32	32	0	0
Spot frozen + conidia	1	4	16	12	0	4
Spot frozen + conidia	3	22	88	44	28	16
Spot frozen + conidia	"outside"	6	24	12	12	0
Spot frozen + conidia	Room temp.[d]	3	12	4	8	0
Cut, held 54 hr. + sclerotia[e]	3	25	100	0	0	100
Cut, held 54 hr. + Mycelial plugs	3	23	92	0	0	92
Cut, held 100 hrs. + sclerotia	3	25	100	0	0	100
Cut, held 100 hrs. + mycelial plugs	3	23	92	0	0	92

[a] Iris variety Cotlet. Plants inoculated at top or knee area.

[b] 25 plants per treatment.

[c] Mean daily max: 9.3° C; Mean daily min: 5.8° C; Mean daily: 7.5° C.

[d] 21-24° C.

[e] After cut wounded, offsets held at 25° C before inoculated.

base, the fungus may have infected the young leaves, buds, or other parts of the rhizome which were in contact with the colonized material.

Offset rhizomes wounded by cutting and allowed to heal for 54 and 100 hours at 25 °C before inoculation resulted in nearly 100 percent infection at the site of inoculation with sclerotia or mycelial plugs. A series of inoculated rhizomes receiving no wounds or incubation at 25 °C were not incorporated into this experiment because results of previous experiments showed such rhizomes became 100 percent infected. All lesions originated at the wounded areas, and large masses of sclerotia were produced at the site of infection. The above data suggest that under field conditions there are three principal modes of infection of iris rhizomes by B. convoluta: (1) offsets may become infected prior to separation from a mother rhizome which is infected, (2) rhizomes may come into contact with sclerotia or colonized plant material, and (3) infection may spread into rhizomes from leaf infections established by conidia. With this in mind, care was taken to distingusish between lesions resulting from one or more of these different modes of infection. This was done by sectioning the rhizomes longitudinally and estimating percentages of necrosed rhizome tissue present according to location, and the presence or absence of an apical bud was also recorded. Estimation of necrosis was done according to

a modified Horsfall and Barratt (1945) system for measuring plant disease and will be referred to as the necrosis severity index.

Results of field studies indicated a large percentage of iris plants used carried latent infection which resulted in extensive decay of rhizomes (Table 10). It was believed this represented latent infection since only rhizomes which were apparently sound were used and they were also surface sterilized in clorox before planting. The soil was eliminated as a source of infections because it had no previous history of iris cultivation and the host range of B. convoluta is limited to irises. Apricot Glory, Sunset Blaze, and Cotlet plants were found to have 67 percent, 84 percent, and 90 percent latent infected, respectively. This high degree of infection made it difficult to determine the infectivity and significance of inocula in the field experiments. Conidia caused significant increases in rot incidence associated with the apical region of rhizomes of Cotlet and Apricot Glory varieties (Table 11). Incidence of infection among Apricot Glory plants inoculated on their leaves only was 19 percent over that of untreated plants, and incidence of infection of plants inoculated over their entire surfaces with conidia was 32 percent more than non-inoculated plants.

Rhizomes inoculated with rolled-oat cultured B. convoluta inoculum failed to increase the incidence of infection when compared to untreated plants. Sclerotia were found to cause an increased

Table 10. Varietal differences in development of latent B. convoluta rhizome rot in symptomless rhizomes.

Variety	Percentage and severity of rot by location on rhizome							
	Total rhizome		Toe rot involved		Top, side, knee rot involved		Apical area involved	
	%	Severity index[a]	%	Severity index	%	Severity index	%	Severity index
	%		%		%		%	
Apricot Glory	67.4[b]	10.8	55.6	5.1	15.5	4.2	17.8	1.5
Cotlet	90.0	21.1	38.8	3.7	29.5	10.5	25.0	6.9
Sunset Blaze	84.0	11.9	67.0	5.2	24.5	5.5	21.2	1.3

[a] Based on average extent of rot per rhizome.

[b] Figures based on averages of two replications of approximately 25 plants each.

Table 11. Infection of iris rhizomes of three varieties inoculated with rolled oat cultures, sclerotia, and conidia of B. convoluta.

Treatment	Variety	Increase in percentage of infection over uninoculated controls
		%
Rolled-oat inoculum		
unwounded rhizomes	Apricot Glory	0[a]
wounded rhizomes [b]	Apricot Glory	6.1
unwounded rhizomes	Cotlet	22.6
wounded rhizomes	Cotlet	0
unwounded rhizomes	Sunset Blaze	0
wounded rhizomes	Sunset Blaze	0
Sclerotia as inoculum		
unwounded rhizome	Apricot Glory	11.3
wounded rhizomes	Apricot Glory	14.3[e]
unwounded rhizomes	Cotlet	0
wounded rhizomes	Cotlet	0
Conidia as inoculum		
leaves only inoculated	Apricot Glory	19.0[c, d]
entire plant inoculated	Apricot Glory	32.2[d]
leaves only inoculated	Cotlet	47.6[e]
entire plant inoculated	Cotlet	39.8

[a] Values based on average of two replications of approximately 25 plants each. Percent increase in infection determined from decay in top, knee and side areas only, i.e.: where rhizomes were inoculated.

[b] Rhizomes wounded by removing tissue from knee area with a potato peeler.

[c] Percent increase in infection determined from decay in apical region only.

[d] Significant at 5% level.

[e] Significant at 10% level.

incidence of infection of 14 percent in wounded Apricot Glory rhizomes; however, results failed to show that wounding rhizomes by cutting tissue off the knee region increased incidence of infection significantly among the other varieties or treatments.

Varietal differences for resistance to Botrytis rhizome rot of iris were observed. Incidence of infection which developed in apparently sound rhizomes and severity of rot differed with the variety (Table 10). Percentage infection and necrosis severity index figures indicate that in each variety most of the plants were infected and rotted most severely in the toe region. Incidence of infection and necrosis severity were generally lower in areas away from the toe region. The Cotlet variety showed the highest incidences of infection and necrosis severity. Apricot Glory showed the least amount of latent infection, which was nevertheless quite high (67 percent).

DISCUSSION

Lilly (1963) succinctly defined the need for basic physiologic studies of pathogens to augment etiological investigations:

> Disease physiology and fungus physiology are related as a building is related to its foundation. . . . it is necessary to know where the pathogen is strong and where it is weak. Much of this information can be learned by studying the pathogen in the laboratory. It should then be easier to study the more complex situation of disease in the greenhouse and in the field.

Such studies should include utility of media, carbon and nitrogen sources, need for vitamins, and the effects of pH, light and temperature.

The results of this study showed that B. convoluta utilized maltose, starch, galactose, glucose, sucrose, and fructose as carbon sources. These saccharides are of common natural occurrence in plants. Utilization of lactose and sorbose was marginal, and sorbose appeared to be inhibitory to growth on BSM agar. Sorbose occurs rarely in nature and is a poor carbon source for many fungi and apparently may be inhibitory to other fungi as well (Lilly and Barnett, 1953). Lactose also appeared to be a poor carbon souce, but it was not inhibitory. Lactose metabolism is infrequent among fungi, and its utilization may be adaptive, requiring longer incubation periods than for more readily metabolized carbon sources (Cochrane, 1963).

B. convoluta utilized casein hydrolysate, asparagine, ammonium tartrate, ammonium sulfate, and glutamate as nitrogen sources. Casein hydrolysate is a mixture of several amino acids and is generally a good nitrogen source for fungi. Potassium nitrate stimulated little growth while glycine and urea appeared to be inhibitory. It is possible that organic nitrogen sources used were also metabolized as carbon sources, masking the real ability of B. convoluta to utilize them as nitrogen sources. On media containing inorganic N, B. convoluta grew better with ammonium forms than with nitrate. Inability to utilize nitrate may simply result from the medium being at a pH level which was not optimal for nitrate uptake. Generally, many fungi utilize ammonium nitrogen, while relative few utilize nitrate nitrogen (Cochrane, 1963).

B. convoluta was shown to be capable of modifying its environment presumably to a more favorable one by altering the initial pH of the system. Optimum initial pH was found to be pH 3 to 4 although it varied with the medium used and the time of harvest. Growth peaks also occurred when the pH of the medium was pH 3 to 4. Alteration of initial pH has been explained as a response of fungal metabolism as it grows (Cochrane, 1963) and may be due to deposition of certain metabolic end products such as oxalic acid into the medium. This is largely presumptive in the case of B. convoluta although the work of Gentile (1954) with Botrytis cinerea suggests

this possibility.

Light in the visable and NUV ranges did stimulate sporulation and more-or-less retarded sclerotium formation in culture, but it had no observable effect on vegetative growth within the intensities used. These findings are substantiated by Jackson (1966) who observed that sclerotia fail to germinate by conidiophore production unless given several days exposure to white light. I made the observation that sclerotia buried in soil sporulated at 5, 7.5, 10 and 15° C; however, these sclerotia were exposed to light between harvest and burial in soil. The adaptive significance of this light stimulus mechanism is obvious if one considers conidia to be the prime inoculum source for new infections. Conidia, for the most part, develop on portions of diseased iris rhizomes or on sclerotia which are at or slightly above the soil surface to be carried about by air currents and splashing water.

Profuse sporulation consistently occurred on media which supported vigorous mycelial growth. Although reproduction by many fungi is initiated as a response to nutrient depletion, this does not seem to be true for B. convoluta. Sporulation under illumination apparently occurred before all nutrients were utilized, since it began while colonies were small and continued until growth covered the culture medium. Cultures grown in complete darkness failed to sporulate; however, they did produce abundant sclerotia.

Sclerotium production in culture, on the other hand, seemed to be more associated with nutrient exhaustion since they were not initiated until well after mycelia had covered the culture plates. Production of sclerotia on rhizomes appears to be similarly induced since they often develop after a rhizome is largely or entirely invaded and rotted, or when an infection is localized by walling off of the infected area.

The optimum temperature for mycelial growth of B. convoluta in culture on several media was 23 to 25° C. The optimum for sclerotium formation in culture was between 10 and 15° C, but the optimum for germination was near 20° C on agar media and on rhizome tissue. Optimum for conidial formation was found by Drayton (1937) to be 20° C and optimum for conidial germination was near 20° C on PDA and in iris rhizome disks. MacWithey (1967a) reported that pathogenesis was optimum at 20° C, falling abruptly at 25° C due to rapid suberization of the rhizomes. Thus optimum temperatures for growth of B. convoluta on PDA or other artificial media are quite distinct from optima for invasion of rhizome tissue; production and germination of sclerotia and conidia. Differences in optima for pathogenesis and growth in culture are frequently observed (Walker, 1957). Temperature plays an important role in Botrytis rhizome rot development because, in late spring, actively growing mycelia in rhizome tissues would be subject to increasingly

higher soil temperatures (above 25° C) which reduce mycelial growth to the point where it becomes inactive, and at the same time rhizome tissue resumes active growth and forms wound periderm around infected areas. The fungus in the walled-off lesions and latent infections may resume activity when temperatures again become more favorable for pathogenesis.

According to Flentje (1959), "the spores of the majority of air-borne fungi appear to germinate satisfactorily in distilled water indicating that no special germination stimulus other than water is required." This was not found to be true with spores of B. convoluta, which required an exogenous carbon source to stimulate germination. These findings are in agreement with those of Orellana and Thomas (1965) who found spores of B. ricini incapable of germination in pure distilled water, but addition of two percent glucose or sucrose resulted in 100 percent germination. Whether or not the inability of B. convoluta spores to germinate in pure distilled water should be considered an ecological and survival advantage is somewhat of an academic question since a film of pure water would not be expected to retain its purity on any surface in nature (soil, plant debris, living plants, etc.), however, competition would be increased by presence of simple carbon sources. Moisture films on plant surfaces have been shown to contain inorganic and organic solutes (sugars, amino acids, etc.) released from the underlying plant tissue

(Brown, 1922b; Kosuge and Hewitt, 1964). Indirect indication of the effect of leaf exudates was observed when B. convoluta spores germinated readily and developed extensive hyphae on surfaces of young iris leaves.

B. convoluta has been considered primarily a wound pathogen by most investigators; however, few have attempted to test the hypothesis that wounds are necessary for pathogenesis. It was shown in this study that wounding may significantly increase disease incidence and disease severity.

Iris rhizomes may also be predisposed to Botrytis rot by cold injury (Dosdall, 1944). In fact, Botrytis rhizome rot has often been confused with or equated to an ambiguous "winter-kill" disease of iris because one subtle sign of Botrytis rot is the failure of plants to emerge in spring, the rhizomes being completely decayed. Freezing of rhizomes, especially buds, may result in dead iris tissue available to B. convoluta for primary colonizations. Presumably B. convoluta may colonize this tissue and use the energy source to invade healthy tissue. In one experiment where offsets were spot frozen with dry ice and inoculated with conidial suspensions, conidia were able to cause infection of offsets, but infections in several cases were somewhat removed from the wound loci. Dead or moribund leaf tissue were evidently colonized by germinating conidia or by mycelium in latent infections and infection of

adjoining bud leaves ensued.

Results of laboratory experiments showed conidia, sclerotia, and leaf tissue colonized by B. convoluta caused infection of iris rhizomes. Sclerotia and mycelial plugs were able to infect both wounded and unwounded rhizomes producing large rotted areas at the point of inoculation. Results of conidial inoculations were less clear cut; however, infection resulted in leaf and rhizome lesions. It was also found that conidia were able to germinate on iris leaves and rhizome sections, and germ tubes grew into the freshly wounded rhizome tissue. Penetration of healthy leaf tissue by germ tubes was not proven although circumstantial evidence pointed toward successful penetration. Stomatal invasion was not observed; however, hyphae were found to grow directly above and parallel to longitudinal cell wall junctions in iris leaf epidermis. In some cases hyphae appeared to lie between cell walls and in other cases terminal germ tube thickenings similar to appressoria were observed.

Field inoculation studies indicated that a high degree of latent infection occurred in noninoculated plants. B. convoluta conidial inoculum, however, was effective in causing significantly more infection of apical areas compared to noninoculated plants when leaves only or entire plants were dipped into a conidial suspension before planting. Sclerotia placed on rhizomes wounded by cutting part of

the knee tissue away caused significantly increased infection incidence over apparently sound noninoculated rhizomes. The effects of other treatments were masked by the high incidence of disease which occurred in noninoculated rhizomes that appeared sound before planting. These infections apparently resulted from latent infections rather than surface contamination since the rhizomes were surface sterilized in clorox and rhizomes with visable lesions were discarded. Also, a soil-borne source of B. convoluta inoculum in the field plots was ruled out because the area had not been under previous iris cultivation and the fungus has a very limited host range.

The establishment of these latent infections is somewhat vague; however, it is quite certain they occurred before the rhizomes were planted in this field study. Uninoculated and visually sound rhizomes were 67-90 percent infected and 38-67 percent of the decay originated in the toe region, as compared to 15-29 percent and 17-25 percent in top and apical areas. At one time or another in the development of an offset into a mature rhizome, each of these areas may have contacted senescent or dead tissue which was colonized by B. convoluta. Dead leaf bases, for example, generally remain attached to rhizomes around the bud and on top of rhizomes for some time. When offsets are removed from the mother rhizome and wound periderm forms, a small amount of parenchymatous storage tissue is left exposed.

This dead or dying parenchyma may have become infected by conidia or other inoculum when the plants were handled. Air-borne conidia may colonize senescent or juvenile leaves as well.

Seasonal timing of infection is perhaps a very important factor in determining amount of infection and extent of disease development. B. convoluta, a cold-weather pathogen, has two principal seasons during which inoculum may be produced and disease development occurs. Infections that occur in fall in the Willamette Valley would be expected to develop uninterrupted and result in noticeable rhizome decay by spring. Infections that occur in spring (or in storage), however, may be curtailed by rising soil and air temperatures and host defense mechanisms. Thus, these undeveloped infections would remain latent and the rhizomes would show no rot symptoms until conditions were favorable for pathogenesis in the fall.

Latent infections undoubtedly play an important role in dissemination of the disease since they were found to occur commonly on visually sound rhizomes and thus would be undetected by the grower, instilling a false sense of security. Also commercially grown iris plants are distributed nationally and internationally, resulting in possible widespread dissemination of the disease also. Chemical control of the disease is made difficult because latent infections in rhizomes are inaccessible to non-systemic fungicides.

Sclerotia placed on wounded and unwounded rhizomes in

laboratory studies caused 100 per cent infection in the areas rhizomes were inoculated; however, in field plantings, only sclerotia placed on wounded Apricot Glory rhizomes caused increased disease incidence and necrosis severity in the areas inoculated. Under controlled conditions, at least, distinction could be made between latent infections and infections resulting from inoculations. Under field conditions, however, identification of infections resulting from inoculations was made extremely difficult by the high incidence of latent infections which tended to mask the treatment effects. Other factors may have been involved which contributed to ineffectiveness of sclerotia in the field inoculation studies. Iris plantings were irrigated regularly to keep the soil moist during late summer and early fall so that moisture would not be a limiting factor. It is possible that sclerotia were ineffectual due to antibiosis which was more pronounced under field conditions than under laboratory conditions, even though soil of the same origin was used in each experiment and kept moist. Rhizomes inoculated with 2-3 sclerotia and incubated at 3°C in a walk-in cooler resulted in a 100 percent infection indicating that at least one sclerotium of each inoculum group was viable and infective. In field inoculations, ten sclerotia were placed on each rhizome before burial. It is possible that either none of the sclerotia were in close enough contact with the rhizomes since an adhesive was not used, or they were rendered ineffective by a

combination of an antagonistic microflora and host response. The latter is very probably because fall temperatures remained high enough in 1967 to permit rapid periderm formation by newly planted rhizomes and to stimulate antagonistic organisms during the 3-4 day lag period necessary for sclerotia to germinate. Susceptibility of sclerotia to microbial attack at 60° F and above will be discussed more fully later, but viability in moist soil fell sharply from 41 percent at 59° F to zero percent at 68° F after 60 days. It is probable that sclerotia play a more significant role in disease development when soil temperatures are nearer 40° F as suggested by the laboratory inoculation results and that disease development is most rapid at cooler temperatures.

Conidia produced on PDA plates were stored in situ at various temperatures and sampled periodically for viability. Conidia kept at 25 or 30° C for more than seven days rapidly lost their viability. Similarly, spores kept frozen at -15° C, slowly thawed and refrozen before harvesting lost their lability, which may have resulted from extracellular ice crystal formation which tends to withdraw water from the spores (Cochrane, 1963). Spore survival was good for 257 days at low temperatures (5 and 10° C). Thus, spores produced in fall and winter from infected irises or sclerotia would remain effective as inoculum until summer. According to Bagga (1967) storage of Botrytis cinerea spores at lower relative humidities (0-25 percent

RH) under refrigeration resulted in greater longevity than spores kept at 50 or 75 percent RH. Assuming this to be true of B. convoluta spores also, then rapid loss of viability at 25°C and especially 30°C should have been due to temperature alone since the relative humidities under these conditions of storage were necessarily low, evidenced by the extreme desiccation of the agar medium at 30°C compared with 5 or 10°C. Freezing in itself did not reduce spore viability since spores held at -70°C remained 70 percent viable after 257 days.

Similar experiments with sclerotia showed that rapid freezing and slow or rapid thawing did not affect viability. After nearly one year, frozen sclerotia remained 73 percent viable. Sclerotia held at temperatures above freezing lost viability with higher temperatures, for example, after 366 days sclerotia held at 5°C were 70 percent viable while those at 30°C were only 30 percent viable. Soil was not used in this particular study and plated sclerotia gave rise to B. convoluta colonies only, indicating that microbial antagonism was not involved in lowered viability. At 30°C mycelium as well as spores and sclerotia of B. convoluta fail to grow or germinate; however, the mechanism of temperature inactivation of mycelium is thought to be somewhat different than that affecting resting structures. Mycelia are generally more sensitive to temperatures above the optimum for maximum growth than spores or sclerotia, and the

sensitivity is presumed due to an imbalance of metabolism rather than to dehydration damage (Cochrane, 1963). Nevertheless, B. convoluta is very sensitive to temperatures between 26 and 30° C, being generally inhibited or killed at temperatures of 30° C or above depending on length of exposure and structures involved.

Sclerotia buried in soil for two months at temperatures above 15° C were less than ten percent viable. Necrobiosis at higher temperatures may have been due to : (1) increased CO_2 concentration due to an active microbial population, or (2) direct antibiotic activities of the microbial population. Antagonistic organisms able to parasitize and destroy B. convoluta sclerotia may have been responsible for their loss in viability. Sclerotia were colonized by several different soil fungi and bacteria; predominantly Trichoderma spp., pythiaceous fungi and a Sepedonium sp. Sclerotia of various fungi as well as other vegetative and reproductive structures have been reported (Barnett, 1963) to be parasitized by a large number of mycoparasites belonging to such diverse taxa as the Chytridiales, Mucorales, Saprolegniales, Peronosporales, and Fungi Imperfecti. Fungicolus fungi have been commonly associated with sclerotia of other species. Trichoderma spp. have been cited as colonizers of weak or moribund sclerotia of Sclerotium rolfsii sclerotia. Some of these were species of Cephalosporium, Penicillium, Aspergillus, Trichoderma, and Fusarium. MacWithey (1967a) also felt that

parasitism by soil organisms limited survival of B. convoluta at soil temperatures above 15° C. It may be possible, judging from the results of MacWithey's and my research that soil temperatures above 15° C would stimulate sclerotia to germinate and become more susceptible to antibiosis. Consistent isolation of Trichoderma spp. from sclerotia buried in moist soil at these temperatures suggest that inactivation of sclerotia may in part be due to direct antagonism or even parasitism of sclerotia and newly formed mycelia, as well as to competition.

Inactivation of B. convoluta sclerotia by increased CO_2 concentrations produced by an active microflora must also be considered. Jackson (1966) found that germination of B. convoluta sclerotia was inhibited in ten percent CO_2 at 5-15° C. It is doubtful that CO_2 concentrations were present in my experiments to completely inactivate the sclerotia.

The above discussion suggests a possible means of controlling the disease in infested fields. Application of organic amendments or crop residues to field soil may prove effective in stimulating organisms antagonistic to B. convoluta, thus inhibiting primary colonization of rhizome tissue or senescent tissue as suggested by MacWithey (1967a). Relevant information in this vein has been reported by Henis and Chet (1968) who found that sclerotia of Sclerotium rolfsii were stimulated to germinate by soil amendation with various

nitrogenous materials. They correlated decrease in germination with increased numbers of antibiotic-producing organisms closely associated with the sclerotia. Inhibition of primary infection or colonization of rhizome tissue is important because once B. convoluta becomes established in iris tissue, growth of the fungus appears to be limited only by ambient temperature and the ability of rhizomes to wall off lesions with suberized barriers. MacWithey (1967a) observed that pure cultures of B. convoluta could be isolated from rhizome lesions that had been in contact with soil for six months or longer. Dosdall (1944) indicated some success had been reported by nurserymen who used a proper balance of crop rotation and use of certain mercurial fungicide dip treatments prior to planting in soil that had a previous history of the disease. The judicious selection of clean stock and planting in clean soil will not prevent Botrytis rhizome rot from occurring since it may be carried over as non-visable latent infections which are not eradicated by surface-active fungicides. Thus, there is clearly a need for an effective systemic fungicide in iris cultivation free of B. convoluta.

This study has provided new information on the disease cycle of Botrytis rhizome rot of iris. B. convoluta is a poor competitive soil saprophyte, so it must exist successfully as dormant sclerotia in dry soil or as mycelium in rhizomes. It was shown that sclerotia lost viability at low temperatures and even more so at higher

temperatures in moist unsterilized soil over a two-month period. However, sclerotia kept in dry storage lost viability more slowly. These data suggest that sclerotia of B. convoluta may not remain viable in field soil more than 2-3 months, depending on soil moisture levels and temperatures. Sclerotia produced in fall would serve to incite new infections only if disturbed and brought into contact with a rhizome before soil temperatures reached above the critical 10-15°C range. Summer survival of sclerotia appears logical at first; however, sufficient soil moisture would be expected to be present part of the time to cause premature germination and/or antagonistic microbial activity. Conidia produced in spring or fall on sclerotia or plant debris may find their way to iris plants, germinate, and colonize senescent tissue or infect juvenile buds directly. Rhizomes infected in fall develop necrotic or rotted areas which may include the entire rhizome, or smaller portions of it and apical buds or flowering buds may be destroyed by spring. Plants in the spring may show little or no effect if necroses are small and weather conditions favorable for rapid iris growth occur. Fungus growth is checked by rising soil temperatures and suberization of a tissue zone around infected areas. Apparently B. convoluta mycelium may remain in a semi-dormant state during summer months in these lesions and begin renewed growth in fall when soil temperatures permit. These latent lesions, especially small inconspicuous

ones, would be the major source of inoculum the following fall and they would also comprise an important means of disease dissemination especially when present on offsets used as propagative material.

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