PATHOGENESIS OF SCLEROTINIA SCLEROTIORUM (LIB.) DE BARY ON POTATO

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ABSTRACT

PATHOGENESIS OF SCLEROTINIA SCLEROTIORUM (LIB.) DE BARY ON POTATO

By

H. Charles Mellinger

The fungus disease of potato caused by Sclerotinia sclerotiorum (Lib.) de Bary was investigated on the Sebago variety. Symptoms are described and aspects of infection and disease development examined. The role of naturally ejected ascospores and mycelium growing from sclerotia in initiating infection was determined.

Stems of plants, infected in the field and greenhouse at the soil level with mycelially colonized corn meal-Perlite (CMP) medium, broke down and collapsed at the inoculation point. Lesions frequently were characterized by a pink border in the vicinity of the lesion perimeter. Five days following stem inoculation at the soil level, apical leaflets of plants showed marginal necrosis and curling although at this time breakdown at the inoculation point was negligible. One week after inoculation when stem breakdown began, leaf chlorosis and necrosis progressed upward from the basal lesions. The pattern of leaf chlorosis was suggestive of the ring rot disease leaf symptoms of potato.

Appearance of leaf lesions caused by mycelium growing from mycelially colonized blossoms was influenced by leaf age. On young leaves (apical plant area) lesions were tan to dark brown, enlarged slowly and were sharply delimited from healthy tissue; on old leaves (basal plant area) lesions were tan and enlarged rapidly; and on middle-aged leaves lesions were tan to brown with a chlorotic zone between the healthy and necrotic tissue.

Naturally ejected ascospores were essentially non-infectious on vigorously growing foliage of whole plants or on detached leaves at environmental combinations of 15 or 20 C, 98-100% relative humidity and at either 50-100 or 400 ft-c light. Ascospores infected potato blossoms as well as chlorotic or senescing leaves. Mycelium, growing either from colonized blossoms or leaves was infectious to vigorously growing leaves, stems and petioles.

The fungus, after initial establishment on either leaves or on stems at the soil level (20 C and high relative humidity) destroyed plant tissue rapidly at temperatures of 20 and 24 C and slower at 15 C. After similar initial establishment the fungus was not halted by low relative humidity, 10%, but advanced through the plant very slowly. Older stems, 6- and 12-weeks-old when inoculated at the soil level, broke down more rapidly than stems of 3-week-old plants. The fungus advanced through mature leaves more rapidly than through young leaves of the apical portion of the plant.

Growth of the fungus in host stems or leaves was localized in the lesion areas. Systemic responses to localized infection included necrotic areas on apical leaves, discolored vascular tissue and plugged xylem vessels which extended from the soil line lesion to the top of the plant. The fungus could not be isolated from any of these necrotic tissues away from the original lesion.

Mycelially germinating sclerotia were tested as inoculum sources both with and without mycelially colonized CMP medium on seed pieces and sprouts in field trials. Sclerotia were either placed on the seed piece surface at planting or 2 to 3 inches directly above the seed piece so that the growing sprout would contact the sclerotia prior to reaching the soil surface. No disease symptoms developed and yields and stand did not differ from noninoculated controls. Infection of stems by sclerotial inoculum could not be demonstrated.

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TO MY PARENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The disease on potato caused by the fungus Sclerotinia sclerotiorum (Lib.) de Bary is normally of minor importance but severe losses have been reported in the United States (Eddins, 1937), Ireland (Johnson, 1902) and Great Britain (Cotton, 1919). The pathogen is broadly distributed in the temperate zone of the Northern hemisphere often inflicting severe losses on a wide range of crops. The disease occurs less frequently in the tropical zone and in the Southern hemisphere temperate zone but occasionally causes material losses on crops of these areas (Reichert, 1958).

The Sclerotinia disease of potato was found in Michigan in 1966 at the Michigan State University Muck Experimental Farm at Bath (Potter and Hooker, 1966). The fungus occurs throughout the state and annually causes disease on many crops, with important losses to celery and lettuce. Modern day cultural practices which include irrigation and increased use of fertilizers, produce luxuriant vine growth creating a microclimate favorable for disease, e.g., high moisture levels, low light penetration, and low soil temperatures. Since this disease is minor on potato in Michigan at present this host may be relatively more resistant than other crops or for some reason has escaped

infection. Because of rapidly changing cultural practices, this disease may become a greater problem in the near future. More information is needed concerning symptoms and factors influencing infection of potato by S. sclerotiorum.

Pethybridge (1911) and Eddins (1937) believed potato plants were infected by Sclerotinia principally by means of airborne ascospores. This belief differed from earlier reports of de Bary (1887) and Johnson (1902) who observed that the fungus mycelium colonized organic matter prior to being able to parasitize and concluded that it must exist saprophytically before being capable of parasitism.

Pethybridge (1911), however concluded from field observations that the fungus infected the potato plant from spores on 1) older, shaded, yellowing leaves, 2) wounds left on the stalks by abscised leaves and 3) leaf axils where water frequently accumulates and where probably the spores could germinate easily. The basis for his disagreement with de Bary (1887) and Johnson (1902) was the frequent occurrence of aerial infection of stems which was not explained by de Bary's views. Pethybridge obtained aerial infection of potato plants in high relative humidity with apothecia suspended over the plants. He observed infection 3-4 weeks later on chlorotic basal leaves. The observations of Eddins (1937) and Moore et al. (1950) are in agreement with the findings of Pethybridge that ascospores cause the major portion of disease.

Eddins (1937) and Niederhauser (1946) observed potato stem infections at or just above the soil surface and the latter thought the infected plants had been wounded or injured. De Bary (1887) however reported that ascospores were noninfectious on wounded plant surfaces. Moore et al. (1950) reported a limited amount of infection directly from soil through colonization of organic matter on the soil surface. Mycelium growing on the soil and on diseased potato foliage (Eddins, 1937) caused disease on healthy plant parts. However, Lockwood (1960) showed that mycelium of S. sclerotiorum does not grow through natural soil in the absence of organic matter. Whether the mycelium in colonized organic matter which caused a limited amount of infection was from ascospores and/or mycelium growing from sclerotia was not determined.

Potato tubers in the field were free of infection even when foliage was severely diseased (Pethybridge, 1914; Cotton, 1919; Eddins, 1937). Bisby (1921) reported tuber breakdown by a sunflower isolate of S. sclerotiorum while Ramsey (1941) under experimental conditions obtained watery decay of tubers with S. intermedia and S. minor but not with S. sclerotiorum. The former organisms have since respectively been renamed S. sclerotiorum "Intermedia" and S. sclerotiorum "Minor" (Purdy, 1955). According to Ramsey (1941), S. minor caused rot of severely wounded tubers of the Bliss Triumph variety under high relative humidity, at 70 F but rarely at 40 and 32 F and S. intermedia caused

tuber decay at 70 and none at 40 or 32 F. Bustamente and Thurston (1964) reported in Colombia, South America that natural tuber infection occurred in the field on var. Tuquerrena and they duplicated the symptoms by planting wounded tubers in sterilized soil infested with S. sclerotiorum. Brown sunken lesions, a few mm to 2-3 cm in diameter, developed around the buds. White mycelium and black sclerotia were visible through breaks in the epidermis. Small tubers attacked by the fungus became mummified. The fungus produced a dry rot similar to that caused by Fusarium caeruleum (Lib.) Sacc. and Phoma faveata Foister. Rot developed conically into the tuber flesh from the bud, the flesh becoming dry and black and sharply delimited from healthy tissue. In Colombia tubers lie dormant in the soil up to 4 months after crop maturation. Long storage after maturity in the field soil may be a factor in the natural occurrence of tuber infection.

Symptoms of Sclerotinia disease on potato have been described in part by a number of workers. A more complete symptom description is desirable. The earliest symptom is a water-soaked lesion on the stem which becomes brown as the lesions enlarge up to 6 inches (Eddins, 1937). The earliest symptom (Pethybridge, 1910; Cotton, 1919; Lachaine, 1922) was development of white patches of mycelium on the stem surface. Under favorable weather conditions the fungus invaded the stalk and destroyed the living stem tissue. The stem cavity was filled with white mycelium and black

sclerotia of the fungus (Pethybridge, 1910; Cotton, 1919; Lachaine, 1922). Johnson (1902) in one of the earliest reports that S. sclerotiorum caused disease on potato observed pith destruction of the stem and the pith cavity later filled by mycelium and sclerotia of the fungus. The entire length of the stem was susceptible to the fungus and lesions formed anywhere on the above ground stalks. Chlorosis of foliage which followed development of stem lesions was uncommon (Pethybridge, 1910; Cotton, 1919; Eddins, 1937). Cotton (1919) noted that, because very little foliage becomes chlorotic in contrast to most stem diseases, infected plants are difficult to detect. Moore et al. (1950) and Lachaine (1922), however, reported some chlorosis of the foliage following invasion by the fungus of the plant stems.

Moore et al. (1950) noted under favorable weather conditions small watery lesions were produced on potato leaves, petioles and stems, which appeared very similar to lesions of the late blight disease. Frequently they found infections were initiated in old leaf spots, e.g., those caused by early and late blight disease of potato, the fungus subsequently producing large quantities of mycelium and sclerotia at the infection loci.

Invasion of plant tissue by the fungus is apparently not systemic and is limited to lesion areas. The fungus was always found on and within the necrotic tissue of stem lesions (Pethybridge, 1910; Lachaine, 1922). Eddins (1937)

reported that all but the lignified tissues of the invaded parts were destroyed and this invaded area subsequently filled with mycelium and sclerotia. Plants wilted when the xylem tissue was destroyed and eventually collapsed (Eddins, 1937). Pethybridge (1911) found that when two lesions occurred on the same potato stalk, the upper lesion was not caused by mycelium which passed through the internal stem tissue, but was a separately initiated infection with the stem tissue between the lesions free of mycelium.

Pethybridge (1911) reported disease development on potato was favored by moist conditions and when foliage was shaded. He also observed (1912) infection occurred during dry weather. Lachaine (1922) and Cotton (1919) reported humid weather favorable for fungus growth during which profuse mycelium and many sclerotia formed both on and within stems. Eddins (1937) reported severe disease in Florida during the cool and rainy growing seasons of 1933 and 1934, but very little loss during the hot and dry season of 1935. The disease advanced (Moore et al., 1950) when temperatures were not too high provided that the foliage was sufficiently full to accumulate and retain moisture. Partyka and Mai (1962) recorded a higher incidence of disease when the air and soil were moist than when the air and soil were dry.

The present investigation concerns certain aspects of infection of potatoes by S. sclerotiorum, including:

1) the relative roles of the ascospores and mycelium growing from sclerotia in pathogenesis, 2) relative susceptibility of stem, leaves and blossoms to infection from ascospores and mycelium, 3) the effect of temperature, relative humidity and plant age on disease development and 4) fungal growth in diseased plants.

Symptoms of disease were observed in the field on naturally and artificially inoculated plants and in the laboratory under controlled environment both with whole plants and detached plant parts.

MATERIALS AND METHODS

Plants were grown from tubers of the Sebago variety in the greenhouse in steamed soil (2:1:1, heavy Miami loam: sand:peat) at 22 C under greenhouse conditions of lighting.

One- to 3-inch sprouts were grown from tubers planted 6 inches deep in moist Sphagnum. Detached leaves were prepared with a 4 cm stem segment attached to the leaf petiole by severing stems between nodes. The stem segments were submerged in vials of water with the leaf extending over the vial lip.

Field plots were on a woody peat soil at the Michigan State University Muck Experimental Station at Bath, Michigan in a randomized block design with 25 tubers for each small plot in 4 replications.

Sclerotinia sclerotiorum isolated from naturally infected potato was maintained at 22 C on potato-dextrose agar (PDA), (200 g potatoes, 20 g dextrose, 20 g agar, distilled water to 1000 ml). The fungus was grown for inoculum either on PDA, on potato-dextrose broth (PDB), similarly prepared without agar, or on corn meal-Perlite (CMP) medium (400 and 250 g respectively in 1000 ml distilled water).

PDA discs, 8 mm diameter and 3 mm thick, of 5-day-old mycelium were cut with a sterile cork borer and placed on potato plant leaves. Mycelial mats from PDB were washed 2 times with sterile distilled water, placed into either fresh PDB broth or sterile distilled water and blended for 30 sec in a Waring blender. Plants were dipped into the suspensions. Mycelium with sclerotia from 10- to 15-day-old CMP cultures was used directly as inoculum.

Sclerotia grown on PDA or CMP medium and maintained at 22 C from 6 to 12 months were either used directly as inoculum or incubated in petri dishes with 15 ml sterile distilled water at 15 C and 400 ft-c continuous light for ascospore production.

In order to obtain foliage infection with ascospores, apothecia were placed adjacent to plants in a polyethylene chamber. Ejection and dissemination of spores were aided by puffs of air across the apothecial cup surface.

Ascospores puffed onto a sterile glass petri dish lid were suspended in a small amount of sterile distilled water. The suspension was atomized onto the plant.

Spore germination on inoculated leaves was determined by staining bleached leaves with Carbol-fuchsin and examining the leaf surface with the light microscope (Daft and Leben, 1966).

Breakdown of stems following inoculation at the soil level with mycelially colonized CMP medium was evaluated

as follows: 0% = healthy, 1-3% = 1-5 mm superficial lesions, 3-10% = 5-15 mm superficial lesions, 10-25% = cortical breakdown, 25-60% = pith showing breakdown and 60-100% = stem almost or completely collapsed.

The fungus was isolated from plants by the following procedure. Segments of leaves and stems were surface sterilized in 0.5% sodium hypochlorite for 1 or 5 min (leaves or stems, respectively); rinsed 2-3 times in sterile distilled water, blotted dry on sterile filter paper and placed on PDA in petri plates.

Tissue sections of the plants were made with the Hooker fresh tissue microtome (Hooker, 1968) and examined by phase and light microscopy.

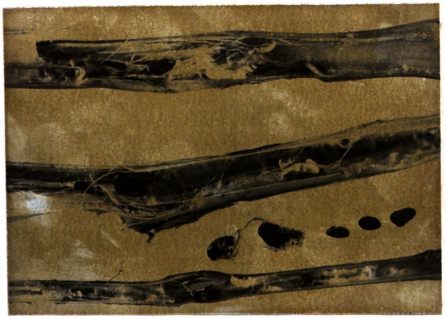
EXPERIMENTAL RESULTS

Symptomatology

Field Symptoms.--Symptoms of naturally infected plants in the field were similar in part to earlier descriptions. Stem lesions were tan to brown, water-soaked, and concentric rings were usually visible within the lesion. Frequently a characteristic pink border outlined the lesion perimeter. In advanced stages the stem became discolored with concentric patterns, disorganized and the pith collapsed (Fig. 1-A). The necrotic area of the stem was hollow, and contained white wefts of mycelium and black sclerotia (Fig. 1-B) as described previously (Pethybridge, 1910; Cotton, 1919; Lachaine, 1922; Eddins, 1937). Leaves on stems with such lesions always became chlorotic and necrotic. A lemon-yellow chlorotic area developed between the healthy and necrotic portion of leaves. This symptom was quite similar to that produced by the ring rot organism.

Stem lesions of plants inoculated in the field with colonized CMP medium resembled those following natural infection. Lesions were initiated 5 days after inoculation as small black flecks enlarging later and becoming tan in the center with a darker-green and water-soaked periphery.

A



B

Fig. 1 Typical symptoms of potato naturally infected by S. sclerotiorum. (A) stem lesions containing the characteristic concentric rings; (B) mycelium and sclerotia within lesions of halved stems.

As lesions enlarged the necrotic stem area became hollow and usually contained white wefts of mycelium and sclerotia of S. sclerotiorum (Fig. 1-A, B).

Seven days after stem inoculation apical leaves became necrotic. Leaflet tips and/or margins became water-soaked and were greenish-black. When dry they were light tan to reddish-brown in color (Fig. 2). Lesions on some leaflets were delimited by the veins. Necrosis advanced to the center and base of the leaflets occasionally involving the entire leaf. Necrotic apical leaflets and leaves were randomly distributed and the number of necrotic leaves varied between plants. Of the 100 inoculated plants, 74 developed apical leaf symptoms, while all non-inoculated plants lacked symptoms.

As cortical decay at the soil-line (Fig. 3) increased leaves progressively became chlorotic from the base to the apex of the plant. Lower leaves initially curled at the margins and tips. This was followed by chlorosis, flaccidity, and eventually total necrosis (Fig. 4-A, B, C). In a few cases stem breakdown was sufficiently rapid so that the stem collapsed when leaves were only slightly chlorotic.

Detached Leaves.--Similar leaf symptoms followed inoculation with mycelially colonized flowers or with agar discs of mycelium. A very small tan lesion formed initially, later enlarging to about 1 cm in diameter,

Fig. 2 Necrosis of apical leaves of potato 5 days after inoculation of potato stems at soil line with S. sclerotiorum.

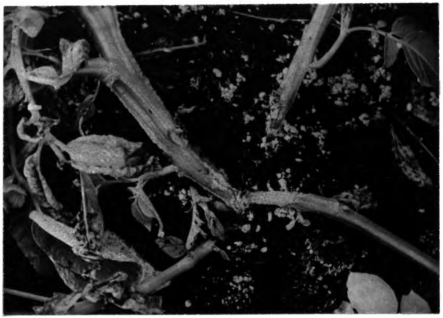


Fig. 3 Lesions 18 days after inoculation of potato stems at the soil level with S. sclerotiorum.

A

B

Fig. 4 Leaf symptoms after artificial inoculation of potato stems at the soil level with S. sclerotiorum. (A) chlorosis and necrosis of leaf 2 weeks after inoculation; (B) diseased plant with some stalks which show extensive chlorosis and necrosis of leaves;

Fig. 4 (cont'd.) (C) chlorosis and necrosis of leaves 1 week after inoculation in greenhouse experiment--noninoculated plant on left, moderately and severely diseased plants, respectively on right.

becoming water-soaked, and tannish-green in color. A narrow darker-green area usually surrounded the central portion if the lesion was water-soaked. The lesion perimeter when dry was brown to black.

Lesions of young leaves enlarged slowly to about 1-2 cm in diameter in 3-5 days, and were dark tan to brown-black in color. Dark concentric rings were frequently visible within the lesions (Fig. 5-A). Lesion enlargement was frequently retarded by veins. Occasionally black, 1 to 3 mm diameter flecks formed in the green leaflet tissue adjoining the lesion.

Lesions of old leaves usually were tan, rarely becoming dark and lacked concentric rings. Lesion enlargement was rapid and unrestricted by veins. The leaf tissue usually became necrotic with little to no chlorosis (Fig. 5-B) but when environmental conditions for disease seemed less than optimum basal leaves were chlorotic.

On leaflets from the middle portion of the plant a chlorotic zone was usually present between the necrotic and healthy leaf areas. The chlorotic zone increased in width on leaflets of leaves nearer the base of the plant and in some cases entire leaflets adjacent to the inoculated leaflet were chlorotic (Fig. 5-B). In leaflets closer to the plant apex the chlorotic zone was less intense and was absent in the uppermost leaves of the plant (Fig. 5-A). Necrotic areas when dry were crinkled and distorted (Fig. 5-B).

Fig. 5 Detached leaves inoculated with PDA discs of S. sclerotiorum. (A) Left: noninoculated leaf; Center: leaf lesions of apical leaf small and sharply defined from healthy tissue; Right: leaf from middle of plant with lesion large and diffuse with chlorotic zone between healthy and necrotic tissue; (B) Left: leaf from middle part of plant; Center: leaf from near base of plant; Right: leaf from base of plant with the entire inoculated leaflet crinkled and distorted and the adjacent leaflets showing chlorosis.

Fig. 5-A

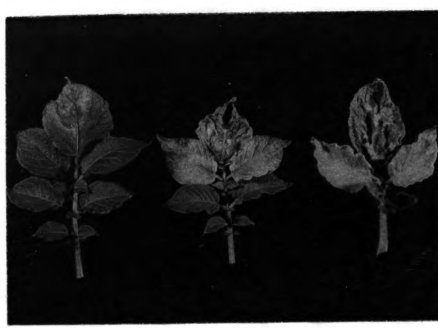


Fig. 5-B

Blossoms.--Attached blossoms were inoculated with naturally ejected ascospores at 20 C in 95-100% relative humidity. The inoculated flowers became senescent approximately twice as rapidly as noninoculated control flowers. Infection and colonization did not hasten blossom drop. First symptoms were distinct brown-black necrotic spots on the petals, later coalescing as mycelial growth became visible. On noninoculated blossoms the entire corolla gradually became dark with the necrotic areas more diffuse than on inoculated blossoms.

Infectivity of Ascospores

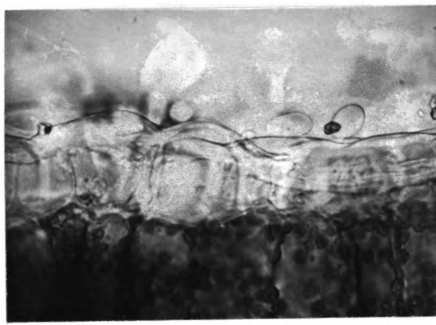
Infection of Vigorously Growing Leaves.--Vigorous plants in the blossoming stage were exposed to infection by naturally ejected ascospores and subsequently maintained in a polyethylene moist chamber at 20 or 25 C for 12 days. The experiment was repeated 3 times with 20 plants inoculated per experiment. No lesions formed. Ascospore germination and infection of leaves were further studied with detached leaves.

Apothecia placed on leaves so the hymenium was perpendicular to the leaf surface ejected ascospores over the surface thus creating an ascospore concentration gradient. Noninoculated leaves were compared for symptom development and senescence.

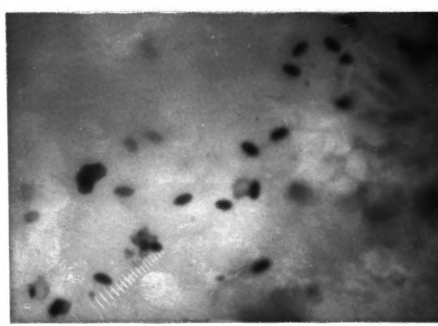
Spore germination on detached leaves from vigorously growing plants after examination at 24, 48, 96, 144 and

in a few cases 240 hr was extremely low varying from < 1 to 3% (Fig. 6-A, B, C). In contrast spores in glass distilled water germinated 90-95% within 12 hr. Spore penetration was observed either on bleached whole leaves or on leaf cross sections. Penetration was less frequent than germination. Approximately 1 out of 200-400 spores penetrated, or 1 out of every 2-4 spores germinating (Fig. 6-D). Germination and penetration was studied at 50-100 and 400 ft-c during a 10 hr photoperiod, 15 and 20 C, and 20-25 and 98-100% relative humidities. For the treatments of high light and high relative humidity, at least 200 spores were observed in 3 different areas/leaf for each environmental condition. Fifty spores in 3 different leaf areas were observed in the remainder of environmental conditions.

The germinability of the spores on the leaf surface was determined. Pieces, 1 cm sq, were cut from leaves which had been exposed to ascospores for varying lengths of time, and were submerged in sterile distilled water for 24 hr, then sectioned with the microtome and observed microscopically. Ascospore germination increased to at least 50% and penetration increased 10-20% (Fig. 6-E) following submergence. These rates were similar regardless of the environmental conditions (described above) in which the inoculated leaves were maintained prior to immergence. Germination and penetration rates did not differ in intact nonimmerged control leaves. When a glucose solution, 320 ppm, was substituted in the 24 hr distilled water

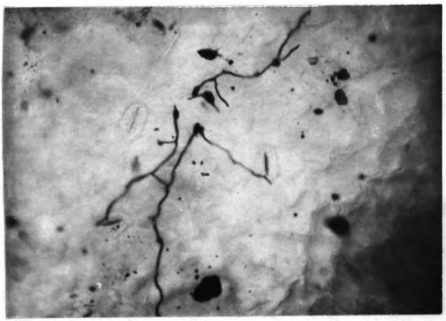


A

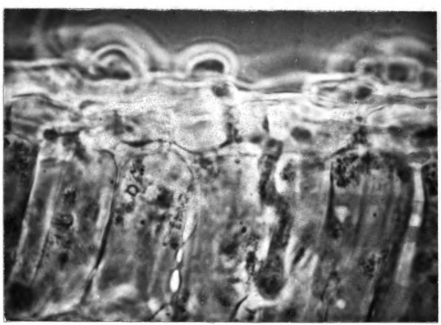


B

Fig. 6 Detached potato leaves inoculated with naturally ejected ascospores of S. sclerotiorum. (A) cross-section of leaf with ungerminated ascospores; (B) ungerminated ascospores on bleached leaf surface stained with Carbol-fuchsin;



C



D

Fig. 6 (cont'd.) (C) germinated ascospores on bleached leaf surface stained with Carbol-fuchsin; (D) cross-section of leaf showing penetration of hyphae from germinated ascospore;

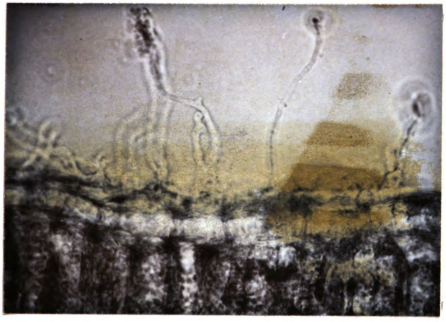


Fig. 6 (cont'd.) (E) germination and penetration of ascospores on section of inoculated leaf.

immergence treatment germination percentage could not be determined because of vigorous mycelial growth from the germinated spores. Germ tubes from ascospores penetrated the leaf tissue and severe infection followed with production of hyphae throughout the epidermis and palisade cells.

Infection of Wounded Vigorously Growing Leaves.--Leaves, stems or petioles of 10 plants were wounded by partially breaking them. Wounds were inoculated with naturally ejected ascospores and maintained in a polyethylene moist chamber at 20 C, 95-100% relative humidity and 300-500 ft-c continuous light. Ten nonwounded plants were exposed to ascospores and maintained with the wounded plants. Infection developed at nearly all the wounded sites. Mycelium grew on the wounded areas and produced lesions and typical symptoms. No infection was observed on the nonwounded plants.

Infection of Weakened Leaves.--In two trials, 6- to 8-week-old plants with chlorotic naturally senescing leaves attached to the lower stem and/or similar leaves which had abscised were exposed to ejected spores and maintained in a polyethylene moist chamber at 22 C.

Three to 4 days after inoculating leaves of 30 plants, considerable mycelium was visible on chlorotic attached leaves and on abscised leaves on the soil

surface. Attached leaves almost always abscised after colonization.

Plants predisposed in low light, 5-15 ft-c, for 5 days before inoculation with a spore suspension developed many chlorotic leaves and leaves were readily colonized by Sclerotinia. Stems became infected at the soil-line by mycelium from colonized leaves when these leaves abscised and fell to the soil surface or, if the colonized leaves remained attached, at the area of contact with the stem.

Leaf Infection From Colonized Blossoms.--The role of flowers in disease development was investigated using both detached and attached flowers in full bloom. Detached flowers inoculated by naturally ejected ascospores and noninoculated flowers were placed on detached leaves from vigorously growing plants in two trials. In one trial at 20 C, 98-100% relative humidity and 50-70 ft-c continuous light 100% of the leaves became diseased and in the other at 400 ft-c per 14 hr day 63% infection was obtained. Leaves with noninoculated flowers placed on them were healthy.

Further evidence that colonized blossoms served as inoculum sources was obtained by naturally ejecting ascospores onto 20 6-week-old plants either with blossoms or with only buds and maintaining them in a polyethylene moist chamber at 20-25 C.

Colonized blossoms dropped onto the leaves and lesions developed on the leaf at all the sites of the fallen blossoms (Fig. 7-A, B). Lesions were absent on leaves with noninoculated blossoms or buds and where blossoms were absent.

In another experiment using blossoming plants a distilled water suspension of ascospores was atomized onto 1) only the foliage, 2) only the blossoms, and 3) the foliage and the blossoms of 6-week-old plants. Plants atomized with distilled water were maintained as controls for each type inoculation. Inoculated plants were maintained in the same environment as the previous experiment. Lesions did not develop when only the foliage was atomized with ascospores. When only blossoms were inoculated, infection consistently developed at the landing site of the abscised blossoms on the leaf (Fig. 7-A, B). Mycelium growing from the inoculated blossoms of treatments 2 and 3 caused infection when the blossoms were placed on the foliage of noninoculated plants. Lesions did not occur when noninoculated blossoms were placed on inoculated or noninoculated foliage.

Effect of Temperature on Disease Development.--Potato plants 6-weeks-old were exposed to infection by placing 4-5 g of CMP medium around the base of the plants maintained at temperatures of 15, 20 or 25 C and a relative

A

B

Fig. 7 Disease by mycelium of S. sclerotiorum from colonized blossom on terminal leaflet. (A) chlorosis and necrosis of leaflets 5 days after terminal leaflet inoculation by the colonized blossom; (B) stem collapse at the node of the blossom inoculated leaf 8 days after terminal leaflet inoculation by the colonized blossom.

humidity of 95-100%. The soil was maintained at maximum water holding capacity.

The first symptoms of disease appeared in the stem region in contact with the inoculum 3-4 days later as small black flecks which increased in size with time as the fungus invaded the stem tissues. In severely diseased stems the tissue of the basal stem became partially or completely disorganized and frequently collapsed. The foliage became chlorotic and necrotic varying in amount with severity of the basal stem lesion. (For a detailed account of the symptom pattern refer to the section on Symptomatology.)

Disease severity increased as temperatures rose (Table 1). At 15 C lesions were small (1-10 mm) and usually superficial. A blackened area usually surrounded the slightly sunken tan center of the lesion. Lesions of 11 of the 15 inoculated plants appeared walled-off and the fungal growth apparently halted. Stem lesions at 20 C were more extensive than at 15 C with 80% of stems girdled and 60% collapsed. The disease was most severe at 25 C with 70% of the stems collapsed. The lesions were more water-soaked than at 15 and 20 C and had a brownish-green color. The walled-off lesions were slightly larger at 20 and 25 C than at 15 C.

Disease development as influenced by temperature was also studied by placing blossoms colonized by mycelium or PDA discs of mycelium on leaflets of whole plants. After the fungus was established in leaflets, the plants were

TABLE 1. Influence of temperature on S. sclerotiorum infection of potato stems inoculated with colonized CMP medium.

	Severity[a]			No. stems with lesion advance restricted		
	15 C	20 C	25 C	15 C	20 C	25 C
Inoculated	25	60	70	11	3	3
Noninoculated	0	0	0	0	0	0

[a]Severity rating is average of 15 plants 13 days after inoculation.

Disease Index: 0% = healthy; 1-3% = small superficial lesions (1-5 mm); 3-10% = 5-15 mm superficial lesions; 10-25% = cortical breakdown; 25-60% = pith becoming disorganized; 60-100% = stem almost or completely collapsed.

transferred to temperature rooms and observed at 15, 20 and 25 C. Optimum environment for disease was 20 or 25 C at 95-100% relative humidity. The fungus spread, within 48 hr, from 1 infected leaflet to the whole leaf, incited a stem lesion, and the diseased leaf abscised. The abscised diseased leaf later dropped onto healthy foliage and mycelium from the infection subsequently caused disease on this foliage. Within the next 48-96 hr the entire stem collapsed. Almost all foliage was either chlorotic or necrotic and the stem lesion had advanced nearly to the soil line. This was essentially typical although rate of disease progression varied slightly in 4 similar experiments. At 15 C the fungus grew more vigorously on plants than at 20 or 25 C but tissue breakdown was less than at the higher temperatures. Plants with the fungus well-established in leaflets were transferred from an environment of 20 C and 95-100% relative humidity to an environment of 15 C with water misting 25 of every 30 min. At 15 C vigorous mycelial growth on the plants produced colonies with hyphae frequently extending outward 1-2 cm from the plant. Tissue breakdown was usually superficial and only occasionally extended into the cortex and vascular area.

Effect of Relative Humidity on Disease Development.--Infection followed inoculations with mycelially colonized CMP at high relative humidity, 95-100%. Plants were not

infected at 10% relative humidity at 20 C. When a plant infected at the leaflet, pedicel, or stem was transferred from 95-100% to a 10% relative humidity the rate of lesion enlargement decreased but was not halted. The following observations are based on disease development in three experiments at 20 C and each involving 10-20 plants.

Infection was established in the attached flowers and pedicels 4 days after being dipped into a mycelial suspension and subsequently enclosed in a polyethylene bag with damp paper towels to provide high humidity. When mycelium was visible on the flowers and pedicels, bags were removed and spread of the disease observed at approximately 10% relative humidity. In infected 6-week-old plants, approximately 24 inches tall, at least 30 days were required for killing the plant. Often disease was slower with only the upper-half of the plant necrotic after one month and in a few plants the fungus advanced only about 7 cm beyond the flowers and pedicels. In part this variation in rate of lesion enlargement may have been due to the density of the mycelium of the colonized flowers and pedicels.

Responses were similar when plants with infected leaflets or leaves were transferred to 10% relative humidity. The lesion advanced up and down the stem frequently collapsing the portion of the plant above the lesion.

Susceptibility of Plants and Leaves of Different Ages.--Stems of 3-, 6- and 12-week-old plants were inoculated at the soil line with mycelially colonized CMP medium. However a higher concentration of inoculum, approximately a 4 fold increase, was used for 3-week-old plants. Collapse was very rapid, 100% after 6 days (Table 2), and the invaded area extensive in the 12-week-aged group of plants. Plants 6-week-old were only partially broken down by this time and the lesion size was smaller than those of the 12-week-group. In a later trial with 3-week-old plant stems the rate of breakdown was slightly more rapid than that of the 6-week-old plants. Control plants of each age group were healthy.

Severity of disease on inoculated leaves varied with leaf maturity. Plants were dipped into mycelium suspended in fresh PDB and subsequently incubated in the polyethylene moist chambers at 20 C. The mean number of basal chlorotic leaves per plant at 2, 4, and 6 days after inoculation was 2.2, 3.1 and 5.0, respectively, on the 30 inoculated plants and 0, 2.2 and 2.2, respectively, on the 20 plants dipped into only PDB. Chlorosis and subsequent abscission beginning with the lower leaves continued up the plant and the number of chlorotic leaves increased with time. Small lesions, 1-3 mm, developed on the leaves of the middle portion of plants and some enlarged later to 1 cm. Apical leaves of inoculated plants were not visibly infected but

TABLE 2. Influence of plant age on severity of potato stem collapse by S. sclerotiorum in CMP medium.[a]

Age (weeks)	No. of plants inoculated	Severity[b]	
		6 days after inoculation	12 days after inoculation
		%	%
3[c]	18	52	88
6	14	40	64
12	16	100	100

[a]Inoculated plants maintained at 20 C and 95-100% relative humidity.

[b]Disease Index: 0% = healthy; 1-3% = small superficial lesions (1-5 mm); 3-10% = 5-15 mm superficial lesions; 10-25% = cortical breakdown; 25-60% = pith becoming disorganized; 60-100% = stem almost or completely collapsed.

[c]Inoculum concentration for 3 week plant was approximately 4 times that used for the 6 and 12 week evaluation.

basal leaves were generally chlorotic and abscised more rapidly than the noninoculated ones.

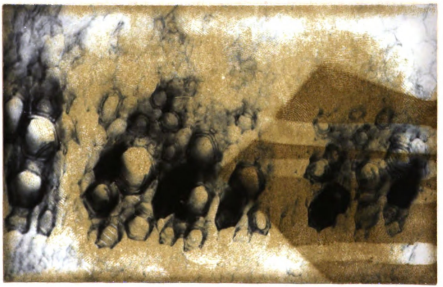
The influence of leaf age on symptom type was further studied with detached leaves inoculated with PDA discs of mycelium or ascospore-inoculated blossoms incubated at 20 C and 98-100% relative humidity. Both inoculum types were infectious (Table 3). Leaf age did not influence frequency of infection. Lesion enlargement subsequent to infection initiation was influenced by leaf age. Lesions on younger leaves in the upper part of the plant were dark brown to black, the lesion periphery was quite distinct and a chlorotic margin around the lesion was usually absent while lesions of older leaves in the lower part of the plant were more tan in color and quite diffuse with sometimes large areas of chlorosis between the necrotic and healthy leaf areas (Fig. 5-A, B).

Fungal Development.--About 50% of nonwounded plants with 2-5 cm stem lesions 6 days following soil line inoculations contained discolored vascular tissue and plugged xylem vessels which extended from the soil line lesion to the top of the plant (Fig. 8-A, B). At this time many leaves were chlorotic and flagging. S. sclerotiorum could not be isolated on PDA medium from fragments of the discolored vascular tissue.

TABLE 3. Infection of detached leaves inoculated by PDA discs of S. sclerotiorum mycelium or ascospore inoculated flowers.

Inoculum type	Inoculated leaves No.	Infected leaves	
		5 days	10 days
		No.	No.
Infected Blossoms			
Inoculated	23	7	16
Noninoculated	15	0	0
PDA discs			
Inoculated	120	96	119
Noninoculated	30	0	0

A



B

Fig. 8 Development of S. sclerotiorum and resulting symptoms within the plant. (A) pith and vascular necrosis of stems 6 days after inoculation at soil level; (B) cross-section of petiole of apical leaf with necrotic and plugged xylem vessels 6 days after soil line inoculation.

S. sclerotiorum was not isolated from cross-sections except within 2.3 cm of the periphery of the basal lesion, even though all but the apical leaves were chlorotic or necrotic. Isolations were attempted on PDA medium from stem sections 2, 8 and 15 cm above the basal lesion, the apical area, petioles and chlorotic and necrotic leaves.

In the field where symptoms developed on the apical leaves almost concurrently with the basal lesion development, no S. sclerotiorum was obtained from the chlorotic and necrotic area of the leaves when they were placed in petri plate moist chambers.

In leaf lesions the fungus grew only from the tan-green to tan-brown lesion center and not in the black-green peripheral areas of the lesions. Hyphae grew directly above and beneath the epidermis causing gradual dissociation of the cells (Fig. 9). Hyphae were not observed inter- or intra-cellularly in the mesophyll until the tissue was almost completely disorganized. In the dark peripheral zone the cells had not lost integrity but showed signs of necrosis. A narrow band of shrunken, heavily pigmented cells separated the dark peripheral zone from the healthy tissue.

Distinctive peripheral zones were inconspicuous or absent around some lesions. Hyphae in this lesion type advanced above and beneath the epidermis with chloroplasts of adjacent palisade cells gradually darkening imparting a tan-brown color to the entire lesion.

Fig. 9 Hyphae of S. sclerotiorum growing between the epidermis and mesophyll in leaf cross-section inoculated with agar disc of mycelium.

Infected leaves generally abscised within 5 days after inoculation or became chlorotic and abscised when the petiole base was in the path of an advancing stem lesion. Neither cellular necrosis nor fungus hyphae were observed when cross-sections of these petioles were examined with the microscope.

Infectivity of Mycelium Growing from Sclerotia

Seed Piece Infection.--Preliminary experiments of seed piece inoculation with mycelial suspensions were conducted in the laboratory. Tubers were surface sterilized in 1.0% sodium hypochlorite for 20 min, rinsed 3 times in sterile distilled water and sliced 8 mm thick. The slices, placed into sterile petri plate moisture chambers containing filter paper and 3-5 ml distilled water, were exposed to infection by placing either mycelium suspended in 0.5 ml fresh PDB or in 0.5 ml water in the center of the slice.

Diseased slices developed a watery mass of dissociated cells and dense mycelium grew on the slice surface. Brown pigmented areas were scattered in the inoculated tissue. Decreased mycelial growth was frequently associated with an increased area of brown pigmented cells.

Infection was influenced by temperature although results were somewhat erratic. Tuber slices inoculated with mycelium suspended in distilled water usually suberized, wound healed, and infection was infrequent. Mycelium

suspended in PDB produced extensive dissociation of cells of the tuber tissue. Tissue dissociation was rapid at 20 and 25 C and reduced at 6 and 15 C (Table 4). Controls of either PDB alone or only distilled water on tuber slices appeared healthy. Slices from tubers stored up to 8 months were generally more susceptible than fresh tubers and were frequently completely broken down in 8 days at the higher temperature.

Since mycelial suspensions were infectious on tuber slices at 15-25 C the possibility existed that mycelium growing from sclerotia could also cause seed piece infection. Sclerotia collected from potato vines were placed between the 2 halves of surface sterilized tubers and held together with rubber bands. The sclerotia were either washed, surface sterilized in 0.5% sodium hypochlorite (1 min) or untreated. After 7 and 21 days no infection had developed and the cut surfaces were suberized and wound-healed. Some mycelium was evident on the healed surfaces but S. sclerotiorum could not be recovered in isolation on PDA from the tuber tissue nor from the healed surface.

In field experiments seed piece surfaces were each placed in contact with 12 culture-grown sclerotia and in another treatment sclerotia were supplemented with 15 g of colonized CMP medium. No symptoms of disease were obtained and yields were not affected (Table 5).

TABLE 4. Susceptibility of tuber slices to dissociation by S. sclerotiorum[a] as influenced by temperature and age.

Temperature	Volume of dissociated tissue[b] in tubers of different ages[c]			
	0 Months	5 Months	6 Months	8 Months
4	0	4.2	0.2	0
15	0	0	1.9	6.2
20	0.5	0	4.9	12.8
25	4.5	1.0	2.6	20.2

[a]Slices inoculated with 0.5 ml mycelium suspended in fresh PD broth.

[b]Dissociation measured 8 days after inoculation. Volume (cm^3) calculated using $V = \pi r^2 h$ where V = volume in cm^3, r = radius on slice of dissociated cells and h = depth on slice of dissociated cells.

[c]Tubers stored after harvest at 4 C until for periods indicated.

TABLE 5. Stand and yield of potatoes following seed piece surface inoculation with S. sclerotiorum sclerotia in muck soil field trials.

Inoculum type	Stand[a]	Yield[b]
	%	lbs.
Noninoculated control	94	111.5
Sclerotia	100	105.0
Sclerotia supplemented with CMP medium	98	122.4

[a]Data were taken one month after planting. Total plants in 4 replicates were 100.

[b]Data were taken 4 1/2 months after planting. Stands and yields were not significantly different between treatments.

Sprout Infection.--Mycelial suspensions in PDB or 1 cm sq PDA blocks of mycelium were placed on sprouts 1-2 inches long. Sprouts were neither discolored nor visibly affected after 6 days although the mycelium was still viable when transferred to fresh PDA. Sprouts wounded by needle scratches and inoculated with the PDA blocks were not visibly different after 6 days from nonwounded inoculated sprouts.

In other trials sprouts were planted in soil or in damp Sphagnum moss infested with the colonized CMP medium. Results were similar in either case. Only data from inoculum incorporated into Sphagnum moss are presented (Table 6). Lesions were 0.1 to 1.0 cm in diameter, black in color, occasionally raised or sunken and very firm (Fig. 10). Apical meristems of sprouts were severely necrotic, and cells were often indistinct containing large concentrations of brown to black pigment. Hyphae were present in the necrotic meristematic cells and pigmented cells in other areas of the infected sprouts (Fig. 11). The pigmented and nonpigmented areas were very distinct.

Approximately 58% of the sprouts were infected after 12 days and sprout growth was reduced by about one-half. Root growth from diseased sprouts was reduced approximately 10 fold. When tubers with infected sprouts were planted in soil, stands were not reduced. Only a slightly longer emergence period was required for diseased sprouts. It is

TABLE 6. Infection of potato sprouts in moistened Sphagnum moss infested with CMP medium colonized by S. sclerotiorum mycelium.

	No. infected sprouts after 12 days[a]	Sprout length	
		6 days	12 days
		cm	cm
Noninoculated[a]	0/37	8.3	13.8
Inoculated[a]	34/58	2.3	8.8

[a]Fractions indicate number of sprouts infected over the number inoculated.

Fig. 10 Infection of tuber sprouts by S. sclerotiorum and reduction of sprout and root growth. Noninoculated tuber on left.

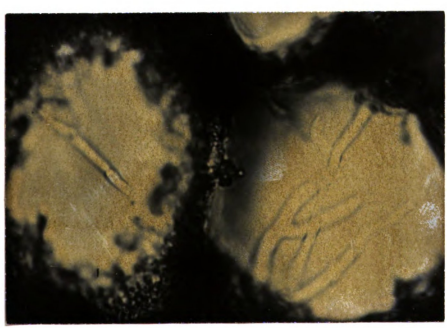


Fig. 11 Hyphae of S. sclerotiorum within cells of tuber sprout.

not known whether the diseased sprouts recovered or new secondary sprouts formed.

Sprout infection in field trials was studied by placing inocula approximately 2 inches directly above the planted seed piece to provide contact with the emerging sprout. For this 12 culture-grown sclerotia were used over each seed piece and in another treatment the 12 sclerotia were supplemented with 15 g of mycelially colonized CMP medium. Inocula were covered with 1-2 inches of soil. No symptoms of disease were observed. Differences were not significant between stand or yield treatments (Table 7).

Ten field collected sclerotia each were placed in contact with 10 1- to 2-inch sprouts in greenhouse experiments. Sclerotia were held against the sprout by the soil. Plants were watered 1-2 times daily. No infection or other features different from the controls was observed.

Stem Infection.--Stems of 4-week-old plants were inoculated in greenhouse tests either at the soil level or 1-2 inches below the soil surface with mycelially colonized CMP medium diluted 1:3 with soil.

Cortical breakdown and collapse of stems was more severe with above- (Fig. 12) than below-ground inoculation. However lesions containing concentrated brown pigments occurred less frequently in above- than below-ground inoculations.

TABLE 7.--Stand and yield when sprouts were exposed to S. sclerotiorum sclerotia in muck soil field experiments.

Inoculum type	Stand[a]	Yield[b]
	%	lbs
Noninoculated control	94	113.2
Sclerotia	95	99.3
Sclerotia supplemented with CMP medium	93	119.6

[a]Data were taken one month after planting. Total plants in 4 replicates were 100.

[b]Data were taken 4 1/2 months after planting. Stand and yields were not significantly different between treatments.

Fig. 12 Stem lesions caused by S. sclerotiorum mycelium. The stem on the left is healthy with severity progressively increasing from left to right.

Generally the amount of chlorotic foliage was correlated with the severity of the basal lesions. This was reflected in the number of chlorotic leaves as compared to the total number of leaves per plant (Table 8).

Three to 5 sclerotia on the soil surface were placed either in contact with stems removed or 1-2 inches from stems of 4- to 6-month-old plants. In the latter case chlorotic and senescent potato leaf litter was placed in contact with both the stems and the sclerotia. In another trial sclerotia wrapped in leaf litter were placed against the stems. Controls of both noninoculated stems and stems with only leaf litter were maintained. The inoculated plants were maintained in a polyethylene moist chamber at 20 C or in an outdoor shade house. Plants in the shade house were misted 3 times daily to maintain moisture. Twenty-two plants were used per treatment and the experiment was repeated with 10 plants per treatment. No infection, mycelium growing from sclerotia, or litter colonization was observed in 1 month.

Litter of a few inoculated plants maintained in the moist chamber was colonized with dense mycelial growth around the stem but no infection occurred. Sclerotia germinating to produce apothecia were common.

TABLE 8. Leaf chlorosis following stem inoculation with S. sclerotiorum[a] either on the soil surface or 1 to 2 inches below the surface.

Inoculum type	Chlorotic / Total Leaves[b]	Disease Index[c]
		%
Noninoculated	0.5/11.0	0
CMP medium at soil line	7.1/9.3	61
CMP medium 1-2 inches below soil line	5.5/11.0	39

[a]Inoculations with CMP medium colonized by mycelium and data was taken 6 days after inoculation.

[b]Data is mean of 15 plants per treatment.

[c]Disease Index: 0% = healthy; 1-3% = small superficial lesions (1-5 mm); 3-10% = 5-15 mm superficial lesions; 10-25% = cortical breakdown; 25-60% = pith becoming disorganized; 60-100% = stem almost or completely collapsed.

DISCUSSION

Symptoms of the disease of potato caused by S. sclerotiorum are described. Chlorosis of foliage was common in the field and always occurred in the greenhouse following stalk lesion development. Pethybridge (1910), Cotton (1919), and Eddins (1937) reported such chlorosis uncommon. However Lachaine (1922) and Moore et al. (1950) reported some chlorosis of foliage following development of the stalk lesion. The relation of the symptom of foliage chlorosis to disease development was not elucidated by either worker. From my trials the pattern of chlorosis was a mottle but at less than optimal conditions the leaves became chlorotic and subsequently necrotic inward from the margin, resembling the foliage symptom of ring rot disease of potato. The leaves progressively became chlorotic from the developing basal lesion to the plant apex.

A symptom not previously reported was blight of apical leaves which appeared 5 days after inoculation of stalks at soil level with S. sclerotiorum. The basal lesion was superficial without obvious tissue breakdown at the time of the blight appearance. Apical leaves became necrotic concurrently with breakdown of stem tissue of the basal lesion.

Apical leaf lesions when watersoaked appeared very similar to leaf symptoms of late blight but the lesions when dried were tan to brown and curled; this stage was clearly distinct. The fungus could not be isolated from apical leaf lesions. Demetriades (1950) and Overell (1952) have, however, reported a toxin is secreted by S. sclerotiorum. The former reported culture filtrates caused rapid irreversible wilting on potato and other crops and leaf curling on fig. Overell reported that only filtrates of cultures older than 17 days contained toxin as determined by estimation of the fresh weight loss of carrot discs and increase of reducing substances and organic acids in the filtrate surrounding them. They made no attempt to demonstrate the toxin in the host-pathogen interaction and therefore evidence for a toxin in this disease is not conclusive. My observations would support the hypothesis that a toxin or an antimetabolite in pathogenesis may be formed in a diseased plant. Although apical leaf necrosis was observed only on artificially inoculated plants and not on naturally infected plants this symptom probably occurs and should be anticipated in a scrutinizing search.

Another unreported symptom was a pink colored ring frequently formed in the vicinity of the perimeter of the stem lesion. The pink color associated with tissue decay is also common in the celery disease caused by S. sclerotiorum (Moore et al., 1949).

Symptoms differed depending on age of leaves and stems. Younger leaves and stems were more resistant to invasion by the fungus since lesions were restricted and small while on older leaves and stems, lesions enlarged rapidly. Severity of leaf chlorosis and necrosis progressively increased with leaf age. Although sprouts and tubers were infected under laboratory conditions with high amounts of inoculum no symptoms of disease were observed in the field.

My observations on the stalk breakdown were similar to earlier observations (Pethybridge, 1911; Cotton, 1919; Lachaine, 1922; Eddins, 1937). Lesions began as small water-soaked areas which formed anywhere on the stem and enlarged and extended into the pith. After the pith has been destroyed the cavity usually was filled with fungus mycelium and sclerotia. The surface of the necrotic area became chalky white to tan in color when dry.

Naturally ejected ascospores rarely infected vigorously growing leaves. Few spores germinated and even fewer penetrated the leaves. This contrasts with results of others who suspended apothecia over potato plants in jars. Pethybridge (1911) observed infection on the stalk after 4 weeks in the jar. This infection time at high humidity was considerably longer than used in my experiments. Eddins (1937) obtained infection in the top of the plant closest to the apothecia. Neither Pethybridge nor Eddins describe vigor of the plants as influenced by length of

time in the jar, light intensity, or aeration. Additional information is desirable to determine if the foliage was weakened or senescent due to less than optimal conditions for plant growth. W. D. Moore, after observing many crops (including potato) infected by S. sclerotiorum, is quoted (McLean, 1958) as having "never seen one case (in hundreds of field observations) where I felt the infection began through direct mycelial (from spores) penetration of clean, freshly growing tissue." This agrees with my observations from carefully controlled greenhouse and laboratory experiments using whole plants and detached leaves that naturally ejected ascospores rarely infected vigorously growing leaves. Purdy and Bardin (1953) reported that tomato is not infected by direct penetration of naturally ejected ascospores under natural conditions.

Purdy (1958) on the other hand found almost 100% of detached leaves of the hosts red clover, ladino clover, lettuce, Brussels sprouts, bean and tomato infected by naturally ejected ascospores of S. sclerotiorum isolates from red clover and ladino clover. Purdy suggests the tomato leaves were probably senescent and thus more susceptible to infection. Other workers have reported naturally ejected ascospore infections on sunflower (Antokolakaya, 1927; Lobik, 1928), clover (Valleau et al., 1933; Loveless, 1951), tomato (Chamberlain, 1932), and Blue Lake bean (Dana and Vaughn, 1949). These workers do not provide data of percentage of spore germination and

penetration to assess the significance of the disease from direct penetration of vigorously growing plants by naturally ejected ascospores.

Infection of wounded vigorously growing foliage by naturally ejected ascospores always occurred in my trials. Pethybridge (1911) thought leaf scars served for entry of the organism although no evidence was provided. Nonwounded leaves remained healthy and wounded broccoli, cauliflower and cabbage were infected by S. sclerotiorum mycelium (McLean, 1958). Only wounded pumpkin fruits (Young, 1936) were susceptible to ascospores.

Weakened or senescent leaf tissues were always infected by naturally ejected ascospores in my trials. This is in agreement with the report of Pethybridge (1911) that chlorotic and senescent leaves are readily infected by naturally ejected ascospores.

The role of potato blossom infectiously ascospores of S. sclerotiorum is reported for the first time. The ascospores germinated and colonized potato flowers and mycelium from colonized blossoms subsequently infected vigorously growing leaves, stems, or petioles. Blossom or flower infection by S. sclerotiorum has been reported on many crops including chrysanthemum (Holcomb and Motsinger, 1968); camellias (Winstead and Haasis, 1954); and gloxinia (Hendrix and Raabe, 1960). Colonized blossoms functioning as intermediaries between S. sclerotiorum spores and infection loci has been reported in certain crucifers (McLean,

1958), tomatoes (Purdy and Bardin, 1953), and apples (Hockey, 1959). And Purdy and Grogan (1952) emphasized the necessity of dead organic matter for lettuce infection.

The severity of infection on the potato plant is apparently determined by either the quantities of nonliving organic matter or wounded, weakened and/or senescent foliage available for ascospore colonization. Ascospores are essentially noninfectious on vigorously growing healthy foliage but all vigorously growing above ground plant parts are apparently susceptible to mycelial inoculum from the colonized intermediary of nonliving, dying, or wounded plant material.

Infection of below ground potato plant parts was lacking or negligible. Results of extensive inoculations of tubers and sprouts with sclerotia and sclerotia supplemented with high concentrations of nutrients indicate a negligible role, if any, for sclerotia in causing disease of underground plant parts. Natural tuber infection by S. sclerotiorum has been reported (Bustemente and Thurston, 1964) in Colombia, South America as field infection of tubers left in the ground during the dormant period. This type of tuber storage is never practiced in Michigan.

This disease of potato may be minor in Michigan perhaps due to the present fungicide spray program for control of the disease. The weekly applications of fungicides may be killing the spores on the weakened host

foliage thus reducing or keeping in check a more severe incidence of disease.

Chlorotic and senescent potato foliage covering the soil surface over sclerotia did not serve as an intermediary for mycelium growing from sclerotia positioned on the soil surface. Potato stalks appropriately situated were not colonized and remained disease free. Sclerotia functioning in this manner in pathogenesis has been reported for lettuce (Purdy and Bardin, 1952; Brown and Butler, 1936) and some other vegetable crops (Moore et al., 1950). Sclerotia functioning in this manner appear to play a minor role in the total disease of potato in Michigan.

It is widely accepted that this disease on all crops develops best at cool temperatures (Eddins, 1937; Moore et al., 1950; Partyka and Mai, 1962; Holcomb and Motsinger, 1968; Brown and Butler, 1936; Moore, 1948 and Moore, 1955). It is important to distinguish between optimal environmental conditions for infection and optimal environmental conditions for subsequent disease development. In our work with the Sebago variety of potato, when infection was established and such plants placed at 15, 20 and 25 C, the pathogen progressed most rapidly through tissues of the plant at the higher temperatures in high relative humidity. Therefore it would be expected with optimum relative humidity, disease would be more destructive at 20 and 25 than at 15 C. A parallel situation exists with late

blight of potato. Although late blight disease is similarly thought to be a cool weather disease if infection is established and relative humidity is high, the foliar destruction is more rapid at higher temperatures than at lower temperatures.

SUMMARY

Some symptoms observed in the pathogenesis by Sclerotinia sclerotiorum (Lib.) de Bary on potato were not previously reported. During very early stages of stalk lesion development, apical leaves frequently became necrotic. As stalk lesions advanced leaves usually became progressively chlorotic from the lesion to the apex of the plant. A pink ring formed in the vicinity of the perimeter of the stalk lesion.

Only senescing flowers and chlorotic or senescing leaves were colonized when foliage was inoculated with naturally ejected ascospores. Primary ascospore infection rarely occurred on vigorously growing leaves. When colonized flowers dropped onto vigorously growing leaves, infection consistently developed spreading into the petiole and eventually causing collapse of the stem. Invasion of senescing and wounded leaves produced a similar pattern of disease development.

High relative humidity 95-100% was required for infection of healthy leaves by colonized blossoms and for colonization of senescing leaves. Tissue breakdown following initial infection at 95% relative humidity was most rapid at 24 C and progressively less at 20 and 15 C.

Tissue breakdown at 20 C was most rapid at 95 to 100% relative humidity and slow at 10% relative humidity.

Older leaves and stems were invaded more rapidly by the fungus than juvenile tissues. Lesions were small and restricted on apical leaves but lesions of basal leaves were large.

The fungus grew only in the necrotic lesion areas and could not be isolated in advance of the leaf or stem lesion even though systemic symptoms, apical leaf blight, necrotic vascular tissue, plugged xylem vessels and pith necrosis were observed in stems with lesions.

Tubers and sprouts inoculated in the field with sclerotia or sclerotia amended with mycelially colonized corn meal-Perlite mixture were not significantly different in terms of yield or stand from noninoculated controls. No disease symptoms were observed. Sclerotia were not infectious on stems with or without senescent leaf amendments.

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