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DISSEMINATION AND SURVIVAL OF SCLEROTINIA SCLEROTIORUM PROPAGULES IN DRY EDIBLE BEAN FIELDS IN WESTERN NEBRASKA

The University of Nebraska - Lincoln PH.D. 1982

DISSEMINATION AND SURVIVAL OF *SCLEROTINIA SCLEROTIORUM* PROPAGULES

in

DRY EDIBLE BEAN FIELDS IN WESTERN NEBRASKA

by

Robert Dale Muckel

A DISSERTATION

Presented to the Faculty of

The Graduate College in the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Life Sciences

Under the Supervision of

Professor James R. Steadman and Professor Wendell Gauger

Lincoln, Nebraska

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TITLE

Dissemination and Survival of Sclerotinia sclerotiorum

Propagules in Dry Edible Bean Fields

in Western Nebraska

BY

Robert D. Muckel

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TABLE OF CONTENTS

PREFACE

This dissertation is divided into a general introduction and literature review, two sections and nine appendices. Sections one and two are to be submitted to Phytopathology for publication and some data presented in the appendices may be incorporated into future publications.

GENERAL INTRODUCTION AND LITERATURE REVIEW

GENERAL INTRODUCTION

Sclerotinia sclerotiorum (Lib.) deBary is the causal agent of diseases of many important crops. As the cause of white mold disease, it is a major limiting factor in dry edible bean production on the high plains. This study is concerned with the dissemination and survival of propagules of this fungus within and near dry edible bean fields on the irrigated semi-arid plains of western Nebraska.

Atmospheric sampling for microorganisms and their propagules has demonstrated that air may serve as a medium for dispersal of fungi of ecological and/or pathological importance (Edmonds 1971; Fulton and Mitchell 1966; Gregory and Hirst 1957; Pady and Kramer 1960; Stakman *et al.* 1923; Walker 1969). Thus atmospheric dissemination of fungal pathogens must be considered along with other means of dispersal in those fungi possessing adaptations for becoming airborne.

Data that demonstrate the atmospheric dissemination of white mold disease in dry edible beans are lacking. Identifying the mode or modes of dispersal of *S. sclerotiorum* propagules is essential to understanding the epidemiology of this disease and in the identification of effective and economical methods of its control. Recent studies have demonstrated the occurrence of *S. sclerotiorum* propagules in the atmosphere above rape fields during wet growing seasons in central Alberta, Canada, but comparable data for dry edible bean fields are unavailable (Stelfox *et al.* 1978; Williams and Stelfox 1979 and 1980). The apparent isolation of newly infested bean fields from other diseased crops has led many investigators to assume, perhaps incorrectly, that atmospheric dissemination of *S. sclerotiorum* is a reality (Bardin 1951; Brown and

Butler 1936; Burke et al. 1957; Stelfox et al. 1978; Suzui and Kobayashi 1972; Williams and Stelfox 1979 and 1980; Zummo and Plakidas 1961). The only concrete evidence on the atmospheric dispersal of S. sclerotiorum ascospores is of questionable relevance since the studies were done in the winter with snow on the ground and in the absence of plant growth (Suzui and Kobayashi 1972).

The microclimatic conditions which are important to the development of white mold disease in the semi-arid conditions of western Nebraska are not well known and knowledge and manipulation of these conditions may prove to be the best means of disease control. Thus a major part of this investigation includes the study of survival of S. sclerotiorum propagules under typical field conditions in western Nebraska.

LITERATURE REVIEW

Taxonomy

The family Sclerotiniaceae was proposed by Whetzel (1945) to accommodate inoperculate discomycetes that produce stromata, stipitate apothecia, ellipsoidal ascospores, and globose spermatia. Sclerotinia was designated the type genus.

Characteristics used by Whetzel and others to distinguish the genus Sclerotinia are a distinct sclerotiorum which develops free from the host tissue, lack of a known functional conidial stage, and hyaline ascospores. Kohn (1979a) further limits this genus to those species in which the outer layer of the apothecium is composed of globose cells in chains oriented perpendicularly to the receptacle surface. Many plant pathologists do not accept this restricted classification of Sclerotinia and include a rather diverse assemblage of organisms in this genus (Dennis 1968).

Since the rules of international botanical nomenclature were not adhered to by Professor Whetzel the genus Sclerotinia was declared an invalid designation by Korf and Dumont (1972). They proposed a new genus, Whetzelinia, in honor of Professor Whetzel's many mycological research contributions. However, many workers accept Whetzel's circumscription and his typification of Sclerotinia with S. sclerotiorum, and a proposal to conserve S. sclerotiorum as the lectotype of Sclerotinia has been approved by the 1981 International Botanical Congress (R. P. Korf, personal communication). It is now correct to refer to Sclerotinia sclerotiorum, and to ignore the generic name Whetzelinia (Kohn 1979a).

Purdy (1955) found as much variation in ascus, ascospore, and sclerotium size among single ascospore isolates within individual species

as between species of Sclerotinia. Thus, he combined S. minor, S. trifoliorum var. fabae, S. intermedia and S. sativa into S. sclerotiorum. Subsequently, several comparative studies have provided ontogenetic, cytological, electrophoretic, and host range evidence that confirms the existence of three major species, S. sclerotiorum, S. trifoliorum, and S. minor (Held and Haenseler 1953; Kohn 1979b; Willetts and Wong 1971; Wong and Willetts 1973, 1974, 1975a, and 1975b).

Life Cycle of S. sclerotiorum

Sclerotia are the primary survival structures of the white mold fungus. A mature sclerotium consists of a darkly pigmented rind 2 to 3 cells thick, a thin-walled hyphal cortex 2 to 4 cells thick and an inner medulla composed of loosely arranged filamentous hyphae (Kosasih and Willetts 1975). They vary considerably in their shape and size. Their dimensions usually range from 2 to 10 by 2 to 5 mm. In western Nebraska 78% of the sclerotia buried at various depths in the soil were found to survive for at least 3 years (Cook et al. 1975). Sclerotia of S. sclerotiorum germinate by either carpogenic or myceliogenic germination. In myceliogenic germination the dense mycelium which erupts from beneath the rind of the sclerotium can directly infect actively growing tissue, and does not require colonization of senescent tissue for penetration. Thus, young seedlings may be directly attacked resulting in damping-off symptoms (Purdy 1979). In carpogenic germination the sclerotia produce apothecia which, when mature, produce ascospores. Sclerotia must undergo a conditioning process to remove their constitutive dormancy to carpogonial germination. This dormancy can persist for 18-210 days (Cook et al. 1975).

A single sclerotium may produce from one to more than a hundred

apothecia (Dickson and Fisher 1923). Schwartz (1977) found that an average of 2.3×10^6 ascospores/apothecium were produced in the laboratory. A range of temperatures and moisture content have been reported to affect the conditioning or physiological maturation process of the sclerotia and their subsequent carpogenic germination (Schwartz 1977).

It is generally thought that white mold of beans is a disease usually initiated by airborne ascospores (Purdy 1979). Ascospores require senescent or necrotic plant parts (blossoms, seeds, leaves, etc.) or an exogenous energy source to infect host tissues (Abawi and Grogan 1975; Abawi et al. 1975; Boyle 1921; Loveless 1951; Purdy 1958; Purdy and Bardin 1953). Ascospores deposited on bean plants need not infect immediately but can survive for a considerable time until the wet conditions and exogenous energy source necessary for infection become available (Grogan and Abawi 1975). Abawi and Grogan (1975) found that 25° C was the optimum temperature for germination after 3 hours of incubation. After 6 hours of incubation, however, the percentage germination was similar between 10° and 30° C. Brooks (1940) and Moore (1955) reported that white mold epidemics are favored by mean temperatures less that 21° C and high humidity or moisture levels. Abawi and Grogan (1975) have found that approximately 48-72 hours of free moisture was required before inoculation of bean plants and/or detached plant organs resulted in visual signs of infection under field conditions.

Germination of ascospores occurs within 6 hours after inoculation of bean blossoms (Abawi et al. 1975). The extensively branched germ tubes produce appressoria which form infection pegs that penetrate the epidermal layer and form vesicles. The vesicles give rise to

secondary hyphae which ramify through the floral parts and cover the flower with a dense cottony mycelial growth. The hyphal strands protruding from the bean blossom produce multi-cellular cushion-shaped appressoria when they come in contact with actively growing green plant tissue. Flattened branches of the appressorial cushion produce infection pegs that disrupt the epidermis and produce hyphae that ramify through the leaf tissue.

S. sclerotiorum can produce microconidia at any stage of its life cycle under proper conditions. These microconidia have not been observed to function during sexual fertilization or host infection (Kosasih and Willetts 1975; Ramsey 1925) and their function remains unknown.

The first visual evidence of white mold disease is the appearance of irregular water soaked spots on branches and leaves. Lesion enlargement is followed by a soft watery rot. Leaves distal to the lesion wilt, become yellow, turn brown, and often abscise. Multiple stem lesions can kill plants (Coley-Smith and Cooke 1971; Cook 1973; Harter and Zaumeyer 1944; Purdy 1979). Abundant white cottony mycelium forms and subsequently produces sclerotia on or within the plant. Sclerotia eventually reach the soil where they are distributed by irrigation run-off or by farming operations that disturb the soil (Brown and Butler 1936; Cook 1973; Cook et al. 1975; Steadman et al. 1975).

The fungus can be distributed throughout a bean producing area by sclerotia- or mycelia-infested seed lots (Blodgett 1946; Hungerford and Pitts 1953; Steadman 1975). Suzui and Kobayashi (1972) reported that 3.2 sclerotia/m^2 caused 60-95% infection in a kidney bean field. A sclerotial population of 0.2/kg soil resulted in a

46% infection of the plant canopy in dry edible beans (Schwartz and Steadman 1978).

Geographical Distribution

S. sclerotiorum on lettuce was studied in Europe as early as 1836 and in the United States by 1890 (Ramsey 1925). White mold disease has been reported from many countries located on all continents (Purdy 1979). It is prevalent in temperate zones of the northern hemisphere, but it can be a problem in tropical or arid climates during cool seasons or at higher elevations under favorable microclimate (Reichert and Palti 1967). It has been a problem in widely separated regions in the United States since the early 1940's (Blodgett 1946; Burke et al. 1957; Starr et al. 1953). Cook (1973) believed that it was a problem in western Nebraska at this time, but it was not a great concern to Nebraska growers until the late 1960's.

Host Range

A recent review of world literature by Schwartz (1977) implicated 399 plants as susceptible hosts to the white mold fungus. These included 374 species of 237 genera in 65 families. Schwartz's review presented data comparable to that reported by P. B. Adams (personal communication in Purdy, 1979). Adams reported the host range as including 64 plant families, 225 genera, 361 species, and 22 others (cultivars, etc.), for a total of 383 species and other categories. Interesting aspects of the host range are that there are 62 host species in 39 genera in the family Compositae; four Gymnosperm hosts in the family Pinacea are included; monocots are rare; and the majority of the hosts are herbaceous.

Crop Losses

Losses of crops to disease caused by S. sclerotiorum are difficult to assess, and loss reports frequently appear to be rather arbitrary. However, a visit to an infected field quickly convinces the observer that crop losses can be significant. Investigators are convinced that serious losses do occur annually in many field and vegetable crops (Zaumeyer and Thomas 1957). Purdy's review (1979) of crop losses of several vegetables indicated annual losses of millions of dollars either in a reduction of yield or of quality. He reported an average annual loss of 3.5% for dry field beans and 2.0% for snapbeans in the United States.

Abawi and Grogan (1975) reported that repeated white mold epidemics in snapbeans in central and western New York caused considerable economic loss. The losses in the fields were added to by truckload rejections at the processing plants with the detection of more than 2% incidence of pod infection.

Estimates of white mold losses in western Nebraska's dry edible bean crop have ranged from 12% to 20% to as high as 65% in some fields (Cook 1973). A 13% decrease in yield was reported for the period 1970-1973 (Kerr et al. 1978).

Control

White mold disease in dry edible beans has not been consistently controlled. Methods of control that offer some promise of success are the use of chemicals, cultural practices (i.e., crop rotation, sanitation, and reduced irrigation), biological control, resistant or tolerant cultivars, and microclimate modification (Steadman 1979).

Benomyl has provided erratic control of white mold disease in dry edible beans (Steadman and Kerr 1972). This inconsistency may be due to their 4 week flowering period and the subsequent difficulty in obtaining coverage of senescent plant blossoms which when colonized by S. sclerotiorum are the major infection sources. Hunter et al. (1978) obtained protection with benomyl from this disease in snapbeans which have a much shorter flowering period (determinate growth habit).

A wide range of chemicals have been found to destroy sclerotia or inhibit their germination in laboratory and greenhouse tests (Steadman and Nickerson 1975). Field tests with pentachloronitrobenzene in dry edible beans resulted in inoculum reduction, but did not produce disease control (Steadman 1979). Gabrielson et al. (1973) however found that ground applications of cyanamide significantly reduced S. sclerotiorum infection in cabbage seed plants.

Cultivars of P. vulgaris showing significant resistance to S. sclerotiorum have not yet been successfully developed. Genetic resistance to S. sclerotiorum, however, has been reported in Phaseolus coccineus (scarlet runner bean) and in cultivars of P. vulgaris (Adams et al. 1973; Anderson et al. 1974; Coyne et al. 1977). Abawi et al. (1978) reported that this resistance appeared to be controlled by a single dominant gene. Hunter et al. (1982) found 24.6% of the F_5 progeny of P. vulgaris x P. coccineus crosses survived limited-term inoculation of stems with S. sclerotiorum. In greenhouse tests they found field resistant cultivars of P. vulgaris to be susceptible to white mold.

Crop rotation has been recommended to bean growers to control white mold disease. However, sclerotia have survived in the soil for at least 3 years and tillage operations and furrow irrigation ensure

their distribution, both vertically and horizontally, in the field (Cook et al. 1975; Schwartz and Steadman 1978). Schwartz and Steadman also found that, in Nebraska, sclerotial populations were similar in all fields sampled despite differences in occurrence of the host in the previous crop history.

In the semi-arid climate of western Nebraska irrigation is essential for dry edible bean production, but evidence shows that reduced irrigation may be used as a means of reducing the severity of white mold disease (Blad et al. 1978; Schwartz and Steadman 1978). Reducing the number of irrigations and especially eliminating mid-August sets can reduce white mold disease and maximize yields.

Sanitation should also be an important consideration for control of white mold disease. Attempts to minimize inoculum should be undertaken. Use of certified seed, crop rotation, and management of reused irrigation water run-off can be of help in reducing white mold disease (Steadman 1975; Steadman et al. 1975).

Plant canopy development has been observed to have an effect on white mold disease in dry edible beans (Coyne et al. 1974; Partyka and Mai 1962; Steadman et al. 1973). Plant canopy density has been manipulated by planting different cultivars or by varying row spacings. These experiments have shown that the more dense plant canopies developed the coolest and wettest microclimate and exhibited the highest disease severity. Microclimate modification may prove to be a valuable method in a coordinated white mold disease control program (Steadman 1979).

Fungal Aerobiology

The science of aerobiology gained its impetus from studies at the Montsouris Observatory in Paris in the late 1800's by Schoenauer

and Miquel (Gregory 1973). The invention of the airplane early in the 20th century enabled comprehensive study of the lower troposphere, but aerobiology progressed slowly until the last three decades.

Studies in fungal aerobiology have primarily involved the determination of numbers and distribution of organisms through atmospheric sampling (Fulton and Mitchell 1966; Gregory and Hirst 1957; Hirst 1953; Pady and Kapica 1955; Pady and Kramer 1960). Such studies demonstrated that air serves as a medium for dispersal of fungi of ecological and/or pathological importance. In addition to many airborne fungal diseases of plants (Edmonds 1971; Waggoner 1952; Walker 1969), several mycoses of respiratory origin in humans and other animals have been discovered (Conant et al. 1954). Stakman et al. (1923) were among the first to provide evidence for the long-range dissemination of a fungal pathogen, Puccinia graminis f. sp. tritici (wheat stem rust). The occurrence of terrestrial fungi in air masses over the Pacific and Atlantic Oceans also provides evidence supporting the occurrence of long-range dissemination of these organisms (Newman 1948; Pady and Kapica 1955). Data supporting atmospheric involvement in long-range dissemination of many types of organisms is shown by Ridley's (1930) work on the recolonization of the Island of Krakatoa after the volcanic eruption. The collection of Ephedra pollen grains over Davenport, Iowa, where no local source is known (Vinje and Vinje 1955), is conclusive evidence on the dispersal of that organism by air.

The author has collected viable fungal propagules up to an altitude of six miles over eastern Colorado and Nebraska. The fungi collected and isolated in these studies included species of Cladosporium,

Alternaria, Penicillium, Aspergillus, Fusarium, and Trichoderma (Muckel and Gillette 1974).

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