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ETIOLOGICAL AND CULTURAL INVESTIGATIONS OF THREE FUNGAL PATHOGENS OF JUNIPERUS SPP.

by

Andrea Ostrofsky

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

School of Life Sciences

Under the Supervision of Professor Glenn W. Peterson

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TITLE

Etiological and Cultural Investigations of Three Fungal Pathogens of Juniperus spp.

BY

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THIS THESIS IS DEDICATED TO
MY HUSBAND
WILLIAM DOW OSTROFSKY

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I. INTRODUCTION

Juniperus virginiana L. (eastern redcedar) and Juniperus scopulorum Sarg. (Rocky Mountain juniper) are important in windbreak, wildlife, and ornamental plantings in the Great Plains. Both species are native to Nebraska, and both are extensively planted throughout the Plains region. Approximately 5.5 million J. virginiana and 0.8 million J. scopulorum were planted in the Plains during the three year period of 1972 to 1974. (Read, 1975). This constituted 44 percent of the conifers planted in the Plains during those years. Both species grow on a wide range of sites and are able to survive extremes of heat, cold, and drought (USDA Forest Service, 1965). Since the junipers are among the most widely used and economically important trees in the Great Plains, pathogens which threaten their survival or reduce their growth should be investigated.

In recent years, branch tips of J. virginiana and J. scopulorum in Nebraska have been damaged by an unknown agent. Sclerophoma pythiophila (Corda) Hoehn. was reported as the cause of tip dieback of J. virginiana in Wisconsin (Brener et al., 1974). Observations of foliage exhibiting dieback were made to determine if S. pythiophila was associated with dieback in Nebraska. Sclerophoma pythiophila was not found on any of the material examined. Kabatina juniperi Schneider and v. Arx was commonly found associated with the damage. Kabatina juniperi and Kabatina thujae Schneider and v. Arx cause dieback of members of the Cupressaceae (Schneider and von Arx, 1966). Both were first found and described in western Europe. Kabatina thujae was found associated with dieback of Chamaecyparis nootkatensis in British

Columbia (Funk and Molnar, 1972). No species of Kabatina has been previously reported in the United States (Ostrofsky and Peterson, 1977). Little is known of the biology of K. juniperi, or of the potential threat it poses to Juniperus species of the Great Plains. Most of the work on the biology of Kabatina species has been conducted in Europe. The main effect of K. juniperi on junipers in Nebraska is reduction in growth due to loss of foliage, and unsightly appearance of trees in springtime. The biology of K. juniperi was investigated by conducting experiments which included:

1. Isolation and maintenance of the fungus in pure culture.
2. Determination of pathogenicity.
3. Determination of conditions necessary for infection of seedlings in the greenhouse.
4. Determination of conditions influencing spore germination in vitro.
5. Determination of conditions influencing growth in vitro.
6. Determination of mode of entry of fungus into plant tissue.
7. Determination of when acervuli occur in nature.
8. Determination of other potential hosts.
9. Comparison of Nebraska isolates to type cultures of K. thujae and K. juniperi, and to S. pythiophila isolate from Wisconsin.

Cercospora sequoiae Ell. and Ev var. juniperi Ell. and Ev. causes a damaging foliar blight of J. virginiana and J. scopulorum. Trees heavily infected by this pathogen for several consecutive years may be killed. Field observations on infection of J. virginiana by this

fungus have been made (Peterson, 1977). Until now, greenhouse inoculations of juniper seedlings have been unsuccessful. A juniper improvement project involving several Great Plains states is being coordinated by the USDA Forest Service at Lincoln, Nebraska. Seeds collected from junipers throughout the Plains will be used for disease resistance investigations. Any information on environmental conditions favoring infection will be valuable to future disease investigations. An objective of this study was to determine conditions necessary for infection of Juniperus spp. by C. sequoiae var. juniperi in the greenhouse. A second objective was to determine the growth rate and optimum temperature for growth of this fungus.

Phomopsis juniperovora Hahn is a common foliar pathogen of Juniperus species in the Great Plains. It is especially damaging in the nursery, and can cause loss of entire beds of seedlings if control measures are not taken. Peterson (1973) found that new, nonwounded foliage of J. virginiana was susceptible to P. juniperovora. Future USDA Forest Service studies will involve screening of seedlings from many seed sources for resistance to P. juniperovora. An objective of this study was to determine cultural conditions which favor sporulation of P. juniperovora in order to establish a consistent source of inoculum for disease investigations. A second objective was to compare the germination of spores on susceptible and nonsusceptible foliage to gain a better understanding of the infection process.

II. LITERATURE REVIEW

Kabatina juniperi

In 1964 Meyer et al. described a dieback of one year old shoots of Juniperus species in a tree nursery in Oldenburg, Germany. The dieback had first been observed in 1962, but was more extensive and severe in the spring of 1964. Juniperus chinensis pfitzeriana aurea and J. squamata were particularly damaged. Juniperus virginiana, J. sabina, J. communis, and other varieties of J. chinensis were also damaged.

The symptoms first appeared in early spring and affected the tips or side shoots of branches. The foliage turned grey-green, lost its glossiness, and finally turned yellow or brown. A brown sunken region was usually present at the base of the one year old foliage. Occasionally individual needles were also brown.

Oval, black acervuli 1 to 2 mm long were found associated with the dieback. The growth and appearance of the fungus in culture was similar to that of Aureobasidium pullulans. The conidia germinated from 5 to 35 C, with an optimum at 25 C. Vegetative growth occurred at temperatures of less than 5 to 35 C, with an optimum of 20 C. Since the fungus was acervular, the authors considered it a species of Kabatiella.

Infection was achieved on freshly emerged plant parts damaged with needle pricks (Meyer et al., 1964). After inoculation, plants were incubated at high relative humidity (>95%) at room temperature for several days.

Although no control tests were conducted, Meyer et al. (1964) recommended thiocarbamate sprays before and during sprouting, and excision of diseased branches.

In 1965 Schneider and Plate reported a disease of Thuja occidentalis similar to that of Juniperus spp. described by Meyer et al. (1964). The disease occurred in a Berlin nursery on 4- to 5-year-old trees. Symptoms were similar, and acervuli 60 - 150 um long originated below the epidermis of dead tissue. Fungal growth in culture was initially similar to that of A. pullulans, but as the colonies grew older coremium-like strands of aerial mycelium were produced. Inoculations were successful on damaged parts of 2-year-old potted T. occidentalis incubated at 20 to 25 C and 90 to 95% RH.

The fungus was considered similar to Kabatiella spp. but further identification was deemed necessary.

Copper fungicide was applied three times to infected nursery stock in May. The disease did not progress, but since no adequate controls were available, the efficacy of the fungicide treatment is not known.

In 1966 Schnieder and von Arx erected a new genus, Kabatina, to accommodate the black acervular fungi found on Juniperus spp. and on T. occidentalis. Fungi of the genus Kabatiella, in which these fungi had been tentatively placed, produce acervuli with broadly rounded conidiogenous cells upon which two or more conidia are formed simultaneously. The conidiogenous cells of Kabatina species are tapered, and produce conidia successively at the cell apex. The type species of the genus is Kabatina thujae Schneider & v. Arx, found on Thuja

occidentalis. The fungus from species of Juniperus was considered sufficiently different in cultural characteristics to be placed in a different species, Kabatina juniperi Schneider & v. Arx.

Kabatina Schneider & v. Arx gen. nov.

Deuteromycetes, Melanconiales amerosporae; Fungi parasitici in plantis vascularibus.

Acervuli primo subepidermide immersi, postea late erumpentes ex hyphis verticalibus, obscuris, cellulis compositis, ad apicem fertilibus formati; conidia, successive blastosporae, ad cellulas apicales conidiophororum oriunda, unicellularia, ellipsoidea vel ovata, hyalina.

Species typica: Kabatina thujae.

Kabatina thujae Schneider & v. Arx spec. nov.

Acervuli in maculis dense dispersi, in et sub epidermide evoluti, pustulati, erumpentes, 50 - 170 µ diam.; strato basili ex hypis verticalibus, brunneis, cellulis compositis, ad apicem fertilibus, cellulis polyedris vel elongatis, 5 - 8 µ diam.; blastosporae ad cellulas apicales successive oriundae, unicellulares, ellipsoidae vel ovatae, hyalinae, 4.8 - 8.0 x 2.3 - 3.5 µ.

Aerium mycelium blastosporas ferente et acervuli tuberculati in vitro producti.

Habitat in ramis Thujae occidentalis L. in Germania, Berlin (Typus in herb. Biologische Bundesanstalt, Berlin, No. 104 18).

Kabatina juniperi Schneider & v. Arx spec. nov.

Species a Kabatina thujae in vitro distincta a ceteri crescentia, submersis blastosporis et aerio mycelio acervuli que nullis, Aureobasidio pullulanti vere similis.

Habitat in ramis Juniperi chinensis L., J. communis L., J. sabinae L., J. squamatae Buch.-Ham., J. virginiana L. in Germainia et Neerlandia (Typus in herb. Biologische Bundesanstalt Berlin, No. 104 17).

Reinkulturen von Kabatina thujae und von Kabatina juniperi befinden sich in den Sammulungen des Centraalbureau voor Schimmelcultures in Baarn. (CBS 238.66 und CBS 239.66).

Hoffman and Fliege (1967) further studied the growth and cultural characteristics of K. juniperi. The fungus grew on bio-malt agar, potato dextrose agar, carrot agar, and wort agar. It formed blastospores but no acervuli in culture. On malt agar colonies were initially light colored, turned a dark brown, and formed aerial mycelium. Sectoring into regions of different color often occurred.

The fungus grew on media with a high sugar content. Colonies were thickest on medium with 10% glucose or lactose. The cream colored colonies growing on media with 30% glucose often formed orange sectors.

The optimum temperature for growth on malt extract agar and on carrot juice agar was 20 to 25 C. On carrot juice agar the fungus grew in a pH range of 3.1 to 9.0, with colony diameter greatest at pH 5 to 7.

Conidia germinated by either germ tubes or by budding. Germination occurred within a few hours at 15 to 20 C, after 31 hours at 30 C, and after 39 hours at 10 C. Conidia germinated in solutions of 0.1 to 4.0 M glucose, with the greatest percentage of conidia germinating at 1 and 2 M sucrose.

Cut branch tips were wounded with fine punctures, sprinkled with a conidial suspension, and left in bowls at room temperature. Browning occurred within 14 to 21 days and acervuli were formed. No fruiting bodies were formed on unwounded foliage. Similar inoculation experiments were successful on foliage of J. communis, J. chinensis pfitzeriana, J. chinensis aurea, J. sabina, and J. squamata meyeri.

Hoffman and Fliege (1967) postulated that the fungus was a wound parasite which probably entered the tissue via wounds from mechanical damage. They suggested that infection occurred in the summer or fall and tissue was destroyed in the winter during host dormancy. Although the new genus and species names were used, they suggested that the taxonomic position of the fungus was not certain, and that more information on the variation of the morphological characteristics was needed. Control recommendations included use of preventive fungicides in the fall, and adequate spacing of juniper plantings.

Morelet (1970) found Kabatina thujae on Cupressus arizonica in France. The fungus was associated with a dieback early in spring. Branch tips turned grey-green, lost their brilliance, and finally turned yellow or brown.

Abundant yellow crystals were formed in the culture medium when the fungus was grown on malt agar. Schneider confirmed that her cultures of K. thujae also formed yellow crystals, while her cultures of K. juniperi did not. Morelet suggested that yellow crystal production could be used as a supplementary characteristic to distinguish between the two species. Morelet (1970) notes that an undetermined species of Kabatina was found by Schneider on Chamaecyparis sp.