Root rot of sweet peas
by Carl M Olsen

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Botany at Montana State College
Montana State University

Abstract:
The soil-borne disease of sweet peas, which manifests itself as a root rot, was investigated in this study. This work was primarily initiated to determine the causal organism and the predisposing factors associated with the disease. Some of the work was also devoted to devising a means of control for this disease.

Many isolations were made during the course of the study, from both cortical and vascular tissues of diseased plants. In all cases species of Fusarium were obtained. Pathogenicity tests were conducted in the greenhouse and in the laboratory with varied results. Three out of a total of thirty Fusarium cultures were pathogenic in three pathogenicity tests, but non-pathogenic in one test, A mixture of 12 cultures, including the three pathogenic ones, was also tested for pathogenicity, A moderate degree of pathogenicity was. expressed in this test.

Three soil disinfecting materials were used for controlling root rot of sweet peas: Vapam 4-S (31% sodium N-methyldithiocarbamate), CBP (chlorobromopropene), and Terrachlor (PCNB - 20% pentachloronitrobenzene).

Sweet pea plots treated with CBP produced the largest proportion of healthy plants. This amounted to 65.3 and 63.5 per cent for 1955 and 1956 respectively. The check plots for the same years produced 55.9 and 46.6 per cent of healthy plants. The plots that were treated with Vapam produced 78.6 and 44.6 per cent healthy plants and the checks produced 31.4 and 15.4 per cent in 1955 and 1956 respectively. Plots treated with PCNB had 74.8 per cent healthy plants as compared with 49.1 per cent in the checks. However, this compound was only used in 1956,

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Approved:

Head, Major Department

Chairman, Examining Committee

Dean, Graduate Division

Bozeman, Montana
June, 1957

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. M. M. Afanasiev, who directed this study and was most helpful in all its phases. The assistance of Professor H. E. Morris, Dr. I. K. Mills, Dr. H. S. MacWithey and other staff members of the Department of Botany and Bacteriology, Montana State College, is also greatly appreciated.

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INTRODUCTION

The city of Bozeman was known in the past as "The Sweet Pea City". This name was applicable as some of the highest quality sweet peas (Lathyrus odoratus L.) in the Northwest were grown in and around Bozeman, Montana. An annual festival called the "Sweet Pea Carnival" was sponsored by community service organizations until 1934. The festival consisted of parades of decorated floats and the presentation of bouquets to passengers on trains passing through the town. During this time many homes and store-fronts were beautifully decorated with arrangements of sweet peas.

In recent years the culture of sweet peas in Bozeman has gradually diminished because of difficulties encountered in producing healthy plants. The Botany and Bacteriology Department of the Agricultural Experiment Station, Montana State College, has received numerous inquiries from residents of the State, especially those living in Bozeman, regarding the trouble in raising sweet peas. Examination of diseased specimens indicated that most of the difficulties in growing them were probably due to one or more soil-borne, root-rot producing organisms which affect the plants. Since sweet peas are usually grown in a permanent bed, it is likely that the continuous propagation in such locations brings about a gradual build-up of the root rot pathogens. The purpose of this study was to investigate this disease, to identify the causal organisms and to develop control measures.

REVIEW OF LITERATURE

Root rots of sweet peas, caused by various fungi, have been reported by several investigators. Most of the available literature merely reports the incidence of the disease with very little description.

Aphanomyces euteiches Drechs. root rot has been reported on the perennial sweet pea (Lathyrus latifolius L.) in Wisconsin and on the annual (L. odoratus L.) in Indiana, Michigan, Wisconsin (8) and in England (2). Since A. euteiches is a cortical tissue inhabitor, the separation of the vascular cylinder from the cortical tissues when pulled from the ground is a quick method of diagnosis of this disease.

Workers in Massachusetts (8) have found Pythium ultimum Trow to be an incitant of another root rot of sweet peas. Drechsler (6), on the other hand, reported P. oligandrum Drechs. to cause decay of mature stems and roots, but only as a secondary organism.

Dodge and Rickett (5) and Taubenhaus (25) state that Rhizoctonia spp. and R. solani Kühn are primarily damping-off incitants, but also cause root rot. This root rot produces a characteristic constriction at the limits of the infected tissues as the disease progresses up the stem. The roots are sparse and brown in color. Post (19) states that R. solani- and Fusarium-incited root rots are impossible to distinguish without isolations.

Ascochyta lathyri Trail foot and root rot has been reported in Argentina (11), England (12) and in Kansas (8). Beaumont (2) reported A. lathyri and its perfect stage, Mycosphaerella pinodes Berk. and Blox., on sweet peas, but added it is "very rare". The symptoms of this disease

are striking and not easily confused with other root rots. Tan, sunken lesions with dark brown margins may be found on the base of the stems. Black, pin-point-sized pycnidia are often conspicuous in and around these lesions.

Dimock (4) reported Verticillium albo-atrum Reinke and Berth. on sweet peas at Cornell University (New York).

Taubenhaus (25) found Chaetomium spirochaete Patt. on samples of root-rot-diseased sweet peas sent him from Cornell University and Illinois. The root system is usually found to be completely or partly destroyed. The disease seems to be primarily of a seedling infecting type. Inoculations of healthy seedlings with pure cultures of fungus proved the organism to be weakly pathogenic, but the pathogenicity is favored by an excess of soil moisture.

A root rot incited by Thielaviopsis basicola Brierley has been reported as occurring on sweet peas generally throughout the United States, especially on the Pacific Coast (8) and in Holland (13). In Holland, the abnormal "soil sickness" is caused by this organism and is responsible for heavy losses in Dutch sweet pea crops. The symptoms of this disease on sweet peas are quite variable. In severe cases, delayed growth is a conspicuous feature and the plants do not exceed 15 to 30 cm. in height. The roots are dark brown and much reduced in length. The formation of adventitious roots retards the death of the plants, which is preceded by gradual wilting. Many individual plants harbor the pathogen without showing noticeable external symptoms. Mooi-Bok (13) states that the fungus has never been detected within the central cylinder or endodermis of even

heavily infected plants. According to Taubenhaus (24) and Dodge and Rickett (5) Thielaviopsis root rot can be easily distinguished by the stubby, charred appearance of the roots.

An insufficient amount of work has been done on the root rot of sweet peas which is incited by Fusarium species. The occurrence of this disease has been reported only in a few cases. Taubenhaus (24, 25) reported Fusarium wilt to be incited by F. lathyri Taub.. He also gave a good account of general symptoms of this disease. Dodge and Rickett (5) also state that Fusarium root rot of sweet peas is due to infection by F. lathyri Taub.. Workers in New York and Florida (8) reported Fusarium spp. as causing stem rot, root rot and wilt. The isolation of Fusarium spp. from sweet peas affected with root rot has been reported in Victoria, B. C. (18). No attempt was made to identify the organism any further in this instance. Beaumont (2) reported the isolation of F. culmorum (Smith) Saccardo from root-rot diseased sweet peas in England.

MATERIALS AND METHODS

An attempt was made to isolate and to prove pathogenicity of the organism or organisms responsible for the root rot of sweet peas. Isolations of fungi were made from diseased sweet pea plants grown in resident-owned plots in and around Bozeman, Montana. Diseased plants were removed from their plots and brought to the laboratory. The roots were washed and bits of diseased vascular or cortical tissue were placed on water agar (2%). Hyphal-tip transfers were made to Potato-Dextrose-Yeast (PDY) medium. This is standard PDA medium with 2 g. of yeast extract (Difco) added per liter.

Thirty cultures of fungi were isolated in this manner. These cultures were allowed to sporulate on three per cent PDY medium and then examined microscopically to identify them. They were next put into nine groups, on the basis of their macroscopic similarities, to facilitate handling.

Single spore isolations of the above cultures were made and the resulting cultures were tested for pathogenicity to sweet peas in the greenhouse and in the laboratory.

In an attempt to find a means of control of the root rot disease of sweet peas, a number of sweet pea plots in Bozeman, known to produce diseased plants, were selected for treatment. The soil in each of these plots was treated with one of the three following soil fumigants.

Vapam 4-S (31% Sodium N-methyldithiocarbamate dihydrate)

Application- 15.2 cc per square foot diluted in water and sprinkled on the soil surface.

Properties-

Solubility- readily soluble in water (72.2 g per 100 cc at 20° C.), moderately soluble in alcohol, sparingly soluble in other common organic solvents.

Phytotoxicity- known to be damaging to roots of established plants and to cause foliage damage by fumes.

CBP (1-chloro-3-bromopropene-1) (OS-840 technical chlorobromopropene)

Application- 1.5 cc per hole spaced one foot apart. A satisfactory hole was made by plunging a one inch diameter, pointed stick into the ground at the prescribed distance to a depth of six inches. Optimum soil temperature for this treatment is 60°F. and soil moisture 50% equivalent moisture (45-70°F. and 20-70%).

Properties-

Solubility- highly soluble in high hydrocarbon solvents, less soluble in water.

Phytotoxicity- Phytotoxicity can be reduced somewhat by shallow planting methods, by the time of application, and by diluting the chemical. Porous and drier soils also help reduce the toxicity.

PCNB- (Terrachlor) (20% pentachloronitrobenzene)

Application- 200 pounds of active dust per acre or 0.0046 pounds per square foot.

The dust was merely worked into the soil to a depth of about six inches.

Properties-

Solubility- soluble in acetone, benzene, toluene, and xylene. Slightly soluble in methyl, ethyl and isopropyl alcohols.

Phytotoxicity- considered safe for use in treating soil (at 50-200 lb. active chemical per acre) just prior to or at the time of planting of most crops.

The application rates of all three materials were made according to the manufacturer's recommendations.

The sweet pea seeds used in these tests were obtained from George J. Ball, Chicago, Illinois, with the exception of one Burpee Seed Company variety.

Eleven varieties, all summer flowering, were used in the field plots. These varieties are:

1. Ball White-white seeded (21982-83)
2. Snow White-black seeded (2142-24)
3. White Blush (3667-103)
4. Cuthbertson Strain, Janet (14047-115)
5. Ruffles (Burpee)
6. Fiesta- Scarlet (165-83)
7. Welcome- Scarlet (12147-14)
8. Geo. J. Ball- Coral Rose (31437-93)
9. Rose-pink (3697-103)
10. Ball Blue Supreme (11995-111)
11. Cuthbertson Strain, Jimmy (11757-103)

These varieties were planted in three groups composed of four varieties each. Each plot was planted with one group (i.e. four varieties) in two rows parallel to the length of the plot. The seeds were planted $\frac{1}{2}$ inch deep and spaced one inch apart. All seeds were Arasan-treated prior to planting. The same planting plan was followed in the check portion of each plot.

EXPERIMENTAL PROCEDURE AND RESULTS

Pathogenicity Tests

Isolations of fungi were made from diseased sweet pea plants throughout the 1955 growing season. These plants usually grow normally in the beginning of the season, but as soon as they begin to bloom a yellowing of the lowest leaves is evident. This yellowing gradually becomes extensive and is followed by a reddish-brown discoloration of the vascular system which is continuous from the tip of the roots to a height of one to two inches above the soil line. The root system is reduced to several dark colored laterals and a short, darkened tap root. In some cases, plants less severely infected frequently produce new lateral roots above the discolored portion of the tap root. These laterals are thicker and shorter than normal. The infected plants may continue to grow until the flower buds begin to open, even though the lower leaves show firing. At this time, the plants are usually rapidly killed. Often infected plants appear stunted and unthrifty in growth, and these may die long before bud formation. In some cases, seedlings only three to six inches in height will succumb when severely infected. The final symptom of this disease is a complete desiccation of the plant.

In making isolations, diseased plants were removed from the plots, their location recorded, and brought to the laboratory. The roots were then washed in mild soapy water and placed in tumblers covered with cheese cloth. Cold tap water was circulated through the jar for two to three hours, followed by several sterile water rinses. This procedure helped to reduce bacterial organisms which were present on the roots as

contaminants. Bits of diseased vascular or cortical tissue were placed in Petri dishes containing water agar (2%) for fungus isolation. Water agar was used to reduce bacterial contaminants. Hyphal-tip transfers were made to Potato-Dextrose-Yeast (PDY) medium which was used as a standard medium for sporulation and growth throughout the experiments.

In an attempt to eliminate the growth of bacteria present as contaminants, lactic acid was added to the warm medium in sufficient amounts to lower the pH to approximately 4.0. It was observed that acidifying the medium to this extent reduced the fungus growth rate somewhat and made zonation of the colonies more prominent and closer together. The retardation of the growth depends on the kind of acid and its concentration according to Smith and Swingle (21). Lewis (10) states that different Fusarium species are not affected to the same degree, some tolerating more acid than others.

Seventeen cultures of Fusarium were isolated in this manner from diseased plants grown in treated and check plots during the summer of 1955 and tested for pathogenicity. Thirteen other cultures of Fusarium, isolated by M. M. Afanasiev, Montana State College, during 1953-1954 were also evaluated for pathogenicity. These 30 cultures of fungi were inoculated onto PDY medium with three per cent agar in test tubes. Slotted, open-faced test tube racks were devised to support these tubes at a 45 degree angle. The racks and tubes were placed in diffuse natural lighting and maintained at room temperature to induce sporulation. Several weeks later, these cultures were examined microscopically and all appeared to belong to the genus Fusarium.

The genus Fusarium belongs to the order Moniliales, the family Tuberculariaceae and the section Pharagmosporae. This genus has sporodochial, coremial or pionnotal type fruiting bodies, however a true coremium was not observed in any of the cultures isolated in this study. Sherbakoff (20) also did not observe a true coremial fructification in his work. Two definite and distinct types of fructification were seen in this study on numerous occasions. The most common fruiting structure observed in this study was a continuous mass of spores, together with their conidiophores, heaped up into a wart-like structure known as a sporodochium. The second type of asexual fruiting structure observed is called a pseudopionnotes, which resembles a true pionnotes in appearance, but differs in origin according to Sherbakoff (20). This structure originates from the production of minute and numerous sporodochia very close to or on the substrate surface, so that they then form a nearly continuous, slimy layer of conidia.

The following characteristics, as outlined by Sherbakoff (20), were noted on the isolated cultures: macroconidial and microconidial size, septations, morphology, and prevalence; chlamydospore prevalence and morphology. Ten conidia, or ten chlamydospores, were measured and the averages of these measurements were recorded. In case of special variability of the material, records were made of 15 or 20 spores.

These isolates were placed in nine groups separated on the basis of similarities in medium discoloration, size and shape of conidia, color of mycelium, and amount of surface or aerial growth.

Group I- Whitish-gray mycelium with yellowish macroconidial masses; little aerial growth, no medium discoloration.

macroconidia- 33.6 x 5.5μ to 60.0 x 6.2μ.

microconidia- 9.3 x 4.0μ to 18.6 x 6.2μ.

Culture numbers:

10, 11, 26-A, 40, 45, 51, 52

Group II- Whitish-gray mycelium with bluish macroconidial masses; little aerial growth; and no medium discoloration.

macroconidia- 24.8 x 4.6μ to 54.0 x 5.5μ.

microconidia- 11.6 x 3.9μ to 22.5 x 5.8μ.

Culture numbers:

21, 34-A

Group III- Whitish mycelium; moderate aerial growth; purplish to reddish medium discoloration.

macroconidia- 31.0 x 6.2μ to 43.4 x 6.2μ.

microconidia- 8.0 x 3.5μ to 24.8 x 4.6μ.

Culture numbers:

14, 22, 24

Group IV- Whitish-gray mycelium; little aerial growth; purple medium discoloration.

macroconidia- 21.5 x 6.1μ to 40.0 x 6.5μ.

microconidia- 6.2 x 4.5μ to 21.3 x 4.6μ.

Culture numbers:

15, 47

Group V- White mycelium; moderate aerial growth; no medium discoloration.

macroconidia- 29.0 x 4.0μ to 41.5 x 5.8μ.

microconidia- 6.4 x 3.3μ to 21.2 x 4.6μ.

Culture numbers:

12, 23, 34-B, 34-C, 46, 50

Group VI- White mycelium; moderate aerial growth with orange tinged macroconidial masses; no medium discoloration.

macroconidia- 32.5 x 4.6μ to 47.5 x 5.6μ.

microconidia- 9.3 x 2.8μ to 15.5 x 3.1μ.

Culture numbers:

13, 26-B, 33-A, 33-B, 42

Group VII- Whitish-gray mycelium; moderate aerial growth; dirty yellow occasional macroconidial masses; no medium discoloration; burnt orange color at junction of aerial mycelium and agar.

macroconidia- 24.8 x 6.2μ to 25.6 x 5.5μ.

microconidia- rare

Culture numbers:

35, 43

Group VIII- Whitish-gray mycelium; little aerial growth; dirty yellow to bluish macrospore masses; no medium discoloration.

macroconidia- 22.2 x 4.6μ to 52.7 x 5.2μ.

microconidia- 9.3 x 3.6μ to 16.5 x 4.2μ.

Culture numbers:

25, 44

Group IX- Whitish mycelium with pinkish cast; much cottony aerial

growth; bright purple medium discoloration.

macroconidia- 56.4 x 4.2μ to 58.9 x 4.6μ.

microconidia- none

Culture number:

49

Single spore isolations were made by a serial dilution method. For this technique, a wire loop 5 mm in diameter was used to transfer spores from an aqueous suspension to tubes containing 10 cc of melted, lukewarm water agar. The spore dilution series was completed by transferring four loop-fulls successively from one tube to the next. A thin film of agar and spores was poured into sterile Petri dishes. After 24 to 36 hours the plates were examined by inverting them on a microscope stage and viewing through the agar with low power (100x). By this procedure, a germinating macroconidium could be found. After marking its position with a dot of India ink on the bottom of the dish, an agar disk including the spore was cut out and transferred to a Petri dish of PDY medium. At least six single spore isolations were made from each Fusarium isolate to reduce the chance of overlooking a mixture of two or more Fusaria. Sherbakoff (20), in his classic works on the Fusaria, found this procedure worth while in only a few cases. However, in one instance, he isolated a pink fungus which on dilution gave rise to a brick red Fusarium and a white one.

In three weeks time the six single spore isolations of each culture were compared. A letter such as A or B was added to the culture number of any variants occurring and they were treated as a distinct culture,

for example 34-A and 34-B.

The following method for testing pathogenicity of the isolated cultures was used in the greenhouse. Eight-inch pots containing three parts soil to one part sand were sterilized in an autoclave for three hours, under 15 pounds pressure in sufficient number for four applications of each culture to be tested. Twelve seeds of Janet variety were planted in each pot and the soil surface was covered with a one-eighth inch layer of sterile sand. A trellis, simply constructed of bamboo poles and string, was used to accommodate the climbing nature of the sweet pea plants (see Figure 1).

One of the single spore cultures, representing the parent type in each series of six tubes, plus any variants that occurred, were selected to prepare the inoculum. A suspension of each culture was made in sterile water blanks and enough of this suspension was poured into a Petri dish to barely cover the hardened PDY medium. After 10 to 14 days the surface growth was used as inoculum. Water suspensions of these cultures were agitated briefly in a Waring Blendor and applied to the surface of the sterilized soil, at the rate of two dishes per pot. The soil was inoculated when the young sweet pea plants were one to three inches high (see Figure 2). It was found that inoculations at the time of seeding produced too poor a stand as most of the radicles of the germinating seeds were severely attacked by the organism. Also, it was noted that the Fusaria colonized the seed coat to produce a whitish mass of mycelium enclosing the entire germinating seed.

Weekly readings were taken and isolations were made from the diseased

Figure 1. Pathogenicity test IV showing plants nearly at maturity and trellis system.

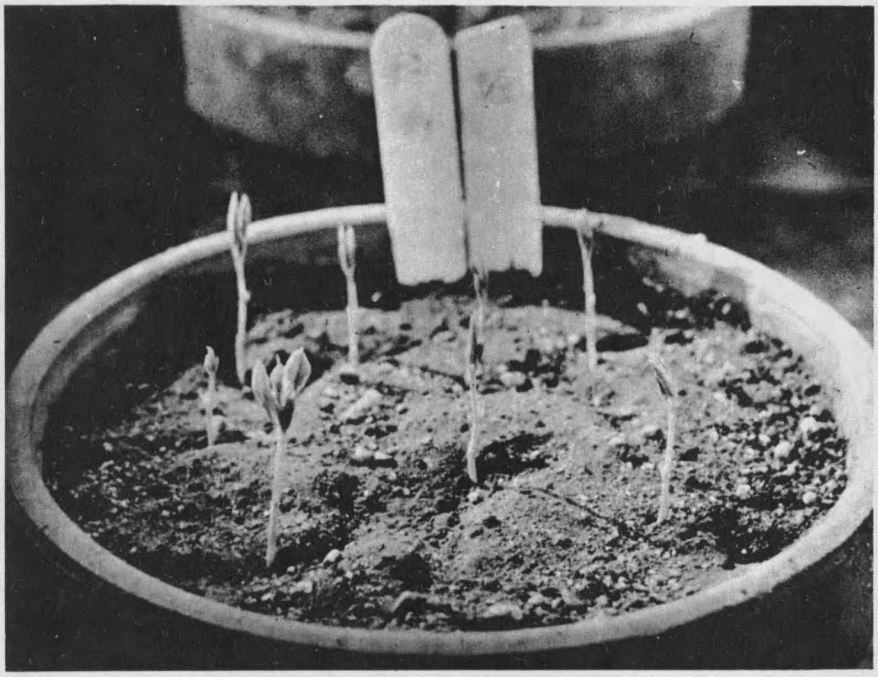

Figure 2. Sweet pea seedlings at stage of inoculation.