Biology and control of Claviceps purpurea (Fr.) Tul. on male sterile wheat and barley
by Shivayogi Basaya Puranik

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Plant Pathology
Montana State University

Abstract:
The seriousness of ergot caused by Claviceps purpurea (Fr.) Tul. in male sterile barley and wheat has prompted further study of the biology of this pathogen. For evaluation of various control measures, a technique of inoculation was developed and involved the removal of the upper 2-4 mm of the glumes prior to inoculation with a conidial suspension using an atomizer. Use of this technique resulted in nearly 1007. head and floret infection whereas previously described methods resulted in 20-607. head infection. Studies on inoculation density revealed that maximum infection occurred with 10^5 or more conidia per ml. Studies on duration of infection period of unfertilized and fertilized ovaries indicated that inoculation during and shortly after anthesis resulted in the highest levels of floret infection. With unfertilized florets, susceptibility declined at 10 days and was lost completely 15 days after the initiation of anthesis. Fertilized ovaries were susceptible right after fertilization. After the ovaries had been fertilized for 4 days susceptibility decreased until no infection occurred 9 days after fertilization. Many chemicals were screened for their effectiveness in inhibiting germination and growth of C. purpurea. Benomyl at 10 ppm inhibited germination of conidia while growth was completely inhibited at 20 ppm. Under field conditions using male sterile barley, 2400 ppm benomyl applied three times just prior to and during anthesis gave some control of ergot.

To determine why benomyl did not give complete control various concentrations of benomyl were applied to florets with the upper portion of their glumes removed which had been previously inoculated with C. purpurea. Using this method, floret infection was reduced from 947. to 11% with 1000 ppm benomyl and to 0% if a wetting agent such as Triton X-77 or Multifilm Buffer X was used along with 1000 ppm benomyl. This indicated that benomyl must reach the surface of the ovary at or before infection to be effective since benomyl did not act as an eradicant against this pathogen. Under natural conditions this will be very difficult to achieve, but various methods of application and different formulations of benomyl may prove to be effective. In male sterile wheat, it was observed that Chris had some degree of resistance in comparison to other varieties tested. While none of the control measures tested was effective by itself, the possibility exists that a combination of one or more of them might prove to be quite effective. This would include the use of resistant germplasm; the use of a variety that produces tillers over a short time period; the use of a good pollinator variety to insure rapid and complete fertilization; and the use of a systemic fungicide to provide protection during 10-15 days after anthesis when the host is most susceptible. The lack of the necessary germplasm at present hinders the testing of these possibilities.

BIOLOGY AND CONTROL OF *CLAVICEPS PURPUREA* (FR.) TUL. ON MALE STERILE WHEAT AND BARLEY

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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in

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ABSTRACT

The seriousness of ergot caused by Claviceps purpurea (Fr.) Tul. in male sterile barley and wheat has prompted further study of the biology of this pathogen. For evaluation of various control measures, a technique of inoculation was developed and involved the removal of the upper 2-4 mm of the glumes prior to inoculation with a conidial suspension using an atomizer. Use of this technique resulted in nearly 100% head and floret infection whereas previously described methods resulted in 20-60% head infection. Studies on inoculation density revealed that maximum infection occurred with 10^5 or more conidia per ml. Studies on duration of infection period of unfertilized and fertilized ovaries indicated that inoculation during and shortly after anthesis resulted in the highest levels of floret infection. With unfertilized florets, susceptibility declined at 10 days and was lost completely 15 days after the initiation of anthesis. Fertilized ovaries were susceptible right after fertilization. After the ovaries had been fertilized for 4 days susceptibility decreased until no infection occurred 9 days after fertilization. Many chemicals were screened for their effectiveness in inhibiting germination and growth of C. purpurea. Benomyl at 10 ppm inhibited germination of conidia while growth was completely inhibited at 20 ppm. Under field conditions using male sterile barley, 2400 ppm benomyl applied three times just prior to and during anthesis gave some control of ergot. To determine why benomyl did not give complete control various concentrations of benomyl were applied to florets with the upper portion of their glumes removed which had been previously inoculated with C. purpurea. Using this method, floret infection was reduced from 94% to 11% with 1000 ppm benomyl and to 0% if a wetting agent such as Triton X-77 or Multifilm Buffer X was used along with 1000 ppm benomyl. This indicated that benomyl must reach the surface of the ovary at or before infection to be effective since benomyl did not act as an eradicant against this pathogen. Under natural conditions this will be very difficult to achieve, but various methods of application and different formulations of benomyl may prove to be effective. In male sterile wheat, it was observed that Chris had some degree of resistance in comparison to other varieties tested. While none of the control measures tested was effective by itself, the possibility exists that a combination of one or more of them might prove to be quite effective. This would include the use of resistant germplasm; the use of a variety that produces tillers over a short time period; the use of a good pollinator variety to insure rapid and complete fertilization; and the use of a systemic fungicide to provide protection during 10-15 days after anthesis when the host is most susceptible. The lack of the necessary germplasm at present hinders the testing of these possibilities.

INTRODUCTION

The substantial increase in yields of corn, sorghum and other crops, by use of hybrid vigor, has stimulated many scientists to propose schemes for production of hybrid barley and wheat (48, 57). Hybrid seed is produced by forcing cross pollination between the two different parent lines. The prevention of self pollination is accomplished by hand emasculation or by use of male sterility. Male sterility may be either genetic or cytoplasmic but to date only genetic male sterility is known in barley (27, 28). One of several procedures described by Ramage (48) for production of hybrid barley seed by use of balanced tertiary trisomics (BTT) is as follows. BTT are tertiary trisomics with the recessive allele on the two normal chromosomes and a dominant allele of a marker gene carried on either the centromere or the interchanged segment of the extra chromosome. The BTT are self fertile and would be maintained by selfing in an isolation block in which both BTT and diploid plants are produced. The BTT and the diploids are separated on the basis of a marker gene, often plant color. The BTT are used for maintenance of the BTT characteristic. The diploids, which are male sterile and serve as the female parents, would be sown between plants that are good pollinators and would thus serve as the male parents. The resulting F_1 seeds are the hybrid seed which would then be sold to the grower. A 20 to 50% increase in yield is foreseen from the use of adopted

hybrids (55). Cytoplasmic male sterility and more recently genetic male sterility in wheat are being developed for hybrid seed production (23).

Since the florets of male sterile lines are open for a long time, they are more susceptible to floral diseases (16, 54). In Montana, male sterile lines of wheat and barley are extremely susceptible to ergot possibly because of low temperatures that occur during flowering thus inhibiting cross-pollination. As a result of these low temperatures, the flowers remain open for a long period of time. Futrell and Webster (22) state that many environmental conditions such as hot or cold temperatures or irradiation can cause pollen to be non-functional.

Atanasoff (2) and Barger (3) have fully reviewed most aspects of ergot. The disease is perpetuated through sclerotia which are viable up to 2 years (2). Even broken and moldy sclerotia can germinate under favorable conditions. This factor is of great significance in the introduction of ergot with seed allowing its dissemination over long distances. Sclerotial germination, longevity of sclerotia, and other related aspects have been studied by various investigators (12, 13, 24, 31, 45, 47). Generally, sclerotia germinate during the flowering period of susceptible crops. The larger and heavier sclerotia produce up to 60 stromatic heads over a long period of time.

Ascospores are best discharged at 75-78% relative humidity (5). Ascospores and conidia are disseminated about 46-92 meters by air currents (3). Primary infection takes place mainly with ascospores disseminated by air currents. Secondary infection is caused mainly by conidia that are carried on insects, by wind, or in honey dew dripping on lower florets of an infected head or neighboring heads (3, 5, 51).

Artificial inoculation of ergot has been accomplished using several methods. Bekesy (36) used a horse drawn multiple injecting apparatus. Heck (36) hand manipulated rye florets causing them to open prematurally and then inoculated them with an aqueous conidial suspension using an atomizer. Usually aqueous conidial suspensions have been used for inoculation (29, 43, 50). However, Lewis (36) used a conidial suspension containing sucrose which would, therefore, be similar to the composition of honey dew. For large scale production of ergot sclerotia conidial suspensions have been sprayed from 2-9 times during the time of flowering of rye (41, 50).

Kirchhoff (30) and Campbell (6) have studied the infection of fertile rye and barley by the ergot fungus Claviceps purpurea (Fr.) Tul. Penetration occurs within 24 hours near the base of the ovule. Conidia and honey dew are produced within 6-8 days after inoculation (6, 46). The infection period on rye appears to last from anthesis

to 15 days after fertilization (3). In wheat, infection can occur from anthesis through the milk stage (49). Sorghum and corn were not infected with ergot after fertilization had occurred (21).

Control measures for ergot that are presently used include use of ergot-free seeds, deep plowing to bury sclerotia, and crop rotation. In addition, to eliminate sources of secondary inoculum, grasses bordering fields can be mowed or maleic hydrazide can be sprayed to delay the flowering of grasses (7, 9, 34).

In the past, chemical control of ergot has not been successful. Recently, however, reports have appeared on chemicals that are inhibitory to C. purpurea. Lindendfelser (38) reported that the antibiotic cinnamycin inhibits the growth of C. purpurea in culture. Maruzzella (42) reported that several perfume oils inhibit Claviceps. In greenhouse trials Sulaiman et al. (53) sprayed 5 ppm of the antibiotic aureofungin on Pennisetum typhoideum Rich which had been inoculated twice with C. microcephala (Wallr.) Tul. This treatment reduced infection by 36% compared to a control. In a field test with fertile barley Timian (56) used 5 systemic fungicides and found that none were effective against C. purpurea.

If the florets are closed at the time of spraying, a systemic fungicide might provide better control than a protective fungicide if it were translocated to the base of the ovule where infection occurs. Delp and Klopping (15) reported that benomyl (methyl-1-

butylcarbamoyl)-2-benzimidazole carbamate) is a systemic fungicide. It prevents Verticillium wilt symptom expression and controls several other diseases such as powdery mildews, apple scab and black spot of roses. In addition several reports have appeared on the use of benomyl for control of various other diseases of fruit, field and vegetable crops, ornamentals, and turf grasses (1, 4, 11, 14, 17, 20, 33, 40). Erwin (18, 19) concluded that benomyl or its break down product was systemic since it reduced the severity of Verticillium wilt of cotton (Gossypium hirsutum L.) when applied to soil prior to inoculation. Its systemicity was further confirmed by bioassay of leaves and stem sections of plants grown in the treated soil. Hardison (25) reported that a water suspension of benomyl applied to soil around Merion bluegrass (Poa pratensis L.) plants controlled ergot and several other diseases, but he did not mention the concentration of the fungicide used and to what extent it controlled ergot. Clemons and Sisler (10) showed that in aqueous solution benomyl breaks down rapidly to bezimidazol carbamate methyl ester which is also toxic to test organisms. Lyda and Burnett (39) found that benomyl completely suppressed the growth of Phymatotrichum omnivorum (Shear) Duggar at 14.5 ppm. However, benomyl at fungitoxic levels did not inhibit the respiration of germinating sclerotia.

Resistance to ergot would provide the best control. Reports from the Mississippi Agricultural Experimental Station (44) indicate that resistance to ergot has been found in hybrids of Dallis grass (Paspalum dilatatum Poir). Resistance to ergot has also been found in the germplasm of Sorghum vulgare Pers. (52).

While several control measures can be used for control of ergot, none is completely effective. Use of ergot-free seed could reduce infection but there may be some sclerotia from a previously infected crop that could serve as inoculum. Inoculum could also come from infected grasses bordering the field. Another control measure would be to grow the crop in an area where development of ergot is restricted by environmental conditions. For instance, very little ergot is observed in Arizona on male sterile barley. However, transportation of hybrid seed from areas such as Arizona to Montana would be economically undesirable. Therefore, if hybrid wheat and barley are to be grown in the northern states, the seed will also have to be produced in these areas. Unfortunately, ergot is prevalent in the northern states, thus necessitating its control through use of chemicals, cultural practices or resistance. In the absence of completely resistant germ plasm, the prevention of infection might offer some control. While protective fungicides have been ineffective in controlling ergot, the use of an effective systemic fungicide

that would reach the site of infection i.e., the base of the ovary, could offer some hope for chemical control.

Considering the above points and the seriousness of ergot in male sterile barley and wheat, the further study of the biology of this pathogen was undertaken. Since previous work did not involve male sterile wheat and barley as hosts, studies were conducted on inoculation technique, infection period, resistance, and chemical control.

MATERIALS AND METHODS

Plant materials.- Seed of the 2 row male sterile balanced tertiary trisomic barley 67-c-383 BTT 2^7 ms and Hypana and Compana fertile barleys were supplied by Prof. R.F. Eslick (Montana State University). Seed of male sterile wheat varieties and elite lines was supplied by Dr. W. W. Roath (Dekalb Agricultural Association, Fargo, North Dakota).

Seeds were planted in sandy loam in 17.5 cm plastic pots, in the greenhouse. Supplemental illumination with incandescent lights was used when necessary to induce heading. Field tests were conducted on the Montana State University Agronomy Farm near Bozeman. Rows 3.5 m long spaced 30 cm apart were used. Four rows of male sterile barley were alternately planted between four guard rows of Hypana fertile barley. Similarly, four rows of the wheat male sterile varieties were planted between 4 rows of fertile wheat or barley.

Cultures.- The culture of C. purpurea used in these studies was isolated from honey dew of naturally infected male sterile barley. It was further maintained on an ammonium citrate agar medium (ACD) slightly modified from that described by Kybal et al (32) and consisted of the following: $Ca(NO_3)_2 \cdot 4\ H_2O$, 1.44 g; $MgSO_4 \cdot 7\ H_2O$, 0.51 g; KCl, 0.125 g; dibasic ammonium citrate, 20 g; Sucrose, 200 g; KH_2PO_4 monobasic, 0.1 g; agar, 12 g; distilled water to 1 liter. In

some studies a calcium nitrate agar medium (CM) described by McCrea (43) was used and contained the following; $Ca(NO_3)_2 \cdot 4\ H_2O$, 1 g; KH_2PO_4 monobasic, 1.25 g; $MgSO_4 \cdot 7\ H_2O$, 0.625 g; Maltose, 30 g; agar, 12 g; water, 1 liter. The cultures were grown at a room temperature of 24-26°C. Unless otherwise indicated, the conidia from 10-12 day old cultures were used.

Conidia were harvested by shaking the cultures with water. The conidia-mycelium suspension was then filtered through 6 layers of cheese cloth. The conidial concentration was determined with a haemocytometer and further diluted with water to the desired conidial concentration. For studies on conidial germination and growth, the conidia were further washed by alternate centrifugation and resuspension in sterile distilled deionized water. Conidia were finally suspended in 5-10 ml of sterile distilled deionized water by use of a loose fitting hand homogenizer and diluted to the required concentration. Aseptic technique was used at all times.

Inoculation techniques.- The florets of male sterile barley open 4-5 days after they emerge from the boot. Generally, heads with florets open were selected for inoculation. The glumes were cut 2-4 mm from the top with scissors. An aqueous conidial suspension of the desired concentration was either dropped into the open florets with a capillary tube or was sprayed onto the florets with an atomizer or a

Sure-Shot sprayer model A (Milwaukee Sprayer Mfg. Co., Milwaukee, Wis.). Inoculated heads were usually covered with glassine bags clipped tightly at the base of the head to maintain high humidity and also to avoid any outside source of inoculum or contamination.

Conidial germination.- Studies on conidial germination were conducted using deep well slides previously soaked in potassium dichromate glass cleaning solution for 12 hours or more and washed thoroughly with detergent and rinsed with deionized water. Moist chambers were prepared by covering the bottom of 9 cm petri dishes with Whatman No. 4 filter paper. After sterilization 4 ml of sterile water were added to each petri dish. Two ml of the test material, 1 ml of aqueous conidial suspension (30-50 conidia per high power field) and 1 ml of either 0.05M citrate phosphate buffer or 0.067M potassium-phosphate buffer of desired pH were mixed thoroughly in sterile test tubes. If buffer was ommited, the test material and conidial suspension were mixed in a 1:1 ratio. A drop of the mixture was then placed in the deep well slides and incubated at room temperature. At least 250 spores were counted in each replication.

Ovary extracts.- A weighed quantity of male sterile barley ovaries minus their stamens were boiled 3 times in 80% ethanol for 30 min and the extracts combined. The combined extracts were reduced in

volume to 10 ml by flash evaporation and then dried completely in a previously weighed beaker under a stream of hot air. The weight of the crude extract was determined. It was then dissolved in 20 ml of distilled deionized water. Five ml of this solution were kept as a crude extract while the remaining portion was passed through Dowex 1-8X (100-200 mesh) formate form and Dowex 50X (100-200 mesh) H^+ form ion exchange resins and the neutral fraction collected. Organic acids were eluted from the Dowex 1 resin with 6 N formic acid while amino acids were eluted from the Dowex 50 resin with 10% NH_4OH. These fractions were dried in preweighed beakers under hot air. They were then dissolved in deionized water and passed through sterilized metricel membrane filters (0.45 μ). The filtrates were kept at -20°C until used.

Ovary washings.- To determine the material present on the exterior of ovaries of male sterile barley, 50-80 ovaries without stamens were placed in 15 ml of distilled deionized water and shaken for 30 min on a wrist-arm shaker. The ovaries were then removed and the washings dried in a preweighed beaker under a stream of hot air. The procedure for fractionation was the same as for the ovary extracts.

Honey dew extract.- Generally, 6 days after infection with C. purpurea, honey dew appears on the surface of the ovaries. Twenty-

three ovaries with honey dew were shaken in 0.85% NaCl to prevent rupture of the conidia. The conidial suspension was then passed through 6 layers of cheese cloth. The filtrate was centrifuged twice, and the spore pellet discarded. The supernatant liquid was then filtered through Whatman No. 1 paper to remove any remaining spores. The filtrate was then evaporated down to 10-12 ml under a hot air stream. The procedure for fractionation was the same as for the ovary extract.

Chromatography.- Descending paper chromatography was used in the analysis of the constituents of the neutral fraction of ovary washings, ovary extracts, and honey dew extracts. Aliquots (5-10 µl) of each solution were spotted on Whatman No. 1 paper. The following solvents were used to develop the chromatograms: Solvent (A) n-butanol: acetic acid: water (4:2:1, v,v,v) or (B) (4:1:5, v,v,v); (C) Ethyl acetate: pyridine: water (8:2:1, v,v,v); (D) Isopropanol: n-butanol: water (14:2:4, v,v,v); (E) Phenol: water (80% liquid phenol: water, 80:10 v,v).

Chemicals.- The systemic fungicide benomyl (=Benlate)(methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate) was supplied as technical material or as a 50% wettable powder by E.I. du Pont de Nemours and Co., Inc., Wilmington, Del. Nabam (=Dithane A 40) (Disodium ethylene-

bisdithiocarbamate, 93% active ingredient) and Dithane M 45 (Manganese^{++} 16%, Zinc^{++} 2% and Ethylene bisdithiocarbamate ion 62%) were supplied by Rohm and Haas Co., Philadelphia, Pa. The other chemicals used were: Ethirimol (5-butyl-2-ethylamino-6-hydroxy-4-methylpyrimidine) 80% active ingredient of Plant Protection Ltd. Surrey, England; F 849 (2-amino-4-methyl-5-thiazolecarboxanilide) and G 696 (2,4-dimethyl-5-thiazolecarboxanilide) of Uniroyal Chemical Co., Bethany, Conn.; Bayer 33172 (2-(2-furyl)-benzimidazole) 97% active ingredient of Chemagro Corporation, Kansas City, Mo.; Captan (N-trichloro methyl mercapto-4-cylohexene-1,2,dicarboximide) 50% wettable powder of Stauffer Chem. Co., San Francisco, Calif.; Thiabendazole (TBZ) [2-(4-thiazolyl) benzimidazole] 60% active ingredient of Merck Chemical Division, Rahway, N.J.

The emulsifiable mineral oils LS-1195, LS-0925, LS-1183 and LS-1197 were supplied by American Oil Co., Whiting, Indiana. Orchex 792 and Orchex 796 were supplied by Esso Research and Engineering Co., Agricultural Products Lab., Linden, N.J. The perfume oils Bouquet No. 22, Bouquet No. 821, Palma Bouquet, Blue Stone Bouquet with trade mark MM&R were supplied by Magnus, MaBee and Reynard Division of BFM Corporation, Paramus, N.J.

The insecticide diazinon AG 500 (o-o-diethyl-o-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiale 48%, xylene 36%) was supplied

by Geigy Chemical Co., Ardsley, N.Y.

Poison food tests.- Benomyl was suspended in 1 ml acetone to which water was added to make the final concentration of benomyl 20 times that required in the growth medium. Nabam and Dithane M 45 were suspended in water. One ml of each concentration of the fungicide was then pipetted into 20 ml of warm melted ACD agar, shaken, and poured immediately into petri dishes. After solidification they were inoculated with a 0.9 mm mycelial disc of a 10 or 12 day old culture of C. purpurea growing on ACD agar. Controls received the appropriate amount of water or acetone. The cultures were maintained at room temperature for 15-20 days and the growth measured from the center of the inoculum disc. The toxicity of the fungicides, perfume oils, and mineral oils was also tested as follows. A conidial suspension of C. purpurea was sprayed on solidified ACD agar or CM agar. Then a sterilized filter paper disc (Difco Laboratories) 7 mm in diameter saturated with various concentrations of the test material suspended in water or acetone, was placed on the inoculated media. Observations on the inhibition of growth around the disc were taken after 25-30 days incubation at room temperature.

The effect of benomyl and Dithane M 45 on growth of C. purpurea was also determined by measuring their effect on mycelial dry weight. When the temperature of sterilized ACD medium without agar was about

40-43°C, 2.5 ml of the appropriate concentration of test material plus 2 ml of a thick conidial suspension were added to 25 ml of media. These flasks were kept stationary at room temperature for 14-20 days. The mycelial mat was filtered on Whatman No. 4 paper, dried overnight at 100°C, and weighed.

Bioassay of benomyl.- Residues of benomyl or its break down product were bioassayed in plant parts using Penicillium expansum Lk. ex Thom. in the same manner as described by Erwin et al (19).

RESULTS

Inoculation technique.- As a basis for studies on chemical control and resistance it was necessary to develop a method of inoculation that would result in maximum per cent infection. In greenhouse tests when male sterile barley plants with florets kept open by various methods, such as placing the plants in humidity chambers, were inoculated by spraying a conidial suspension on the heads, a low infection percentage resulted. However, if the upper 2-4 mm of the glumes was removed followed by inoculation using an atomizer or capillary tube, nearly 100 per cent of the florets were infected. The results shown in Table I indicate that maximum infection was obtained using an atomizer to apply the inoculum. In addition, if only the awns were removed (Fig. 1), the infection percentage was lower and quite variable since the florets open at different times on one head. In field trials, 80-96% of male sterile barley florets with glumes clipped back were infected when inoculated once with a capillary tube and bagged. However, if the florets were not clipped back and not bagged after being inoculated three times using an atomizer or Sure Shot sprayer, 70-75% of the florets became infected.

Inoculum density.- The effect of inoculum density on infection was studied so that a standard inoculum could be used in studies on resistance. Florets of greenhouse grown male sterile barley and Mexican Sterile No. 2 wheat with glumes clipped back were inoculated

TABLE I. A comparison of methods of inoculation of Claviceps purpurea on infection of florets of greenhouse grown male sterile barley.

Floret preparation	Method of inoculation [a/]	% Florets infected
I. Upper 2-4 mm of glumes removed.	1. Capillary	80
	2. Atomizer	93
II. Awns only removed.	1. Capillary	81
	2. Atomizer	83

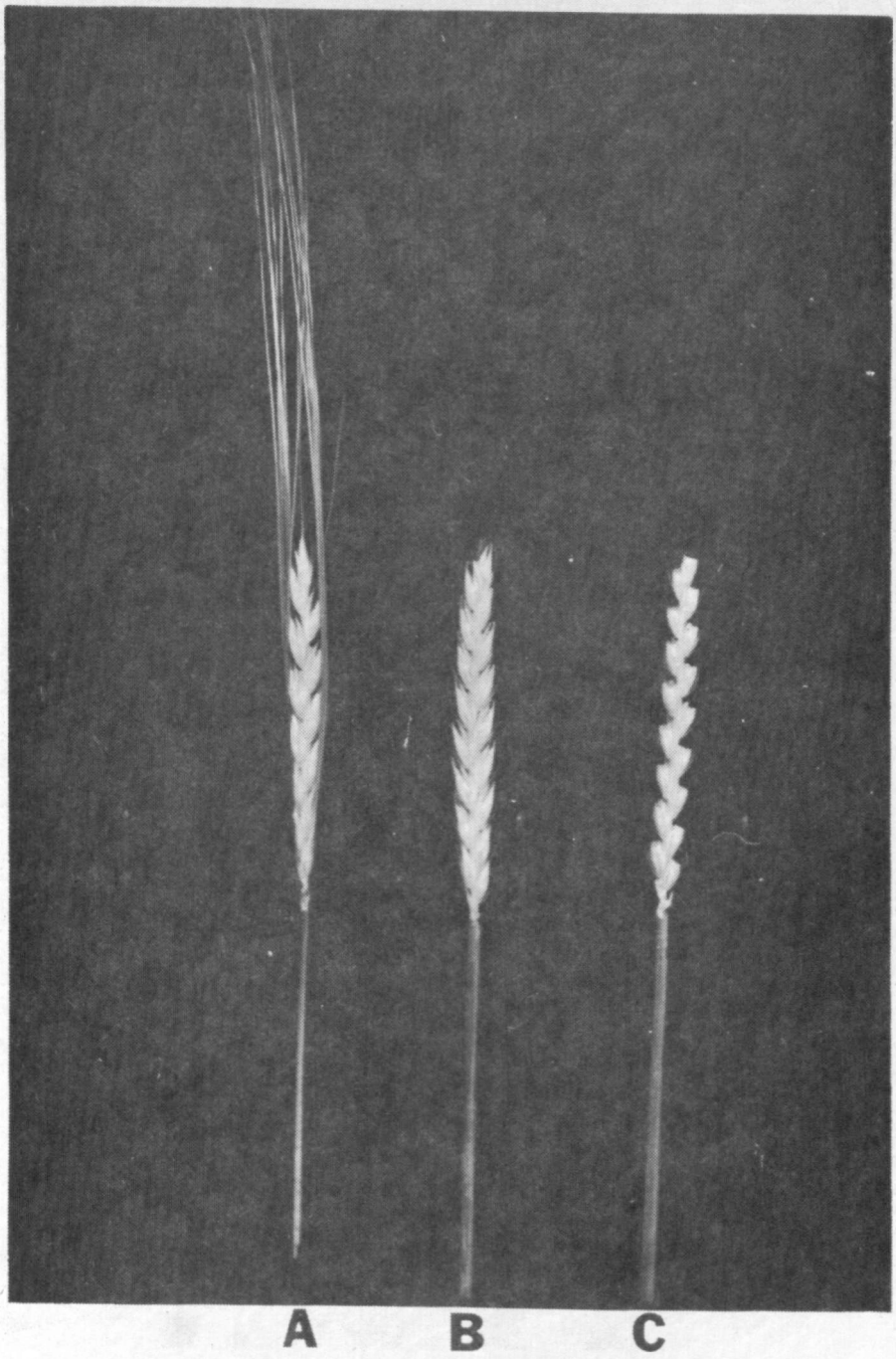
[a/] A conidial suspension of 10^6 conidia per ml was used.

Figure 1. Male sterile barley heads showing the degree of glumes modification used in inoculation studies.

A. Normal

B. Awns only removed.

C. Upper 2-4 mm of glumes removed.


A
B
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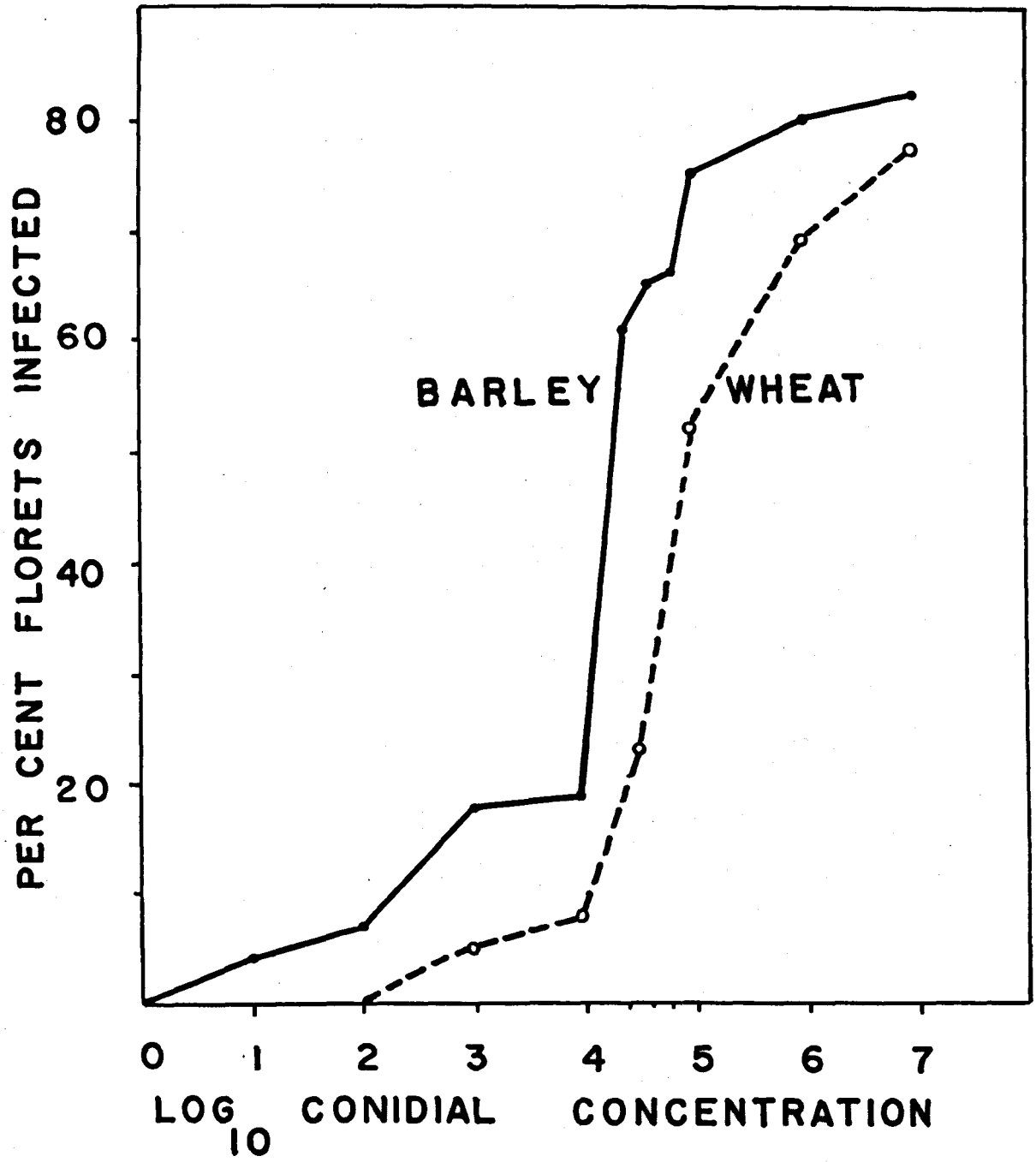
using a capillary tube. About 20-30 µl of a conidial suspension were placed in each floret. Infection could take place with as few as 10 conidia per ml on barley and 1000 conidia per ml in wheat but maximum infection occurred when 10^5 or more conidia per ml were used (Fig. 2). Considering that an average of 25 µl of inoculum was applied to each barley floret, it appears that one conidium can probably initiate infection, with maximum infection occurring when 2500 or more conidia per floret are used. Suspension of the conidia in 2% sucrose rather than in water did not increase the infection percentage in barley. Occasionally infection occurred with no honey dew or sclerotial development. Such ovaries were observed microscopically and many more conidia were found than had been used for inoculation. Moreover such ovaries were shrunken and discolored as compared to healthy ones. Further, some sclerotia were so minute that they were not visible until florets were dissected. Therefore, all the floret infection percentages include these infected shrunken ovaries and minute sclerotia.

Infection period.- To determine the length of the susceptibility period of field grown unfertilized male sterile barley florets, inoculations were made from 5 days prior to anthesis to 25 days after anthesis. An aqueous conidial suspension (10^6 conidia/ml) was atomized onto florets with glumes clipped back which had been previously

Figure 2. The effect of inoculum density of Claviceps purpurea on infection of non-fertilized florets of male sterile barley and wheat.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PER CENT FLORETS INFECTED
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(!"$+ " " ';, #, #0$(! @ #)--!)(") .!(#+#6!& '1)(