Infection and development of *Ustilago syntherismae* in *Digitaria ciliaris*

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Plant Pathology

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August, 1990

Blacksburg, Virginia
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(ABSTRACT)

*Ustilago syntherismae* (Schwein.) Peck, which causes loose smut of crabgrass, infects its host systemically and destroys seed production. Greenhouse experiments were carried out to investigate the mode of infection, the disease incidence that can be obtained by artificial inoculation and the conditions necessary for optimum infection, cross-infection on southern and smooth crabgrass, and the reasons for the late observance of the disease. *U. syntherismae* infected southern crabgrass, *Digitaria ciliaris* (Retz.) Koele, by both seed-borne and soil-borne teliospore inoculum. Disease incidence was high (84–91%) in some treatments. Higher rates of teliospore application resulted in larger percentages of smutted plants. When infesting the potting mixture, the highest percentages of smutted plants were obtained when infestation and planting were done at the same time. Earlier or later planting resulted in fewer smutted plants. When planting at various depths, greater numbers of smutted
plants were obtained when inoculated seed were planted 0.5 to 1.0 cm below the surface versus planting on the surface or at greater depths. The appearance of 50% of infected plants' first panicles (in greenhouse experiments) was delayed by 4.5 weeks compared to the emergence (50%) of first panicles from healthy plants. In cross inoculation experiments, southern and smooth crabgrass plants developed smut only when inoculated with spores collected from large and smooth crabgrass, respectively.
ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to the following individuals:

Dr. Anton B. A. M. Baudoin, for serving as my major professor and for his generous assistance, wise guidance, and encouragement throughout this endeavor.

Dr. Jeffrey F. Derr, for serving as a committee member and providing many very useful good suggestions and encouragement throughout this project.

Dr. Loke T. Kok, for serving as a committee member and also providing many very useful good suggestions and encouragement throughout this project.

The faculty, staff, and graduate students of the Department of Plant Pathology, Physiology, and Weed Science, for providing many needed inputs into this research as well as good moral support.

Ms. Hweiyiing Li, fellow graduate student of the Department, for very unselfishly providing technical, advisory, and moral support to this endeavor.

Dr. Marvin Lentner of the Department of Statistics, for very patiently providing tremendous advice on the statistics of this research.

Mr. Thomas F. Wieboldt of the VPI&SU herbarium, for providing very skillful help with plant identification and taxonomic references.

Ms. Shirley Scott and others at the VPI&SU library, for providing unexcelled assistance in literature searches.

The Bruce W. Perry scholarship committee for awarding me scholarships which encouraged as well as helped sustain me.

And last but not least, my parents, Mr. and Mrs. J. L. Johnson Jr., for their continuing encouragement, guidance, and assistance throughout this work.
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INTRODUCTION

Crabgrass species have a worldwide distribution and are vigorous competitors in many crops (Holm et al. 1977, Holm et al. 1979, Holzner and Numata 1982). In the U.S., crabgrass species are weeds in cultivated fields, gardens, lawns, pastures, roadsides, and waste places (Lorenzi et al. 1987).

Loose smut (Ustilago syntherismae (Schwein.) Peck) is a disease commonly observed in September on the large (Digitaria sanguinalis (L.) Scop.), southern (Digitaria ciliaris (Retz.) Koeler), and smooth (Digitaria ischaemum (Schreb. ex Schweig.) Schreb. ex Muhl.) crabgrass in the Blacksburg area. The smutted panicles of infected plants are not visible until several weeks or longer after the healthy crabgrass has begun to flower. Norton (1895) reported that usually every branch of infected large or smooth crabgrass is smutted although often only a portion of the inflorescence of the latter is infested. Examination of smut sori from large and southern crabgrass sometimes reveals a very small number of seed at the top of sori. Infected plants have been described as more leafy and branched than normal (Norton 1896, Halsted 1899) with shortened leaves and internodes (Gaskin 1958). This disease was recognized long ago as a natural enemy of
crabgrass. Halsted (1898) reported an abundance of this "natural enemy" on the large crabgrass in his New Jersey experimental plots and reported that rarely do infected plants produce seed. Large crabgrass, as is most of the crabgrass found in the U.S., is an annual and relies on seed production for annual propagation. The purpose of this research was to investigate this disease to better estimate its potential for biological control of crabgrass through a reduction in seed production.

*Ustilago syntherismae* has been reported to cause loose smut throughout the world only on various species of *Digitaria* (Zundel 1953). In the United States, it has been recorded on *D. filiformis* (L.) Koeler (slender crabgrass), *D. ischaemum* (smooth crabgrass), and *D. sanguinalis* (large crabgrass) (Farr et al. 1989). In Canada it has been reported on the latter two hosts (Conners 1967), and in Mexico on the first and the last (Duran 1987). The primary *Digitaria* species used in this study was *D. ciliaris* (southern crabgrass). Southern crabgrass is found throughout most of the U.S. (Webster 1987). We found *D. ciliaris* in the Blacksburg area commonly infected with *U. syntherismae*, although there is no previous report of this disease on this host in the U.S. (Farr et al. 1989). Large and southern crabgrass are difficult to distinguish (Webster 1987) and Gould (1975) indicated that in earlier
Digitaria taxonomy (Hitchcock 1971, Fernald 1950), D. ciliaris was classified as D. sanguinalis. Clewell (1985) indicated that until recently plants of D. ciliaris in the Florida panhandle were routinely identified as D. sanguinalis. This change in crabgrass species delineation probably accounts for the lack of reports of U. synthetismae on D. ciliaris.

Fisher (1951), in his guide to the literature of the smut fungi, has outlined much of the work done on U. synthetismae. Investigators have suggested that infection probably takes place early in the plant's life (Norton 1896, Halsted 1899). Spore germination of U. synthetismae has been described by Norton (1896) and Kolk (1943). Kolk (1943) was able to obtain smutted inflorescences after dusting seed of D. sanguinalis with teliospores of U. synthetismae, but presented little information on disease incidence. Gaskin (1958) found U. synthetismae within infected large crabgrass plants to be located only at the nodes and in the inflorescence.

Objectives of this research were: (1) to investigate the mode of infection of U. synthetismae, (2) to determine the disease incidence that can be obtained by artificial inoculation and the conditions necessary for optimum infection in southern crabgrass, (3) to investigate cross-infection of U. synthetismae collected from smooth
and large crabgrass, and (4) to determine whether smutted plants are observed only late in the season because only late germinating seeds become infected, or because the smutted panicles of infected plants emerge late.
MATERIALS AND METHODS

Seed. Southern crabgrass seed was obtained from the Azlin Seed Company (Leland, MS 38756) and used in all experiments unless indicated otherwise. Seeds of both southern and smooth crabgrass were also collected in the Blacksburg area for use in some experiments. *D. ciliaris* was identified using the key and descriptions of Webster (1987). Some of the characteristics of *D. ciliaris* used to distinguish it from *D. sanguinalis* were (1) absence of spicules on the lateral nerves of the lower lemma of spikelets, (2) long cells of the abaxial leaf epidermis less than 60 μm wide, (3) rarely having hairy leaf blades, and (4) rarely having a relatively short second glume. *D. ischaemum* was identified using the keys and descriptions of Gould (1975) and Radford (1968). Seed germination was estimated by sprinkling seed onto wet paper towels. Towels were rolled and placed in a slanted position in clear plastic boxes with the lower towel end in distilled water. Containers were kept in the laboratory, out of direct sunlight, at 25 ± 2 °C.

General procedures. 10-cm round plastic pots were disinfested by submersion in 10% v/v commercial bleach for about 5 minutes and then rinsed. Plants were grown in
these pots in a greenhouse using a soil-less potting mixture consisting of 1/3 each (by volume) Promix (Wetsel Seed Co., Harrisonburg, VA 22801), sand, and expanded shale (Weblite Corp., Roanoke, VA 24011).

Before seeding, the potting mixture was moistened and firmly packed into each pot. A template consisting of a wooden disk with blunt nails extending through it was pressed into the potting mixture surface to make holes for planting the seed. Twenty seeds per pot were planted at a depth of 1 cm unless indicated otherwise. The seeds were individually placed into the holes using forceps. Potting mixture was spread over the surface and firmly packed. Unless indicated otherwise, the pots were placed in pans of water for 1 to 2 days after seeding and then removed to a wire-mesh bench and watered overhead once or twice daily. Pots were returned to pans of water for a 1- to 2-day period when the potting mixture was found to be drying.

Greenhouse experiments were seeded from January through May and greenhouse temperatures varied between 15 and 38 C. Aphids were controlled with 2% insecticidal soap (Safer, Inc., Wellesley, MA 02181). Plants were first fertilized at 3 weeks of age and then every 10 days using approximately 25 ml per pot of a 15-30-15 water soluble fertilizer of 2.6 g/L.

To determine whether additional fertilization would
have an effect on the number of smutted plants, this standard fertilization schedule was compared to a fertilization regime which included fertilization at the time of seeding and then once a week through the third week after which plants were fertilized at 10 day intervals. In this experiment, seeds were inoculated by vacuum-infiltration in an aqueous spore suspension of approximately $3 \times 10^6$ spores/ml. Noninoculated control seed were vacuum-infiltrated with distilled water. Trials (two) were combined for the analysis of smutted plants as well as the analysis of seeding success. Fertilization regime did not affect either the number of smutted plants ($P = 0.19$) or the number of total plants ($P = 0.47$) (data not shown). The amount of smut for the more and less frequently fertilized plants was 69 and 57%, respectively.

**Inoculum.** Smutted southern, large, and smooth crabgrass plants were collected in the Fall of 1987 and 1988 from the Blacksburg area. Plants were allowed to air-dry and the teliospores were collected by crushing the sori. Teliospores were passed through a 60 mesh screen to remove most of the plant debris. Teliospores used as inoculum in the flower inoculation experiments were collected from a stand of predominately southern crabgrass. Teliospores used in all other experiments were collected
from a stand of predominately large crabgrass. Large and southern crabgrass are difficult to distinguish, and many of the plants from which spores were collected had some characteristics of the other species.

Teliospore germination was determined several times during the course of these experiments. Teliospores were suspended in sterile distilled water and drops were placed on water agar containing 50 mg/L streptomycin sulfate. These plates were incubated at 25 C with 12 hours diurnal light supplied by two 4-foot, 40-watt, cool-white fluorescent bulbs.

**Flower inoculation.** Southern crabgrass from locally collected seed was grown in pots in the greenhouse to flowering. Inoculation was done at different stages relative to anthesis. Anthesis occurred at dawn, generally 4 days after the panicle began to emerge from the leaf sheath. The beginning of anthesis was indicated by an open floret with the anthers (and stigmas) newly emergent. Groups of florets nearest the apex of the racemes were the first to flower. Florets closed within several hours. Basipetal groups of florets would flower at dawn for generally 4 to 5 consecutive days until all of the florets of the racemes had flowered. Flowers were inoculated at either (1) the beginning of anthesis, (2) 6 hours after the
beginning of anthesis, or (3) 12 hours after the beginning of anthesis.

For inoculation, panicles with florets at the desired stage of flowering were placed in an inflated plastic bag containing an abundance of teliospores. Ink marks were made at the ends of these groups of florets to identify them from other florets on the racemes. The bag was gently shaken for about 1 minute to create a cloud of spores around the florets. Spores adhered to all parts of the florets including the flowers and hulls.

After inoculation, half of the plants were kept in a south-facing windowsill while the other half were placed for 7 days in a dew chamber. Plants in the dew chamber were kept wet and received 12 hours of diurnal light supplied by eight 4-foot fluorescent bulbs. Plants kept in the windowsill received approximately 12 hours of sunlight each day. The temperature in both environments was \(25 \pm 2\) C.

The flower-inoculated seeds were collected when mature and stored at room temperature for about 11 months until planting. A delay in planting was necessary due to the dormancy of crabgrass seed. The experiment was conducted three times. The third trial included only the dew chamber post-inoculation treatments and used a 4-day dew period. Also in the third trial, the inoculation procedure was
altered by dusting the panicles of a plant with spores on consecutive days as different groups of florets flowered. This alteration of the inoculation procedure permitted the correct inoculation of all the florets of an inflorescence but also increased the spore load on the outside of the developing seed.

Before planting, half of the seeds were subjected to bleach treatment to kill the teliospores found on the surface of the seed and beneath the hulls. Seed were placed for 6 minutes into 15 ml screw cap vials containing 10% v/v commercial bleach. For the first minute the vials were shaken by hand. The caps were loosened, and the vials were placed in a vacuum chamber and subjected to 550 mm Hg of vacuum (210 mm Hg pressure) for the remainder of the 6 minutes. The vacuum chamber was placed on top of the vacuum pump and motor to receive constant vibration. The pressure was quickly released 3 times during the vacuum treatment. The contents were dumped onto filter paper in a Buchner funnel and rinsed for approximately 3 minutes with four 250-ml additions of water. After rinsing, the filter paper with seed was removed and allowed to air dry.

Most spores had lost color but some beneath the hulls were still brown after the bleach treatment. To help insure the destruction of all inoculated teliospores, a second vacuum-infiltrated bleach treatment was done with
630 mm of vacuum. Prior to the second bleach treatment, the seed were presoaked in water for 2 hours to increase the efficacy of the disinfectant and protect the seed from injury (Braun 1920). The water was drained and the seed were vacuum-infiltrated as previously described in 10\% v/v commercial bleach containing 0.01\% v/v Tween 80. The seed were rinsed for 15 minutes in water using three 330-ml additions of water.

Seed were planted 5 and 10 per pot for the first two and third trials, respectively. Seed of the same origin and age as the flower inoculated seed, but stored under refrigeration, were used for controls. Controls consisted of (1) noninoculated, nonbleached seed, (2) noninoculated, vacuum bleach-treated seed, and (3) nonbleached seed vacuum-infiltrated in a spore suspension of approximately 3 X 10^6 spores/ml (procedure described below). Plants were grown in the greenhouse on a mist bench for the first 3 weeks after seeding.

Watering consisted of 15-second bursts of fine mist every 6 minutes between 7:45 AM and 9 PM of each day. Plants watered in this fashion were exposed to an oversupply of water along with increased leaching of nutrients which slowed the growth and development of the seedlings. For the second trial the pots were watered as described in "General Procedures" but with an initial
soaking of 7 days.

**Vacuum-infiltration of seed with teliospores.** 6.25, 25, or 100 mg (1:4:16 ratio of weights) of spores (screened spores included small amounts of impurities) were gently pressed between layers of weighing paper using a round glass vial to loosen any clusters of spores. The spores were added to 10 ml of water in 15-ml screw cap vials and shaken by hand for 20 minutes to produce a suspension of predominately single spores. Resulting spore concentrations were determined with a hemacytometer and were approximately $7 \times 10^5$, $3 \times 10^6$, and $1 \times 10^7$ spores/ml.

Crabgrass seed (0.44 g) was added to the suspension and the vials were shaken for 1 minute by hand. After loosening the cap, the vial was placed in an Erlenmeyer flask with sidearm and subjected to 630 mm of vacuum for the remainder of 30 minutes. This was done to help get the spores beneath the hulls and closer to the caryopsis. The flask was placed on top of the vacuum pump and motor so that the seed and spore suspension would receive constant vibration. The vacuum was quickly released three times at 7-to 8-minute intervals. The vial was temporarily removed after the first 15 minutes and gently shaken to redistribute spores from the bottom. At the completion of the vacuum-infiltration, the vial was removed and the
contents emptied onto paper towels and allowed to dry.

The number of spores adhering to the inoculated seeds was determined for each concentration used. Inoculated seeds (0.160 g used on the average) were placed into 125 ml Erlenmeyer flasks. Ten ml of water containing 0.1% Tween 80 was added. Flasks were shaken for 2 hours on an oscillating shaker at 150 rpm. The teliospore concentrations of the suspensions were determined using a hemacytometer. The number of spores adhering to each seed was calculated and found to be approximately 750, 3,500, and 17,000 for seed inoculated in teliospore suspensions of $7 \times 10^5$, $3 \times 10^6$, and $1 \times 10^7$ spores/ml, respectively.

Suspension and dry inoculations compared with vacuum-infiltration. Seed were vacuum-infiltrated using the three inoculum concentrations described above. Noninoculated control seed were vacuum-infiltrated with water. For spore suspension inoculations without vacuum-infiltration, 0.44 g of seed were added to identical spore suspensions as those used in the vacuum-infiltration and shaken on an oscillating shaker at 100 rpm for 10 minutes. Vials were emptied onto paper towels and the contents allowed to dry. Noninoculated control seed were shaken in water. The number of spores adhering to the seed was determined as previously described and was the same as
that determined for the vacuum-infiltrated seed.

In a separate experiment, dry inoculation was compared to vacuum-infiltration. Seed were vacuum-infiltrated in an aqueous spore suspension containing approximately $3 \times 10^6$ spores/ml. Dry-inoculated seed were prepared by placing seed in a 30-ml vial with an excess of spores and shaking by hand for about 10 minutes. Excess spores were removed from the dry-inoculated seed during planting by tapping the forceps used to hold the seed. Control seed were not vacuum-infiltrated. Mist bench watering was used for the first 3 weeks after seeding. A second trial utilized the standard watering procedure as described in "General Procedures". The number of spores adhering to the seed was not determined, but, judging from the darkness of the seed after inoculation, was considerably higher than that obtained with the other inoculation procedures.

**Effect of planting depth and seed inoculation rate on infection.** Seed were vacuum-infiltrated using the three inoculum concentrations previously described. Noninoculated control seed were vacuum infiltrated with distilled water. Seed were planted on the potting mixture surface and at 0.5, 1, 2, and 3 cm below the surface. The planting procedure described in "General Procedures" was used for seeding at the 0.5 and 1 cm depths. At depths of
2 and 3 cm, the template was not used but instead the pots were first partially filled with potting mixture and firmly packed. The seed were placed on this surface and the pot was filled and firmly packed with potting mixture to obtain planting depths of 2 and 3 cm. Twenty seed were planted per pot at the 0, 0.5, and 1 cm depths. Thirty and forty seed were planted at the 2 and 3 cm depths, respectively, to compensate for the poorer emergence of southern crabgrass at these depths.

**Cross inoculation of southern and smooth crabgrass.**

All seed used in this experiment were collected locally. Southern and smooth crabgrass seeds were inoculated with teliospores collected from smooth and large crabgrass by vacuum-infiltration in spore suspensions of approximately 3 \( \times 10^6 \) spores/ml. Noninoculated southern and smooth crabgrass seeds were vacuum-infiltrated with distilled water for use as controls. The number of spores adhering to the smooth crabgrass seed was determined as previously described and was approximately 5,500 spores per seed.

**Potting mixture infestation: effect of host age.** Pots were seeded at 21, 14, 7, 4, 2, and 0 days before potting mixture infestation. Seeded pots were placed on a mist bench until the time of infestation. Six ml of a spore
suspension, either $3 \times 10^5$, $1 \times 10^6$, or $6 \times 10^6$ spores/ml (prepared respectively from a 1:5:25 ratio of spore weights), were applied evenly to the potting mixture surface (approximately 74 cm$^2$) in an overlapping spiral pattern using a pipette. After infestation, care was taken not to overwater the pots or water with too much force.

**Potting mixture infestation: effect of delayed planting.** Spore suspensions were applied to the potting mixture surface as described above. After infestation, the pots were watered as described in the standard watering procedure in "General Procedures." Care was taken not to overwater the pots or water with too much force. Randomly selected pots of each infestation rate were seeded at 0, 3, 7, 14, 21, 28, and 56 days after infestation. Noninfested pots were prepared and seeded at the time of each planting for use as controls. Seeded pots were placed on a mist bench for the first 3 weeks after planting. A second trial utilized the standard watering procedure as described in "General Procedures" for the seeded as well as the unseeded pots. All seeded pots were randomized and then rerandomized as new pots were seeded. Pots seeded 56 days after soil infestation were kept separate from the others to prevent excessive shading.
Experimental design, data collection, and analysis.
All experiments were arranged in a completely randomized design. Each pot represented a treatment replication. Treatments were replicated 4 or 5 times within an experiment. All experiments were repeated (repetitions referred to as trials) using 3 replications of treatments.

Data were collected as the panicles, healthy or smutted, became visible. With few exceptions, the panicles of a plant were either all smutted or all healthy. The number of smutted plants was expressed as a proportion of the total number of plants in the replicate. Noninoculated control treatments (all nonsmutted) were not included in the statistical analysis of smutted plants. Replicates with less than four total plants (healthy plus smutted) were not used in the smutted plant analysis since such a low total number of plants may not represent the treatment effect well and result in misleading percentages of smutted plants. The number of these replications removed from the smutted plant analysis is indicated in the results of each experiment if any replications were removed. These proportions were arcsine transformed and analyzed using the GLM (General Linear Models) analysis of variance procedure of SAS (SAS Institute Inc., Cary, N.C. 27511-8000). Trials were combined for analysis when the results were similar and the variances were homogeneous by the variance-ratio
test (Li 1964). Where appropriate, LSD tests (P=0.05) and single degree of freedom contrasts (P=0.05) were used to separate means. The LSD mean separation was only used if the ANOVA indicated significant effects for the variable.

The number of total plants (healthy plus smutted) in a replication was converted to a proportion of the number of seeds planted. These proportions, referred to as seeding success, were arcsine transformed and analyzed using the SAS procedures previously described.
RESULTS

Inoculum and seed viability. At 25 C, teliospores collected from southern crabgrass began germinating between 24 and 36 hours after plating. Most spores germinated within 72 hours. At 72 hours, germination was 70% or higher for both 4- and 14-month old spores. Very rarely were sporidia observed.

Germination of southern crabgrass seed in rolled paper towels averaged 75% over the course of these experiments. Smooth crabgrass seed had a 70% rate of germination in rolled paper towels.

Flower inoculation. Nineteen of 116 pots (replications) had less than four plants and were eliminated from the smutted plant analysis. A minimum of three plants per replication, instead of four, was used in this analysis due to low numbers of plants in the first two trials. Plants grown from flower-inoculated, bleach-treated seed did not become smutted. Flower-inoculated seed which had not been bleach-treated produced no smutted plants in the first trial, 13% in the second, and 36% in the third. In the second trial, nonbleached seed which had been flower-inoculated at 0, 6, and 12 hours after the beginning of anthesis produced no
differences \( (P = 0.25) \) in the amount of smutted plants (18, 4, and 16\%, respectively). In the third trial, the timing of inoculation with the nonbleached seed did affect \( (P = 0.002) \) the percentage of smutted plants such that florets inoculated at anthesis produced a higher percentage \( (P < 0.05) \) of smutted plants (55\%) than those inoculated at 6 hours (20\%) or 12 hours (32\%) after the beginning of anthesis. Percent smutted plants obtained from flower-inoculated nonbleached seeds with post-inoculation dew periods versus dry post-inoculation periods could be compared only in the second experiment; there was no difference \( (\text{dew} = 14\%, \text{dry} = 11\%) \) \( (P = 0.75) \). In the three trials, the combined total number of plants grown to maturity from seed inoculated at 0, 6, and 12 hours after the beginning of anthesis was 241, 201, and 180 plants, respectively. About half of these plants were grown from bleach-treated and half from nonbleached seed. The seed-inoculated controls had an average percent smut of 30, 40, and 80\% for the three trials, respectively.

Bleach treatment reduced the seeding success of the flower-inoculated seed in the first two trials. In the first trial, there was a difference \( (P = 0.02) \) between seeding success for nonbleached flower-inoculated seed (85\%) and bleach-treated flower-inoculated seed (68\%). In the second trial, there was also a difference \( (P = 0.04) \)
between seeding success for nonbleached flower-inoculated seed (67%) and bleach-treated flower-inoculated seed (52%). In the third trial, seeding success for bleach-treated (92%) and nonbleached (95%) flower-inoculated seed was not different (P = 0.30). A combined analysis of the control seed of all three trials indicated that seeding success appeared to be enhanced (P = 0.051) by bleach treatment (94%) when compared to nonbleached seeds (89%).

Comparison of seed inoculation by spore-suspension with and without vacuum-infiltration. Trials were combined for the analysis of smutted plants as well as the analysis of seeding success. One of 42 pots (replications) had less than four plants and was eliminated from the smutted plant analysis. Inoculation by spore suspension with and without vacuum-infiltration did not affect (P = 0.85) the occurrence of smutted plants, 65 and 66% respectively. The percent smut increased (P = 0.0006) as the inoculum concentration was increased (Table 3.1).

Seeding success decreased (P = 0.004) as the inoculum concentration was increased (Table 3.1). In addition, single degree of freedom contrasts indicated differences in seeding success between noninoculated controls and those inoculated at 3 x 10⁶ spores/ml (P = 0.03) and between noninoculated controls and those inoculated at 1 x 10⁷
Table 3.1 Effect of inoculum concentration on the percentage seeding success and the percentage of smutted plants grown from southern crabgrass seed inoculated in three teliospore suspensions of *Ustilago synertherismae*.

<table>
<thead>
<tr>
<th>Inoculum Concentration (spores/ml)</th>
<th>Seeding Success* (%)</th>
<th>Smutted Plants* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>noninoculated</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>$7 \times 10^5$</td>
<td>45 ($P = 0.9272$)</td>
<td>48</td>
</tr>
<tr>
<td>$3 \times 10^6$</td>
<td>$36^* (P = 0.0260)$</td>
<td>66</td>
</tr>
<tr>
<td>$1 \times 10^7$</td>
<td>$33^* (P = 0.0023)$</td>
<td>83</td>
</tr>
</tbody>
</table>

* Data represent a combination of two trials and are combined from seed inoculated by spore suspension with and without vacuum-infiltration.

* Seeding success is the total number of plants in the replication (healthy + smutted) divided by the number of seed planted.

* Smutted plants is the number of plants in the replication with smutted panicles divided by the total number of plants (healthy + smutted) in the replication.

* * indicates that the mean is significantly different from the noninoculated control according to single degree of freedom contrasts.
spores/ml \( (P = 0.002) \).

**Comparison of inoculation by vacuum-infiltration and dry spores.** Trials were combined for the analysis of smutted plants as well as the analysis of seeding success. One of 16 pots (replications) had less than four plants and was eliminated from the smutted plant analysis. There was no difference \( (P = 0.68) \) in the efficacy of the two inoculation techniques: both vacuum-infiltrated and dry inoculated seed produced 82% smutted plants.

Inoculation influenced \( (P = 0.04) \) seeding success such that vacuum-infiltrated inoculated seed produced fewer plants (31% seeding success) \( (P < 0.05) \) than did non-inoculated nonvacuum-infiltrated control seed (43% seeding success). Dry-inoculated seed produced 34% plants, which was not different \( (P < 0.05) \) from the controls or the vacuum-infiltrated inoculated seed.

**Effect of planting depth and seed inoculation rate.** Trials were combined for the analysis of smutted plants as well as the analysis of seeding success. Nine of 120 pots (replications) had less than four plants and were eliminated from the smutted plant analysis. Appendix Figure A.1 presents the smutted plant results of the arcsine transformed data. Planting depth influenced \( (P = \)
the number of smutted plants such that the highest percentage of smutted plants was obtained when seeds were planted at depths of 0.5 and 1 cm (Fig. 3.1). The mean percentage of smutted plants increased \( P = 0.0001 \) from 22 to 52% as the inoculum concentration was increased from the low to the high rate. The response of planting depth and inoculum concentration on the number of smutted plants did not depend \( P = 0.08 \) on the level of the other factor. The highest treatment mean percentage of smutted plants (84%) for the combined trials occurred when seed were inoculated in a suspension of \( 1 \times 10^7 \) spores/ml and planted at a depth of 0.5 cm (see Fig. 3.1).

Seeding success was 54% when seed were planted on the surface and decreased \( P = 0.0001 \) to 43, 45, 34, and 15% at 0.5, 1.0, 2.0, and 3.0 cm planting depths, respectively. Seeding success decreased \( P = 0.0001 \) when seeds were inoculated in the more concentrated spore suspensions (decreased from 41 to 32% and 50 to 27%, for each trial respectively). The effect of planting depth and inoculum concentration on seeding success did not depend \( P = 0.57 \) on the level of the other factor.

**Effect of infection on the emergence of panicles.** The appearance of the first panicles from both healthy and infected plants in the planting depth experiment was
Fig. 3.1. Effect of planting depth on the percentage of smutted plants grown from southern crabgrass seed (0.44 g) vacuum-infiltrated in three *Ustilago syntherismae* teliospore concentrations. Data represent a combination of two trials.

\[ \nabla = 7 \times 10^5, \quad \Box = 3 \times 10^6, \quad \blacksquare = 1 \times 10^7 \text{ spores per ml} \]
recorded weekly. The first smut sori were not visible until 3 or 4 weeks after the appearance of the first healthy panicles. Fifty percent (average of both trials) of both the healthy and smutted plants had at least one emerged panicle within 3.5 and 8 weeks, respectively, of the first healthy panicle appearance.

**Cross inoculation of southern and smooth crabgrass.**
Southern and smooth crabgrass plants developed smut (75 and 48%, respectively, mean of combined trials) only when inoculated with spores collected from large and smooth crabgrass, respectively. No smut developed in either species when southern and smooth crabgrass plants were inoculated with spores collected from smooth and large crabgrass, respectively. An analysis of each trial indicated that inoculation did not affect (P = 0.35 and P = 0.67, respectively) seeding success.

All of the first emerging panicles of infected smooth crabgrass in this experiment were smutted. However, some of the subsequently appearing panicles of the infected plants were either only partially smutted or not smutted at all and produced seed. With southern crabgrass, however, only very rarely would a plant have both healthy and smutted panicles. Smutted panicles of smooth crabgrass often emerged above the flag leaf while those of southern
crabgrass usually remained below the flag leaf and erupted through the leaf sheath.

**Effect of host age at the time of potting mixture infestation.** The variances of the two trials were not homogeneous for the smutted plant analysis, although both trials demonstrated similar trends. The smutted plant results presented are those of the first trial. Appendix Figure A.2 presents the smutted plant results of the arcsine transformed data. Both the seeding date \( (P = 0.0001) \) and the infestation rate \( (P = 0.002) \) influenced the percent smutted plants. Infestation at the time of seeding resulted in the highest percentage \( (P < 0.05) \) of smutted plants (mean of all rates, 71%) (Fig. 3.2). Seeding 7 days or more before inoculation resulted in very few or no smutted plants. Averaged over all seeding dates, the high and medium inoculation rates produced the same amount of smut (29%), whereas the low inoculation rate produced less (18% smutted) \( (P < 0.05) \). The response of seeding date and infestation rate on the number of smutted plants did not depend \( (P = 0.14) \) on the level of the other factor. Combining trials, seeding date \( (P = 0.18) \) and infestation rate \( (P = 0.91) \) did not affect seeding success.
Fig. 3.2. Effect of planting date, in days before infestation, on the percentage of smutted plants grown from southern crabgrass seed sown 1 cm deep into potting mixture surface infested at three rates with teliospores of *Ustilago sphenidosa*.

\[\n\n\text{\textbullet} = 1.8 \times 10^6, \quad \square = 7.0 \times 10^6, \quad \text{and} \quad \blacksquare = 3.6 \times 10^7 \text{ spores per 10-cm pot.} \n\]
Potting mixture infestation: effect of delayed planting. Trials were combined for the analysis of smutted plants. Fourteen of 168 pots (replications) had less than three plants and were eliminated from the smutted plant analysis. A minimum of three plants per replication, instead of four, was used in this analysis due to low numbers of plants in the more delayed plantings. Fewer plants in the more delayed plantings were probably the result of roach and mice infestations and possibly shading by the plants of the earlier plantings. Appendix Figure A.3 presents the smutted plant results of the arcsine transformed data. Infestation concentration affected \( P = 0.0001 \) the number of smutted plants such that the highest percentage \( P < 0.05 \) of smutted plants (30%, mean of all rates) occurred when seeds were planted at the time of potting mixture infestation (Fig. 3.3). The percentage of smutted plants decreased to low levels (5% or less) when seeds were planted 7 days after mixture infestation. Planting from 14 to 56 days after infestation at the high rate resulted in 10 to 19% smutted plants. However, seeds planted during this same time period into potting mixture infested at the low and medium rates produced few or no smutted plants.

Mean percent smut increased \( P = 0.0001 \) as the infestation rate was increased from the low (3%) to the
Fig. 3.3. Effect of planting date, in days after infestation, on the percentage of smutted plants grown from southern crabgrass seed sown 1 cm deep into potting mixture surface infested at three rates with teliospores of *Ustilago synerghismae*. Data represent a combination of two trials. Bar represents the standard error based on the mean square error of all data combined into one analysis. ▼ = 1.8 x 10^6, □ = 7.0 x 10^6, and ■ = 3.6 x 10^7 spores per 10-cm pot.
medium (8%) rate and from the medium (8%) to the high (22%) rate. Percent smut increased as the rate was increased from low to medium primarily at the seeding dates of 0 and 3 days after infestation. The response of seeding date and infestation rate on the number of smutted plants depended (P = 0.003) on the level of the other factor, as is evident in the following two observations. (1) The large reduction in smutted plants from seeds planted 0 and 3 days after infestation for the medium and high rates but not the low rate (Fig. 3.3) was observed in both trials. At the low rate, smut in one trial increased from 4 to 6% while in the other trial decreased from 8 to 7%. (2) The percentage of smutted plants for the high rate of infestation increased for 2 days after day 14, whereas with the medium and low rates of infestation it immediately decreased.

The seeding success in both trials was very similar, but the results were not combined and the results of the second trial are presented since only the second trial had controls at every seeding date. Apparently due to the pests and possible shading problems previously mentioned, seeding success was higher (P = 0.0001) when planting was done closer to the time of infestation (treatment means range of 28-55%). Infestation rate had no effect (P = 0.15) on seeding success.
DISCUSSION

Southern crabgrass seedlings were readily infected (84-91%) by *U. syntherismae* from soil-borne inoculum and seed-borne inoculum. Using soil-borne inoculum, such high rates of infection were found to occur during preemergence. This ability to infect from both types of inoculum is typical of many seedling-infecting smuts. Exceptions include the head smut of corn (*Sporisorium holci-sorghii* (Rivolta) K. Vanky) and the dwarf bunt of wheat (*Tilletia controversa* Kuhn in Rabenh.), which infect very little when seed are artificially inoculated, but which readily infect from soil-borne inoculum under natural conditions (Fischer and Holton 1957).

In the flower-inoculation experiment, smut was obtained only from seeds which had not been treated with bleach after the inoculation. The known embryo-infecting smut, *Ustilago tritici* (Pers.) Rostr. of wheat and barley, is not controlled by surface treatments such as formaldehyde and copper carbonate treatments which will control many seedling infecting smuts (Tapke 1931). References describing the effects of bleach treatment on smut pathogens in seed embryos were not found, but inactivation of the smut pathogen if in the embryo without damage to the seed seems unlikely. Most likely there was no embryo
infection of the seed after inoculation since none of the bleach treated seeds grew into smutted plants. The smutted plants grown from the nonbleached seeds most likely were infected by sporidia or mycelium produced from the teliospores resting on the surface or beneath the hulls of the seeds after the seeds were planted. Since none of the bleach-treated flower-inoculated southern crabgrass seed produced smutted panicles under apparently favorable conditions, embryo infection by *U. syntherismae* appears unlikely.

These experiments cannot rule out completely the possibility of embryo infection by *U. syntherismae*. However, the embryo-infecting smut has not been shown to infect its hosts using common seed inoculation or soil infestation procedures (Fischer and Holton 1957), whereas *U. syntherismae* can readily infect by both of these techniques. Furthermore, the appearance of the smutted panicles of barley and wheat plants infected with *U. tritici* coincide with the flowering of the noninfected plants (Freeman and Johnson 1909). Such correspondence between the appearance of smutted panicles of crabgrass infected with *U. syntherismae* and the panicles of healthy crabgrass plants was not observed. As previously mentioned, the panicles of crabgrass with loose smut are usually not observed until several weeks or longer after
the majority of healthy plants have begun to flower. Also, conditions in these experiments should have been favorable for embryo infection because half of the plants were kept in a humid environment for either 4 or 7 days after inoculation. Infection by the embryo-infecting smut, *Ustilago tritici*, on wheat and barley was greatly increased when plants were kept at high humidity after inoculation (Tapke 1931). In addition, flowers were inoculated during a period (0 to 12 hours after the beginning of anthesis) which is part of the optimum period of infection for embryo infecting smut pathogens (Fischer and Holton 1957, Freeman and Johnson 1909).

Two other modes of infection with some smuts are local infection and shoot infection. Local infection is indicated by nonsystemic development of the pathogen such that the mycelium and sporulation occur only in the region or organ of initial penetration (Fisher and Holton 1957). No evidence of local infection was observed with *U. syntherismae* on southern or smooth crabgrass and Gaskin (1958) has found the mycelium to be systemic in the host. In the flower inoculation experiment, no local smut occurred in the florets after they were inoculated, indicating the absence of local infection in a meristematic region where it might have occurred if local infection were a valid mode for *U. syntherismae*.
Shoot (bud) infection is apparently the single mode of infection for some smuts while for others it is an accessory mode (Fisher and Holton 1957). These experiments can not rule out the possibility of shoot infection. However, field and greenhouse observations of southern and smooth crabgrass during this research indicate that almost exclusively all of the tillers of an infected plant are smutted (at least at first) or the plant is not smutted at all. This evidence suggests that the new tillers forming from a noninfected crabgrass plant are not readily shoot-infected at their nodes. Leach and colleagues (Leach *et al.* 1946) demonstrated infection by *Ustilago striiformis* (Westend.) Niessl on Kentucky bluegrass (*Poa pratensis* L.) after dipping roots and tillers of plants in a suspension of teliospores. This inoculation procedure was not done with *U. syntherismae* on crabgrass, but if shoot infection occurred readily with *U. syntherismae*, then smutted plants would be expected to occur in the pots of the host age experiment inoculated 21, 14, and 7 days after planting (Fig. 3.2). These treatments included seedlings ranging from newly emergent to 5 cm tall at the 2-true leaf stage. A few smutted plants did occur for some of the rates at some of these times (see Fig. 3.2). These small numbers of smutted plants probably are the result of infection of seeds which emerged after infestation of the potting mix.
Much emergence of this type was observed. Seeds emerging after infestation had a greater chance of becoming infected.

The increases in smutted plants demonstrated by these experiments with increased rates of *U. synderismae* are similar to trends for other seedling-infecting smuts such as rough-spored bunt and common bunt on wheat caused by *Tilletia caries* (DC.) Tul. & C. Tul. and *T. laevis* Kuhn in Rabenh., respectively (Heald 1921, Leukel 1937, Fischer and Holton 1957). High percentages of smutted crabgrass plants resulted from seed inoculation by either shaking in a spore suspension, vacuum-infiltrating in a spore suspension, or dusting with dry spores. This range of effective, artificial, seed-inoculation techniques is common for many of the seedling infecting smuts, although there are exceptions, such as the covered smut of oats and barley (*U. hordei* (Pers.) Lagerh.) and the loose smut of oats (*U. avenae* (Pers.) Rostr.). To obtain maximum infection when artificially inoculating with these barley (Tisdale 1923) and oat smuts (Johnston 1927, Stanton *et al.* 1930) generally requires methods which place spores beneath the hulls; when intact seeds with hulls were dusted with dry spores, much less smut was produced.

In the case of seedling infection by seed-borne or soil-borne spores, proper timing between seed and spore
germination is essential because with most examples of this type of infection the young seedling is vulnerable only during the earliest stages in its development (Fischer and Holton 1957). The host age experiment with *U. syntherismae* demonstrated that maximum infection occurred when sowing and infesting were done at the same time. Emergence of southern crabgrass seedlings in the host age experiment was usually first seen 5 days after sowing, with 56% of the total emergence by day 7 and 90% by day 14. Since the majority of spores germinated between the second and the third day in the laboratory (which may be different than in the greenhouse because of the different environments), this experiment indicates that infection decreased rapidly if the majority of spores had not germinated 2 to 3 days before seedlings began emerging.

When seeding was done after potting mixture infestation, moderate amounts of infection (10-19%) were obtained with the highest infestation rate for seeds sown 2, 3, 4, and even 8 weeks after infestation (Fig. 3.3). This trend occurred in both trials as did also the sharp decrease in smutted plants for all seeds planted 7 days after infestation. Possibly the low amounts of smutted plants from seeds planted after infestation are due to (1) spores being washed from the depth of seeding (1 cm) during watering, (2) spores germinating and shortly thereafter
dying, and (3) spores losing viability in the soil. The subsequent reoccurrence in smutted plants at the highest rate when seeding 14 or more days after infestation may be due to (1) infection after mycelium had grown or spores diffused (or moved with evaporating water) back, after displacement by watering, to the depth of the seed (1 cm) and (2) a delay in the germination, of at least part, of the applied teliospores. The rapid decline in infection as planting was delayed after teliospore application raises the question whether inoculum can survive in the soil to any appreciable extent. Field studies would be needed to better quantify the range of effective teliospore application times relative to seed germination.

Inoculated seeds planted either on the surface or 2 and 3 cm below the surface of noninfested potting mix developed into fewer smutted plants than those seeded 0.5 or 1.0 cm deep. This suggests that spores of *U. syntherismae* which are deeper in the profile (2-3 cm) do not play a large role in the infection of southern crabgrass. However, experiments with noninoculated seeds planted at various depths in infested soil may more clearly depict the effect that depth would have under field conditions. Studies on dwarf bunt of wheat caused by *Tilletia controversa* Kuhn in Rabenh. in naturally infested field plots demonstrated a decrease of dwarf bunt at greater seeding depths (Meiners
et al. 1956). Conversely, the covered and semi-loose smuts of barley (U. hordei and U. avenae, respectively) (Taylor and Zehner 1931) caused more smut in naturally infested plots when seeds were planted at greater depths.

Sometimes similar treatments among trials and experiments produced different levels of smutted plants (for example, infestation at the time of planting in Figures 3.2 and 3.3), suggesting that factors other than those monitored influenced infection. Literature of other smut pathogens suggests such factors as soil temperature and soil moisture (Tapke 1948) whose effects on infection have been shown to be interrelated (Faris 1924, Reed and Faris 1924, Reed and Faris 1924a). These or other variables may have influenced infection due to their effects on the spores or the crabgrass seedlings.

The results of three of the experiments indicated that inoculation reduced the number of plants (seeding success). Inoculation had no significant effect on seeding success in the other four experiments where seeding success was examined. These results raise the possibility that the number of smutted plants observed at the end of an experiment may be fewer than the number infected because of mortality. This would make the percentage of smutted plants a conservative estimate of the effect of inoculation on crabgrass.
The delay in appearance of panicles of infected southern crabgrass plants observed in these greenhouse studies apparently explains, at least in part, the late appearance of smut observed in the field. The amount of delay seen in the field may be different from that observed in the greenhouse due to possible developmental differences in the crabgrass caused by differences in environmental factors such as temperature, sunlight, pests, wind, and movement.

Pathogenic specialization is common among the cereal smuts (Fisher and Holton 1957). Varietal or species' differential reaction to various collections of a smut has led to the division of smuts into pathological races (Fischer and Holton 1957). Pathogenic specialization in \textit{U. syntherismae} was demonstrated based on the reaction of smooth and southern crabgrass to inoculum collected from large and smooth crabgrass, respectively. That southern crabgrass was readily infected from inoculum collected from predominately large crabgrass indicates a lack of pathogenic specialization in \textit{U. syntherismae} between these two species.

Before a pathogen is used as a biological control agent, the host range must be investigated to determine the risk to nontarget plants. The hosts reported for \textit{U. syntherismae} worldwide are annual \textit{Digitaria} species (the
type of growth and significance of one of these, *Digitaria bifasciculata* (Trin.) Henr., has not been located) which are often not considered productive enough to be used in cultivation and are mostly weeds, although they can be nutritious and can be used for grazing and hay (Bogdan 1977, Holm *et al.* 1977). One host, *D. sanguinalis*, in many places is a serious weed, but in Kashmir it is a minor cereal (Dahlgren *et al.* 1985). Another host, *D. divaricatissima* (R. Br.) Hughes, is reported as an important native pasture grass in Australia (Whittet 1969). Many other *Digitaria* species are important economically as crops, cereals, or lawns (Bogdan 1977, Dahlgren *et al.* 1985) although no reports have been found citing these as hosts of *U. syntherismae*.

*U. syntherismae* is distributed worldwide and it stands to reason that it must be widely adaptable to various environments. This indicates that, if *U. syntherismae* were to be developed as a biological control agent, it probably could be used in many different climates. Since *U. syntherismae* apparently infects only at the very early stages of plant development, this could be a limiting factor in biological control. If inoculum would persist in the soil when applied preemergence, this rather limited time for host infection may become less significant. The pathological specialization demonstrated for *U.*
syntherismae can be beneficial since pathological specialization may allow use of U. syntherismae on undesirable hosts near taxonomically related desirable plants. Pathological specialization could also be troublesome and necessitate the use of different inoculum to control various troublesome Digitaria species. Further investigation with varieties and species to estimate the degree of specialization which has developed in U. syntherismae is needed to confirm these results and evaluate the likelihood of increased resistance developing in Digitaria species.

These experiments demonstrate that U. syntherismae has potential as a biological control agent of crabgrass. High rates of infection (84-91%) were obtained in some treatments when applying teliospores to the surface of the potting mixture and when inoculating seeds with dry spores and spore suspensions with and without vacuum-infiltration. Field tests are needed to investigate more closely this disease in more natural conditions. A key factor is how far in advance of seed germination could an application of spores be made to the soil and remain effective.

In a stand of crabgrass, infection of some plants by U. syntherismae reducing crabgrass seed production does not necessarily result in reduced crabgrass competition for all crops since crabgrass can quickly grow and overcome a
reduction of its plant density. If a build up of inoculum in the soil could occur, either from reapplication or production from infected plants, this may reduce seed production enough, and hence deplete the seed reserve in the soil, to significantly reduce crabgrass competition or eradicate the crabgrass from the site. Yearly application of inoculum to crabgrass seeds while attached to their racemes may also provide effective infection percentages such that the crabgrass may be greatly reduced or eliminated from the site.
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Fig. A.1. Effect of planting depth on the arcsine transformation (degrees) of the percentage of smutted plants grown from southern crabgrass seed (0.44 g) vacuum-infiltrated in three *Ustilago syneresismae* teliospore concentrations. Data represent a combination of two trials. Bar represents the standard error based on the mean square error of all data (arcsine transformed) combined in one analysis.

\[ \nabla = 7 \times 10^5, \quad \square = 3 \times 10^6, \quad \blacksquare = 1 \times 10^7 \text{ spores per ml.} \]
Fig. A.2. Effect of planting date, in days before infestation, on the arcsine transformation (degrees) of the percentage of smutted plants grown from southern crabgrass seed sown 1 cm deep into potting mixture surface infested at three rates with teliospores of *Ustilago syntherismae*. Bar represents the standard error based on the mean square error of all data (arcsine transformed) combined into one analysis. ▼ = 1.8 x 10^6, □ = 7.0 x 10^6, and ■ = 3.6 x 10^7 spores per 10-cm pot.
Fig. A.3. Effect of planting date, in days after infestation, on the arcsine transformation (degrees) of the percentage of smutted plants grown from southern crabgrass seed sown 1 cm deep into potting mixture surface infested at three rates with teliospores of *Ustilago syntherismae*. Data represent a combination of two trials. Bar represents the standard error based on the mean square error of all data (arcsine transformed) combined into one analysis. ▼ = 1.8 x 10⁶, □ = 7.0 x 10⁶, and ■ = 3.6 x 10⁷ spores per 10-cm pot.
VITA

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