THE FIELD INOCULATION OF RYE WITH CLAVICEPS PURPUREA (Fr.) Tul.

by

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A THESIS

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I Introduction

This paper presents a new technique for the inoculation of rye with Claviceps purpurea (Fr.) Tul. The method consists primarily of producing an artificial honeydew by mixing saprophytically grown spores with a strong sugar solution. In this solution the spores can be stored for months without a great loss of viability. They do not die when drops of the sugar-spore-suspension are allowed to dry out, and will germinate rapidly when water is added. In these respects the suspension resembles natural honeydew. A special sprayer was devised to apply the diluted spore suspension to the blooming rye plants.

The results of this work may well find application in the commercial production of ergot and in many scientific problems. As improvements in agriculture spread it is entirely possible that there will cease to be enough natural ergot to supply the world markets with this drug. Dr. E. H. Lucas* who, during the last twenty years, has observed an enormous decrease in the amount of ergot in Germany, Austria, Hungary and the Balkan countries due to improved agricultural practices, agrees with Barger (3; page 102) who states that "----- improvements in agriculture make the drug more and more scarce so that, like other drugs, it may ultimately have to be cultivated,-----."

Undoubtedly the results presented here will find greater use in scientific work than in commercial production of ergot. This technique will enable extensive inoculations, with a minimum of labor, so that the

*Verbal communication. Dr. E. H. Lucas, now Assistant Professor of Horticulture at Michigan State College, was Director of the Plant Breeding Station for Small Grains at Rohran (near Vienna), Austria.

questions of ergot species and biologic races can now be studied without the tedium and uncertainty of single flower inoculations. Many possible problems have opened up during this work as to the possibility of using the methods herein described as the basis for quantitative studies on insect transmission of this disease. This work also suggests the possibility of applying the technique here developed to other diseases. In nature the spores of many fungi, Colletotrichum, Septoria, Cytospora, Endothia, etc., are produced in a matrix of some sort. It may well be that the spores of such fungi can be handled in a manner similar to this method of handling ergot spores. By mixing spores grown in culture with a matrix similar to that in which they are produced in nature, it may be possible to bring about inoculations by fungi which in the past failed to produce ready infection, or it may enable infection to take place without careful control of the moisture relationship by protecting the spores from death by desiccation. Bacterial pathogenes may also respond to similar techniques. Further studies along these lines may show that substances which will destroy or break down the natural matrix surrounding spores or bacteria may assist in their destruction.

Surely the most useful scientific outlet for this method of inoculating grasses with ergot will be in breeding for resistance to ergot. The breeder will find the early preparation and storage of spores very convenient because this work can be gotten out of the way during the winter before the spring planting begins. With ergot a serious problem in pasture grasses, because of its poisonous nature and because the sclerotia are difficult to separate from the seed, the use of this

technique of inoculation with ergot could well become a part of any grass breeding program.

II. Review of the Literature Dealing with the Large Scale Inoculation of Rye with Ergot.

Fairly successful methods for the large scale inoculation of rye with ergot have been worked out by Bekesy(4) and Hecke(6 and 7). Bekesy's method is based on the use of a horse drawn, multiple injecting apparatus which deposits spores inside the closed flowers, while Hecke's method depends upon a considerable amount of hand manipulation to cause the flowers to open and to apply the spores. Hecke's method could not be used successfully for commercial production of ergot in the United States because it requires too much hand labor. Bekesy's apparatus might be adapted to commercial production provided the machine does not cause too much injury to the rye, provided it will stand the wear and tear of much use, and provided most of the infections arise from the initial inoculations. Any method depending upon natural spread is not reliable enough for the propagation of ergot in most locations where rye is grown.

McCrea(11), Hynes(9), and Thomas and Ramakrishnan(12) have tried large scale inoculations by spraying aqueous spore suspensions on the rye when the plants were in bloom. McCrea's results using a horse drawn sprayer were so inconclusive that she says in her summary, "Field demonstrations have shown it to be improbable that parasitic culture of C. purpurea on a large scale would be desirable, under prevailing conditions in Southern Michigan." Hynes says of the results secured by application of an aqueous spore suspension by various means to 200 acres of rye: "Dry seasonal conditions generally were adverse to ergot formation. Consequently, yields were very low ($\frac{1}{2}$ lb. to 20 lb. per acre), but ergots

did develop in most instances--even in the driest areas sown to rye." The plots of Thomas and Ramakrishnan covered 2½ acres. They sprayed their plots twice and a few infections occurred after the first treatment. Ergot subsequently spread to the whole field, even to the untreated plots. Their results were better than those of McCrea and Hynes, but most of their sclerotia apparently arose from secondary infections, hence a good yield would be dependent upon favorable weather.

When inoculating small plots, many investigators have had good success by spraying on spores in an aqueous suspension. The results of Fron(5) and Hynes(9) illustrate this. Heckt(8) was granted German and U. S. patents on a hand operated, multiple-injecting apparatus for inoculating rye through the palets and lemmas of closed flowers. He claims that large scale inoculations can be made, but here, again, large amounts of hand labor would be necessary. Bekesy's apparatus is a large scale application of this multiple injecting method set forth by Heckt, or vice versa.

Barger(3) and Bekesy(4) give good reviews of the literature dealing with attempts at large scale inoculations with ergot. They also discuss an important phase of the problem not touched upon in this work: the use of more susceptible varieties of rye in order to get maximum infection with minimum inoculation effort.

III. Search for a Spore Matrix.

Almost all papers dealing with ergot mention the honeydew in which the asexual spores are produced in countless numbers, yet none of the investigators, attempting to induce the disease, have tried to duplicate, in the laboratory, the matrix found in nature which protects and assists in dissemination of these spores. Upon observing the insects visiting the drops of honeydew and after demonstrating the germination of the spores imbedded for days in the dried honeydew, it is surprising that some previous scientist should not have discovered the method herein described. Kirchoff (10) even determined that the undiluted honeydew corresponded to 2.33 molar sugar solution. A brief recounting of the circuitous thought path by which I arrived at the basic hypothesis of this paper is presented at the end of the section on "Preliminary Trials With Field Inoculation" (see page 22).

Early research aimed to discover a substance in which the spores from saprophytic cultures could be suspended so as (1) to prevent immediate germination, (2) to prolong the life of the spores after being sprayed on the host plants, (3) to remain soft so insect dissemination could take place, and (4) to allow germination once the spores had come in contact with the pistils of the rye flowers.

The first substances tested were: maple sirup, honey, a concentrated solution of beet sugar, light and dark corn sirup. The suspensions of spores in these substances, except the concentrated beet sugar, became contaminated on the first day. After three days the spores in the concentrated beet sugar solution, upon dilution, germinated an estimated 15%.

The second test using bacteriological techniques, sterilized solutions and glassware, gave very promising results. A spore suspension in water was prepared from a corn meal culture, the suspension poured into test tubes, centrifuged and decanted. To these tubes containing spores were added the various matrix solutions. From time to time small amounts of the matrix suspension of spores were removed, diluted, incubated in hanging drops, and examined for spore germination. The matrix suspensions were also examined. Tables I and II give estimated percents of germination in the undiluted and in the diluted suspensions. Two identical sets of tubes were prepared, one kept at room temperature, the other in the refrigerator. To test the germinability of the spores in these different matrices a small amount of the suspension was transferred on the end of a needle to a hanging drop of sterile water and set up to prevent evaporation of the water. After one and a half to two days, the drops were examined with the microscope and the percent of germination estimated.

In Table I it can be seen that all of the matrices, except water, at both room and refrigerator temperatures, inhibit germination of the spores. The important fact brought out in Table II is that a large proportion of the spores will live without germinating, for a considerable time in a concentrated beet sugar solution. The other matrices treated were of no value except, possibly, light corn sirup. In the light of later work it is difficult to account for such a rapid drop in germination of spores in the beet sugar suspension. No contamination was observed, but tests were not made to be sure of this.

Because of their non-drying properties, tests were made using glycerine and some related substances as matrices. These substances

Table I

Germination of Spores in Undiluted Matrices

Stored at Room and Refrigerator Temperatures.

Matrices:	Days After Experiment Set Up			
	1	3	6	10
	Estimated Percent Germination			
Stored at Room Temperature				
Honey	0	0	0	0
Conc. beet sugar*	0	0	0	0
Dark corn sirup**	0	0	0	0
Light corn sirup**	0	0	0	0
Tap water	10	80	80	--
Stored in Refrigerator				
Honey	--	--	0	0
Conc. beet sugar*	--	--	0	0
Dark corn sirup**	--	--	0	0
Light corn sirup**	--	--	0	0
Tap water	--	--	0	50

*The concentrated beet sugar was approximately a 60% solution.

** Karo brand.

Table II

Germination at Room Temperature after Dilution of Spores from Different Matrices Kept at Room and Refrigerator Temperatures.

Spore suspensions kept at room temperature								
	Days after spores put in matrix							
	1	2	3	5	10	13	17	21
Matrices:	Estimated Percent of Germination							
Honey	0	0	0	0	0	--	--	--
Conc. beet sugar	0	10	40	40	0	0	0	0
Dark corn sirup	0	Tr*	Tr	Tr	0	--	--	--
Light corn sirup	0	10	30	30	0	0	0	0
Tap water	0	0	80	80	--	--	--	--
Spore suspension kept in the refrigerator								
Honey	--	--	0	Tr	0	0	--	--
Conc. beet sugar	--	--	60	60	30	50	40	10
Dark corn sirup	--	--	0	0	0	--	--	--
Light corn sirup	--	--	0	Tr	0	--	--	--
Tap water	--	--	Tr	30	50	--	--	--

* Tr means less than 10% germination.

Note: These are the same suspensions as in Table I.

were almost immediately toxic, so were undesirable.

The results recorded in Tables I and II led directly to other questions in need of answering. Would the spores live in the matrix after it had been air dried for a few days? How high a concentration of sugar was necessary? An experiment was run using five different concentrations of beet sugar. Germination percentages were estimated on spores taken from the matrices and diluted to allow germination. Table III gives the concentration by weight of the sugar solutions and the percent of germination up to 10 days after the experiment was started. Table IV gives the percent of germination in air dried and calcium chloride dried drops of the sugar-spore-suspension. After drying, water was added to these drops to allow germination.

During this experiment a "sitting-drop" technique was devised to replace the hanging drop for the germination of spores. It proved to be so much faster and easier to manipulate that it was used in all work until the smear method was inaugurated as an outgrowth of the sitting-drop method. Slides were prepared with a thin grease film, by rubbing with the fingers, and sterilized. One of these slides was placed in a Petri dish with a piece of wet paper toweling in the bottom. A very small drop of the spore suspension was put on the slide and a large drop of sterile water added. The dish was then covered and incubated at room temperature or in a 26° C oven. The oven proved better because good germination was secured in 16 to 24 hours. An estimate of the germination was made by examining the drops with the microscope.

Under the conditions of the experiment it can be said, from the results given in Table III, that sugar concentrations from 34% to 66%

inhibit germination of ergot spores without greatly decreasing their ability to germinate when the suspension is diluted. It is possible that some of the lower values can be accounted for by contamination in the germination drops. It is useful to know that spores will tolerate a high concentration of sugar because, if this method of inoculating rye with ergot becomes a commercial process, it will be necessary to work with non-sterile suspensions, and, under these conditions, a highly concentrated sugar solution will keep contaminants in check.

The results given in Table IV are germination percentages of a large number of drops which were placed on slides, dried either in air or over calcium chloride for one to five days before the water was added to the drops of suspension and to the paper in the bottom of the Petri dish. These drops, when dry, consisted of a tough film or of a much thickened mass containing sugar crystals. Neither condition seemed to have any pronounced effect on the germination of the spores. Spores germinated just about as readily after having been dried in the matrix as those kept wet. This is an important fact. It means that spores applied to the plants in dry weather will still be viable after at least five days and thus be on hand for insect transmission and to bring about infection, if, in the opening of the grass flower, the stigma should come in contact with a dew-diluted droplet of the suspension.

Table III

Estimated Percent of Germination of Spores in Sterile Beet Sugar Suspensions of Different Concentrations Kept at Room Temperature.

Number of days spores in suspension before germination determinations made	Concentration of Sugar Solutions				
	66%	60%	52%	43%	34%
	Percent of Germination				
1	50	40	70	50	30
2	70	70	70	70	70
4	70	70	70	70	70
5	70	60	30	60	80
6	70	60	70	50	60
7	70	70	30	70	70
8	50	60	20	60	60
9	50	60	50	70	60
10	50	50	60	70	70

Note: Spores were removed and germination tested as described in text. No germination occurred in any of the sugar-spore-suspensions before they were diluted.

Table IV

Estimated Percent of Germination of Spores in Sterile Beet Sugar Suspensions of Different Concentrations which Had Been Air or Calcium Chloride Dried Before Diluting to Allow Germination

No. of days the spore suspensions were allowed to dry before water was added	Concentration of sugar solutions				
	66%	60%	52%	43%	34%
	Percent of germination				
Spore suspension drops air dried					
1	20	30	30	30	40
3	50	70	50	--	70
4	40	40	20	30	50
Spore suspension drops dried over calcium chloride					
1	50	30	30	70	40
2	70	70	70	70	50
5	40	20	40	50	70

Note: No germination occurred in any of the sugar suspensions before they were diluted.

IV. Laboratory Methods.

A pure culture of Claviceps purpurea was isolated from a sclerotium. Stock cultures were carried on potato-dextrose agar slants. For the quantity production of conidia, a number of different media were used. The earliest cultures were on corn meal slants in quart milk bottles. Carrot cubes, potato cubes, corn meal cubes, cracked corn and a mixture of corn meal and oats were also tried. It was finally decided that the method of Hynes(9) produced the greatest number of conidia per unit volume, with the least amount of labor.

Two hundred and fifty ml. of wheat and 250 ml. of water were added to a quart milk bottle, plugged with cotton and allowed to stand overnight. After autoclaving for at least an hour, the bottles were allowed to cool until they could be handled with gloves, then pounded on a rubber stopper and shaken until the grains did not cling together in large masses. If the bottles were not pounded, a large part of the grains would form a solid mass at the bottom into which the fungus would not readily penetrate. Pure wheat was found to be better than a wheat and oat mixture.

Attempts were made to enhance the spore production of the wheat cultures by the addition of various amounts ($\frac{1}{2}$, 1, 2, 3, 4, & 5%) of sucrose with and without a nutrient solution (Coon's synthetic, without the sugar). The sucrose favored greater and prolonged vegetative development; the nutrient solution alone had no observable effect. Because there appeared to be no advantage gained in spore production, these fortified cultures were not used.

Most of the quart cultures were inoculated by pouring in about 50 ml.

of a heavy spore suspension. The spores were from especially prepared wheat-medium cultures which were sub-cultures from the stock cultures. After the spores were added, the culture was pounded, and thoroughly shaken so as to spread the spores throughout the mass of wheat grains. Later in this work it was found that if the agar slant stock cultures were allowed to grow for about four weeks until large numbers of conidia had been formed, about one sq. cm. of the fungus would furnish ample spores to inoculate the whole quart culture. The piece of stock culture was added to the wheat medium without any water. By thoroughly pounding and shaking the bottle, the spores were spread well through the wheat. This method is better because it eliminates a number of chances for contamination.

The exact age at which the optimum number and quality of spores were present in the quart cultures was not determined. All cultures were grown at room temperature and because of this, at least in part, different lots varied in their time of development. Most cultures were harvested at the end of five to six weeks because this was the period just before the large masses of spores began to germinate.

When ready to harvest the cultures were mixed with an equal volume of tap water and beaten in a blendor. This method of preparing inoculum has been described by Andrus(1). After blending for about two minutes, the thick mass was screened through a sixteen mesh and then a forty mesh screen to remove all particles which might plug a sprayer. To this thick, water suspension of spores, medium, and mycelium, an equal weight of beet sugar was added and stirred until dissolved. Five gallon honey

cans were used for storage at - 18°C and 0°C. Ten quarts of culture made about 4½ gal. of the sugar-spore-suspension. No precautions, except clean utensils, were taken to prevent contamination while the suspension was being prepared.

All spore germination tests were made by the smear method except those reported in Tables I, II, III & IV. Because the smear method was simple to set up and because an accurate count of the germinated and ungerminated spores could be easily made, it was used for all the work reported henceforth in this paper. A drop of spore suspension was placed on the end of a carefully cleaned, sterile slide and smeared with the end of another sterile slide to form a thin film. The smeared slide was placed on a wet circle of paper in a Petri dish. No water was added to the slide because condensation water in the dish diluted the spore suspension sufficiently to allow the spores to germinate. Best results were obtained if the slide was incubated overnight at 28°C.

At the end of about eighteen hours the slides were removed and allowed to dry. A cover slip with a small drop of water was placed on the slide. If the right amount of water were used, the spores did not float around and interfere with the counting as was sure to take place when the sitting-drop method was used. Ten high power fields were counted, combined, and the percent of germination computed.

V. Storage of Spore Suspensions.

Considerable success in keeping the sugar-spore-suspensions over long periods was attained by storing the material at 0°C and at - 18°C. Difficulties were also encountered and many questions left to be answered by further experimentation.

For the results reported in Table V quart bottles of 50% beet sugar-spore-suspension were used. The spores came from wheat cultures which were seven weeks old. While the suspension was being prepared no attempt was made to protect it from contamination. The No. 1 bottles were filled to the top and the No. 2 bottles were half filled; both were stoppered with corks. Pairs of bottles were kept at different temperatures. In the 30°C oven, spoilage was very rapid with a consequent loss of viability of the spores in less than 11 days. At room temperature, which fluctuated considerably, there was also a rapid destruction of spores within the same length of time, but not quite so rapidly. Spores kept in a hallway that was about 10 degrees cooler than room temperature remained viable in considerable numbers up to 21 days. These bottles, at the aforementioned temperatures, became contaminated with bacteria in a few days and, as the contamination increased, the germination decreased. It is obvious from the table that storage at 0°C and - 18°C was by far the best. Except for one bottle, there was a high percentage of germination even after being in storage for 128 days. Which of these temperatures is better cannot be said for sure, but - 18°C appears to be.

Other germination tests were run on large lots (1 to 5 gals.) of

Table V

Percent of Germination of Samples Taken From

Sugar-Spore-Suspension in Storage at Various Temperatures

Days suspension in storage before samples taken	Storage Temperatures									
	-18°		0°C		12-16°C		20-26°C		30°C	
	Bottle Number									
	1	2	1	2	1	2	1	2	1	2
3*	60	60	80	80						
4					60	60	70	80	60	60
6	70	70	70	60	60	70	70	70	5	5
11	50	50	70	70	70	70	0	0	0	0
14	80	80	80	80	80	80	0	0	0	0
18	40	50	60	60	50	50				
21	30	50	60	50	10	20				
24	30	30	60	60	Tr	Tr				
27	60	60	70	70	Tr	Tr				
32	70	60	70	70	Tr	Tr				
38*	70	60	70	70	0	0				
42	40		40							
47	46		34							
50	41		32							
61	36	45	56	30						
68	39	36	47	47						
72	34	41		34						
77	30	25	42	40						
84	29	29	38	52						
90	26	24	32	52						
97	35	43	19	39						
128	38	53	8	30						

* Percentages of germination for samples stored from three to 42 days were estimated; all others were by actual count.

suspension which was prepared for field inoculation work. In Table VI are given the germination percentages of samples taken from two 5 gal. lots stored at 0° C. On the forty-third day after the storage period began ten samples were taken from bottle 1 and the germination percentage determined. There was a variation from 14 to 33 percent. It is regrettable that available time did not allow a statistical study to be made to determine how much faith could be placed in a single count or how many counts were necessary to get an accurate picture of the germinability of a 5 gal. lot of spores. There appears to be a decrease in the percent of germination in both of these bottles which was probably due to contamination, but this was not proven.

Table VII lists the percentages of germination of samples taken from 5 gal. lots of 50% sugar-spore-suspension. Six cans were stored at 0°C, 4 at -18°C. In most of the cans there is little drop in the percentage of germination for the short period over which these tests were run. However, cans 2, 6 and 8 gave a considerably lower count on their last test than they did at first. This may be real or due to sampling. It was true that almost every low germination count, for all experiments, was accompanied by large numbers of bacteria on the slide. The converse is not true, however, for often high germination counts were made on slides on which there were large numbers of bacteria. Probably some bacteria are capable of destroying or, at least, inhibiting the germination of spores while others are capable of living alongside the spores without reducing their power to germinate.

Table VI

Germination of Spores from 50%

Sugar-Spore-Suspensions Stored at 0° C.

No. of days after suspension prepared that sample was taken	Percent of germination	
	Bottle 1	Bottle 2
5	36	33
7	32	32
14	30	22
21	28	39
25	31	32
36	19	21
40	13	13
44	30	
48	24	
53	25, 17, 24, 20, 16, 14, 21, 19, 33, 16	
62	15	

Table VII

Germination of Spores From 50%

Sugar-Spore-Suspensions Stored at 0° and - 18°C.

No. of days after suspension prepared that sample was taken	Storage Temperatures									
	0°C						-18°C			
	Can 1*	Can 2	Can 3	Can 4	Can 5	Can 6	Can 7	Can 8	Can 9	Can 10
	Percent									
1		35	37			12		30	21	12
2		33		16				40		
3						18				16
5							17			
6					3					
7				12			14			
9									31	
10			36							
13	13						13			
15		5						18		
17						8				14
22	8						11			
24					7					
25				11						

* Each can contained approximately 5 gallons of suspension.

VI. Preliminary Trials with Field Inoculation

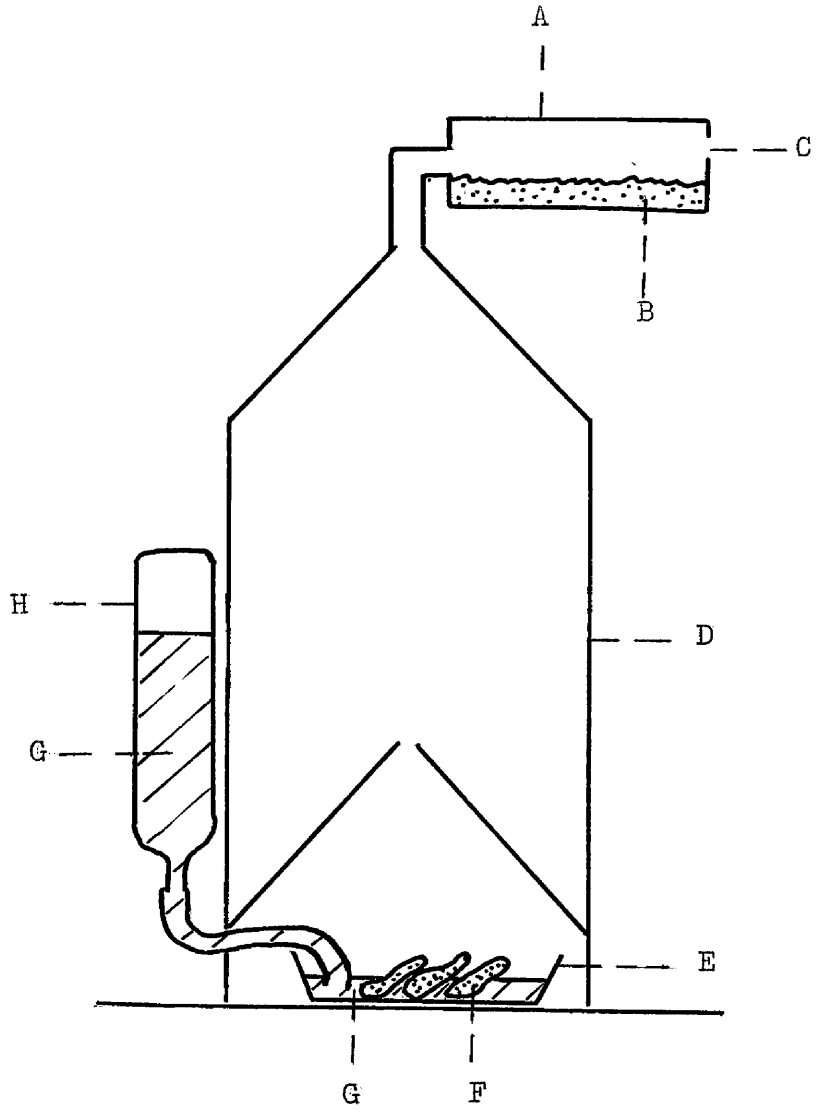
1941 Plot

The first field trial in 1941 was an attempt to trap flies and then release them after they had been forced to walk over a sporulating culture of Claviceps purpurea. In this way, it was thought, the spores could be widely and easily disseminated. Two fly traps like Fig. 1 were placed in a rye field just as it was starting to bloom. The flies were attracted by the decaying liver in the pan underneath the trap; they moved up into the body of the trap, out its top, through the tube containing the culture and finally were liberated, presumably carrying spores on their bodies. A provision was made to keep the liver wet, and hence in an odoriferous condition, by use of a bottle so attached that a constant water level was maintained in the liver pan. Unfortunately no provision was made to maintain the moisture of the corn meal culture which was placed in the culture tube (A) at the top of the trap. The culture became dry and hard after one day in the hot sun so that probably the spores were killed by desiccation. No ergot resulted from this attempt although the traps were very successful in catching and releasing large numbers of flies. It is possible that such an arrangement might be used to study other insect borne diseases.

While observing the failure of this experiment, I was trying to imagine some way by which a good culture of the fungus could be maintained in the field without drying out. In order for the spores to be picked up and carried away by the flies, they must be kept moist. How would that be possible without encouraging the growth of contaminants which would overrun the culture and destroy the spores? At the very moment I was pondering

Fly Trap for the

Dissemmation of Spores



A. Transparent tube of pyroxlin in which flies come in contact with the fungus culture.

B. Culture of C. purpurea.

C. Exit opening for flies leaving the trap.

D. Screen fly trap.

E. Pan

F. Liver.

G. Water.

H. Water reservoir.

Figure 1.

this question, I stood looking at drops of honeydew falling from a head of rye. Would it be possible to imbed the spores in a substance that would not spoil, yet would stick to the flies' feet as they walked over it? Would honey do? Honeydew? In that instant was conceived the hypothesis which is the basis for this paper. As everyone knew, the asexual spores of the ergot fungus are produced in a sweetish, sticky matrix which protected the spores and aided in dissemination. Could not I copy nature and embed spores, grown saprophytically, in a honeydew of my own concoction? Out of this few minutes of pondering grew the work described under Section V, Search for a Spore Matrix, with a consequent discarding of further attempts to spread ergot by the use of fly traps.

The 1942 Plots

The first attempt to inoculate rye with a "synthetic" honeydew was made in 1942. Three strips of spring rye were planted at different dates. Of these only the second and third plantings were used, and results were secured only from the middle planting, made April 29, because the second group of plots was trampled by cattle. The plots were 12 feet long by 6 feet wide, in the same drill row, containing 11 rows 7 inches apart and separated by 50 to 100 feet of untreated rye. The control plots were in the same drill row and the same size as the treated plots, separated from them by three feet of untreated rye.

The treated plots were sprayed with a sugar-spore-suspension containing about 50% beet sugar by weight on the following dates: June 15, 19, 21, and 22. Each application was made with a three gallon hand sprayer at the rate of approximately 150 gallons per acre. Poor coverage was secured because such a strong sugar solution does not spray well. This did not seem important at the time because it was thought that insects would do the disseminating.

On June 17 a pan of liver was put in Plot 1 to attract flies. It was arranged with a resevoir of water so there was a half inch of water in the dish all the time in order to keep the liver in a putrid condition. On June 21 there were hundreds of flies on Plot 1 and only a few on the other plots. By June 22 thousands of flies were present on Plots 3 and 4 and almost no flies on the control plots. All plots had thousands of flies (there appeared to be 2-3 flies for each head) on June 24; they were no more numerous on Plot 1 than on the others even though the liver was still

giving off plenty of putrid odors; the control areas had only a few flies here and there. On June 26 the number of flies had dropped to hundreds on Plot 1 with tens on the rest.

Rains, during the treatment period, came so that they washed the suspension off within one day after each application. This was undoubtedly detrimental and reduced the possibilities for infection. TableVIIIgives the amount and dates of the rains as well as the dates of treatment.

From Table IX it can be seen that there was a pronounced difference in the number of sclerotia that developed between the treated and untreated plots. The differences are large enough so that there is no doubt that this method was effective in bringing about a large number of infections. The lower results in plots 3 and 4 were probably due to the trampling they got when a herd of cattle broke into the field. There is not sufficient evidence here presented to make any conclusions about the effect of insects on the number of infections, but from the general experience gained during the work, it was decided that insects were of secondary importance and that the important feature was to get the spray directly into the opened flowers by pointing the spray downward into the tops of the heads.

Table VIII

Rainfall and the Dates of Application
of the Spore Suspension on the 1942 Plot.

Date	Treatment No.	Amount of Rainfall
June 15	1	0
16		Tr
17		0
18		.22
19	2	0
20		1.83
21	3	.01
22	4	0
23		Tr
24		.03

Table IX

Number of Heads Infected with Ergot in 1942 Plots.

Plot No.	1	Con-trol	2	Con-trol	3*	Con-trol	4*	Con-trol
Total No. of heads	1710	1568	1630	1439	1257	1127	1419	1127
No. of heads infected	206	24	178	20	87	9	107	13
Total No. of sclerotia	245	41	239	19	97	9	139	19
Percent of heads in-fected	12	1.5	10.9	1.4	7.0	0.8	7.5	1.2

*Partially trampled by cattle just before harvest.

VII. The 1943 Field Inoculations

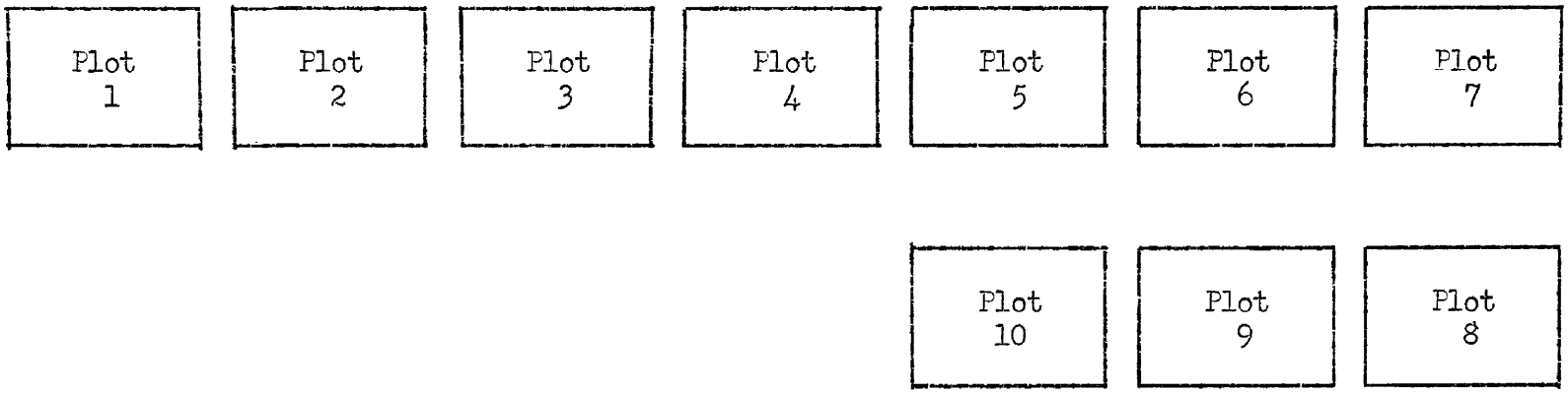
Rye in four different fields on the Michigan State College farm was used for inoculation tests during the 1943 season. The four fields were: the Botany Field, the Clark Field, the Soils Field and the Crops Field. Each field will be described and discussed separately. Plots in two of these fields were treated with a hand sprayer, in the other two with a power sprayer. No attempt was made to lay out plots for statistical analysis because the differences desired were so great that analysis would be unnecessary and because too little was known about carry-over from plot to plot and about time and rate of blooming. The primary concern was to get differences between the treated and untreated plots.

Most of the suspensions used on these fields had been prepared within 4 weeks of the time of application. A germination test was run at the time of preparation and this was considered, for want of more evidence, to be a measure of the germinability. Earlier prepared suspensions (see Table VII.), and all others, which dropped below 10% germination were discarded. Any suspension with a germination of 40% or above was considered to be good quality, with a germination of 10—30% was considered to be of inferior quality. When not stated, the suspension was of good quality.

The Botany Field I

The Rosen rye in these plots was broadcast August 20, 1942. The layout of the plots and other information are given in Figure 2. The plots were sprayed during the first days of the heavy blooming period. At the

Layout and Treatment Information of Botany Field I, 1943



Size of plots: 10x22 ft.

Kind of sprayer: 3 gal. hand sprayer

No. of treatments: three

Dates and times of treatments: June 4, 5:00 P.M.

June 5, 11:00 A.M.

June 9, 10:00 A.M.

Rate of application: Approximately 100 gal. per acre

Figure 2

time this work was done it was not known what time of day was best for applying the spores, so care was not taken to apply them when most of the flowers were open. The best time of application is still not proven, but it is believed that careful spraying when most of the flowers are open will give the best results.

From June 6 to 9 the weather was very cool with rain and mist, most of the time. The suspension of the first two applications was completely washed off. A few small flies were present on the treated plots June 6. On June 10, a bright warm day, hundreds of flies were present on Plots 1, 3, 5, and 10, the plots with the strongest suspensions, while there were only a few on the others.

Table X , gives the results from the Botany Field I plots. It can be seen that all of the treated plots developed more sclerotia than did the untreated. It is also important to note that the number of sclerotia produced in Plots 5 and 8, sprayed with the weakest spore suspension, was about equal to those plots sprayed with the stronger suspensions. This means that a weak suspension, which is much more economical to use, can probably be used in place of the more concentrated suspensions.

Table X .

Sclerotia Produced in Botany Field I

Plot No.	Strength of suspension*	Total no. of heads**	No. heads with sclerotia	Total no. of sclerotia	Percent of heads infected
1	1:3	654	129	209	20
2	1:7	502	68	108	14
3	1:1	577	77	81	13
4	untreated	486	0	0	0
5	1:15	527	74	101	14
6	1:7	520	50	62	10
7	1:1	520	54	73	10
8	1:15	426	79	117	19
9	untreated	530	2	2	0.4
10	1:3	488	71	98	16

* Spore suspensions as prepared for storage (see section VI) diluted with water in the ratios indicated.

** Samples 3 by 11 ft. were taken across each plot for the data presented in this table.

Botany Field II.

A plot of rye broadcast October 15, 1942 was in full bloom June 13, 1943. On this day two strips 6 ft. wide were sprayed three times with a power sprayer (See discussion of the Clark Field for a description of this sprayer.) between 10:00 and 12:00 A.M. A 1:7 dilution of the stock spore suspension was used and applied, each time over, at the rate of 100 to 150 gal. per acre. Each application was made when large numbers of flowers were open.

The results from this plot are given in Table XI. Plants on an area 1½ by 5 ft. were taken as a sample from two places in the treated plots and from two places in adjacent untreated areas. Here again, there was a pronounced difference between the treated and untreated plots. As can be seen by comparing the number of heads with sclerotia with the total number of sclerotia, there were, on the average, 1.7 sclerotia per infected head.

If commercial production of ergot does develop from the methods described in this paper, the results from this field may be useful. Further tests may prove, as indicated here, that several applications on a single day are just as efficient in producing sclerotia as the same number of applications applied one each day. The costs of inoculation will be reduced if this proves to be true.

Table XI .

Results from Plots on Botany Field II.

Sample*	Total no. of heads	No. heads with sclerotia	Total no. of sclerotia	Percent of heads infected
Treated	183	66	136	36
Treated	185	61	87	33
Untreated	175	13	20	7
Untreated	202	7	7	3

* The samples consisted of the plants on an area $1\frac{1}{2}$ by 5 ft.

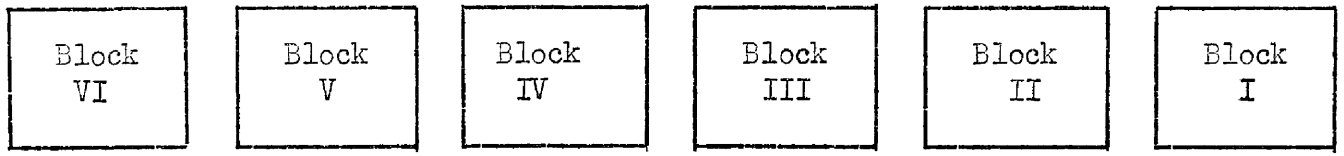
The Clark Field

Rosen rye was broadcast on this field about October 15, 1942. The layout is given in Figure 3. The six blocks, 80 x 48 ft., were broken up longitudinally into strips six feet wide; three of these strips were used for roadways leaving five plots in each block. The blocks were separated from each other by 20 ft. of rye which was broken down by the turning around of the truck. To the south of the blocks was a continuation of the rye field of which these blocks were a part. To the north was a strip of sod and weeds 50 ft. wide which extended to the railroad track. See Figure 4 for a photograph of a part of this field. It was taken standing in the south side Block IV looking slightly north of due west.

The spraying was done with the sprayer pictured in Figures 5 and 6. This was a small orchard sprayer to which was attached a side boom to support the nozzles and the guides for pulling the heads together under the nozzles. The boom was constructed with a hinge so when not in use, it could be tipped back onto the top of the truck. The guides were made of sheet metal. Their construction and arrangement can best be learned by studying the pictures. The forward tips were about twenty inches apart so that the total width of rye taken in was approximately 5 ft. At the time the Clark Field was being sprayed there was only one nozzle between each pair of guides, not two as can be seen in the pictures. The single nozzle was adjusted to point downward and back so that the spray would shoot directly at the tops of most of the heads as they passed between the guides.

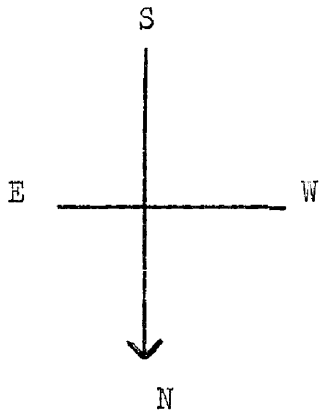
In TableXII is listed the information concerning the number, date

The Clark Field Layout

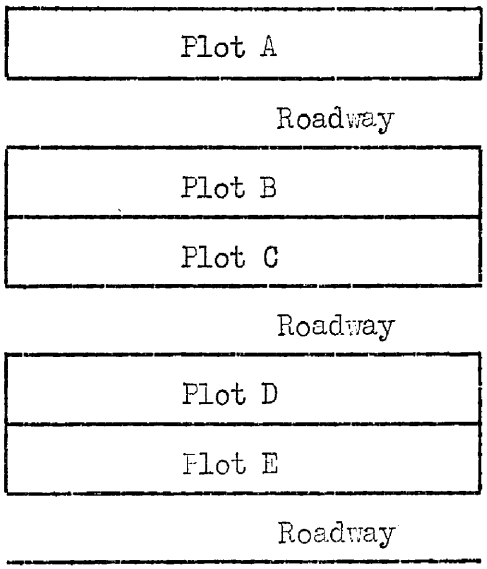


Each block 80 x 48 ft.

Turn around areas between blocks 20 x 48 ft.



Plot Layout Within Each Block



Each plot is 80 x 6 ft.

Figure 3

The west half of the Clark Field

and part of the adjacent rye field

Figure 4

Power sprayer used on the Clark Field, Crops Field and Botany Field II

Figure 5

A close-up of the boom on the above sprayer

Figure 6

and time of application, the quality of suspension used, and the dilution of the original suspension (See Part V for the method of preparation.) used for each plot. All the plots in Blocks V and VI were sprayed with the same dilution of inferior quality suspension except, of course, the controls. Block V differs from Block VI only in that Block V had one more treatment. The suspension used on all the other blocks was of good quality. Block I received one more treatment than did II and IV. In Blocks I, II and IV there are duplicate plots for each dilution of suspension used.

Between July 9 to 14 samples were collected from all plots, the heads counted, the infected heads sorted out and counted, and the number of sclerotia per head counted. The samples consisted of three strips 1 by 5 ft. across each plot. These samples were taken approximately 20, 40 and 60 ft. from one end of the block so that the samples from all plots formed three strips across each block. Block III was untreated so samples were taken from Plot C only.

In TableXIIIall six of the samples of the same treatment have been added together for each block and the percent of heads, containing at least one sclerotium, computed. In every block regardless of the kind of treatment there is a great difference between the treated and untreated plots. Even from these data, it is probably safe to say that the lower quality spore suspension is less efficient in producing infections than is the better quality. From a practical point of view it is good to note that the dilution of 1:15 was just as efficient as the more concentrated suspensions. This same fact was also observed in Botany Field I.

From the layout of the plots it can be seen that Plot C, the control

Table XII

Treatment of Plots in the Clark Field

Block No.	No. of treatments	Date & time of applications	Quality of suspension	Plot	Strength of suspension
I	4	June 8, 10 A.M. 9, 2 P.M. 10, 7 A.M. 11, 10 A.M.	40-60 percent germination	B&E A&D C	1:1 1:3 untreated
II	3	June 8, 10 A.M. 9, 2 P.M. 10, 7 A.M.	40-60 percent germination	B&E A&D C	1:1 1:3 untreated
III	0				
IV	3	As for Block II	40-60 percent germination	B&E A&D C	1:7 1:15 untreated
V	4	As for Block I	10-30 percent germination	A,B, D&E C	1:1 untreated
VI	3	As for Block II	10-30 percent germination	A,B, D&E C	1:1 untreated

plot, in all treated blocks was in immediate contact with Plot B along one side and separated from Plot D by a 6 ft. roadway. Carry-over from the treated plots was very small, but probably accounts for a part of the infection present on the untreated plots. In the middle of the large rye field south of the area used for the experiment, it was with difficulty that an ergot sclerotium could be found. It is difficult to account for the higher percentage of ergot in the untreated block than in the control plots in each of the treated blocks. It may have been due to the location of this block in a lower spot in the field.

From the point of view of the would-be commercial grower of ergot, the results from this field are especially interesting because the treated area was more than a quarter of an acre. This work has demonstrated that a rapidly moving sprayer, which can cover large areas, is capable, by using a sugar-spore-suspension inoculum, of causing a fairly high percentage of infection. It should be remembered that this was the first attempt to apply a sugar-spore-suspension with a power sprayer and, of course, the whole operation was crude when considered in the light of the experience gained. Without considering any of the other variables, it is believed that a specially constructed sprayer could almost double the number of infections. This, however, must await future research.

Flies were almost absent on the Clark Field. Honey bees, however, were quite plentiful during the treating period, especially on those plots with the highest concentrations of suspension. Ergot beetles (Acylomus ergoti Casey) were first seen in Block I on June 20 and continued to be present throughout the field while the sclerotia developed. At no time were they as plentiful as on the Soils Plot. From the few observations

Table XIII

Results from the Clark Field

Block No.	Plot*	Number of heads in sample areas	Number of heads with sclerotia	Number of sclerotia	Percent of heads infected
I	C	365	20	28	5
	B&E	646	280	556	43
	A&D	702	270	489	38
II	C	301	10	16	3
	B&E	579	214	376	36
	A&D	731	216	408	30
III	C	226	18	29	8
IV	C	329	11	21	3
	B&E	657	216	398	33
	A&D	603	213	418	35
V	C	336	20	32	6
	A,B, D&E	1247	312	521	25
VI	C	357	20	29	6
	A,B, D&E	1305	285	413	22

*See Table IX for the treatments applied to these plots.

made, nothing can be said about the relationship of insects in this field to the number of infections. It is probably true that insects are not a very important factor in the spread of ergot if the rye blooms and ripens evenly over the whole field.

Weather data for June 4 to 25, 1943 are given in Table XIV. These data are from the Monthly Meteorological Summary of the U. S. Dept. of Commerce Weather Bureau, Lansing, Michigan,Station. The days covered include the days on which the following fields, all within 3 miles of the Station, were treated: Botany Fields I and II, the Clark Field, the Soils Field and the Crops Field. It can be seen from this report that there was almost no rain on the 4 days, June 8, 9, 10 and 11, that treatments were applied to the Clark Field and that the days were largely bright and warm. These facts lead one to believe that this method of inoculating rye is not dependent for its success on hot, humid weather which is usually thought to be necessary for the development of a natural epidemic.

Table XIV

Weather Data for June 4 to 25, 1943

Collected at the Lansing, Michigan, Station of the Weather Bureau

Date	Max. Temp. °F	Min. Temp. °F	Mean Temp. °F	Total (inches) Precip-itation	% of possible sunshine	Relative Humidity* 1:30 am	7:30 am	1:30 pm	7:30 pm
4	81	59	70	0	71	84	88	63	58
5	70	53	62	0	72	80	72	55	62
6	66	56	61	.17	8	79	87	82	94
7	62	55	58	.02	1	94	100	84	84
8	63	50	56	T	38	92	86	59	63
9	70	45	58	0	88	86	74	39	56
10	76	57	66	0	88	81	81	64	46
11	73	50	62	0	76	81	78	54	68
12	87	60	74	.05	75	71	86	64	72
13	81	68	74	0	100	89	87	61	59
14	85	65	75	T	50	89	87	64	81
15	87	70	78	T	70	88	85	55	73
16	77	64	70	.13	38	97	86	93	78
17	76	62	69	.05	70	98	86	55	63
18	81	59	70	0	100	94	80	54	57
19	86	61	74	0	100	71	67	50	56
20	83	65	74	0	90	73	73	62	63
21	87	63	75	.19	63	90	92	61	78
22	84	63	74	0	99	74	82	56	49
23	83	58	70	0	100	70	74	40	55
24	87	62	74	0	92	81	78	50	45

* Relative humidity data from the Lansing Airport, about 10 miles from the plots.

The Soils Field

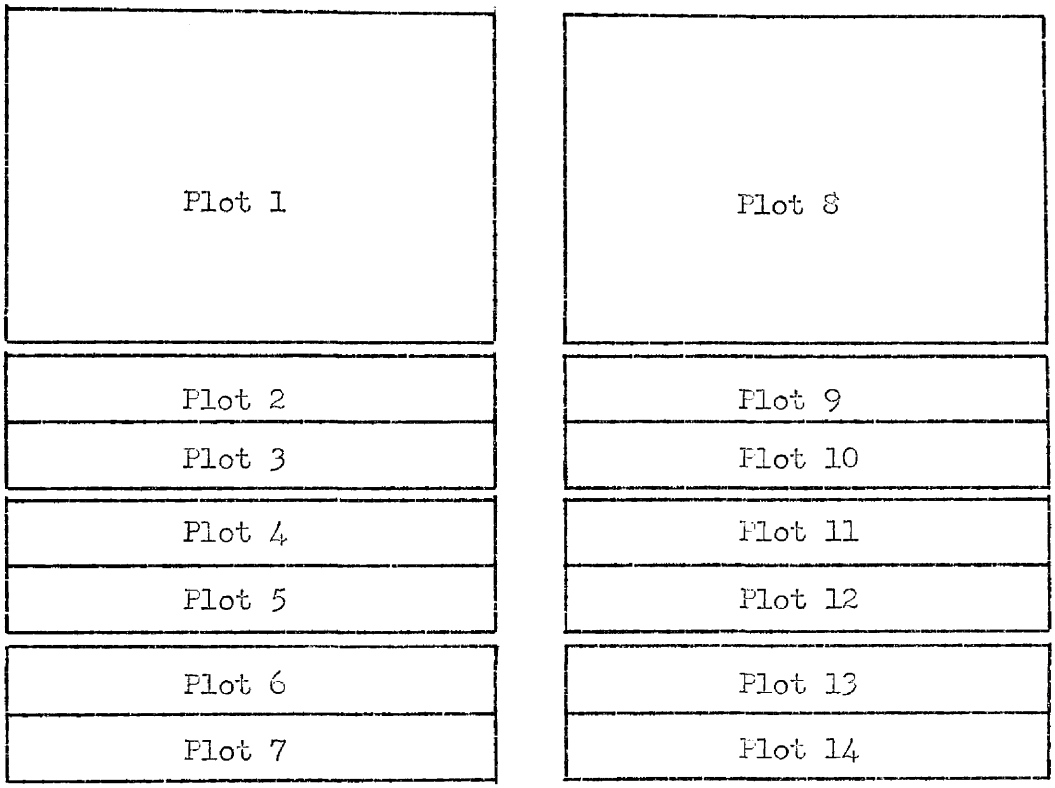
This experiment was set up, primarily, to test the spread of ergot from fall rye to later-blooming, spring rye, but the results also gave a better idea of the possibilities of bringing about infection by use of a hand sprayer, than did those for Botany Field I.

All of the rye was planted with a regular field drill which planted eleven rows of rye seven inches apart. See Fig. 7 for the layout of these plots. The fall rye, Rosen, Plots 1 and 8, was planted October 19, 1942; the spring rye, April 17, 1943. Plot 1, the only treated plot, was sprayed with a three gallon hand sprayer using a 1:1 dilution of high grade spore suspension. One application at the rate of 150 gal. per acre was made between 8:00 and 10:00 A.M. during the period of most rapid blooming, on each of the following days: June 12, 14, 15, 16 and 17. The spring rye began to flower June 22 and continued in full bloom for about 6 days during the period when lots of honeydew was being produced on Plot 1. These plots were bounded by 20 foot strips of beans on the east and west and by clipped roadways on the north and south.

In order to determine the amount of infection two sample strips 2 feet wide were harvested. These strips ran through Plots 1 to 7, ten feet and 20 feet respectively, from the south side. Two like strips were harvested through Plots 8 to 14. The total number of heads, the number of heads infected and the total number of sclerotia are given in Table XV for the samples taken from these plots.

The five treatments given to Plot I produced a high percentage of infection with an average of 1.4 ergots per head. The amount of ergot which developed in the untreated fall rye plot which bloomed at the same

Layout of the Soils Field



Plots 1 & 8: 15 x 25 ft, 2 drill widths.
Plots 2-7 and 9-14: each $3\frac{1}{4}$ x 25 ft., $\frac{1}{2}$ of a drill width wide.
Plots 1 and 8 are Fall rye (Rosen); the others are spring rye.

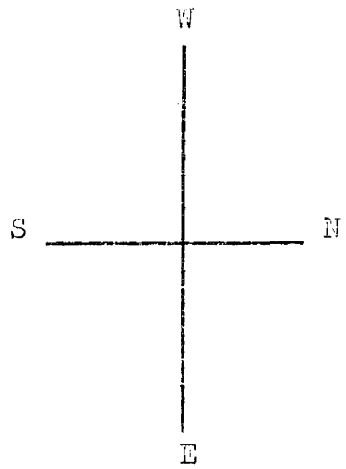


Figure 7

Table XV

Results from the Soils Field

Plot No.	Total No. of heads	No. of infected heads	Total No. of sclerotia	Percent of heads infected
1*†	787	477	1103	61
2	118	53	95	45
3	85	39	64	44
4	125	37	76	30
5	150	37	56	25
6	114	18	26	17
7	137	39	72	29
8†	861	83	139	10
9	123	31	45	25
10	155	43	72	28
11	154	26	45	17
12	163	44	64	27
13	143	26	39	18
14	166	62	116	37

* Plot 1 treated; all others untreated.

† Plots 1 and 8 fall rye, all others spring rye.

Note: See Figure 6 for the layout of these plots.

time as the treated plot was considerable, but small when compared to the treated plot or to the later blooming spring rye adjacent to the treated plot. There was considerable spread from the treated fall rye to the spring rye, especially to those areas nearest the treated plot. The higher percentage of infection in Plots 7 and 14 was probably due to the "border effect" which lengthens blooming period, introduces larger numbers of insects and possibly other factors.

Ergot beetles (Acylomus ergoti Casey) began to appear in Plot 1 in large numbers on June 16. There appeared to be as many beetles as there were heads; on one head six beetles were counted, on others there were none. The number of beetles continued to be high through June 22 and were present in decreasing numbers until July 10 when none could be found. Only an occasional beetle was found in the spring rye plots. The significance of these beetles in the spread of ergot cannot even be guessed at from the few observations made during this work. Future studies may shed some light on this subject.

The Crops Field

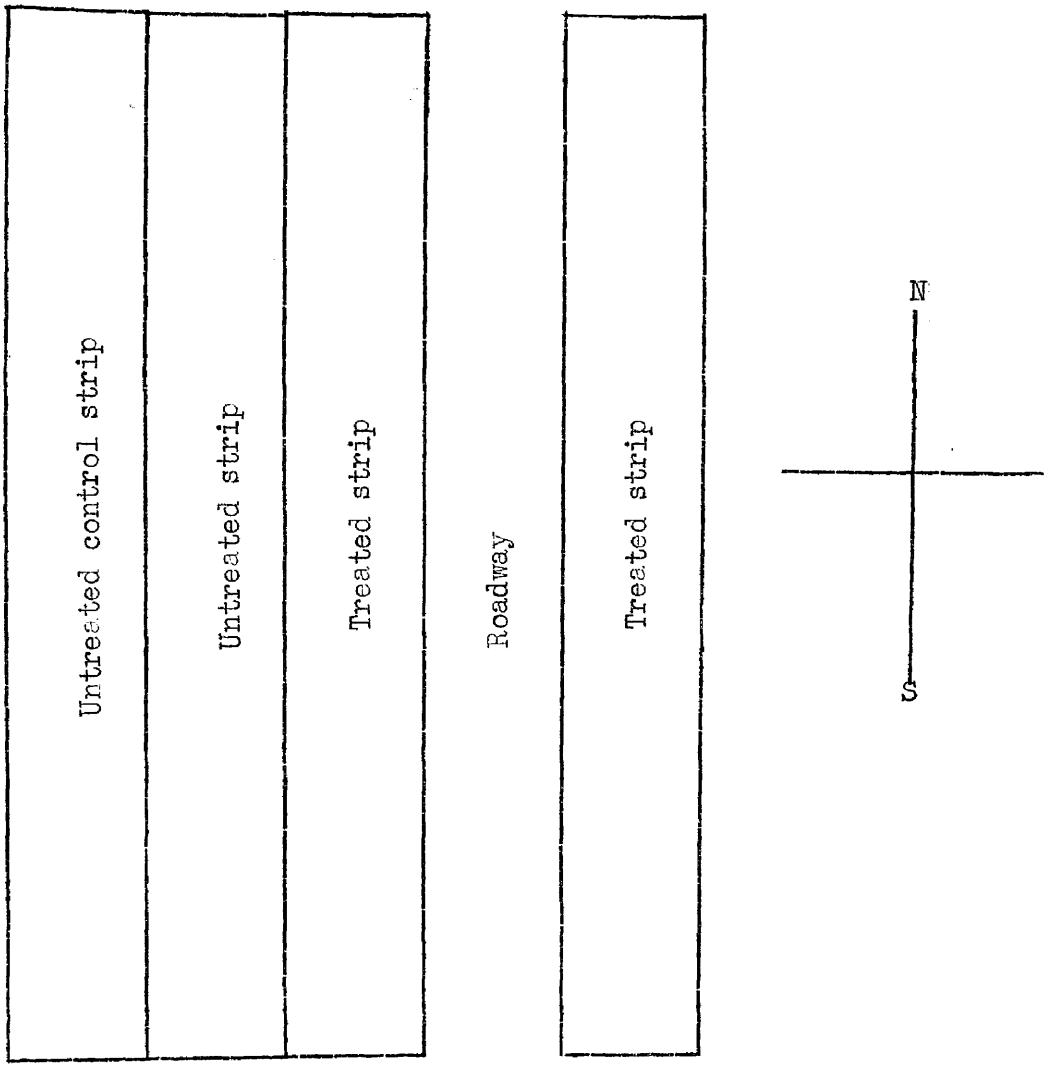
Five drill widths of spring rye were planted April 17, 1943. The layout is given in Fig. 8. Each side of the roadway was treated and the strip on the west side of the plot was used as a control. This field was surrounded by fall rye on the west and spring rye on the other three sides.

The two strips on either side of the roadway were sprayed three times, with a 1:7 suspension on each of the following days: June 20, 21, 23, at the rate of 150 gal. per acre. On the first two days a high grade spore suspension was applied; on the last day a low grade suspension. The power sprayer used is described and pictured in the section on the Clark Field. Two nozzles were used, for each pair of guards, instead of one as used in the Clark Field.

Three samples 1 by 5 ft. were taken from each of the treated strips and three from the control strip. The tabulated results from these samples are given in Table XVI .

Considering the number of treatments the amount of infection on this field was small. This can be accounted for in several ways. Spring rye does poorly in Michigan and this field was no exception. The plants were quite small and the grain that developed was small and shrunken. Most of the sclerotia were small, but only those were counted which protruded enough to be seen without breaking away the palet and the lemma. Probably there was a blasting of many flowers although no count was made to be sure of this. The last day's sprayings were made with a low grade suspension of spores. All of these factors contributed to give a rather small percentage of infections in spite of the large number of treatments.

Layout of the Crops Field



Dimensions of each strip: 6 x 80 ft.

Figure 8

Table XVI

The Amount of Infection in the Plots of the Crops Field.

Plot	Total no. of heads	No. of infected heads	Total no. of sclerotia	Percent of heads infected
Treated	755	441	915*	60
Untreated	299	40	60	13

* An average of 2.1 ergots per infected head.

VIII. General Discussion

By weighing a number of medium sized sclerotia it was calculated that there are approximately 5000 sclerotia per pound. On the basis of 1,200,000 heads of rye per acre the approximate yield of ergot in lbs. per acre can be computed by multiplying 1,200,000 by the percent of heads infected, multiplying by the average number of ergots per head and dividing by 5000. The figures thus arrived at may be misleading from a commercial point of view because a goodly number of sclerotia are bound to be lost in any harvesting operation, especially if machinery must be used. It was felt wise to leave the yield per acre estimates until later work with large plots would actually show that a certain number of pounds of ergot could be harvested from large areas.

The problem of weather in relation to this method of inoculating rye with ergot has not been solved by the experiments presented, however, two questions appear to be partially answered. Rain is definitely detrimental. It washes off the sticky droplets of suspension so they have no chance of coming in contact with the pistils of later opening flowers. This was observed in the 1942 plots, in Botany Field I and in the Soils Field. Bright, clear weather appears to be favorable. Three of the four days during the treatment period on the Clark Field were bright; the first day had a trace of rain, but there was enough sunshine to dry it quite rapidly. It is possible that the small amounts of rain which fell in the five days following the treatments were important in causing a high percentage of infection, yet from observations on other plots of the effect of rain, probably these rains were detrimental. Further research will be necessary to secure definite answers to the many questions about the weather in

relation to this method of inoculating with ergot.

It is obvious from information secured in the Clark Field that ergot does not spread far in a field of rye which blooms evenly during bright weather. The control strips which were adjacent to treated strips had a small percentage of infection, most of which could have been caused by blowing of the spray at the time of treatment. Probably the best evidence of failure to spread was obtained by examining the field of which these plots were a part. Only by careful searching could a sclerotium be found even within ten feet of the treated areas. Many authors have made note of this, but it bears repeating, because it is one of the first questions which comes up when one thinks of growing ergot artificially. It is obvious from the results of the Soils Field that if later blooming rye is nearby there may be considerable spread. This will undoubtedly be true of grasses also which are susceptible to the biologic races of ergot which attack rye.

The problem of the blooming of rye in relation to the time of application of the spore suspension remains to be fully answered. Rye blooms periodically over a whole field. The first wave of blooming starts quite early, but varies from day to day. A wave of blooming lasts about fifteen minutes and appears to recur about every hour. In late morning or early afternoon there is a lessening in the numbers of flowers that open, until in mid afternoon, blooming almost ceases. Cloudy weather and temperature changes influence the rhythm of bloom. The observations have not been carefully checked and are of a tentative nature. However, based on these observations it is believed that the best time to spray is between 7 and 11 in the morning.

A number of factors need further research before it can be said that

the full capacity of this method to produce ergot is reached. Some of these are: (1) the most efficient method of applying the spore suspension, (2) the strength of the spore suspension, (3) the strength of the sugar solution used for the suspension, (4) the time of application, (5) the rate of application, (6) the number of applications, (7) the best age of culture to harvest for spores, (8) the best strain of the fungus, and (9) whether or not all applications should be made on one day or on different days. Add to these some factors which are general to this problem— (1) the variety of rye, (2) the site of the field, (3) the geographical locations of the fields, (4) machinery for harvesting the ergots, etc.— and one can see that the possibilities of producing ergot artificially are good if the optima for these variables are determined.

IX. Summary

A method is described for the preparation of an ergot spore suspension which can be kept in cold storage for weeks and probably months, then diluted and used to inoculate rye plants in the field by spraying on the plants at blooming time. The suspension consisted of beaten and screened cultures to which was added an equal weight of beet sugar. A machine for the application of the spores is described. The results indicate that this is a good and relatively simple method of inoculating rye with ergot.

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