NOTES ON GREENHOUSE CULTURE METHODS USED IN RUST INVESTIGATIONS.[1]

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The methods necessary for the production of infections with rusts are relatively simple. Yet, the more intensive and detailed studies, especially in connection with specialized strains and physiological relations, have necessitated certain refinements. These are being developed in various ways in laboratories where such investigations are conducted. In the investigation of the leaf rusts of wheat, *Puccinia triticina* Eriks., rye, *P. dispersa* Eriks., barley, *P. simplex* (Körn) Eriks. & Henn., and corn, *P. Sorghi* Schw., in progress at the Purdue University Agricultural Experiment Station, co-operating with the Office of Cereal Investigations, of the Bureau of Plant Industry, U. S. Department of Agriculture, since 1918, it has been necessary to modify certain established culture methods to meet conditions peculiar to the problem.

This paper has been written primarily with the object of describing such modifications. However, with the idea that it might be made more generally useful, especially for students desiring to undertake some culture work in laboratories where little or no rust research is carried on, descriptions of these modifications, together with reference to methods developed in other laboratories, are incorporated in an account of the general culture procedure employed in this laboratory. This procedure is believed to be similar to that used in most other laboratories where such studies are in progress. Descriptions of rust-culture methods have been published in numerous journals and bulletins dealing with the results of investigations of many different species of rusts. Papers by Carleton (1903),[2] Melhus (1912) and Fromme (1913), however, deal primarily with culture methods for this group of parasites.

1. Collection and Storage of Inoculum. Spores for inoculation purposes are relatively easily obtainable. Where the aecia or uredinia occur locally, rusted plants are brought into the laboratory and sowings are made with the fresh spores. When the problem involves the study of a rust from various sections of the country as for example the investigation of the occurrence and characteristics of different strains of *Puccinia triticina*, the fungus causing the leaf rust of wheat, the inoculum may be in transit several days. In the case of this rust, and many of the grass rusts, the spores will remain viable several days if rusted leaves simply are placed in heavy Manila envelopes and mailed.

It is a mistake to tightly wrap rusted plants in moist or paraffin paper in order to prevent desiccation. Under such conditions, especially during hot weather, more or less heating, fermentation, and molding of the material takes place, which is very detrimental to the viability of

[1] Contribution from the Botanical Department of the Purdue University Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, co-operating.

[2] Reference, by date, to "Literature Cited."

"Proc. Ind. Acad. Sci., vol. 33, 1923 (1924)."

the spores. This has been found to be especially true in the case of the aecia of the leafrust of wild grasses, *Puccinia Clematidis*. If the latter are gathered when thoroughly mature, they can be sent long distances, drying out on the way, and satisfactory cultures may be obtained in most cases. Aeciospores of *Puccinia Impatientis* have been dried rapidly for two days in a plant press heated to a temperature of 27° C., and excellent germination obtained from them. Aeciospores of this same species also have given good germination after dry storage in a packet at room temperature for 25 days. Aeciospores of *Puccinia triticina*, similarly kept, gave excellent germination at the end of 5 days, a slight percentage of germination still being obtained at the end of 28 days.

Uredinial material of *Puccinia triticina* has been sent by mail from many points over the United States and no difficulty has been experienced in obtaining good cultures. Wheat leaves infected with this rust were collected in 1923 at Hanford, Calif., on June 7; Knoxville, Tenn., June 12; Washington, D. C., June 16; Harmony, Wash., June 21; Urbana, Ill., June 27; Bloomington, Ill., July 5; Wanatah, Ind., July 9; Madison, Wis., July 23; and St. Paul, Minn., on July 24, and mailed to Lafayette, Ind., as described above. They were kept in an ice-box at approximately 9 to 12° C., until Nov. 13, when sowings were made from them on wheat, a fair amount of infection being obtained in each case. In other words, urediniospores of *Puccinia triticina*, 159 days after collection, were still able to give infection.

On Jan. 26, 1921, urediniospores of *Puccinia Sorghi* and *Puccinia triticina* were collected from greenhouse material and distributed in small cotton-stoppered vials at temperatures of 10, 15, 20, 25, 30 and 35° C., and outdoors. The germination of these was determined at various times until March 1. Both rusts showed a gradual reduction in germination after storage at the higher temperature, 35, 30, 25° C. About the same or slightly less germination was maintained at 20, 15, 10° C., while spores stored outdoors showed even better germination on March 1, when last examined. Peltier (1922) has investigated this question extensively for *Puccinia graminis*. Fromme (1913), Melhus and Durrell (1919), Peltier (1922), and Doran (1922) give data obtained by themselves and other investigators showing that the germinability of aeciospores and urediniospores of a number of species of rusts may be retained from 30 to 112 days. In general, germinability is retained longest at lower temperatures. Storing these spores dry at the cool temperature and moderate humidity of a refrigerator, either on the dry host or in vials, may be a useful method of carrying rusts over the summer, when it is not desirable or impossible to carry on investigations because of high greenhouse temperatures.

Teliospores may be collected and mailed in a similar manner. As in many species these are resting spores, the usual difficulty is not to prevent loss of viability but to bring about germination. The viability of the teliospores of those species which germinate at once may be prolonged, however, by refrigeration. Those which usually germinate only after overwintering are hung out of doors in cheesecloth bags within a foot or so of the ground. In this way, they are brought into a ger-

minable condition in the spring when their alternate hosts are in the best condition for infection.

2. **Germinability of Spores.** In general, aeciospores and urediniospores are capable of germinating when they naturally become detached from the spore chain or pedicel, spores not germinating being considered either immature or too old. A number of the rusts under investigation in this laboratory, however, have given only fair to moderate germination of urediniospores under conditions which indicate other factors of importance. Urediniospores of *Puccinia Sorghi* and *Puccinia triticina*, collected from greenhouse material on Jan. 26 and Feb. 1, 1921, and stored out of doors in vials, showed an increase in germination when tested Feb. 11 and March 1. We have found that drying for several days greatly increases germination of such spores. Melhus and Durrell (1919) have noted a similar condition for the urediniospores of *Puccinia coronata*. They found that storage of these spores in vials resulted in a marked increase in germination, indicating a maturation of the spores, even after detachment from the pedicel. It is very likely that the conditions under which these spores are formed have an important bearing upon their viability. But little seems to be known concerning this question.

On the other hand, the factors bringing about germinability in teliospores have received more attention. This probably is due to the fact that the teliospores of many species function as resting spores and usually are germinable only after weathering, generally over winter. This has stimulated investigation as to the factors concerned. Klebahn (1914), Maneval (1922), and the writer (1916) have shown that the teliospores of such species may be brought into a condition for germination by alternate wetting and drying or by more or less prolonged soaking without exposure to winter conditions. Thiel and Weiss (1920) have been able to bring about a condition for germination by treatment of teliospores of *Puccinia graminis* with citric acid. These methods may be found helpful in some investigations, as teliospores made germinable in the fall or early winter enable work to be carried on at a time of year when greenhouse conditions are more easily controlled. However, if the alternate host is a perennial, complications are introduced, as it will be necessary to bring it out of its resting period and into a vigorous growing condition.

That, even in nature, conditions sometimes may occur which bring about a considerable deviation from the typical situation, was noted in 1920. Thus two collections of *Puccinia montanensis* Ellis, two of *P. Koeleriae* Arth., five of *P. Clematidis* (DC.) Lagerh., eight of *P. procera* Dietel and Holw., three of *P. apocrypta* Ellis and Tracy, and fourteen of *P. triticina* showed teliospores germinating vigorously when brought into the greenhouse on Dec. 10 and tested. Collections of teliospores of these species tested the two previous seasons during the fall gave no germination and it has been generally considered that the teliospores of these species are germinable only after overwintering. All of the above collections were placed outside again and overwintered, and seventeen of them were still germinable when tested during March. On the other

hand, teliospores of a species which usually gives germination the fall of the year in which they mature also may germinate the following spring. In such a rust, *Puccinia dispersa*, twelve collections showed teliospores germinable between Aug. 19 and Nov. 25, 1919. Two additional collections gave germination in December. Of these 14 collections, five still gave germination the following spring on April 7, 1920.

3. **Methods of Testing the Germination of Spores.** It is evident that, before inoculating with teliospores, it is of considerable importance to know whether they are germinable. In the case of aeciospores and urediniospores it is not so essential as they usually can be relied on to give some germination from fresh material. With old inoculum, or where the plants to be inoculated are uncertain hosts, it is well to make germination tests in order to give proper value to negative results.

Fig. 1. Methods of testing spore germination. A. Petri dish; B. Syracuse watch glasses; C. Modified Van Tieghem cells.

Several different methods of making spore germination tests have been in use in this laboratory, being more or less modifications of those used by a number of other investigators. Modified Van Tieghem cells (fig. 1, C) were first used. In this method a drop of water is placed upon a cover glass and the spores are added to this drop. Water is placed also in the bottom of the cell which consists of a glass ring sealed onto a glass slide. The cover glass, with drop, is inverted and sealed with vaseline to the upper side of the ring.

A metal microscope-slide case was found very convenient in handling germination tests of 200 to 300 collections of teliospores, made about once a week during the spring months. Every other tray was removed and this gave plenty of room for the Van Tieghem cells, allowing them to be stored in a small space. These cells make easily possible the examination of the spores with the 4 mm. objective of the microscope without disturbing the humidity. Where frequent and numerous tests are

being made this method has the objection that considerable time and work is necessary to prepare the cells and remove the vaseline from the cover glasses in preparation for another test.

A less laborious method is to use Syracuse watch glasses (fig. 1, B) with ground glass margins. The bottom of the watch glass is covered with water on which the aeciospores or urediniospores are floated. The accession number of the collection or other identification mark is written on the ground glass margin. Another dish is placed on top of this in which another collection of spores is tested. A number of watch glasses can be stacked thus and a number of such stacks placed in shallow dishes for convenience in carrying from place to place.

Hanging drops also can be made with these watch glasses by placing the spores in one or more drops of water on the bottom of a watch glass, inverting it and placing it on another inverted watch glass which has been covered with water. The beveled edge of one glass fits into a groove in the bottom of the other so that there is little danger of slipping. The hanging drop is by far the best method with teliospores of *Puccinia triticina*, for they otherwise are likely to sink to the bottom of the drop and either not germinate or give abnormal germination. In this method, however, the drops can be examined to advantage only under the lower powers of the microscope. This method has the advantage of requiring but little time in the preparation and labeling of the tests. Hursh (1922) also has used and described this method.

Still another method is to make hanging drops on the lid of a petri dish, the water in the bottom providing the humidity. Spores have been tested also by placing the drops on glass slides in petri dishes raised above the wet substratum (moistened filter paper or paper toweling) by glass slips (fig. 1, A) as described by Melhus (1915) and Melhus and Durrell (1919). The petri dishes take up somewhat more room than watch glasses, but the latter method has the advantage that a cover glass can be placed on the drop, enabling examination under the higher powers of the microscope. Each method has features of advantage in different types of investigations.

4. Environmental Conditions Governing Spore Germination. As inoculation with the rust fungi depends upon the germination of their spores, the conditions influencing spore germination must be taken into consideration in devising methods for the culture of these fungi. Perhaps the most essential condition is a humidity of 100 per cent. In many rusts, the spores must also be in contact with water. While spores may germinate through a fairly wide range of temperatures, for many rusts a temperature of 16-20° C., is most favorable for germination, few spores germinating below 5° C., or above 30° C. Different species, however, vary somewhat as to the cardinal temperatures for spore germination. Fromme (1913), Doran (1922) and Maneval (1922) give summaries of the results obtained by themselves and other investigators in the studies of the effect of various conditions on the spore germination of a number of rusts.

While these are the principal factors governing germination, still other factors are of some importance in the greenhouse investigations.

One of these is the toxicity of the water. At this laboratory, no detrimental effect has been obtained from the use of tap water. Melhus and Durrell (1919), however, found tap water toxic to urediniospores of *Puccinia coronata* at Ames, Iowa, and used distilled water in their inoculations. Another factor of importance is the submersion of the spores. Aeciospores and urediniospores of *Puccinia triticina* give little or no germination if submerged and the teliospores, if they germinate at all, produce long tubes without forming basidia. This has been noted by a number of investigators (Melhus & Durrell, 1919; Melhus, Durrell & Kirby, 1920; and Doran, 1921).

5. **Inoculation—Urediniospores.** The inoculation method used depends upon the type of investigation being conducted. Where either a few plants are being inoculated, or the amount of inoculum is small, or where different strains of rust are being studied, necessitating carefully controlled conditions, each plant is inoculated with spores applied with a scalpel. The plants are first wet by atomizing with a De Vilbiss atomizer as recommended by Melhus (1912). In the case of the cereals, a waxy coating on the leaves prevents water from adhering. This is overcome by gently drawing the leaves between wet fingers, as recommended by Melchers (1915), until water adheres.

Spores are removed from the rusted plants with a straight-edged scalpel and spread on the wetted leaves. It has not been found necessary to trim the inoculated plants of all leaves except those inoculated and no difficulty has been found in interpreting the results. Inoculations with the leafrusts of wheat, rye, barley, and corn give practically as good results when the spores are placed on one surface of the leaf as on the other. This enables rapid sowing of spores, as a number of plants may be inoculated by holding the leaves by the tips so that they are in one layer and covering them all with spores at the same time. Hungerford and Owens (1923) state that in the case of *Puccinia glumarum* good infection is obtained only when spores are sown on the upper side of the wheat leaf. Melchers (1915) recommends the sowing of *Puccinia graminis* on the lower side of the leaf. Where more than one culture of the same rust is being studied the usual precautions of sterilizing the instruments used must be taken.

Where plenty of viable urediniospores are available and only one culture of a species is under greenhouse investigation, dusting with spores has been found the most effective method of inoculating large numbers of plants in a manner similar to that recommended by Fromme (1913). The plants are first atomized and then heavily rusted plants are shaken over them, after which they are placed in a moist incubation chamber. By this method the urediniospores are well scattered and the exposed plant parts usually are fairly uniformly inoculated. Where this method can be used, it has the advantage that a large number of plants can be inoculated quickly.

Aeciospores. Similar methods are used with aeciospores. There are several modifications, however, which have been found very serviceable. As aeciospores are catenulate, the lower spores being immature when the upper are ripe, scraping the aecia off with a scalpel removes many immature spores. When aecia have been produced in the green-

house, the spores are collected by fastening paraffined paper loosely around the infected parts of the plants in such a way that there are several openings for ventilation. The ripe spores which fall in this loose paper bag are scraped off and applied with a scalpel to the plants under investigation. Where sufficient inoculum is collected, it is dusted by using the glass blower described by Durrell and Parker (1920). Oftentimes in the greenhouse the peridia of aecia do not open but project out as a long cylindric column from which no spores are shed. If some of the infected plant parts bearing such aecia are placed upon waxed paper in a moist chamber over night, the peridial cells will fall apart and the mass of spores can be scraped from the paper for inoculating.

Teliospores. In those rusts where the teliospores are deciduous, dusting can be employed very effectively, as, for example, in *Kunkelia nitens*. However, in most species of the genus Puccinia, the teliospores are not deciduous and therefore must either be scraped off and applied with a scalpel or the plant parts bearing the teliospores laid on or around the plants to be inoculated in such a way that the basidiospores will reach the parts to be infected. Where sufficient material is available, the latter method probably is the better, as Melhus, Durrell, and Kirby (1920) point out that the teliospores of *Puccinia graminis* germinate better if not detached from the straw.

6. Condition of Plant Inoculated. In general, the plants to be inoculated should be in the best of growing conditions. It is a mistaken idea that weaker plants are more subject to rust. Plants which are in a weak and stunted condition, poorly developed from any cause do not readily infect or if infection takes place the rust does not develop well, the uredinia are fewer and smaller and often paler in color than when the host is developing vigorously. This also is true for parts of the same plant. Usually the young rapidly-developing plant parts are most readily and vigorously infected, the older, especially the senescent leaves and stems, showing much less infection. Not only is this true in case of the development of uredinia, but especially in the case of aecia.

The aecia of *Puccinia Clematidis* developing on the young, rapidly-growing stem of *Clematis virginiana* often produce marked hypertrophies and galls with the production of a large number of pycnia and aecia. Infection on very old leaves of the same plant usually produces only a few pycnia or possibly also a few aecia. On the other hand, telia usually develop when metabolism and growth are slowed up as in the case of ripening grain, under poor conditions of light and growth during midwinter in the greenhouse, or on old senescent leaves. However, the species of rusts vary considerably in this regard. Thus while *Puccinia montanensis* upon seedling grasses in the greenhouse in midwinter goes to telia so readily that it is sometimes difficult to keep it in culture, *Puccinia triticina* upon wheat seedlings has never been seen by the writer to produce telia under such conditions even on old dying leaves, but does produce some telia on old leaves when the wheat plants approach maturity.

7. Inoculation Chambers. Any sort of an enclosure which will permit the maintaining of a saturated humidity and a temperature optimum for the rust under investigation, will do for an inoculation chamber. When seedlings are to be inoculated sufficient light should be admitted to prevent excessive etiolation during the inoculation period. Unless provision for cooling the chamber is made, direct sunlight is to be avoided on account of the resulting high temperatures. Belljars have been quite generally used and are very satisfactory where the number of plants being inoculated is small. In this laboratory these are placed on a layer of wet sphagnum under the greenhouse bench where they

Fig. 2. Plant Covers which may be used either for inoculation chambers or for carrying of rust cultures.

receive diffused light. They have the disadvantage, however, of being easily broken and costly to replace.

As a substitute for belljars a modification of a plant cover (fig. 2) such as is used to shelter early planted cucumbers or melons has been found very satisfactory. These plant covers, as usually constructed, are too low for plants in pots. As a consequence it is necessary to have them made to order. Two sizes are constructed, one 21 inches high for 9-inch pots, and the other 14 inches high for 3- or 4-inch pots. The accompanying illustration (fig. 2) shows the general character of these plant covers. They are of cypress, except for two panes of glass which serve for the roof, and in consequence are very durable. If the glass is broken it usually can be replaced by cutting down broken greenhouse panes. These plant covers are used not only for inoculation chambers,

but have been found very useful for separating the various cultures of *Puccinia triticina* and other species in the study of specialized strains. As the panes of glass slide up and down in a groove, plants can be removed without lifting the cover and also various degrees of ventilation can be employed.

For still larger number of plants, galvanized-iron tanks, similar to those described by Parker (1918), Melchers and Parker (1922) and other investigators, are used. These are covered with lids in which panes of glass are inset, allowing entrance of light. Wet sphagnum in the bottom of the tank aids in keeping up the humidity. These tanks are kept under the greenhouse bench. The floor being of cement, they are easily pulled out to open and easily push back out of the way when closed.

One of the principal difficulties of successful culture in late spring, summer, and early fall is the high temperature of the greenhouse. By installing a system of spray nozzles beneath the greenhouse bench, the tanks, belljars and plant covers are cooled by a fine spray of water. By this means inoculation has been successfully carried out during the hot weather. When this system is used the tank covers are removed about twice a day for a few minutes and the plants are thoroughly moistened by leaving them exposed to the atomized spray. Thus the plants are aerated and covered with a fine dew, and the humidity of the chamber is renewed all in one operation. Belljars and plant covers are sprayed by means of a De Vilbiss atomizer. Inoculation chambers of the icelessrefrigerator type, described by Hunt (1919) also have been used in greenhouses where no spray system was available, cooling being obtained by evaporation from the wet muslin cover of the chamber. Melhus, Dietz and Willey (1922) also have described a series of muslin compartments in which their cultures are both kept and inoculated and which are cooled by a fine spray of water.

Where only one culture of a rust is being used in a greenhouse and the number of plants being inoculated is very large, as in the F_2 generation of a hybrid, the plants are dusted as described above and, after atomizing, a moist chamber is created by covering the pots of plants in place on the bench with heavy muslin. This is kept from resting on the plants by stakes placed at intervals in the bed. The muslin is kept wet by spraying as often as necessary during the day. This in reality is a very flexible moist chamber. It is especially useful where the plants to be inoculated are grown in soil on a greenhouse bench. In this way, thousands of seedlings of corn have been inoculated, notes taken, further selections of corn planted, inoculated, etc., all within a comparatively short time and with much less labor than if planted in pots and inoculated by hand.

8. General Treatment of Cultures. Inoculated plants usually are left in the moist chambers for about 48 hours. It is not advisable, when removing, to place them on the greenhouse bench in direct sunlight while tender from confinement in the moist chambers as the plants are likely to scald. It, therefore, is best to remove them in the late afternoon.

The incubation period of the rusts varies somewhat with the spe-

cies. In the case of *Puccinia triticina*, uredinia appear in 6-10 days after inoculation, development being most rapid in warm, sunny weather, and slowest in cool, cloudy weather. In the case of leaf rusts of wheat, rye, and barley the heavily infected leaves of seedlings die in about two weeks after the uredinia appear, and the plants thus become free of the rust unless reinoculated. If the leaves have to support only a small quantity of rust, infection may persist for some time, new uredinia being produced toward the border of the mycelium until there are two or even three concentric circles of uredinia around the uredinium first produced. As these rusts usually are carried on seedlings upon which it is desirable to produce as much infection as possible, it is necessary to transfer cultures about once every three or four weeks.

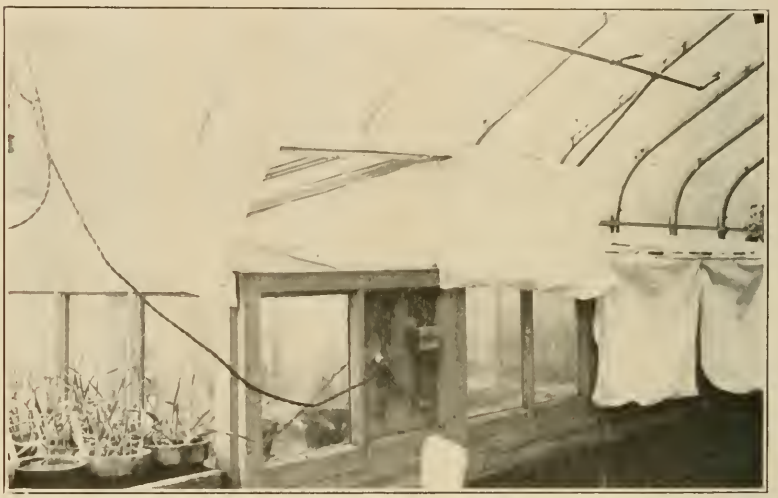

Fig. 3. Series of Wardian chambers for separation of rust cultures, showing right-hand two with sash down as used with spray and left-hand two with sash up and curtained off with muslin.

In the case of the rust of snapdragon, *Puccinia Antirrhini*, where the infected stems may not die for some time, the rust may continue to sporulate for two or three months, until the infected parts are killed. Corn rust, *Puccinia Sorghi*, maintains itself in the greenhouse as the funnel of unrolling young leaves makes an ideal moist chamber for spore germination. Whenever plants or plant parts overlap so that water persists on the lower leaves and a high humidity exists, the rusts will continue to reinoculate, provided temperatures are favorable for spore germination. Where comparative studies of different cultures are being made it usually is more desirable to limit the inoculation only to certain plants and therefore by proper spacing and care in watering, the rusts are prevented from reinoculating.

9. Comparative Study of Cultures. Where more than one culture of the same species of rust are being studied in order to determine if specialized races or strains exist and their behavior upon different spe-

cies or varieties of plants, it is very important that precautions be taken to prevent contamination of the cultures. Plants upon which these cultures are to be sown are grown in a greenhouse or compartment where the rust under investigation is not present. If the rust is very prevalent out of doors, as is the case with *Puccinia triticina* in the summer, it is necessary to grow such plants under very confined conditions, such as in a Wardian case (fig. 3), in order to protect them from wind-blown spores from without.

It would be highly desirable in such studies if methods as refined as those used in bacteriology could be employed. However, no nutrient medium has been devised upon which a rust can be grown. It is necessary to grow it on its living host and under the conditions which are favorable for the growth of such a host. Usually the confined conditions necessary to be absolutely sure there is no chance for contamination are not favorable for the best development of the host. In consequence, various expedients have to be employed. As the rusts primarily are wind-borne diseases, it is essential that direct air currents be avoided.

For this purpose, the partitions on the greenhouse bench made of a double thickness of muslin, and the cages of similar material used by Stakman, Piemeisel, and Levine (1918) have been found good. However, for *Puccinia triticina* the plant covers (fig. 2) described above have been found better, there being less chance of contamination. Where larger numbers of plants are being inoculated than these plant covers will hold, Wardian chambers (fig. 3) are used. These are made entirely of glass, the top being on the order of a hotbed sash so that a spray of water can be run on the chambers in the summer in order to lower the temperature. During the winter the sash usually is raised, the individual compartments being curtained off with muslin.

In the study of *Puccinia triticina* for the presence of specialized strains, some method was sought which would permit the growing of a fairly large number of single-spore cultures of the rust organism within a small space. The plan finally adopted was to sow wheat in four-inch pots filled with soil to the top. A lantern globe, covered over the top with a thin layer of cotton held in place with cheesecloth, was then pressed down into the soil as deeply as possible (fig. 4, D). The wheat coming up within this lantern globe is protected from wind-blown spores. Cultures of rust in such lantern globes can be carried from place to place without distributing spores or contaminating the cultures themselves.

During the winter months such leaf-rust cultures are maintained in good condition and transfers are made about every three or four weeks. During the summer, however, the high humidity and temperatures in such lantern globes are very favorable for the development of other fungi, especially Helminthosporium, which often kills the wheat and makes it difficult to carry the cultures through the summer. If only a few pots of seedlings are to be inoculated the spores developed in these lantern globes are sufficient. Where it is desirable to inoculate a large number of varieties at one time, so as to have all under as nearly the same set of conditions as possible, it is necessary to multiply the culture. The rust spores are first sown on wheat under another lantern globe in order that a pure culture may always be available. The remain-

ing inoculum is sown on as many pots of wheat as possible in plant covers or Wardian chambers. From the infection obtained, the set of varieties is inoculated.

After inoculating, the plants are placed on the greenhouse bench, possibly beside a set inoculated with another culture. As the humidity of the greenhouse does not reach the saturation point, and the seedlings are not grown thickly enough to provide such a condition among them, no infection results from one culture to the other on the bench. After notes are taken, the plants are discarded. If further investigations are carried out, the rust inoculum is obtained again from the lantern globe.

10. Pure Lines of the Rust Fungus. In the course of the investigations of the leaf rust of wheat *Puccinia triticina*, it became evident

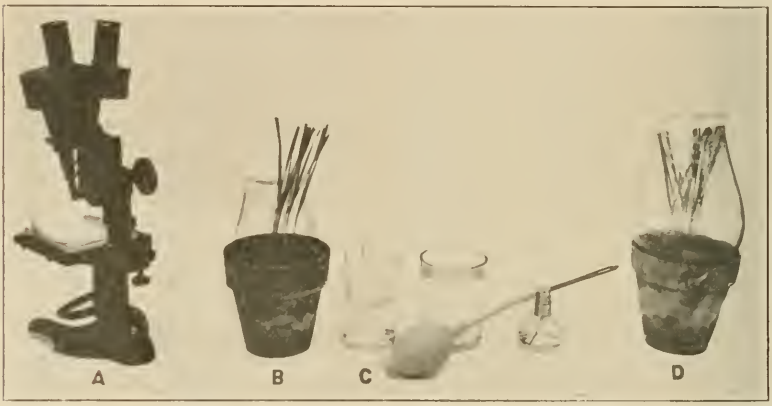

Fig. 4. Single spore cultures. Spore picked up from petri dish on binocular stage (A) by wet glass needles (C) and placed on wheat seedling (B). Culture covered with lantern globe (D).

that the rust organism as collected consisted of more than one strain. In order to be sure that the results obtained were characteristic of one strain of the organism and not due to a mixture of several strains, it became necessary to devise a method for obtaining a pure culture or pure line of the rust fungus. This can be done, with certainty only, by starting from a single spore. After a number of unsuccessful attempts to obtain such cultures the following method was found to work very successfully.

Urediniospores of a collection of the rust are gently dusted into a petri dish (fig. 4). This dish is placed under the highest powers of a binocular and a single urediniospore located, considerably separated from others. This is picked up by touching it with a wet glass rod drawn out to a fine point. By means of this glass needle, the spore is transferred to the moistened blade of a wheat seedling. Usually ten such plants, which have been grown under a lantern globe as mentioned above, are so inoculated. The culture is observed daily and as soon as the first

sign of a uredinium shows, all other plants are removed leaving the one plant with the one uredinium under the lantern globe. From this the culture is multiplied.

Another method, more easily used, consists in lightly dusting some spores on a pot of plants grown under a lantern globe. When uredinia first show, but before they have broken the epidermis, a plant is selected showing only one uredinium, all the rest being eliminated. If no such plant is found, but a plant is found having only one uredinium on a leaf, all the leaves but that one are cut off. By such a method one is not quite so certain of having a pure line as in the former, but in many cases it is practically as good.

11. Cultures of Rust Free from Other Fungi. In the usual culture work with rusts it is not necessary to exclude other fungi, especially saprophytes, yet if investigations looking toward the growing of these parasites on artificial media are undertaken, the rusts must be free from saprophytic fungi which otherwise will rapidly overgrow the cultures. The writer (1917) has elsewhere described such a method.

To recapitulate briefly, urediniospores of *Puccinia Sorghi* are sown on the upper side of leaves of corn plants grown in large sterile test tubes, from seed disinfected with corrosive sublimate. The first uredinia occur on the lower side of the leaves, and spores from these are sown on similar test-tube cultures of corn plants. Thus the rust might be said to have been strained through the corn leaf. Another method is to float small pieces of leaves from sterile corn plants on sterile sugar solution in small capsules. These pieces of leaves will take up the sugar solution and live for a considerable period. Spores taken from a freshly opened uredinium are used to inoculate the pieces of leaves in a number of these capsules. Spores from uredinia developed in those capsules which do not show saprophytic fungi are then used to inoculate a further series.

12. Recording Data. When only a few individuals of a small number of species or varieties are being studied, it is relatively easy to record in detail the symptoms produced when infected with rust. If an attempt is made to describe in detail all the symptoms shown by a large number of varieties, the time necessary for such notes becomes a serious limiting factor. To meet this difficulty a system of symbols has been in use in this laboratory, thus reducing the time necessary for note taking. These symbols indicate the terms applicable to the symptoms. They are as follows:

i = immune, no signs of infection.
f = flecks.
k = killed or necrotic areas.
M = mottled or chlorotic areas.
s = susceptible, no signs of resistance.
c = uredinia in concentric circles.
g = green islands of tissue surrounded by chlorotic.

The size of the uredinia is denoted as follows:

m = minute.

m = moderate in size.
$\overline{l}$ = large.
l = very large.

In resistant varieties where flecks, necrotic or chlorotic areas are produced, uredinia may or may not develop. This is denoted by the numeral, sub 1, following the symbol. When both areas with and without uredinia are found, the relative prevalence is denoted by writing the more numerous first, ex., ff_1 denotes flecks without uredinia more numerous than those with uredinia.

The susceptibility as illustrated in Figure 5, A would be recorded thus, s_1lc; B as s_1l; C as M_1l; D as s_1lk_1m; E as $s_1\underline{l}g$; F as k_1l; G as k_1km; H as $k\dot{k}_1m$; I as f.

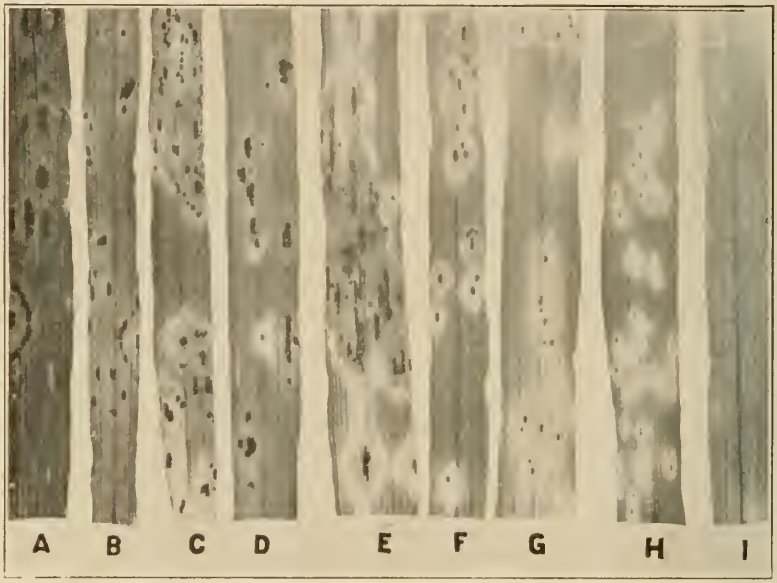

Fig. 5. Types of susceptibility shown by rye to leafrust, *Puccinia dispersa*.

Such a system can be arranged to meet the needs of the individual investigator. It should be emphasized that this is in no way a system of classification of susceptibility. It is a rapid method for the recording of symptoms from which the individual plant or the variety can later be placed in its proper class of susceptibility when enough types have been studied upon which to base such a classification.

The relative quantity of uredinia produced has been denoted in our notes by a corresponding segment of a circle surrounding the + mark denoting infection. This, however, should be replaced by some more suitable symbol. The number of plants infected out of the number inoculated is denoted by a fraction, the denominator giving the number inoculated and the numerator those showing infection, a method used by a number of investigators.

While detailed notes are necessary when an investigation is in progress, they would only be confusing in a publication of results. At this stage, however, the various types of susceptibility can be placed in a relatively few classes with some degree of accuracy. Vavilow (1913) following Eriksson has employed a classification based largely on the amount of uredinia developed on grain in the field. Stakman and Levine (1922) and a number of other investigators have used a similar system for their greenhouse investigations of *Puccinia graminis*, symptoms as well as quantity of uredinia being used as criteria. Mains and Leighty (1923) have used a similar classification for *Puccinia dispersa*. This may be arranged as follows for the leafrusts of wheat and rye:

0. No uredinia formed; hypersensitive areas sometimes present and definite, sometimes faint or absent. Fig. 5, I.

1. Uredinia few, minute, in the center of definite hypersensitive areas; few to many hypersensitive areas without uredinia. Fig. 5, H, G.

2. Uredinia fairly abundant, moderate in size but always surrounded by hypersensitive areas, hypersensitive areas seldom without uredinia. Fig. 5, F.

3. Uredinia abundant, moderate in size, without hypersensitive areas but in some cases surrounded by slightly chlorotic tissue. Fig. 5, C, B.

4. Uredinia abundant, very large, hypersensitiveness absent but uredinia occasionally in green islands. Fig. 5, D.

Where time is available, perhaps the best method of taking notes is to make herbarium specimens, especially where there is likely to be need of critical comparison later on. If infected leaves are dried quickly under moderate pressure, they will lose very little in color or other characters and will serve for several years at least as good material for reference. The plant press described by Jackson (1921) has been found especially good for this purpose, as the drying is very rapid, 12 hours usually being sufficient.

Photographs also are very important records, especially when publishing results. Many of the rusts are very difficult to photograph. This is especially true in the case of *Puccinia triticina*, where the uredinia are orange and the leaf is green in color. By the use of Wratten & Wainwright panchromatic plates and the B (green) Wratten M filter and enlarging two or three times, the uredinia photograph very well. Where a number of different types of susceptibility are to be photographed together, filters usually cut down the contrast on some of the types. It has been found that photographing by both reflected and transmitted light shows practically all the characters well defined, the uredinia appearing darker on account of transmitting less light than the leaf and the chlorotic areas, necrotic areas, and flecks appear lighter because they transmit more light.

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