T H E S I S

SWEET PEA MILDEW AND ITS CONTROL UNDER GREENHOUSE CONDITIONS

Submitted by

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for the Degree of Master of Science

Colorado Agricultural College

Fort Collins, Colorado

May 1, 1932

COLORADO AGRICULTURAL COLLEGE

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GRADUATE WORK

May 23 1932

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Kenneth W. Taylor ENTITLED "SWEET PEA MILDEW AND ITS CONTROL UNDER GREENHOUSE CONDITIONS" BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science Majoring in Botany

~~CREDITS~~ 20

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The writer wishes to express his appreciation for the help given him by all the members of the department and is especially grateful to Dr. L. W. Durrell for his helpful criticism and interest in this work.

This is to certify that Mr. K. W. Taylor has translated for me assigned passages of technical French bearing upon his graduate Botanical work.

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GREENHOUSE CONDITIONS

INTRODUCTION

In the Rocky Mountain area the growing of sweet peas has become a very important greenhouse crop in recent years. Sweet peas, however, are subject to severe attacks of mildew caused by the fungus Erysiphe communis. The control of mildew under glass has been generally affected through the application of sulfur. This material, while an excellent control under ordinary conditions, is not wholly satisfactory because it frequently burns the leaves and flowers.

With the hope of finding a better control the work reported in this thesis was undertaken. Results are given of experiments with different kinds of sulfurs, copper carbonate, "Grape Dust" and copper oxy-chloride as control measures. In order to better understand the action of the causal organism and the condition of its spread and reproduction, studies were also made of factors pertaining to spore production and spore germination and their bearing on control under greenhouse conditions.

LITERATURE REVIEW

The origin of the sweet pea is divided between Sicy and Ceylon (11). The original purple variety is said to be indigenous to Sicily and Sardinia. The original pink and white variety, known as the Painted Lady, is credited to Ceylon. There are other reports from different sources as to the origin of the sweet pea, but the consensus of opinion seems to be that Ceylon and Sicily are the native habitats. The flower was first cultivated by Father Franciscus Cupani (11), an Italian monk and naturalist at Panormus, Sicily in 1699. The seed of the purple variety was distributed by him to England and other countries in that same year. Dr. Uvedale at Enfield, England, grew plants from this seed and from there the plant has been spread to nearly all civilized countries.

Joannes Bauhinus (11) gave the first description of the sweet pea in his "Historia Plantarum" published in 1651. Later several botanists classified it and finally Linnaeus (11) in his "Systema Plantarum Europae" (1753) termed it "Lathyrus odorata". That nomenclature has persisted until the present time.

Erysiphaceae

There are several diseases occurring on sweet pea, paramount among which is the very common powdery mildew.

The powdery mildew belongs to a large family all of which are parasitic. The members of the family Erysiphaceae are easily recognized by the powdery white conidia, conidiophores, and mycelium that coat the surface of their hosts, giving them an appearance as though lightly dusted with flour. Their description is well given by Stevens(26)as follows: Later in the season the white patches are more or less liberally sprinkled with black perithecia. The mycelia in all genera except Phyllactina is entirely superficial, hyaline, branched and, septate. The cells of the mycelium are uni-nucleate. The fungus anchors itself to the host tissue and obtains its nourishment therefrom by special adaptive structures termed haustoria. These are of three main types, those arising directly from the lower surface of the mycelium; those arising from the sides of the mycelium hyphae and occurring as small, semi-circular processes, and those arising from a more or less deeply lobed, lateral swelling of the mycelium (25).

"Only in Erysiphe graminis do the haustoria grow laterally to long, filiform lobes". The tuberiform or saccate knot is the usual form of haustorium in this genus (14).

Reproduction in the mildews is carried on by the means of conidia which are produced in chains (14). These conidia are produced by the successive constriction of a series of daughter cells. Sexual reproduction is brought about thru the formation of perithecia. These bodies are subspherical, dark to black and reticulate at maturity. They are without ostioles, but are provided with various types of appendages. These appendages aid in the dissemination of the fungus by their hygroscopic movements and also serve to indicate generic differences. The asi in the perithecia vary from solitary to numerous. This peculiarity aids in specific characterization. Each ascus bears two to eight hyaline spores (14).

In Erysiphe the conidia were supposed to be a species of the form genus "Oidium" and only the perithecia were assigned to Erysiphe. The researcher Tulasne first came to a true understanding of the real consideration of the case (9).

The formation of perithecia is rather interesting from a morphological standpoint. The antheridium and oogonium arise directly from the mycelium and fertilization occurs by the union of two gametic nuclei. The oospore nucleus divides and the oogonium develops into a short bent tube containing five to eight nuclei. Septa now appear cutting off cells, which may be uni-nucleate or multi-nucleate. The ascogenous hyphae develop a knot and divide into two or three cells each. These eventually give rise to the asci which are bi-nucleate in the beginning (26).

In some species the sexual fusion is considered non-essential. This is true in E. communis. Although both antheridia and ascogenous hyphae are present fertilization is absent and the male nucleus degenerates in the antheridium (14).

The perithecia of Erysiphe are formed in June and July and are able to cause infection only after the winter rest period. The production of perithecia is attributed to the sudden alternation of high and low temperatures (16).

DeBary (9) quotes Wolff as saying, "one finds that the perithecia (of Erysiphe) arrive at maturity with the termination of the vegetation (of the host) and that they separate from the substratum and enter the period of winter rest with the asci and spores formed on the inside."

The genus Erysiphe is characterized by several asci to each perithecium, the appendages of the perithecia are flexuous, hyphae-like and generally unbranched (17).

The species causing mildew in sweet peas is variously known as E. communis, E. martii, E. pisi, and E. polygoni.

According to Engler and Prantl (12), E. communis has many unbranched or slightly branching haustoria with the (perithecial) appendages formed by a webbing up of the mycelium. It is a common sort, found on many host plants, namely Ranunculaceae, Leguminosae, Polygonaceae, Compositae, Scrophulariaceae, etc. They (Engler and Prantl (12) note that Schroeder called the species by its oldest name, E. polygoni (E. C.) Schrot. This name is not descriptive and it would seem better to use the usual name, E. communis. E. pisi D. C. (E. martii Lev.) also has lapped haustoria and mycelium webbed appendages on the fruiting body. It is abundant on the stems and leaves of many plants especially on legumes in the north temperate zone.

All these names are used synonymously by Gäuman (14) and the writer believes the name E. communis to be best terminology, and that name will be used throughout this paper.

Control -

Sweet peas have very delicate blossoms and foliage. Because of this fact it is necessary that any agent that may be used to control mildew on them, be one that will neither burn the foliage nor stain the flowers.

In the past, several fungicides have been used to control mildew with varying degrees of success. Among them are flowers of sulfur, lime sulfur, applied both as a spray and as a dust, liver of sulfur, copper sulfate in a weak bordeaux mixture and "Grape Dust" (8). None of these compounds have been entirely successful.

Copper sulfate in a bordeaux mixture is obviously unfit for use as a control because of the staining of the flowers. "Grape Dust" is probably more widely used in greenhouses at present than any other fungicide and is very effective in so far as the foliage is concerned. When blooming begins, however, the grower must cease applications or discard part of the flowers as being unfit for the market as they will be badly stained and blotched by the copper sulfate which is one of the ingredients of the dust.

Perhaps the first fungicide to be used in controlling powdery mildew was sulfur or some one of its compounds. The first mention of a control in literature was in 1821 long before there was any accurate knowledge of the nature of the disease (22). At that time, Robertson (22), a peach grower of Georgia, found that flowers of sulfur were effective as a control for mildew on his peaches. In fact, he goes further and says that sulfur is the <u>only</u> specific remedy. He applied the material in the form of a thin paste or thick spray prepared by mixing flowers of sulfur with a solution of soap suds. This mixture he applied to the diseased trees by dashing it <u>violently</u> on the leaves with a rose syringe.

This proved to be a fairly effective control measure, but usually resulted in burning the foliage and oft-times scabbed the fruit.

Since Robertson's first work sulfur has been very widely used as a fungicide. There have been numerous attempts to secure a compound or form of sulfur which can be successfully used, without incurring any of its objectionable qualities. Ground sulfur, iron sulfate, liver of sulfur, lime sulfur, and several so-called wettable or colloidal sulfurs have been used in these attempts.

Nearly all of the above compounds and mixtures have certain qualities which prohibit their universal use as fungicides. Finely ground sulfurs and flowers of sulfur have a tendency to ball up when mixed with water (22). Robertson, as stated above, attempted to overcome this condition by the addition of soapsuds. He succeeded to a certain degree, but the ultimate result was a burning. More recently many of the commercial dusting sulfurs have been found to contain small amounts of inert materials as kaolin, casein, and others (28). These have evidently been added to overcome or prevent this balling tendency and to improve the qualities of adhesion.

Iron sulfate and liver of sulfur have been used in the past, but are little used at present because of their tendency to burn. Liver of sulfur, which is a mixture of various polysulfides of potassium (13) and is so named because of its color, burns because of the liberation of free alkali upon being wetted.'

Lime sulfur is highly recommended as a fungicide and is successfully used as a dormant spray under out-of-doors conditions (22). With the appearance of flowers and foliage its use must be discontinued because of the severe burning. This same quality renders it unfit for greenhouse use.

Many attempts have been made to produce sulfur of a "wettable" or colloidal nature. Such sulfurs would result in a greater fineness of particles and a more even distribution over the sprayed surface. In so far as chemical and physical analyses show, these sulfurs have been prepared by grinding crystalline or amorphus sulfur together with various protective colloidal substances and spreaders in colloidal mills of the Plauson type (22). Young (30) prepared a "colloidal" sulfur by the addition of sulfuric acid to a concentrated solution of sodium thiosulphate by which means sulfur dioxide and free sulfur are produced. The free sulfur is thrown out in a finely divided form which remains well in suspension. Such a system, however, is not truly colloidal.

One such colloidal sulfur is produced by the precipitation of extremely minute sulfur particles from H_2S into an aqueous solution containing a protective colloid. The details of the preparation have not been divulged as yet, but if the product proves successful it will be produced on a commercial scale.

There are several so-called "wettable" sulfurs prepared on a commercial scale at present and some of them have proven very good fungicides. One of these is manufactured and distributed by the Southern Acid and Sulfur Company of Saint Louis, Missouri. It goes readily into suspension when mixed with water and requires but little agitation to keep it in that state. Its definite composition is a trade secret and its complete chemical constituency is not known. This is the material that was used throughout the author's experimental work.

Many theories as to the fungicidal action of sulfur have been advanced. Some of them are quaint and obviously impossible; however, it is believed that it is worth while to incorporate a brief discussion of these theories in this paper.

Mangini (22), in 1871 brought forward the idea that the production of electricity thru the contact of the sulfur particles with the plant tissue might prove toxic to the fungus. So far as is known he did no experimental work on this theory and it was later discarded as being valueless.

Mach (22) suggested an optical action in which the small particles of sulfur acted as minute lenses focusing the sun's rays on the fungus and killing it by burning. This is obviously impossible and the theory was subject to the scorn of his contemporaries.

Pollaci (22) did some experimental work with sulfur and came to the conclusion that sulfuretted hydrogen is the toxic agent. Barker (3) and Foreman (13) in England and Wilcoxon and McCallon (29) in America support this statement, but Eyre and Salmon (10) and Young (30) have attempted to disprove the theory by their experimental work.

In the same year that he advanced his electric theory, Mangini (22) put forth another argument to the effect that under the influence of direct sunlight sulfur dioxide is formed and is the toxic agent. It would seem as though this statement did not cover all cases as it is known that sulfur is also active as a fungicide where direct sunlight is lacking. Marcilli (22) found sulfur trioxide present in all sulfur sprays and without a great deal of proof came to the conclusion that that compound was the one to which sulfur owed its fungicidal property. Doran (8) showed that to form an inhibition of spore germination in the presence of sulfur, oxygen must also be present, and that in the absence of oxygen no inhibitive effect was apparent.

Vogt (22) worked with elemental sulfur out-of-doors to determine, if possible, what were the decomposition products. During the course of his experiments he was unable to produce evidence of the oxidation of sulfur to form SO_2 under the action of humidity and light. All of his experiments were carried on in the absence of plant material, however. In view of the fact that sulfur dioxide, even at extreme dilutions, is toxic to vegetation it is improbable that it is produced, for if it were reports of sulfur injury would be more frequent.

Young has considered that the toxic property of sulfur is due to pentathionic acid (30), but Debus (5) says this is highly improbable, as pentathionic acid is one of the acids of Wackenroder's solution, and these acids are not volatile. He assumes, apparently, that volatility is one of the factors upon which toxicity is dependent. Since then Young has advanced no proof of its volatility and Wilcoxen and McCallan (29) have actually grown colonies of _Botrytis_ sp. on it, so it is indeed difficult to see how pentathionic acid can be the fungicidal agent.

Wilcoxon and McCallan (29) in their recent work established very good evidence to the fact that it is H_2S which is valuable as a fungicide. They made a comparison of the toxicity of pentathionic acid, sulfuric acid, and H_2S. They found that both acids exhibit typical toxicity within the error of the experiment, and that the toxicity of the acids was apparently due to the H-ion concentration. Their experimental data shows that H_2S is 6 to 200 times as toxic as either of the acids considered. The sulfur samples they used gave aqueous extracts of pentathionic acid which were not toxic under the conditions of the experiment.

Sestini and Mori (22) point out that the vapours which arise from sulfur may act in a toxic manner to fungal hyphae and spores.

All the above hypotheses conceive the agency of a gaseous derivative of sulfur as being the toxic property, but Barker, Gimmingham, and Wiltshire (1) have shown that the toxic property can be removed by filtration thru a cellulose pad. They believe that the sulfur acts as a finely divided sulfur which they designate as "particulate" sulfur. This, they say, does not mean that the particulate sulfur is directly toxic to the fungus but may be more reactive chemically and hence yield the toxic agent more readily.

Salmon's work (24) out-of-doors indicates that sulfur acts only when in direct contact with the fungus.

Conidiophores of the fungus (Sphaerotheca humuli) shrivel on contact with a sulfur particle, but an adjacent chain of spores only 1/800 of an inch away remained unaffected. In a later work Goodwin and Salmon (15) more completely substantiate this theory.

Liming (21), in the most recent work on the toxic factor of sulfur, very conclusively proves that penta-thionic acid is that factor, but that it is not sufficiently volatile to prove toxic from any great distance. The toxic factor from a distance is attributed to the vaporization of the sulfur and the condensed vapor resulting in the formation of the toxic factor.

For the purpose of this paper, then, pentathionic acid will be considered to be the toxic factor.

It was in 1882 that Millardet (22) stumbled across the possible use of copper sulfate as a mildew control. Utilizing a chance observation he perfected a specific remedy for mildew which he termed Bordeaux mixture. This mixture is so familiar that a detailed discussion is not necessary here.

Martin (22) concludes his discussion of the fungicidal action of Bordeaux by the statement, " we may ----- conclude that the fungicidal action of Bordeaux is to be attributed to the formation of soluble copper, presumably as the electro-positive ion from the insoluble copper precipitate, that the copper is brought into solution by any of the three main agencies:

1) the atmospheric carbon dioxide and the ammonium salts dissolved in the rain water or dew;

2) secretions from or the solvent action of the fungus spore;

3) secretions from the healthy or wounded surface of the host plant."

The soluble copper may cause the death of the fungus direct, or may indirectly, by absorption into the host tissue, bring about a failure of the fungus to establish infection (2).

Methods and Materials -

As infection is dependent on spore production and spore germination, a series of tests was undertaken in order to determine what factors limit spore production and upon what conditions their germination is dependent.

In so far as the writer has been able to discover in his literature review of the Erysiphaceae no work has been done on the factors limiting spore production. Accordingly the following factors were taken into consideration; light, temperature, humidity, and oxygen.

In carrying out the above experiments heavily infected leaves which still maintained a firm connection with the parent plant were chosen. The spores present were carefully removed by brushing lightly with a camel's hair brush. This was done so as to remove all the spores and still not injure the mycelium. Five leaves were placed in each petri dish and sufficient water added to insure a constant supply to prevent wilting. These petri plates were divided into groups of five plates each. Each group of plates was subjected to a different treatment of light, temperature, humidity and so forth. After 12 hours the dishes were removed from the different treatments and the spores per unit area estimated. Temperatures were controlled by a Freas electric oven and specially built tanks in which the temperature was controlled by the use of a thermostat (Fig. 1). (4) Relative humidity was controlled by different concentrations of sulfuric acid as described by Stevens (27). An abundance of light was obtained by placing the petri dishes in the greenhouse. A complete exclusion of light was obtained by placing the test leaves in the photographic dark room. Oxygen was removed from the presence of the leaves to be tested by placing them in a dessicator in the basal part of which there was sodium hydroxide and pyrogallic acid.

Methods of Determining Amounts of Spore Production -

Several methods of determining the number of spores per unit area were attempted. These included ocular observation, picking the spores, in which they were sucked up into a pipette, and picking with a glass rod moistened

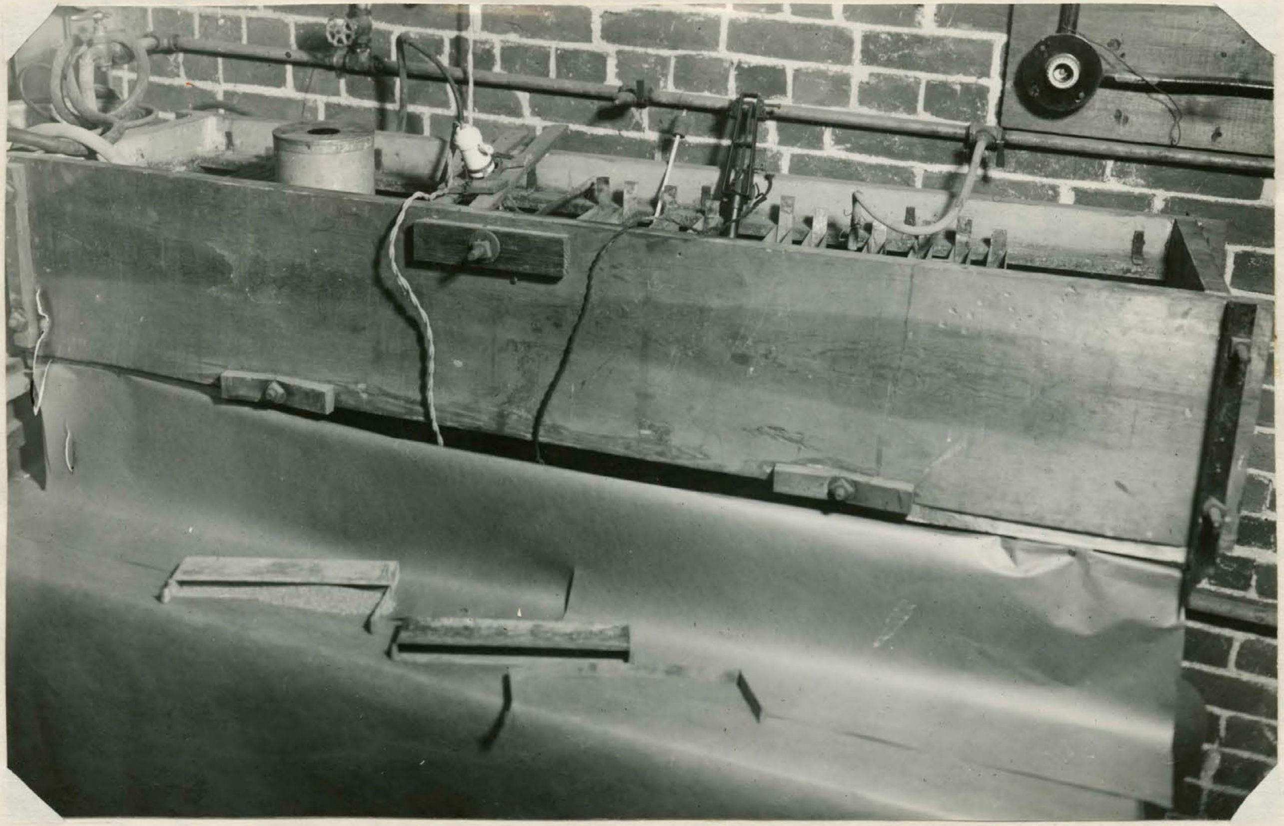


Fig. 1.--Temperature tank and thermostat used for the tests on spore production and germination at low temperatures. The arrow indicates the cylinder tanks containing culture plates.

in a sugar solution. Ocular observation proved to be of no value because not all of the spores were visible, (Fig. 2) due to the manner in which they are borne on the hyphae. Picking with a pipette was inaccurate as only the fully matured spores could be obtained. The last method, that of picking the spores on the end of a glass rod was found to be the most accurate. A detailed description of that method follows.

The exact diameter of the end of a glass rod, which was cut squarely across, was obtained by the use of a micrometer and the area calculated. The end of this glass rod was barely moistened in a sticky solution made by dissolving five grams of sucrose in 10 c.c. water. This sticky end was pressed firmly and evenly down upon an infected leaf surface, then washed off in a drop of water placed in a hollow ground culture slide. The number of spores in this drop were then counted with the aid of a microscope. This was done three times for each leaf in each experiment, the average taken and the standard deviation applied. The result was the number of spores produced on the area of the leaf surface which is represented by the area of the end of the glass rod. Microscopic examination revealed that all spores and even part of the mycelium was removed from the leaf surface.

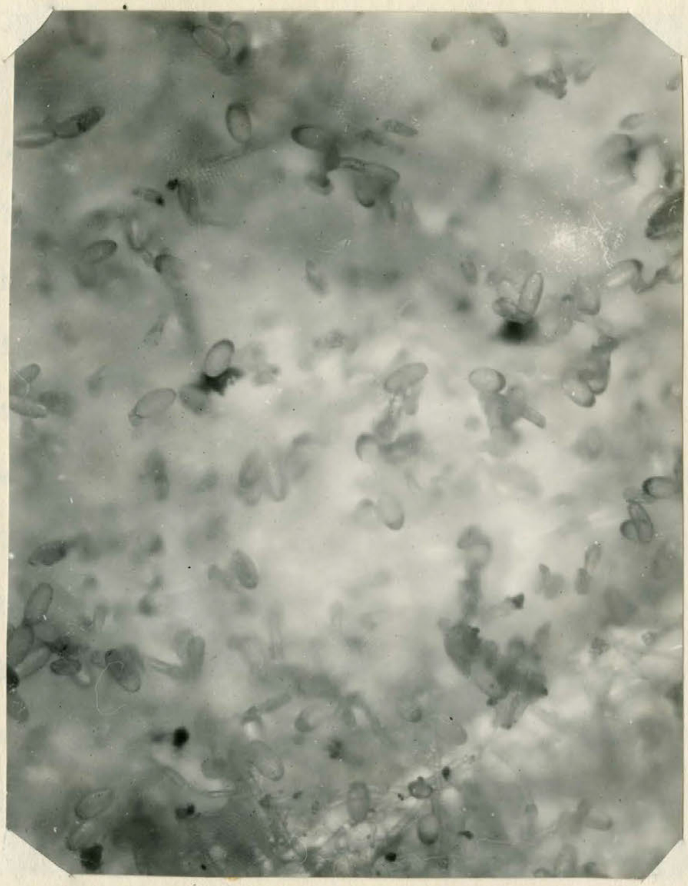


Fig. 2.--Photomicrograph showing the large numbers of conidia on a heavily infected leaf and illustrating the impossibility of counting in such a confused mass.

Spore germination -

Debary (9), Gwynne-Vaughn (16), Gaumann (14) and many others have spoken repeatedly of the fact that the spores of E. communis germinate immediately after their formation, but in no instance was the author able to find a definite statement that they had germinated these spores under artificial conditions. As it was desirable to determine how the fungicides affected the germination of the spores an attempt was made to germinate them. Before completion the work appeared to be more complex than was first thought. A description of the methods used by the writer follows: Van Tieghem cells covered with cover glasses on which were hanging drops; van Tieghem cells covered with cellophane, inverted and the spores placed on the cellophane; the hanging drop method as described by Melhus and Durrell(23), and the dry slide method were used. In this latter method a piece of filter paper was placed in the bottom of a petri dish and moistened with sterile water. Above the filter prper was placed a dry slide supported by two small blocks of glass (18), (19). The spores were dusted carefully onto the surface of the slide and the whole covered with the top of the petri dish. An attempt was made to use special culture slides, but these were abandoned as useless.

Methods of determining the per cent of germination

In determining the percentage of spore germination three average fields of each slide were chosen and the spores therein counted by microscopic examination.

Cultural methods and methods of inoculation

Two greenhouse benches were planted to sweet peas of the Ball variety on September 16. The seeds were obtained from the Colorado Seed Company of Denver. Usual cultural methods were followed until the peas attained a height of 6 to 8 inches. Although later tests showed that the spores would not germinate in water, an attempt was made to inoculate the plants with a spore dilution. As shown by work on spore germination this proved of no value. Inoculation was finally accomplished by shaking the diseased material over the plants. By slightly raising the humidity, by repeated spraying with a fine mist, a heavy infection was soon produced.

Control materials.

When the peas reached a height of 18 to 20 inches and were heavily infected with mildew, treatments were begun. The following compounds were used in the treatment of the disease: 1. Copper carbonate in the form of Copper Carb used in the control of stinking smut which contains about 18 per cent chemically active copper carbonate;

2. a so called colloidal or Wettable sulfur prepared by the Southern Acid and Sulfur Company of St.Louis; 3. Flowers of sulfur; 4. a commercial mildew control sold under the trade name of Grape Dust; 5. a copperoxy-chloride imported from Germany.

The Copper Carb was applied as a dust. The wettable sulfur was applied both as a dust and as a spray. When used in spray form one ounce to a gallon of water was used. This material when put into water disperses to a finely divided suspensoid state with but little agitation needed. The Copper oxy-chloride is a recently prepared compound imported by Jungmann and Company of New York and courteously supplied by them gratis.

Methods of Treatment

All dusting was done by the use of a bellows-acting, hand-operated duster (Fig. 3) of the Calispray Knapsack Duster type loaned by B. J. Thornton of the department. Sprays were put on by the use of a five gallon hand-operated pressure sprayer (Fig. 4) whose use was granted thru the courtesy of Dewey Stewart of the U. S. Department of Agriculture.

Methods of applying controls

The disease was permitted to run its course untreated until an extremely heavy infection was obtained.

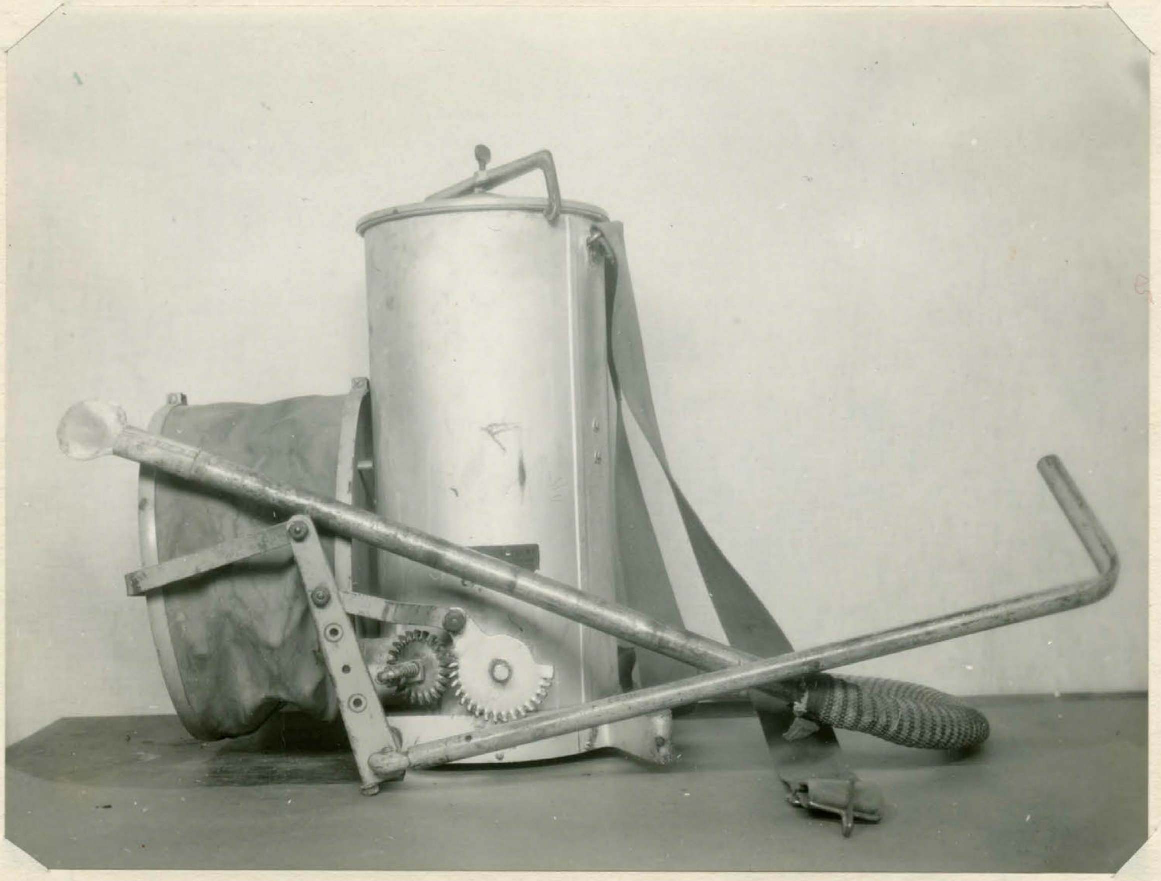


Fig. 3.--The duster used for making dust applications.

Fig. 4.---Sprayer used in making spray treatments.

Each bench was divided into five plots of equal area. Each of these plots received a different control treatment except number five which was used as a check. During the course of treatment each plot was separated from its neighbors by tye use of a heavy canvas tarpaulin to form a screen about the plot which was being treated. During the intervals between treatment the plots remained unseparated.

The plots were treated in the following manner: on bench 1 plot 1 was dusted with Copper Carb, plot 2 was sprayed with wettable sulfur, plot three was dusted with this same material, plot 4 was dusted with flowers of sulfur, plot 5 was retained as a check. On bench 2, only 1 plot was treated and that with Grape Dust. The remainder of bench 2 was to be used as a check and as a supply of spores for future spore work.

Flower treatment materials

In addition to those materials which were used in control work two other compounds were used with a view to their possible use as effective controls. They were organic mercury compounds similar to many of those now on the markets which are used in sterilization and seed treatment. These were furnished thru the courtesy of the Depont Manufacturing Company.

Flower treatment methods

Inasmuch as during the period of treatment in the greenhouse the sweet peas were not yet in bloom it was necessary to run an entirely separate set of experiments in order to determine the effect of different control treatments on the blossoms, their color, and keeping qualities. In order to accomplish this bunches of flowers were treated with the different chemicals used for dusts or sprays. The flowers were subdivided irrespective of color into bunches and kept fresh by placing the stems in water. One lot from each group was maintained without treatment under identical conditions to determine whether there were extraneous influences which might cause a staining or premature wilting. The second lot of each group was treated dry. The third lot, because flowers are usually dampened in packing for shipment to prevent wilting in transit, was sprayed with an atomizer before treatment.

EXPERIMENTAL DATA

Powdery mildew may make its appearance on sweet peas ten days to two weeks after planting. The first symptom is a small weft of white mycelium usually occurring on the upper surface of the leaf. This small mat of mycelium (Fig. 5) radiates from a common center where the conidium caused the original infection.

Fig. 5.---Beginning of infection of sweet pea leaf by *Erysiphe communis*. Masses of the superficial mycelium may be noted. About eight times original size.

Conidia may be produced as soon as the area of infection reaches a diameter of three to five millimeters. They are disseminated by air currents, water, and cultural methods and will germinate immediately under optimum conditions of temperature and humidity. Conditions of not less than 18°C. and 40 per cent relative humidity are essential for germination. It is apparent that relative humidity is a factor to be considered in the control of powdery mildew.

The lower leaves are the first affected. As the disease runs its course the leaf becomes entirely covered with a dense spread of hyphae and conidia until, on the upper surface no green coloration is distinguishable (Fig. 6). The lower surface becomes faded and chlorotic in appearance. As soon as this lower surface is completely yellowed premature abscission and subsequent dropping occurs. This process continues until the whole plant may be completely defoliated.

The dissemination of the disease throughout the range is very rapid under optimum conditions. Complete infection may occur in a week's time. Heavy infections slow up flower production and may even cause a complete cessation of blooming if control methods are not applied.

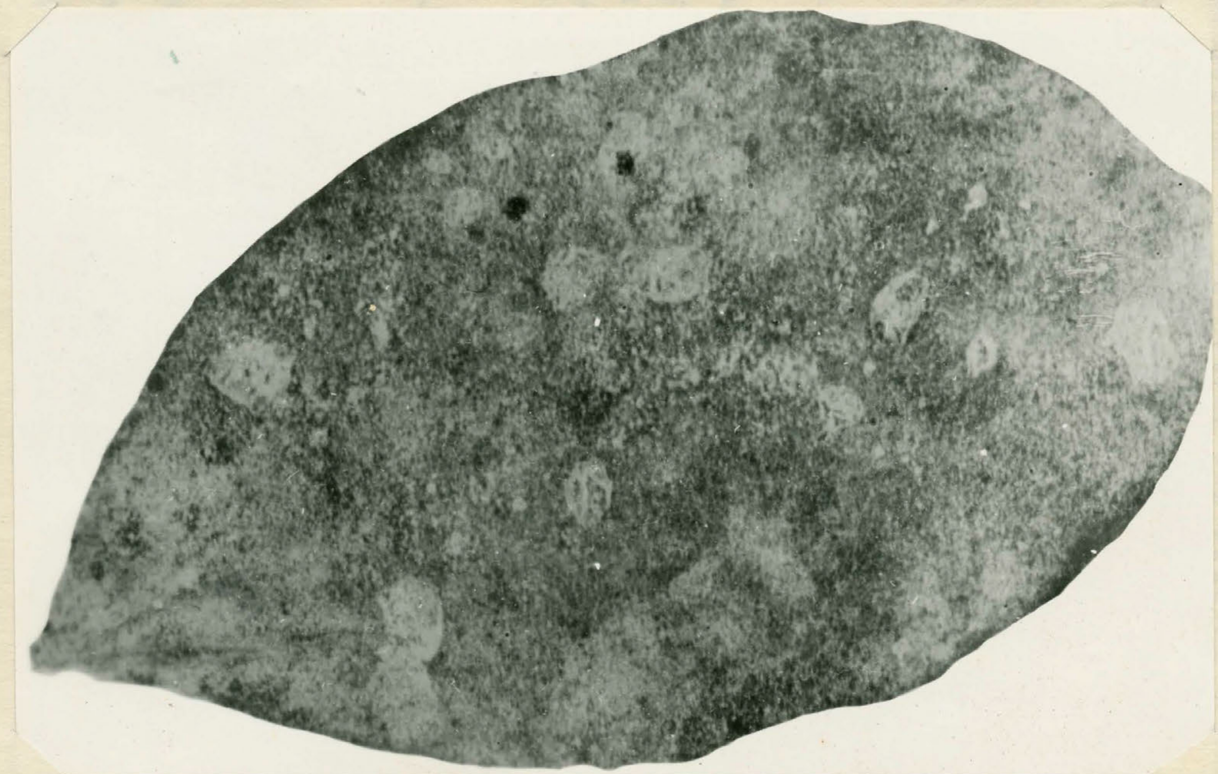


Fig. 6.--A sweet pea leaf heavily infected with *Erysiphe communis*. Magnified about eight times.

It is evident from even a casual observation that the degree of infection in the case of *E. communis* is definitely effected by spore production. It is important and interesting to determine the factors governing this activity.

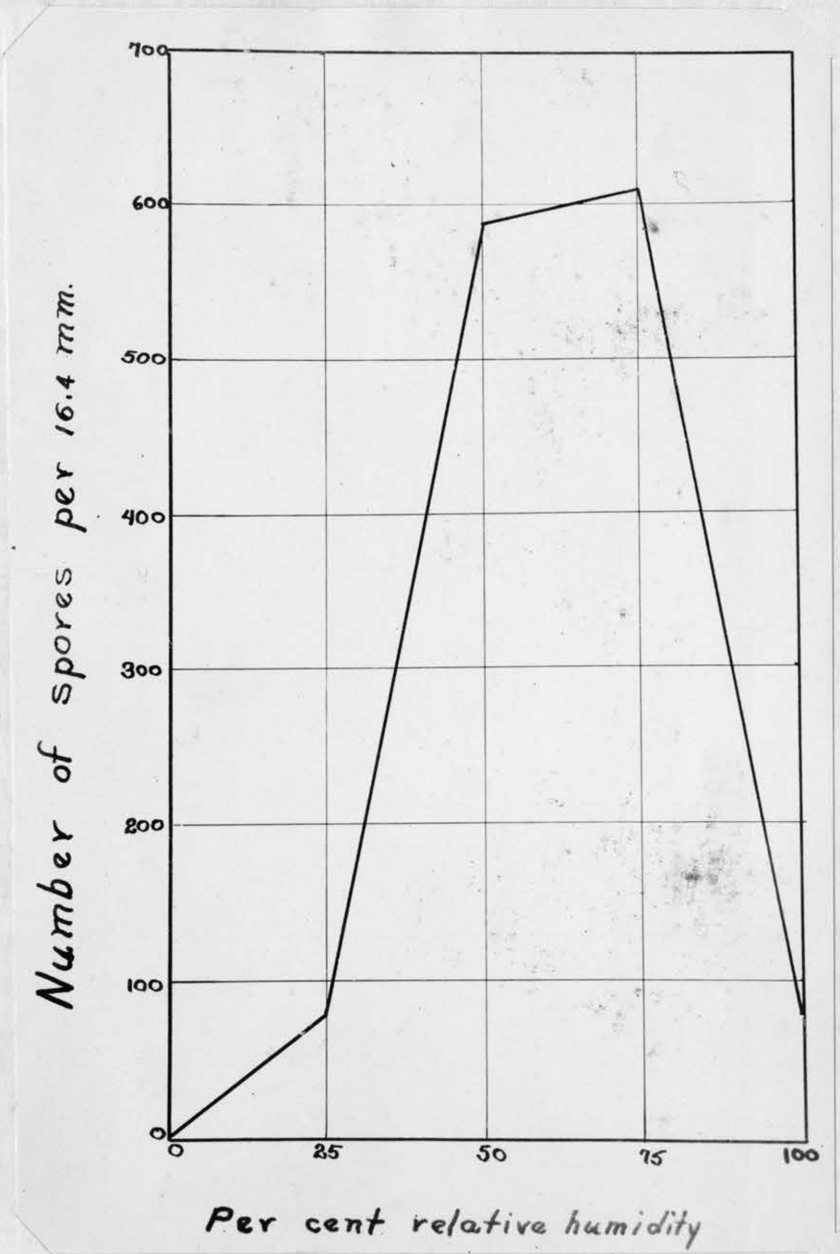
Using the methods described, a series of experiments were carried out to determine the factors limiting conidial production.

Table 1 and Graph 1 show the effect of relative humidity on the production of spores.

Table I.--The effect of relative humidity on spore production.

No. of plates	Relative humidity	Leaves per plate	Counts per leaf	Total No. counts	Number spores	Average spores
5	0	5	3	75	20	0
5	25	5	3	75	5,552	74
5	50	5	3	75	44,300	592
5	75	5	3	75	45,411	608
5	100	5	3	75	2,259	30

Both Table 1 and Graph 1 illustrate the fact that with a relative humidity of 100 per cent there is only a scant production of conidia, while if a relative humidity of zero per cent is maintained there is no spore production what-so-ever . In the latter case it is probable that under the conditions of the test where rapid drying of the leaves and fungus occurred, no spores can be produced. It seems likely that if the leaves were attached to the parent plant and the atmospheric humidity were reduced



Graph 1.--The effect of relative humidity on spore production.

to zero per cent, there would be sufficient moisture obtainable from the host plant to enable the fungus to produce an abundance of spores. In all the experiments below 25 per cent relative humidity a great deal of difficulty was experienced in preventing the leaves from drying out and it is probable that the drying curtailed spore production.

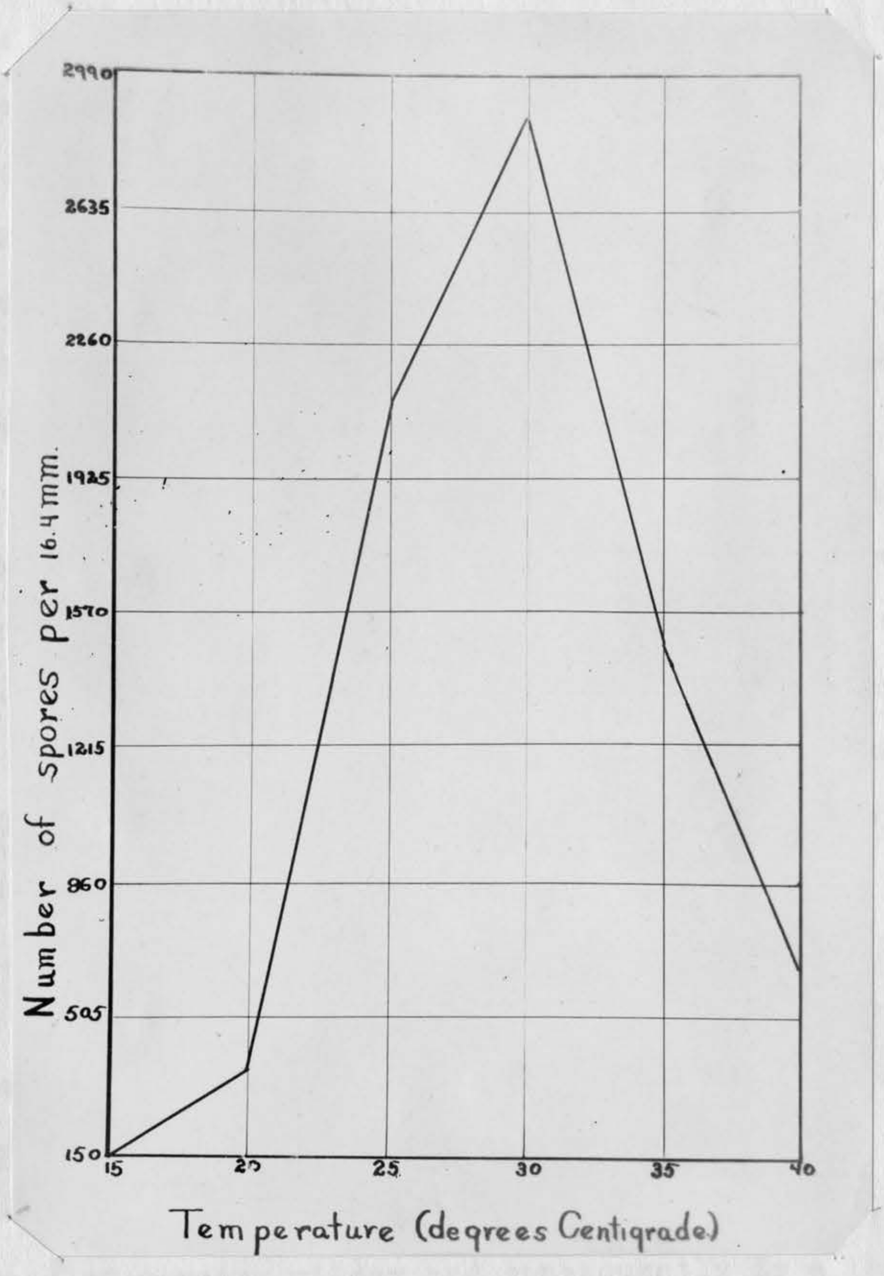
It may be seen in Table I that there is an abundance of spore production in an atmosphere of 50 to 75 per cent relative humidity.

Temperature, as well as humidity, is a factor governing spore production. Table 2 contains the experimental evidence on the relation of temperature to spore production.

Table 2.--The effect of temperature upon spore production in E. communis.

No. of plates	Temperature °C.	Leaves per plate	Counts per leaf	No. of counts	No. of spores	Average spores
2	15	5	3	30	172	6
2	20	5	3	30	270	9
2	25	5	3	30	2,129	71
2	30	5	3	30	2,888	96
2	35	5	3	30	1,533	51
2	40	5	3	30	0	0

It may be seen in the above table that temperature is also a factor which must be considered in spore production and spread of mildew. The optimum temperatures for spore production correspond very closely to those



Graph 2.--The effect of temperature on spore production.

requisite for spore germination. The greatest number of conidia was produced at temperatures of 25°C. and 30°C. The upper limit for conidial production was 35°C., while the lowest temperature at which any spores were produced was 15°C.

Experiments were also carried out to determine the effect of oxygen on spore production. This was accomplished by placing the test leaves over pyrogallic acid and sodium hydroxide.

The evidence obtained from this experiment indicates that oxygen is a factor of vital significance in spore production as in those cases where oxygen had been removed no spores were produced. On the other hand, leaves under similar conditions of temperature and humidity, but which had a plentiful supply of oxygen produced a great many spores.

Light apparently is an inconsequential factor as regards spore production as leaves in the dark and in the light produced spores with equal facility.

Spore germination -

Spore germination is important in consideration of the spread of powdery mildew and consequently is a factor to be considered in its control. For this reason it was thought important to make a study of the factors bearing on spore germination.

The first method of germinating spores which was tried was that of a hanging drop inverted over a van Tieghem cell. No germination of spores was obtained. The second method consisted of the double slide method and was adopted because it was thought that the van Tieghem cells used in the first part might cause so great an exclusion of oxygen as to prevent germination. This, however, was apparently not the case and again no spore germination was obtained. The writer then attempted spore germination by adding a certain amount of nutrient solution to the culture drop. This nutrient was made up of a 2 per cent sugar solution. At temperatures of 30° and 35° C. a few spores were observed to have sent out short germ tubes. In these cases those spores which had germinated were seen to have been pushed up above the surface of the drop by their underlying fellows. This phenomenon led to the belief that the sinking of the spores in the drop had caused a complete exclusion of oxygen (6) thus preventing their germination. Another method which would obviate this was then tried. This involved the scattering of the spores over the dry surface of a slide and placing this slide in a petri dish in the bottom of which was placed a moistened filter paper. It was thought that the evaporation from the filter paper would supply sufficient moisture to enable the spores to germinate without entailing the exclusion of the oxygen by their submergence in a drop. In this case again there was a trace of germination at temperatures of 25° to 30° C. This trace could not be termed entirely successful as in no case did the germination amount to more than 2 per cent.

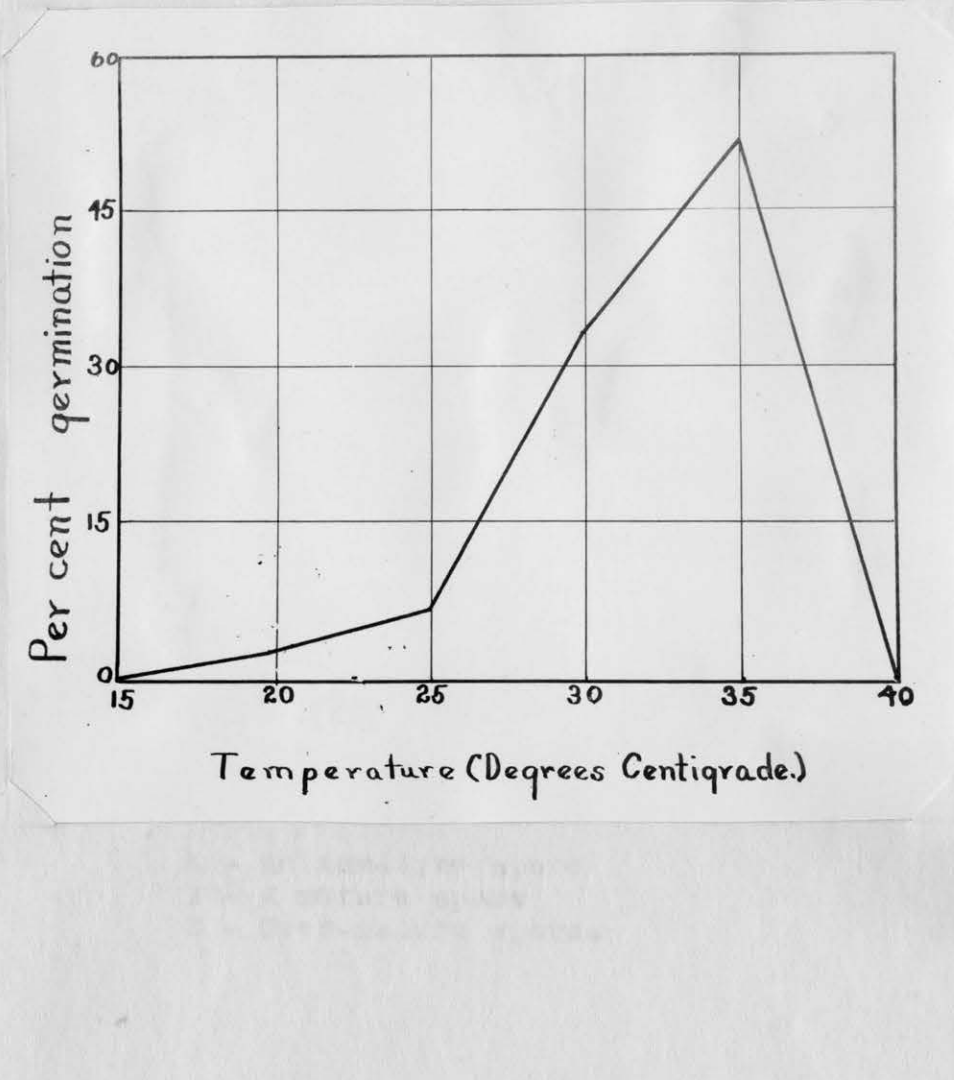
The situation was reviewed and compared with spore germination as described by Howe and Durrell (19). (23). From this review the writer arrived at the conclusion that he had failed to meet one condition, namely, the source of supply of carbon dioxide such as normally occurs on a leaf surface (19). As a result another attempt was made, using the dry slide method as described above, except that a small piece of apple was included in the petri dish to provide a source of carbon dioxide. This method was quite successful. The spores germinated well and tests could be made of the effect of temperature and other factors influencing spore germination. The following table shows the effect of temperature on the germination of the spores.

Table 3.--The effect of temperature on the germination of conidia of E. communis.

Temperature °C.	No. of slides	No. of field per slide	Total no. spores counted	No. of spores germinated	Percentage of germination
15	10	3	1,080	0	0
20	10	3	1,073	8	T
25	10	3	940	38	4%
30	10	3	1,113	378	34%
35	10	3	600	318	53%
40	10	3	709	0	0

The fact that no germination greater than 53 per cent was obtained is due to the mixture of young and old spores in the culture drops. Under normal conditions the younger spores are not readily dehiscent from the parent hypha, but in the above the spores were removed from the leaf and transferred to those pieces of apparatus used, by the means of a camel's hair brush. This is a more stringent action than that of the wind or other natural means of dissemination and it resulted in a complete removal of spores from the leaf surface in microscopic examination revealed. The younger spores were considerable smaller than those which had germinated (Fig. 8) while the older spores had a rather wrinkled and shrivelled appearance (Fig. 7). In no case was one of these wrinkled spores observed to have germinated. The spores which germinated were, without fail, full and plump.

While the production of a germ tube may take place at any point in the periphery of the spore, it usually occurs apically. The germination apparently occurs through a weakening of the spore wall and the subsequent extrusion of the germ tube through this weakened portion. The extruded part is quite slender, rarely reaching a diameter greater than 2 microns, but may attain a length of 16 microns. See Figure 8 . It is by the penetration of this germ tube into the epidermal cells of the host that



Graph 3.---The effect of temperature on spore germination.

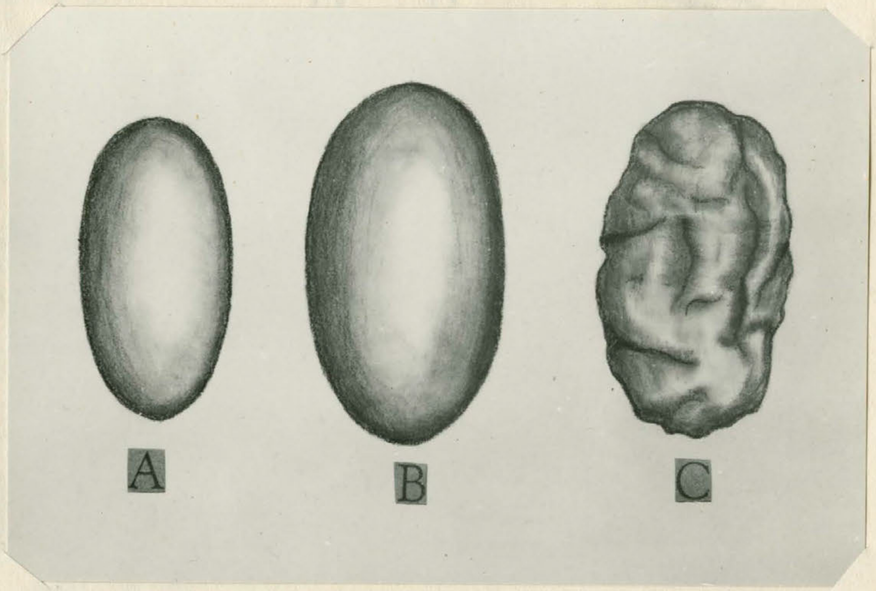


Fig. 7.--Spores of Erysiphe communis

A - An immature spore
B - A mature spore
C - Over-mature spore.

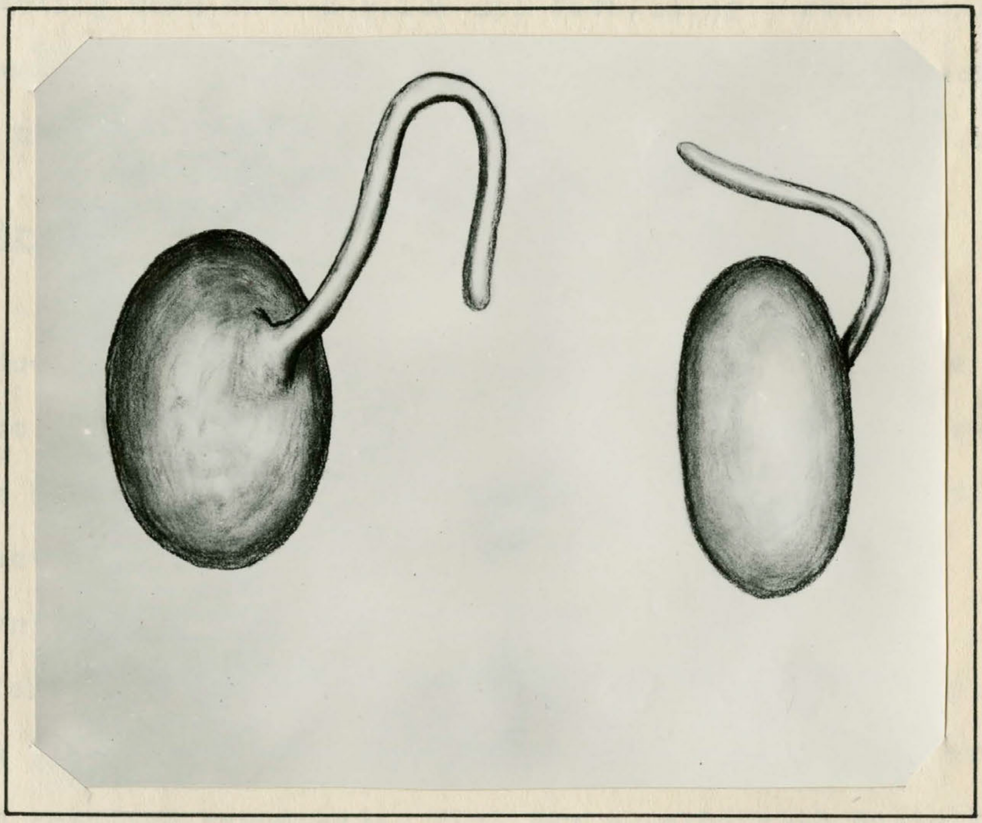


Fig. 8.---Germinating spores of <u>Erysiphe</u> <u>communis</u>.

infection occurs.

An attempt was made to germinate the spores by pipetting them onto a slide and furnishing carbon dioxide. It was not successful and seems to corroborate DeFrance's (6) theory that oxygen is essential to spore germination.

Control results -

Using the control measures as described under methods, daily observations were made to determine the effect of the various fungicides on the infection of sweet peas by E. communis. All experimental plots were heavily infected at the time of treatment. At the end of the seventh day after treatment, there was an appreciable difference in the degree of infection on the various plots. Plot one on bench one which had been dusted with Copper Carb remained unchanged. Plot two sprayed with wettable sulfur appeared to be less heavily infected on the outer leaves, but the under foliage evidenced no change in the degree of infection. Plot three was dusted with wettable sulfur. It was decidedly brighter in the color of the foliage than the preceding two plots and there was a lessening in the amount of mildew present. Plot four dusted with flowers of sulfur showed about the same degree of cessation of fungal activity as did plot three, but also showed a very marked burning of the foliage . Plot five retained as a check, was apparently unchanged. Plot one on bench two which had been dusted withGrape Dust showed little or no change, while the remainder of the bench which was untreated showed the same degree of infection as it had before.

The fact that in plot two on bench one only the outer leaves appeared to have a lessened infection may be accounted for by the fact that in spraying only those leaves which were open and exposed were well covered as the penetration of the pressure spray was not great enough to reach the under foliage.

The burning observed in plot four was due to the use of flowers of sulfur. The sulfur particles were so large that an excessive amount of the toxic factor was evolved, resulting in a burning of the foliage in addition to the lethal effect on the fungus.

After an interval of 10 days a second application was made. At the end of the fourth day after this treatment there was a very decided cessation of the growth of the mildew on all the sulfur treated plots. Plot four which had been treated with flowers of sulfur , showed a very bad burning and in many instances the leaves had yellowed and fallen. In addition to the fact that all sulfur treated plots showed a definite decrease in the amount of mildew present, the remaining plots, including the check plots, were of a brighter color. At the end of the 10th day after the second application was made the degree of infection was very definitely decreased throughout the range. Fifteen days after the second application showed a very nearly complete controlling of the mildew.

This almost complete cessation of fungal activity can be attributed to the fact that the wettable sulfur used as a dust was so fine as to be spread all over the greenhouse in spite of the tarpaulin which was used to separate the plots undergoing treatment. These small sulfur particles could be seen throughout the greenhouse with the aid of a hand lens.

It was thought necessary to further establish the value of wettable sulfur as a control agent and due to the almost total absence of mildew on the plants used in the preceding experiment cooperation with one of the local greenhouses was sought. The Espelin Floral Company offered their support and volunteered to carry out dusting experiments under the author's direction. Accordingly a sufficient amount of wettable sulfur and a duster were furnished. A considerable quantity of mildew was present in the range and the first application was made rather liberally. Favorable results were obtained within a week and a second application resulted in complete control. One decided fault was evident, however. This was a serious staining of the flowers and bursting buds. This staining was so severe as to render the flowers unfit for market. Obviously this would prove detrimental to the wide spread usage of wettable sulfur as a fungicide in floral treatment during the flowering period.

It was planned to follow up this proof of the effectiveness of wettable sulfur with a series of experiments with Copper Carb and copper oxy-chloride to determine their respective values as fungicidal agents. In light of the fact that the mildew had been destroyed in this experimental range a certain amount of the two materials was sent to some of the greenhouses in Denver. Conley's range in the northwestern part of the city used the Copper Carb for an entire growing season. The material proved to be a fair control, but was much slower in its action than the wettable sulfur. Whenever the flowers were wetted in watering there was a sufficient staining to render the flowers unfit for marketing purposes. Inasmuch as in a commercial establishment the flowers are found at a considerable height from the ground, they are not often wetted in ordinary watering processes. We may conclude from this experiment that Copper Carb is effective as a mildew control if applied from the time the plants make their appearance above the surface of the soil. The flowers, however, must be kept dry if the material is to be used as a control during blooming. Once the mildew has made its invasion Copper Carb is too slow in its action to be recommended for greenhouse use.

Two applications of copper oxy-chloride were made at Hollberg's range and a slight controlling of mildew resulted. Due to the fact that the plants had passed their useful period of blooming and that he needed the space for other crops, he was unable to carry on sufficient experimental work to permit accurate conclusions to be drawn. From the very limited data available, it would appear that copper oxy-chloride might be of value in preventing infection, but if infection were already present in the range other agents would have to be used as controls.

Flower treatments -

At the time when the spray and dust treatments were being carried out on the plants in the experimental greenhouse, the flowers had not yet come into bloom, consequently it was impossible to determine what effect the agents had upon the flowers. In order to determine these effects a number of experiments were carried out with chemicals on the flowers.

Wettable sulfur, whether wet or dry, caused a premature wilting and slight staining within 12 hours after application, while check blossoms retained nearly all their original freshness after 36 hours exposure to the same atmospheric conditions (Fig. 9). Copper Carb, if not wetted, caused no deleterious effects, but resulted in a staining of the red flowers at their margins when wetted. (Fig. 10) This staining did not cause a wilting as in the case of the wettable sulfur and as the staining occurred in the case of the red and pink blossoms its action was attributed to the acid value of the cell sap. Flowers of sulfur resulted in a premature wilting and staining in much the same way as did the wettable sulfur except that the effect was noticeable sooner after application, being in evidence within six hours after treating (Fig. 11).

Grape Dust caused a blotching and discoloration and in all cases rendered the flowers unfit for market.

The copper oxy-chloride imported from Germany caused no wilting, staining or other unwelcome effects, until 36 hours when there was a slight wilting. This, however was not considered to be significant as the check bunches were also beginning to lose their fresh appearance at this time.

In addition to the treatments listed in Table 4 two organic mercury compounds were used on the flowers

Fig. 9.---Photograph illustrating the effect of wettable sulfur on sweet pea blossoms. Note the wilting of the petals.

Fig. 10.--Sweet pea blossoms discolored by copper carb. Arrow indicates staining.

Fig. 11.--A photograph showing sweet pea blossoms burned with flowers of sulfur. Note wilted petals.

with a view to their possible use as control agents. They were utterly useless, however, and resulted in a rapid and complete necrosis of the flower tissue. This was characterized by a rapid wilting and apparent breaking down of the cell walls. The final appearance of the flowers was a shiny, homogeneous mass. In all cases the original color was reduced to a dirty grey.

Table 4 sets forth the comparative values of the different treatments and the effects on the flowers and foliage.

It seems evident from the limited experiments herein recorded that mildew on sweet peas is most successfully controlled by dusting with "wettable sulfur". Applications of the sulfur should be commenced when the plants reach a height of 8 to 10 inches and should continue at intervals of every two weeks until the blooming of the flowers. If the range is kept clean until the flowers begin to bloom there is little danger of subsequent infection. In the event of a light infection after flowering starts the flowers and well opened buds should be cut and the plants heavily dusted with "wettable sulfur". This procedure should be continued at intervals until control is obtained. This may mean the loss of some flowers from the wilting caused by the sulfur, but is better than suffering continued losses from mildew.

Table 4.--Results of control treatments

Treatment	Degree of control	Speed of action	Effect	
			On leaves	On flowers
Copper Carb	Fair if begun early and continued	Slow	No burning	Red flowers stained when wetted
Flowers of sulfur	Good in case of heavy infection, but burns	Fast	Some burning	Severe burning of all flowers
Wettable sulfur	Good, burns flowers	Fast	No burning	Burns all flowers
Grape Dust	Fair, if begun early. Stains and burns flowers	Slow	No burning	All colors staining and burning
Copper oxy-chloride	Too slow for plants already infected	Very slow	No burning	No burning

SUMMARY AND CONCLUSIONS

1. The shortcomings of flowers of sulfur led to a study of other possible fungicides for the controlling powdery mildew (<u>Erysiphe communis</u>) on sweet pea (<u>Lathyrus odorata</u>) under greenhouse conditions.

2. In this study a fairly complete literature review was made of work on the causal organism and control methods.

3. Factors governing spore production and infection were studied. It was found that on the host leaves spores were produced most abundantly at temperatures of 25° to 35°C. and 50 to 75 per cent relative humidity. The maximum temperature for spore production lies between 35° to 40°C. while the lowest temperature at which spores are produced is 15°C. A small percentage of spore germination occurs at 100 per cent relative humidity, but at zero per cent no spores are produced.

4. It was further found that germination of the conidia occurred under similar conditions. The optimum temperature for germination ranges from 25° to 35°C. The maximum lies between 35° and 40°C. The minimum is near 20°C. Spore germination required an abundant supply of carbon dioxide, contrary to usual experience . Spores would not germinate in a spore dilution.

5. Various commercial fungicides were tested on mildew under greenhouse conditions with the results that all worked, but all but one caused a staining or wilting of the blossoms. This one, copper oxy-chloride, is apparently unfit for greenhouse usage because of the slowness of its action. The most effective control found, was "Wettable sulfur", a commercial colloidal sulfur.

Wettable sulfur should be applied liberally from the time the plants are small until flowering begins. There is little danger of infection after the plants mature. In case of infection at maturity cut flowers closely and dust with wettable sulfur until controlling is complete.

Spore production and germination both play a part in infection, but from a review of the factors influencing these phenomena, it is evident that the requirements for mildew growth correspond so closely to those for successful sweet pea growth, that they have no value as control measures.

The most important consideration regarding mildew on sweet peas is that prevention is better than control. It may be very successfully prevented by dusting the peas with wettable sulfur from the time the plants are small until flowering begins. If the range is free from disease at the time of flowering it is unlikely that mildew infection will be of economic importance.

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