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A STUDY OF SOME LEGUMINOUS ASCOCHYTAE.

A dissertation submitted in partial
fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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by

Roderick Sprague
B.S. Washington State College 1924.
M.S. Washington State College 1925.

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Roderick Sprague.

INTRODUCTION.

The recent revision of the so-called Ascochyta disease of peas throws into uncertainty the host ranges of the forms studied, and opens a field for a critical consideration of the genus Ascochyta on a wide range of our common herbaceous legumes. It was shown by Linford and Sprague(23)(24) and Jones(18)(19) that Mycosphaerella pinodes(Berk. and Blox.) Stone had a sphaeropsidaceou stage clearly distinct from Ascochyta pisi Lib. which was shown to be imperfect. Therefore since literature is replete with citations of the occurrence of Ascochyta pisi Lib. on a wide range of hosts (Vicia spp., Lathyrus spp., Cicer spp., Phaseolus spp. etc.) the question occurs as to whether the forms on these hosts are related to M. pinodes, to A. pisi, or are distinct. The study in all its ramifications must consider many named Ascochyta species and forms, and becomes a general study of the genus on the family Leguminosae.

The work at the start is additionally confused by the finding in recent study(14)(24) of a small-spored Ascochyta similar in symptomatology with M. pinodes, and which Linford and Sprague(24) tentatively referred to as M. pinodes micro form?. More recently Jones(19) has given it the name Ascochyta pinodella L.K. Jones.

In this country the work of Stone (39) and of Davis(9) has caused most workers to group the majority of the forms on Vicia, Lathyrus, Lupinus and Pisum under Ascochyta pisi Lib. considered in its old sense.

To serve as a guide to the reader the following table summarizes the hosts and forms chiefly studied in the work given in this paper

TABLE I.

Host.	Forms in Literature.	Occurrence	Found by Writer. (Forms)
Vicia faba L.	Ascochyta fabae Sp. A. boltshauseri Sac A. pisi Lib.	South Amer. General North Amer.	A. pisi Lib.
Vicia villosa Roth	Ascochyta viciae A. pisi Lib. Mycosphaerella pinodes.	General. General North Amer.	A. pisi
Vicia angustifolia V. sativa L.	A. viciae Lib. A. pisi Lib.	General General	A. pisi
V.hirsuta S.F.Grey	A. ervicola Sydow A. pisi A. viciae	Europe Americas. General	A. pisi
Vicia lathyroidis L.	A. vicia-lathyroidis Sydow. A. viciae	Europe Europe	A. pisi
Lathyrus odoratus L.	A. lathyri Trail. M. ontarionensis M. pinodes	Europe North America	A. lathyri M.pinodes
Lathyrus tingitanus L.	Ascochyta sp.	United States of America.	M. pinodes
Medicago sativa L.	A. imperfecta Peck A. pisi var. medicaginis	North Amer. North Amer.	A.imperfecta (Eur. forms on M.sativa not studied
Medicago lupulina L.	A. medicaginis Bres.	Europe	A. medicaginis
Phaseolus vulgaris L.	Phyllosticta phaseolina Sacc. A.phaseolorum Sacc A. boltshauseri Sacc.	General General European.	Same as in literature.

While the writer's work is admittedly far from being exhaustive and unquestionably forms occur that are not listed in the above table, it has become evident that there is considerable

unnecessary overlapping of names in the literature. This will be especially clearly shown in consideration of the vetches, on which forms of Ascochyta pisi Lib. have proved to be the predominating causal organisms.

GENERAL METHODS AND PROCEDURE.

In studying the confusing array of literature upon this subject one is struck with the preponderance of work that relies only on an examination of fresh or usually dried material for the erection of new species on hosts already listed as having one to several distinct forms. Only a few workers(39)(40)(24)(19) have attempted to employ methods that would tend to throw light on host range and life histories of the types involved.

In the studies recorded the following methods have been employed:

a.Microscopic study of all available horbarium material of Ascochyta in this country and some foreign,where necessary.

b.Personal collections and studies of forms in the field.

c.Camera lucida drawings and spore measurements(50 spores from a collection) of all material seen.

d. Isolation of Ascochyta from various hosts from pod,leaf, stem and hypocotyl parts.

e.Maintenance of cultures in sporulating condition.

f.Study of cultures of Ascochyta on various media including potato-1%dextrose agar(24), Difco potato-dextrose, Difco corn meal agar, Difco lima bean agar, Difco bean pod agar,pea soup agar[1]

g.Inoculation studies, using various isolations and hosts on both aerial and subterranean parts.

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[1]Pea soup agar was made by adding the contents of an eight ounce

Potato-1% dextrose agar made according to the method given in previous work(24) was satisfactory for all forms except where the pea soup agar was employed as mentioned later. The other media listed proved to force vegetative growth rather than sporulative.

The writer disagrees with those workers, in various countries who disclaim any taxonomic value in media work. Keeping in mind that the conditions may cause peculiar spore forms the writer has notwithstanding found media of great value in macroscopic determination of differences in plate cultures.

METHODS OF INOCULATION.

The methods of all inoculation work on the various hosts used in the study will be here given. In the work as many of the economic legumes grown in this country as time would permit were employed, including practically all those for food and forage, as well as a number of ornamental types.

A. Inoculations of Aerial Parts.

The plants were grown from seed in freshly sifted clay-loam soil in four inch clay pots. They were sub-irrigated in sphagnum-packed benches in the greenhouse. Seed was obtained from reliable sources and inspected for purity and freedom from disease before planting. This was especially necessary in growing the vetches as mixtures of several varieties were common. The number of seeds to a pot varied according to the type of plant. Small-seeded vetches(e.g. Vicia villosa Roth.) were planted twelve seeds to a pot; Pisum spp. 8; clovers, medics, etc. 20-30; beans and similar comparatively large seeds 4-6 to a pot.

When the plants were about three weeks old they were moistened

under the tap and then thoroughly sprayed by means of a hand atomizer with a pycnospore suspension in sterile distilled water. The spores were obtained from freshly sporulating cultures on potato-1% dextrose agar.

Immediately after inoculation the plants were placed in a spray chamber modeled after the type used at Wisconsin during the past few years(20). Two Wardian cases served as frame. Anti-drip screens, ventilation, run-off, spraying apparatus, and inside cover were similar to the Wisconsin equipment.

It was found that a temperature of 18-23° C was best for infection purposes. Lower temperatures inhibited the organisms under study and allowed Botrytis to grow; higher temperatures obscured their symptoms by favoring damping off forms of various types. Temperature conditions were partially controlled by various expedients, such as adjacent steam pipes, within limits favorable for infection.

Inoculated plants were left in the chamber 48-60 hours and then left in a damp place for symptoms to develop. Sometimes a large number of different hosts were inoculated with the same organism for one running of the chamber. This required a burdensome number of check plants and so where possible plants of the same host inoculated with a number of different cultures were run at a time instead. Care was taken to keep pots of such series separate in the chamber. In all of the work extending over some fifteen months of study the writer had but two instances of checks showing infection and one case where there seemed to be mixed infection. These series were discarded and repeated.

Reisolations were made, after symptoms and fruiting were observed and recorded. Unless cultural, pycnial and spore characters

tallied, positive results were not recorded.Frequently faint positive symptoms were obtained on the hosts but reisolations were negative. Such cases received attention until occurrence or lack of pathogenicity had been determined.

On account of their voluminous nature, the positive(+) or negative(-) results of the separate aerial inoculations will be given in tabular form.(Table II) Each sign indicates the results of the inoculation of a single pot.Where necessary discussion will come under the individual host headings.

B.Inoculations of Subterranean Parts.

The methods used in this work were similar to those employed in former footrot studies(24). One departure from the schedule of the former work lay in employing two types of checks:

1. Seeds of each host were planted in quadruplicate in sterile pots of soil.

2.Seeds of each host were planted in quadruplicate in sterile pots of soil to which sterilized powdered peas were added as in the inoculation tests.

The cultures were grown on sterile steamed peas and were from the same stock as the ones used in aerial inoculations.Inoculations were attempted on Pisum sativum L.Trifolium sp.,Medicago sativaL, Melilotus alba Desr., Cicer arietinum L.,Lathyrus spp.,Vicia spp., and Phaseolus spp.

It is a well known fact that soil sterilized and left to stand in the air is quickly invaded by organisms that run riot thru it unhampered by the competitive types that once flourished there. Again, workers are aware that peas and other legumes do not take kindly to steam sterilized soil. Therefore while sterile conditions may hold at the start of a footrot experiment such

is not the case at the end. The abnormal conditions give a chance for various weak parasites to invade the roots of inoculated plants. Also the mass of powdered inoculum (page 388 of citation 24) of steamed peas and fungus tissue tends to afford better conditions for fungi and hampers the growth of the plant at the same time.

RESULTS OF INOCULATIONS.

A. Aerial parts.

The tabular results previously referred to are given in the following pages.

B. Subterranean inoculations.

The results of several experiments with 400 separate pots were not sufficiently decisive to record in detail. Mycosphaerella pinodes and Ascochyta pinodella L.K. Jones caused severe injury to Horsford's market garden peas (P. sativum L) but less on other hosts except the chick pea (Cicer arietinum L) which was very susceptible. A. pinodella caused moderate injury to the hypocotyl region of Lathyrus odoratus L., L. sativus L. and L. tingitanus; also a slight ammount on Vicia villosa Roth. and V. ervilia Willd. M. pinodes acts similarly though less severely on these hosts.

Ascochyta pisi Lib. isolated from pea and also forms of the same species taken from vetch (Vicia spp.) did not cause any evident footrot, altho a red cortical injury of minor nature was indicated in some instances from the Vicia inhabiting forms.

TABLE II.
Result of all inoculations on aerial parts of hosts.

HOST.(BINOMIAL).	HORTICULTURAL NAME.	SOURCE OF SEED.
Arachis hypogaea L.	Spanish peanut	Michigan.
Cicer arietinum L.	Chick pea	California.
Dolichos lablab L.	Hyacinth bean	Mass.
Dolichos lignosus L.	Austrl. pea vine	Mass.
Lathyrus clymaceum	French pea	Calif.(Agronomy U. of Cal)
Lathyrus latifolius L.	Everlasting pea.	New York.
Lathyrus odoratus L.	Sweet pea	New York.
Lathyrus sativus L.	Grass pea	California
Lathyrus tingitanus L.	Tangier pea	U.S.D.A.
Lens esculenta Moench.	Lentil	Ohio.
Lespedeza stipulaceae	Korean clover	Virginia.
Lespedeza striata Hook. and Ar.	Japan clover	Virginia.
Lupinus hartwegii Lindl.	Lupine	Calif.
Lupinus polyphyllus Lindl.	Lupine	Ohio.
Medicago hispida Gaertn. var. denticulata	Bur clover	Calif.
Medicago lupulina L.	Black medick	Alabama.
Medicago sativa L.	Common alfalfa	Ohio
Medicago sativa L.	Grimm alfalfa	Northern grown.
Medicago sativa L.	Non-hardy, smooth and hairy Peruvian	California
Medicago sativa L.	Turkestan alfalfa	Calif.
Melilotus alba Desr. var. annua Coe;	Hubam clover.	Ohio.
Melilotus indica All.	Bitter clover	Calif.
Melilotus officinalis Lam.	Yellow clover	Ohio.
Onobrychis viciaefolia Scop.	Sainfoin	U.S.D.A.
Phaseolus aconitifolius Jacq.	Moth or mat bean	Calif.

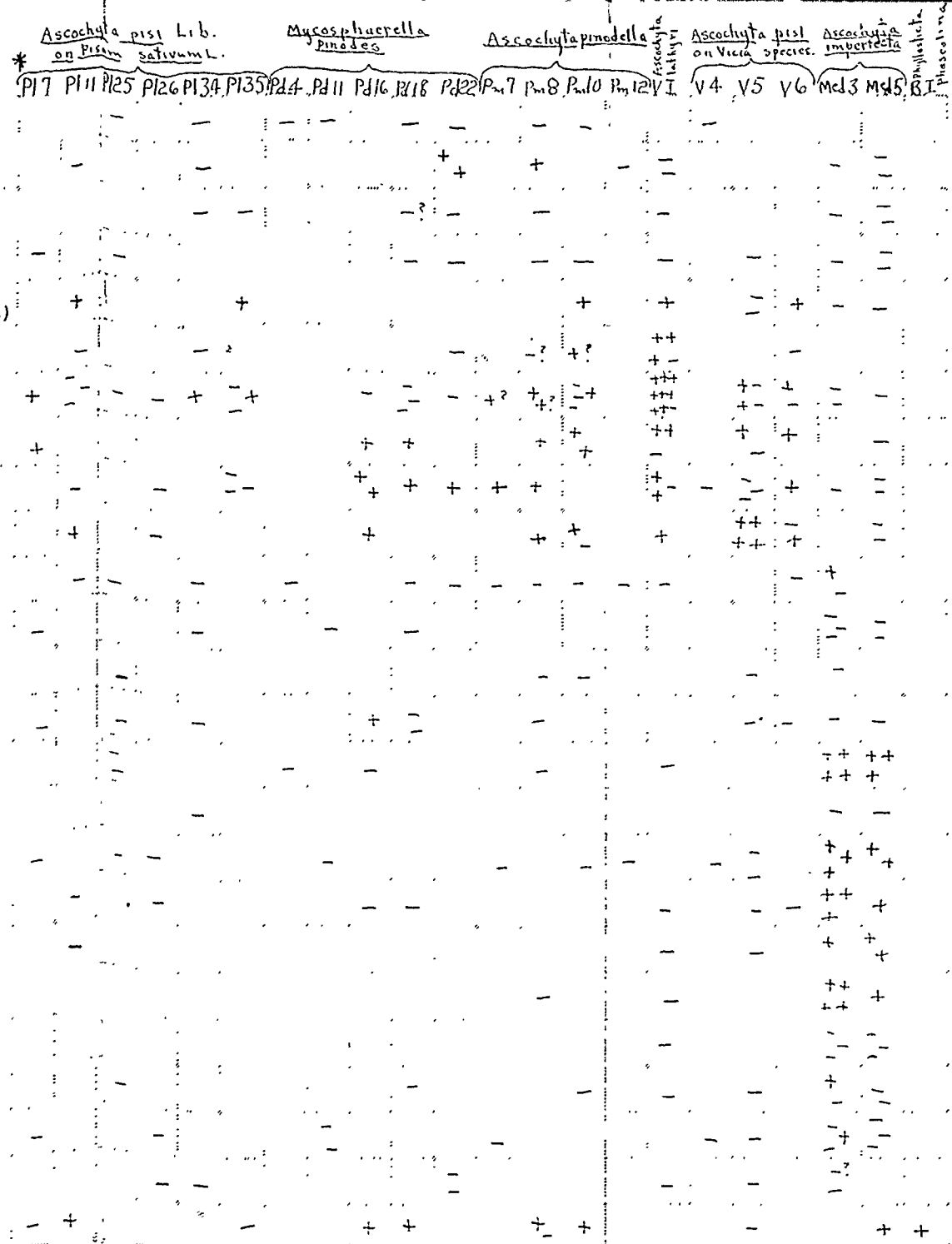

Table II Contd.

HOST (BINOMIAL).	HORTICULTURAL NAME	SOURCE OF SEED
Phaseolus angularis Wight.	Adzuki bean	Calif.
Phaseolus aureus Roxb.	Mung bean	Georgia.
Phaseolus calcaratus Roxb.	Rice bean	Calif.
Phaseolus lunatus L.	Sieva bean	New York
P. limensis Macf. (P. lunatus var.)	Lima bean	New York
Phaseolus mungo L.	Urd bean	Calif.
P. multiflorus Willd.	Scarlet runner bean	Ohio.
P. vulgaris L.	Black valentine bean	Ohio.
P. vulgaris L.	California red bean	Calif.
P. vulgaris L.	Lazy wife bean	Ohio.
P. vulgaris L.	Refugee	New York.
Pisum sativum L.	Amer. wonder pea	Ohio.
P. sativum L.	Alaska pea	Idaho.
P. sativum L.	Little marvel pea	New York
P. sativum L.	Horsford's market gard.	Wisconsin.
P. sativum L. var. arvense	Austr. winter pea	Imported.
P. sativum L. var. arvense	Canada field pea	Ohio.
Pueraria thunbergiana Benth.	Kudzu vine	Southern
Soja max Piper	Black Wilson soy bean	Penn.
Soja max Piper	Pine dell soy bean	Virginia.
Stizolobium deeringianum Bort.	Early black Miss. velvet bean	Virginia.
Trifolium hybridum L.	Alsike clover	Ohio.
Trifolium incarnatum L.	Crimson clover	Virginia.
T. pratense L.	Red clover	Washington
T repens L.	White clover	Wash.

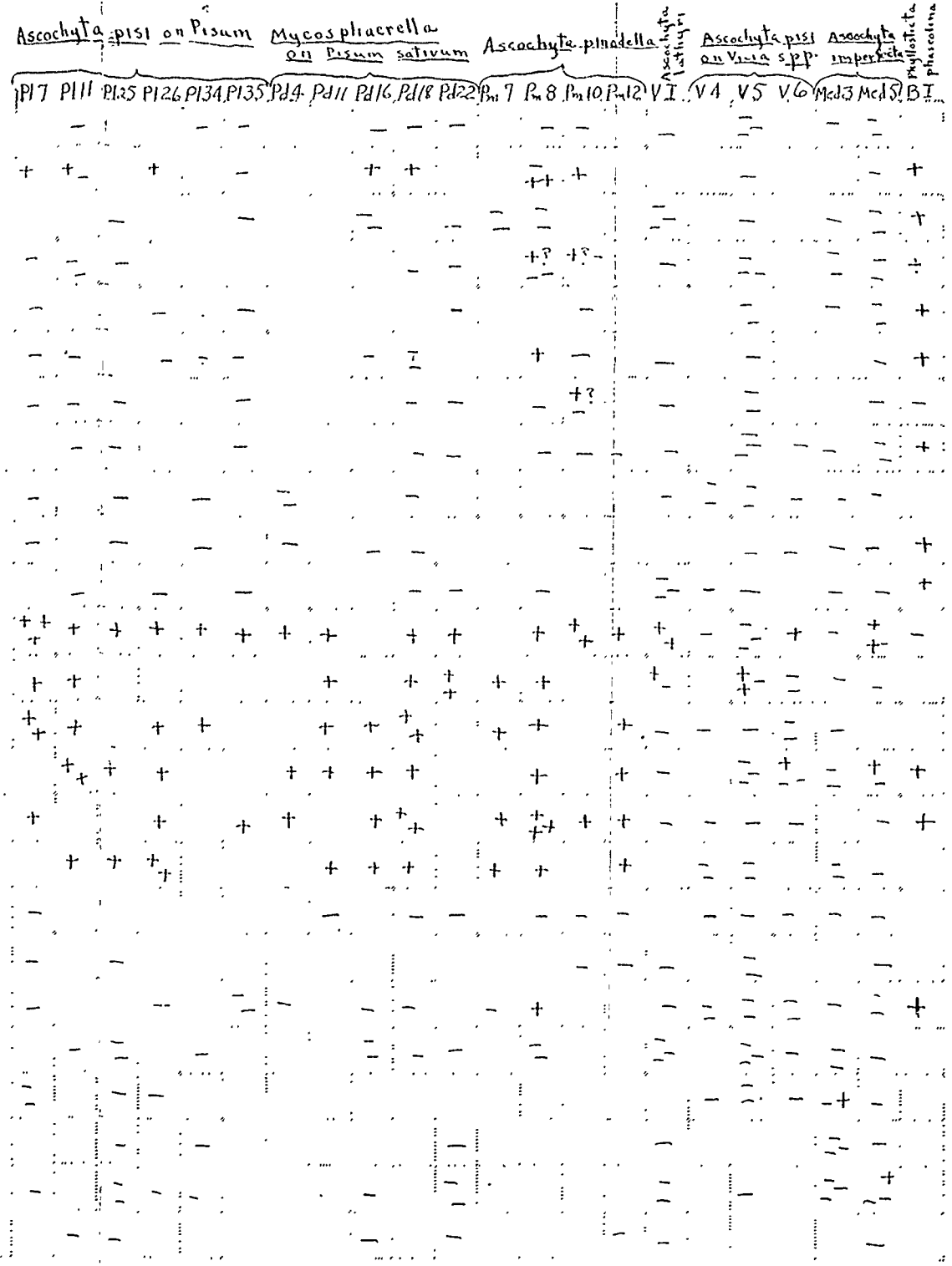

Table II Cont'd

HOST(BINOMIAL).	HORTICULTURAL NAME	SOURCE OF SEED.
Trigonella foenum-graecum L.	Fenugreek	Calif.
Vicia angustifolia (L).Reich.	Narrow leaved vetch	Minn.
V. atropurpurea Desf.	Purple vetch	Calif.
V. cracca L.	Bird seed vetch	Oregon.
V. dasycarpa	Wooly podded vetch	Calif.
V. ervilia Willd.	Bitter vetch	Calif.
V. faba L.	Broad Windsor bean	Mass.
V. faba L.	Small seeded horse bean	U.S.D.A.
V. monanthos Desf.	Monanthos lentil	Calif.
V. pannonica	Hungarian vetch	Wash.
V. villosa Roth.	Hairy vetch	Wash.
V. sativa L.	Spring or common vetch	Ohio.
V. sativa L.	Oregon or winter vetch.	Wash.
Vigna sinensis Endl.	Cowpea	Georgia.

Ascochyta pisi Lib. from pea. | Mycosphaerella pinodes | Ascochyta pinodella | Ascochyta Trifolii | Ascochyta pisi from vetch. | Ascochyta imperfecta | Phyllosticta phaseolina

PL 7 | PL 11 | PL 25 | PL 26 | PL 34 | PL 35 | Pd 4 | Pd 11 | Pd 16 | Pd 18 | Pd 22 | Pn 7 | Pn 8 | Pn 10 | Pn 12 | V 1 | V 4 | V 5 | V 6 | M 13 | M 15 | B I

• Footnote.

The figures are the writer's numbers for the separate isolations.

Pl-Ascochyta pisi from Pisum sativum as follows,

Pl 7,11,25,26 from infected seeds, Wisconsin grown.

Pl 34, 35, from infected seeds, Ohio grown.

Pd- Mycosphaerella pinodes from P. sativum , all from infected Wisconsin seed except Pd 18 which was isolated from leaf spot material collected at Red Lodge Montana by M.B. Linford 1928.

Pm-Ascochyta pinodella L.K. Jones

Pm 7-Footrot from Pisum sativum L. Rochelle Ill.

Pm8,10- Footrot of P. sativum ,Wisconsin.

Pm 12-Footrot of field peas (P. sativum var.) from Mississippi.

V1-Ascochyta lathyri from infected seed of Lathyrus odoratus. Wisconsin.

V4- Ascochyta pisi from leaf spot on Vicia villosa collected at Ullman Wash.

V5-A. pisi from 9 year old California borad bean seed(Vicia faba).

V6-A.pisi from leaf spot of V. villosa collected at Cincinnati O.

Med. 3- Ascochyta imperfecta from leaf spot on alfalfa,Lewiston Idaho.

Med. 5- A. imperfecta from leaf spot on alfalfa,Cincinnati O.

B1- Phyllosticta phaseolina from leaf spot of snap beans (phaseolus vulgaris). Hazelwood Ohio.

STUDIES OF ASCOCHYTA PISI LIBERT.

Introductory.

As a means of clearing the ground for his general study on a wider range of hosts the writer has made further studies on Ascochyta pisi Lib. as it occurs on the pea(Pisum sativum L.), The writer has been unable to develop an ascigerous stage of A. pisi on sterile Melilotus stems;and inoculated plants subjected to various conditions including overwintering have not shown anything more than an abundant pycnidial growth in most cases. This contrasts with common occurrence of peritnecia from M. pinodes under the same conditions.

L.K. Jones(19) points out that four out of five of the leaf spot inoculations of A. pisi that he made gave spore samples with a mean spore length above the 13.5μ maximum given by Linford and Sprague(24).Jones does not make clear the source of the inoculum but presumably it was from New York grown seed. In Linford's and Sprague's work(24) spores were measured from twenty leaf spot specimens from scattered parts of the world and, as was pointed out at the time, only two such samples were above the maximum named.Recent measurements of spores from twelve specimens from this country show one specimen with a mean spore length of 14μ; another 13.8μ: two 13.6: and the rest below 13.5μ.Spores from the twelve specimens averaged 13.1μ in length.In measuring these spores one must use care to select mature spots and to measure all septate spores that appear mature. The writer considers that the figure as given(13.5μ) is, in the main of value in identification but the possibility of occasionally larger specimens should be considered. Jones' measurements seem to be of spores scarcely typical.

Historical.

In the main this paper deals with available North American specimens and in the historical review of Ascochyta pisi Lib. many familiar foreign hosts for the fungus will be omitted.

Libert(22) gave to Crypt.III no. 356 on Vicia sepium L. the name Ascochyta viciae thus in her mind considering it distinct from A. pisi on pea. The description of the vetch invader was appended on the packet. It caused a sub-rotund spot with a darker margin than the paler interior and showed epiphyllous, aggregated pycnidia(90-100μ) containing hyaline 1-septate spores,12-15 x4-5μ, a description that reminds us at once of Ascochyta pisi(24).

Cooke(8) placed Libert's specimen together with Phyllosticta ervi West under his new P. viciae.Saccardo(33) gives both A. viciae Lib. and Cooke's combination, the latter without measurements of any sort. It seems to be the opinion of most mycologists since Cooke's time that he was working with an immature Ascochyta.

Trail(44) describes an Ascochyta spot on pods and leaves of V. sepium from Scotland. This Ascochyta viciae(A. vicicola(Trail) Sacc.has a similarity to Libert's description- spots pale, irregular with reddish border- but the spores are slenderer, 13-16x2.5-3μ. Recently Petrak(28) referred Ascochyta vicicola (Trail)Sacc. to the genus Ascochytella which differs from Ascochyta chiefly in the supposedly olive-green tinged spores.Petrak left A. viciae Lib. unchanged.Bubak's description of Ascochyta vicia-pisiformis(5) with the "leather colored ", usually concentrically zoned spots reads somewhat differently from those given above.

Sydow(43) names Ascochyta vicia-lathyroidis from Germany. It has spores 16-20x4-6μ contained in pycnidia 150-180μ in diameter.

The same writer (42) describes Ascochyta ervicola on Vicia(Ervum) hirsuta S.F. Gray.It causes small spots on the aerial parts; pycnidia 150-200μ, spores 12-14x3-3.5μ.

Spegazzini(38) describes a dark yellowish to brown leaf spot on Vicia faba L.(Faba vulgaris) from a garden in Argentine. His species Ascochyta fabae has large spores and pycnidia;spores 20-25x5-6μ.

Stone (39) gives both A. viciae and A. vicicola under M. pinodes.

Scalia(37) groups practically all the above mentioned forms under Ascochyta pisi Lib.

It was pointed out by Linford and Sprague(24) that an Ascochyta spot was of common occurrence on Vicia angustifolia (L.)Reich. in Wisconsin, and that host and culture symptoms (potato-2% dextrose agar) bore close resemblance to A. pisi Lib. and were totally unlike the cultures of M. pinodes.

Further minor historical data will be brought up in discussions to follow.

Ascochyta pisi Lib. on the genus Vicia.

1.On Vicia faba L.

In 1918 Ascochyta pisi was found by various workers on the broad bean as a leaf and pod spot in California. Recently some of that year's seed was sent to the writer by Dr. F.R. Jones. Most of them bore large, sunken, brown spots(some were black) 6-12mm. in diameter, on their sides and ends. These seeds after being surface-sterilized and planted out on nutrient agar(24) and left in the ice box for three weeks developed a fluffy white (later buff) growth similar to that made by Ascochyta pisi on the same medium(24). Spore and pycnidial production from tissue transfers was slight. A few comparatively large bright pink spore masses of one-septate(1-4) spores developed.The spores

measured 14-21 x 4-6 μ (Plate I fig. c). Inoculated plants showed spores with a mean spore size of 18.2x 5.3μ (Plate I fig.B). Also somewhat immature material collected by Roland McKee from Chico, California showed spores 17.3x 4.7, mean spore size and a mature packet gathered at Corvallis, Oregon by F.R. Jones has a mean spore size of 20.2 x 4.3μ(Plate I fig. A.). These and spores from another inoculation gave an average for all host material of 18.8 x 4.6μ mean spore size, which does not fall short of the mean spore length one might expect from Spegazzini's measurements. The symptomatology of these collections tallies with the description of A. fabae Speg. and while the type material has not been seen there is little doubt that the west coast material is identical with that which Spegazzini described.

The symptomatology of the form on Vicia faba is somewhat different from that of A. pisi on peas. There is a light-centered spot, in which mature material eventually tends to fall out. Large pycnidia are prominently arranged in circles on the spot which is surrounded by a discolored to black, sometimes red border. The spots are very often large, sometimes extending for several centimeters, which is possible because of the large host parts. In inoculations on this host it was found that Ascochyta pisi isolated from Pisum sativum L. caused a similar spot to that brought about by the form isolated directly from the broad bean. It was also found that bacterial injury, mechanical tears, drought, or any disturbance resulting in injury to the parts of the bean caused the injured members to turn black. It may be likened to the habit of some herbarium material such as Baptisia bracteata (Muhl.) Ell.(false indigo) turning black on curing. Therefore the black to yellowish border that we find around the type of spot known as A. fabae Speg. differs from

A. pisi Lib. on pea in its symptoms chiefly on account of differences in host reaction. This is further substantiated by symptoms caused by A. fabae Speg.(?) on other hosts, chiefly small leaved vetches. On these it develops a light-centered spot with a deep red border, very similar to that found on Vicia sativa L. and other hosts in this country.

Spore production on potato-1%dextrose agar is scanty unless a c.c. of a spore suspension (1 loopful of spores to 10 cc.sterile water) is added to the surface of a solidified petri dish of agar. Pea soup agar served best for this purpose. Large masses of bright pink spores nearly covered the surface of the dish in about a week at laboratory temperature.Ascochyta pisi developed in a similar manner by this method.The unusual behavior of the organisms under the conditions can probably be explained by the work of Leonian(21). He believes that a large number of pycnidial initials form in the thin film of water during the adverse conditions and then as soon as the spores or their germ tubes reach the rich medium, growth of a fruiting and not a mycelial nature occurs.

Rate of growth on various media between Ascochyta pisi Lib. isolated from pea and the form isolated from the broad bean showed close parallel for ten days at room temperature.

The period of incubation on various hosts is the same for the pea and the broad bean forms just mentioned.

The spores of the broad bean organism are large for an Ascochyta(Compare figs. C and F of Plate I).Saccardo and Marchal (36) mention A. pisi f. foliicola with spores 15-18x 4-5μ on peas. Saccardo later thinks that this might be related to

Ascochyta boltshauseri Sacc., a form usually found on beans (Phaseolus spp.) in Europe (33). This latter is given as occurring on Vicia faba in Denmark(30). The spores are larger (Plate III fig. P) than those of the California material and the brown distinctly zonate spot caused by A. boltshauseri on beans is not at all like the form on the broad bean, even allowing for differences in host reactions. The beans(Phaseolus spp.) were also highly resistant, in fact practically immune, to any injury by Ascochyta from broad bean.

One finds reference to a number of large-spored collections of Ascochyta on vetches.Jaap(16) gives A. viciae Lib. on Vicia sepium L. with spores roughly given as 18 x5μ. Ascochyta orobi Sacc. as interpreted by European mycologists is a large-spored form with variable spores which seem to merge on one hand with A. pisi Lib. and with A. fabae Speg. on the other. Petrak(26)(page 52) in mentioning this form on Lathyrus vernus Bernh. notes that the conidia are extremely variable, 15-27(mostly 18-23)x 4.5-7μ(5-6). The writer has examined material on this host collected by Oertel(Sydow Myc. Ger. 530) and except for a larger number of non-septate spores(Plate I fig. 1) and a smaller, less definite type of spot it might well be a transitional form between the large-spored form on the broad bean and the smaller-spored form on the pea. Transitional types are noted on Vicia sativa(Plate II fig. D,E,F). The forms on Vicia angustifolia (L.) Reich.(Plate II fig. G) are commonly found with a considerable number of spores from 15-25μ in length and often with two or more septa(see next sub-heading for further detail).

Pycnidia of the form on Vicia faba L. are variable on host material. Under the oil immersion lens the pycnidium is light golden in color and made up of intertwining mycelium which is not quite as compact as we find in the firmly knit pycnidium

of Mycosphaerella pinodes (Berk. and Blox.) Stone(pycnidial stage) or of Ascochyta pisi on peas. The outer wall is not marked off in regular four or five-sided segments but appears more indefinitely developed. There is a tendency for the ostiole to be loosely bounded with few none of the definite pigmented cells common to firmer types. These fruiting bodies approach the pseudopycnidia of Potebnia(29), Diedicke(13) and others.Diedicke bases his genus Stagonosporopsis on the presence of a loosely structured pycnidium, sometimes acervulus-like in nature, and the production of a variable number of several-septate spores, mingled with the one-septate majority. The differentiation from Ascochyta on these frail variations has little if any basis. A study of many cultures and of host material of Ascochyta, examination of herbaria material; and a study of Ascochyta boltshauseri Sacc. that has been referred to Stagonosporopsis by Diedicke(12) and Petrak(27); leads the writer to believe that in the case of the Ascochyta and the so-called Stagonosporopsis forms on legumes, the term pseudopycnidium is not valid. This loosely developed type of pycnidium seems associated with the larger size of the fruiting bodies and may be due in part to excessive development of the reserve food of the stromatic layer(in the sense of Archer)(1) with consequent thinning and underdevelopment of the pycnidial wall.

The writer has shown that the period of incubation, the symptoms, and characters on media are all identical for Ascochyta pisi Lib. on pea and the Ascochyta on V. faba.He has also shown that all transitions may occur in size of spore between the two and there seems little doubt that both belong in the same species.At the same time since this form on Ascochyta pisi Lib. seems to fall under the genus Stagonosporopsis it indicates that Stagonosporopsis

is not distinct from Ascochyta. The pycnidial and spore characters used to separate this genus from Ascochyta are not distinct or definite enough to be of generic value.

It will be shown more conclusively later that if one is to separate the Ascochyta or Ascochytae forming the light, definitely -bordered spots on vetch into named forms and varieties of Ascochyta pisi Lib., it would be along arbitrary lines, as transitional types are very frequent. Therefore A. pisi Lib. on Vicia faba L. will not be regarded as a separate species of the variety.

2. On Vicia villosa Roth., Vicia sativa L., V. angustifolia (L.) Reich.

All of the collections examined to date on these three hosts show light buff to white spots, usually 4-10mm. in diameter, one or two to a leaf and surrounded by a definite red to brown border. On Vicia villosa Roth. the border is more often brown than red; on the others, usually red. Spots are most commonly found on the leaf and pod, in the more severe infections, on the stem. Pycnidia are very prominent against the light background of the spot. They are usually concentrically arranged. They vary in size from 40μ to over 200μ in diameter but the most common type averages 70-120 μ. They appear golden under the microscope with darker cells about the ostiole. Some have the loose-knit character of Ascochyta pisi on broad beans (California material); in fact most of the large pycnidia in any collection, as has been mentioned, have this character.

On account of the confusion in this group it seems well to list a considerable number of specimens with brief notes where it is deemed advisable. Measurements are of mean spore size when not otherwise given.

VICIA VILLOSA ROTH.

J.M. Bates(U.S.D.A.) Nebr. 1897. Spores broader proportionately than most of the collections to follow, 14.6x4.3μ;Vestergrun, Microm. rar. sel. 1537. Suecia1898, slender, cylindrical spores with homegenous contents, 15.3x3.7μ; Barth. Fungi Columb.1506, Alabama 1901, pod spot, 14.6x4.1μ from specimen at Ohio StateUniv., 12.7x.3.2μ from specimen at Wash. State College;Barth. Fungi Columb. 2305(The host Vicia americana Muhl. seems to be Vicia villosa Roth. from material in the bureau of Plant Industry Herbarium at Washington D.C., 13.7x4.3μ;Clinton Mass.1906, spores cylindrical with homogenous contents 12.7x3.9μ;Clinton, Granby Conn.1907, 13.8x4μ;J.J. Davis, Wis.191413.6x3.7μ, spots seem immature with greenish, water soaked appearance noted in early stages of inoculated infection development;R.M. McKee(U.S.D.A.) Fayetteville Ark.1919,pycnidia 70-100, spores 13.2x3.6μ;RM. McKee, Georgia,1921, 14.8x 3.9μ;J.H. Miller, Georgia 1923, leaf spot, spores cylindrical,contents clear, hyaline 13.8x4μ;Linford, N.J. 1928.;R.F. Pocle,N.J. 1924, pod spot, spores cylindrical, blunt ends, 13.6x 3.6μ; Kienholz, Pullman Wash. 1928,leaf spot, spores 12.5x4.3μ, pod spot spores 13.1x4.2μ; Sprague, Ohio, 1928, 13. 4x4.3μ;Sprague,Ohio. 1928, 13.4x4.5μ;Wolf,N.Y. spores slender, 12.9x3.4μ.

VICIA SATIVA L.

Dearness, Ontario, 1897, spores clear, cylindrical, 15x4.5μ, pycnidia 60-100μ diameter; Tranzschel et S. Myc. Rossica 37.pod spot, pycnidia arranged concentrically, spores large, frequently several septate, similar to material from Mississippi, 16.6x 4.7μ; J.H. Miller, Ga.;L.E. Miles, Miss. 1922, 14.1x4.3μ; H.H. Wedgeworth, Miss. 1927, spores large, variable, cylindrical, somewhat bulgy, especially the frequent two or more septate ones, 16.1x 4.6μ(13-19x3.5-6μ), pycnidial walls loosely structured, light golden, pycnidia of comparatively large size.

VICIA ANGUSTIFOLIA (L.) REICH. AND VARIETIES.

H.M. Barre, Clemson College S.C. 1909, 13.4x4.1μ;Kabat et Bubak Fungi Imp. Ex. Ger.(Labelled Ascochyta vicia-lathyroidis Syd., 13.7x4.3μ;R.M. McKee(U.S.D.A.) Ga. 1921, 15.4x4.1μ,many are more than one septate and range up to 22μ in length;J.J. Davis Barneveld Wis.,1921, spores cylindrical, not constricted in some, strongly so in others,14.2x 4.2μ;Davis,Ridgeway Wis.,1921, spores cylindrical, 13.4x4.2μ; L.E. Miles Miss. 1922, 13x4.5μ;J.H. Miller Georgia, 1923, 15.6x4.1μ;Linford, Wis. 1924,spores cylindrical, some two septate, 15x4μ, in culture on potato-2% dextrose agar this resembled Ascochyta pisi Lib. from the pea.

While all variations in size may be found, even in the same collection, the forms on the vetches range in spores size above the average for A. pisi on the pea. On V. villosa they are commonly nearer the same size as on the last mentioned host but on the other two Viciae and at times on V. villosa they are much

larger and sometimes reach the minimum for Ascochyta pisi on the broad bean. The last-named form is usually one or two microns longer(mean spore length) than the largest types on other vetches and seems to have more claim to distinction than other vetch-invading Ascochytae(Compare figs. F,G,H,I,J,K,L,M, of Plate I and Figs. B to H inclusive of Plate II).

A study of isolations from Vicia villosa Roth. leaf spot material collected in New Jersey,Ohio and Washington State shows a deep buff to fluffy olive-grey growth that in some petri dish cultures is nearly white and appears like an occasional culture of Ascochyta pisi Lib. from pea(24).However it is darker than most of the latter on potato-dextrose agar. The rate of growth under laboratory conditions of temperature and light is the same for both types. Pycnidial development is scanty in the vetch forms.On agar long-cylindric spores are produced quite in contrast to the short-cylindric ones developed on the host(Compare figs. E,F, H,J,K,M of Plate I). Inoculation into various hosts resulted in production of the same short-cylindric ones(Plate I fig. E). The writer has noted that the various forms retain their general characteristics through several cycles of inoculations. While the morphology may be fairly definite the differences are too slight to consider farther.

Inoculations show that the forms on V. villosa are more aggressively parasitic on this host than are any other Ascochytae, to most of which it has proven decidedly resistant.

A culture of a light-spot forming type from V. angustifolia mentioned in a previous article(24) died out before inoculation work could be done with it. It produced a white to buff fluffy growth that resembled the pea-inhabiting Ascochyta pisi.

Morphologically, as has been intimated, the forms on the three vetches in spite of fairly well marked minor differences that show some indication of stability, are not distinct enough from A. pisi Lib. to warrant specific rank. Physiologically there is a well marked difference. While isolations from vetches caused slight but consistent injury on vetches and likewise forms from peas were able to attack the latter host it was found that A. pisi from pea could make little headway on the vetches and the pea itself was almost immune to attack from the light-spot forms from vetches.

TABLE III.

Summarized table of all inoculation and cross inoculation results between forms of Ascochyta pisi Lib. on Pisum sativum L. and Vicia spp. Data taken from Table II.

Host.	+ [3]	-	Comparative injury.
A. pisi from pea inoculated onto pea.	25	0	Moderate to severe
A. pisi from pea inoc. onto Vicia spp.(TableII).	22	40	Slight.
A. pisi isolated from Vicia spp. inoculated onto Vicia spp.	42	18	Variable.
A. pisi isolated from Vicia spp. inoc. onto pea.	4	23	Very slight.

[3] Figures indicate number of pots of plants inoculated in each case.

3.On Vicia hirsuta S.F. Grey and Vicia lathyroides L.

The following specimens semm to be clearly forms of A. pisi Lib.;

1.Barber, Orono, Maine. Pod spot on Vicia hirsuta.Dark brown bordered, lighter in center containing pycnidia with spores having a mean size of 12.8x3.5μ.This resembles material on V. villosa (Plate II fig.H.)

2.Ascochyta ervicola Sydow on Vicia hirsuta, type material.This seems to be slightly immature; large dark pycnidia, confined largely to stem in fragment seen. Leaf spots were few and very similar to Barbers specimen. Spore characters also similar.

3.Ascochyta vicia-lathyroidis Sydow. Type material. Very similar to leaf spot material on most of V. sativa collections seen.Spores are of the Ascochyta orobi Sacc. type. This seems to be a large- spored form of A. pisi and is no different from most of the collections on V. sativa and V.angustifolia in this country.

4.Dubious Ascochyta pisi forms on Vicia spp.

Three collections sent the writer by Dr. J.J.Davis labelled A. pisi were studied with results as follows;

1. On Vicia caroliniana Walt. ,July 3,1919, Fish Creek Wis.Mean spore size 22.3x3.4μ. Many spores were multiseptate, long and slender, borne in pycnidia on vague, indefintely-bordered, leathery, discolored spots.

2.On Vicia americana Muhl. Aug. 25, 1919 from Tomahawk Wis. Spores 19.3x3.1μ(Plate II fig.A). Davis(11) mentions the fact that the ostioles of the large pycnidia(90-190μ) are hypophyllous in the form on V. caroliniana and epiphyllous on V. americana.

3. ON Vicia americana Muhl.Dr. Davis recently sent the writer a collection similar in all respects to the previously mentioned specimen except that the spores were not hyaline but brown, short cylindric and contained large oil globules. The collector states that the material was gathered in an open burn and he considers it only an abnormal type of Ascochyta in an exposed location.

Until something more than observational data relative to

dried material is available the writer would hesitate to place these collections with Ascochyta pisi Lib. They emphasize the need for further study on these types.

Ascochyta pisi Lib. on hosts other than Vicia and Pisum.

The number of hosts in literature for this species is considerably reduced now that A. pisi and M. pinodes are shown to be distinct. It has been mentioned that Ascochyta orobi Sacc. on Lathyrus spp. is in part at least a large-spored variable form of Ascochyta pisi.

In inoculation studies of aerial parts A. pisi is found to occasionally attack Lathyrus spp., some of the oriental Phaseolus spp., lentils and a number of others. The range is not as great as might be expected. From all information assembled Ascochyta pisi Lib. seems to be chiefly confined to Pisum and Vicia but may be expected on a number of other genera in this country and abroad. Ascochyta pisi Lib. is therefore considered by the writer to be made up of several to many closely grading forms capable of infecting a range of hosts and showing well marked physiological forms in some instances.

Technical Description of Ascochyta Pisi Lib.

It seems advisable to give a revision or possibly an extension of the description of Ascochyta pisi to cover the range indicated in this paper. On account of small differences noted throughout the descriptions on various hosts it will be necessary to divide this voluminous description into three parts for the sake of clearness and usefulness The writer cannot see the value of naming these forms since all minor divisions in fungi are so notoriously variable.

ASCOCHYTA PISI LIB.

On Pisum sativum L. and varieties.

Spots definite, circular on leaves and pods, elongate on stems, yellowish brown with dark brown to reddish border which is often prominent, sometimes whitish in center, spots not defined on mature plant parts; pycnidia gregarious at center or in circular zones, somewhat prominent, depressed-globose, light golden brown to darker brown, ostiolate, 75-225μ; spores hyaline with homogenous contents and occasional oil drop, oblong, ends rounded, straight or somewhat curved, 1(1-3) septate, somewhat constricted, 10-14(6-19)x3-5(2.5-6)μ. Mean spore length less than 13.5μ(12-15μ).

On leaves, stems, pods and seeds of Pisum sativum L. and on aerial parts of Lathyrus spp. and Vicia spp. by artificial inoculation.

On Vicia species.

Spots definite, circular, occasionally elongate on leaves and pods, elongate on stems, at first yellowish brown to green, later white with dark red to brown border which is always prominent when mature, spots not defined on mature or senile parts; pycnidia gregarious at center or in circular zones, very prominent, depressed-globose, hyaline(light golden) to dark brown(pycnidia on stem parts often dark brown), ostiolate, 60-225; spores hyaline, contents when mature homogenous with rare oil drops, 1(1-4) septate, cylindrical, sometimes curved, ends rounded, scarcely to somewhat constricted, 10-17(8-25) x 2.5-5(2-6), mean spore length variable.

On leaves, stems, pods and seeds of Vicia spp. and by inoculation on aerial parts of Lathyrus spp. and slightly on Pisum sativum L.

On Vicia faba L.

Spots usually definite, circular to elongate on leaves and pods, elongate on stems, at first dark brown or olive to black, later yellowish to white in center with dark brown to red border which is often prominent tho sometimes diffused; pycnidia gregarious near center or in circular zones, very prominent, depressed globose, light brown, sometimes darker, parenchyma cells of walls usually not closely packed, ostiolate, 80-250μ; spores hyaline, contents homogenous except frequent medium to large oil drops and crystalline-like material, 1(1-4) septate, cylindrical, straight or somewhat curved, constricted at septum(sharply constricted in spores that are more than one septate), ends rounded, 14-25(12-28) x 3.5-6(3-7) μ, mean spore length usually greater than 17μ when mature.

On leaves, stems, pods and seeds of Vicia faba L. from Oregon and California and by artificial infection on aerial parts of other Vicia species.

STUDIES OF MYCOSPHAERELLA PINODES (BERK. and BLOX.) STONE.

Historical.

Previous work(19) (24) has shown the position of M. pinodes

as separate from A. pisi Lib.Both Stone(39) and Atkinson(2) found the former on hairy vetch(V. villosa) and most general.texts give M. pinodes on vetches as well as peas. Literature and common acceptance still leaves the organism somewhat ill defined as to host range.

On Vicia species.

To date the writer has been unable to develop an ascigerous stage from any material isolated from vetch and all specimens encountered personally by the writer have been unquestionably A. pisi. The only collection that the writer has viewed that hinted of M. pinodes was a collection on dead stems of Vicia dasycarpa from Virginia (R.M. McKee). This had a note appended stating that a small ammount of an ascomycete,Sphaerella viciae Schroet. was present together with Ascochyta viciae Lib. Both must have been destroyed in former mounting as the writer could not locate mature material of either.

The writer has been unable to obtain material of Stone's work(39) on the vetches.

The writer has recently found a very destructive, dark zonate leaf spot of vetch(Vicia villosa Roth.). It was apparently due to a Colletotrichum. Symptoms were not dissimilar to the type of injury caused by M. pinodes on peas.

Through inoculation work on both aerial and subterranean parts it is shown that M. pinodes can attack a variety of vetches tho some of them such as M. villosa are very resistant.

Since most of the collections examined by the writer were made by workers during the growing season when plants were in full vigor the writer feels that no doubt M. pinodes is destructive ~~very~~ later in the season and that probably

Stone(39) and Atkinson(2) found it in a saprophytic condition on dead overwintering parts or late summer plants. Ascochyta as a genus is readily adaptable to the saprophytic life. Jarius(17), Jones(14) and others have clearly shown that a small ammount of Ascochyta in a field may spread over extensive areas toward harvest time. Rainy weather may result at this time in severe blighting in the case of the pea.

On Lathyrus species.

Some late fall material of Ascochyta on pods of Lathyrus odoratus L. sent from Madison Wis. by Dr. F.R. Jones showed one pod overrun with M. pinodes. Isolations were obtained from infected seed and on potato-2% dextrose agar growth was identical to that described for M. pinodes from pea in a previous article(24). This particular culture died out before being entered in to inoculation studies. Inoculations on aerial parts of this host with M. pinodes pycnospores taken from Pisum were negative. Other Lathyri were in part susceptible.

It will be shown(page 28) that the most common type of Ascochyta on sweet pea in this country and , from literature, in Europe also, is a small-spored type referred to Ascochyta lathyri Trail.

Dr. J.L. Weimer sent the writer cultures of Ascochyta isolated from footrot material of Lathyrus tingitanus L.(Tangier pea) grown in experimental plots in Mississippi. These later developed perithecia in culture on Melilotus stems. Morphological characters were the same as for M. pinodes. This, along with cultural characters on various media, definitely showed that L. tingitanus was another host for this organism. A study of inoculation rsults bears this out very clearly.

STUDIES OF ASCOCHYTA PINODELLA L.K. JONES.

Introductory .

Through considerable study carried over a period of years Ascochyta pinodella L.K. Jones has remained a distinct and imperfect form. For this reason, along with those differences pointed out by various workers including Jones,(19) (24) (14) the writer agrees with Jones' naming of this organism. The cumbersome M. pinodes micro form should be dropped.

From extensive collections sent the writer recently by M.B. Linford and others the prevalence of A. pinodella in this country is an established fact. It seems to be most common in the arid region of the western United States where it exceeds all other Ascochyta in abundance tho in the east where Ascochyta diseases are far more abundant it is frequently found. Both this form and A. pisi were found associated with Peronospora viciae (Berk.) De Bary in a number of Linford's collections. The occurrence of Phoma or Phoma-like forms on old leaves or on mature spots was occasionally noted also. Non-pathogenicity of a number of these was clearly shown. Their presence may confuse.

Asochyta pinodella L.K. Jones on hosts other than Pisum.

The writer has found no collections of this form on vetches in any herbaria examined. This is surprising in view of the fact that some of the vetches are extremely susceptible to the organism under greenhouse inoculation conditions. While hairy vetch and Hungarian vetch and some others show a high degree of resistance Vicia sativa L. is nearly always killed to the ground by an inoculation of spores. Pycnidia develop scatteringly in some cases, abundantly in others.

The very obscure nature of the common symptoms, that of

Ascochyta's depredations to pass unobserved.

STUDIES ON ASCOCHYTA LATHYRI TRAIL.

Historical.

Trail (44) gives Ascochyta lathyri as a new species on Lathyrus silvestris L. from Scotland. It causes an ill-defined spot that tends to cover the entire leaf. The pycnidia are numerous, globose, depressed and 50-100 μ in diameter; spores small, 8-10x2.5μ. Saccardo, in examining material from Italy, finds larger pycnidia 100-125μ and smaller spores, 5-8x2-3μ(34). Stone mentions the small spores and from his description of the Ascochyta connected with his Mycosphaerella ontarionensis on Lathyrus sativus L. it seems fairly certain that he was working with Trail's and Saccardo's type (40).

Saccardo(32) describes Ascochyta orobi on Lathyrus vernus Bernh. and L. orobus. Material seen of this (not type material) is a large-spored form of A. pisi as mentioned above.

Harkness(15) gives Ascochyta pisi as the causal organism for a leaf spot of Lathyrus palustris L. in California and Gilbert and Davis (10) list the same host and organism at Madison Wis. None of this type have been seen by the writer.

Bubák and Kabát (7) described Ascochyta lathyri var. lathyri-odorati as the cause of light colored to brownish spots of a general indefinite nature on the leaves of the sweet pea. Spores are small 8-14 x 2.5-3.5μ.

Studies on Lathyrus odoratus L.

The bulk of the work with A. lathyri Trail lies with the host Lathyrus odoratus L, the cultivated sweet pea. Repeated attempts to connect this species on the sweet pea with an ascigerous

stage (*M. ontarionensis* Stone) were unsuccessful. Stone mentions (40) that he was unable to develop a perithecial stage in culture. Work on inoculated hosts has not progressed far enough to record at this time.Outside of this gap in the life cycle the form on the sweet pea, or at least the commonest form in this country will be shown to be very clearly related or identical to Trail's type.

A number of collections on sweet pea from this country are typified by one from Hopkins Minnesota by Vande Vere. This shows a purple to brown stem lesion with many black-brown small pycnidia containing small, plump, cylindrical to faintly fusiform spores having a mean size of 9.4x3.6μ(Plate II fig.I).

Infected pod material from Madison (F.R. Jones) showed a form similar to the Minnesota material (Plate II fig. M). Isolations on potato- 1% dextrose agar were obtained from both spores and from infected seed (Plate II fig. K).

On the potato agar the fungus grew rapidly forming a flat grey growth with frequent so-called saltations of lighter and darker strips and the production of abundant small black pycnidia buried in the substratum, with only the upper half protruding. Microscopical examination of this substratum showed it to be made up of chlamydospores and dark compacted hyphae. The pycnidia did not usually exude spore masses; a few developed tendrils of spores, pale flesh to grey in color. The spores were either non-septate or abortively septate (Plate II fig. K). On a number of different kinds of media the rate of growth of this culture was slightly faster than the average for several isolations of *A. pinodella* (To compare *A. pinodella* and *A. lathyri* as to spore character see Plate II fig. I and Plate III figs.F and G.).

The host range in inoculations of A. lathyri from sweet pea is practically as extensive as that for A. pinodella but the symptoms are more variable. Sometimes it causes dark brown lesions and leaf spots similar to the other; again on such hosts as the sweet pea it causes indefinite leaf and stem tip injury in a manner similar to that described for Ascochyta lathyri Trail in literature. Injury of this type seems to be due to a rapid spread of the fungus after entrance is gained at the tip of the leaf or on the stem back of the tip.

The period of incubation of the sweet pea organism is longer than that for A. pinodella in most cases, 24-36 hours more being required for symptoms to develop to the same severity.

Although similar to Ascochyta pinodella L.K. Jones in many respects, the somewhat different spore characters, together with those other differences mentioned, separate the two and place our collections with Ascochyta lathyri Trail.

STUDIES ON ASCOCHYTA IMPERFECTA PK.

Historical.

According to Davis (9) there are two forms in this country on Medicago, Ascochyta imperfecta Pk. on Medicago sativa L. and A. medicaginis Bres. on Medicago lupulina L. The former, described by Peck (25) from material gathered by Stewart and workers (41) is characterized by having variable spores ranging from 6 to 15μ in length with frequently a large number of non-septate ones present. It is thus distinguished from A. medicaginis Bres. which has spores measuring 18-23x3.5-5μ.

Rostrup (31) has mentioned A. pisi Lib. on alfalfa.

Saccardo (35) describes A. pisi var. medicaginis on living stems of alfalfa from Spokane, Washington. Spores 14x5μ.

On alfalfa (Medicago sativa L).

Examination of material from Missouri, New York, Ohio, Colorado, Maryland, Idaho, Washington, Utah, California, Wyoming and Kentucky show the spores of A. imperfecta on alfalfa to average 10.6x 2.8μ to 13.4x 4.3μ for mean spore size of specimens, with a grand average mean spore size for fifteen collections of 12.1x 3.4μ.

The appearance of the spores of A. imperfecta varies considerably. Material from Ohio when mature showed rather cylindrical spores, which were mainly septate with homogenous contents. Material from the arid regions of the West gave a larger percentage of immature and non-septate pycnospores (Plate III figs. A,B,C,E). Co-type material collected by French resembles this western material closely. Immaturity through unfavorable conditions such as dry weather or removal of the cuttings seems to account for some of the non-septate spores.

Stagonospora sp. (usually referred to S. carpatica Bauml.) found in a white, faintly bordered spot on the leaf tip or margin is often associated with Ascochyta on alfalfa. The lighter, larger spots with prominent pycnidia distinguish it macroscopically from the Ascochyta spot. Immature Stagonospora spores are frequently one-septate and might be mistaken for A. medicaginis Bres.

Isolations made by the writer from leaf spot material collected by Linford in Utah and Maryland and from personally collected material in Ohio and Idaho, while showing all the variations mentioned above for A. imperfecta, were indistinguishable on potato-1% dextrose agar. On this medium the fungus develops a flat grey felty growth with olive-brown patches. The felty growth is gradually superseded by a darker, flatter, carbonaceous growth in which numerous small black pycnidia are imbedded. This substratum is made up of a mass of chlamydospores and stromatic hyphae.

The remains of the original felty growth plus additional later tissue obscures the pycnidia with a thin evanescent mycelial felt. Viewed from beneath, a plate culture has an olive- brown appearance. Pycnidia are black to lighter brown; they exude pale pink spore masses made up largely of non-septate spores (Plate III fig. D). The rate of growth on media is rapid, practically the same as for A. pinodella and far more rapid than for cultures of A. pisi .

On Melilotus stems in culture A.imperfecta does not produce any cottony or fluffy mycelium to speak of but red-brown carbonaceous pycnidial masses cover the stem, producing spores in great numbers.

By means of water inoculations on the surface of pea soup agar a large number of light golden to brown pycnidia are secured with little production of mycelium. Spores are produced in quantities.

Inoculation results with Ascochyta imperfecta Pk. were always positive on all forms of alfalfa but were extremely variable and inexplicable on a number of other hosts. It seemed to be able to cause leaf tip injury and sometimes spotting of the leaves of a number of Trifolium, Melilotus and Medicago species and at times was able to invade the leaves and petioles of the pea(Pisum sativum L.). It was noticed however that only the alfalfa and sometimes the bur clover (Medicago hispida Gaertn. var.) showed mature spots in greenhouse inoculation studies. The injury on other hosts showed more as a scalding or blighting of the leaves. Since the specimens inoculated were young it is not remarkable that some injury was encountered and might possibly at times be found in young fields of some of these hosts, although too obscure to attract attention.From

the ready greenhouse infection on the bur clover it is indicated that this host might be found infected in the field.

The perfect stage of the alfalfa organism has never been found and all attempts to induce it have failed. The fungus was watched also on naturally infedted plants thruout the year with no indication of any ascigerous stage. A vigorous development of saprophytic condition on the dead leaves and stems of overwintering plants was noted. Leaf infection develops from this material about the crown of the plant in early spring. Collections made locally showed developing pycnidia on spots in early March(vicinity of Cincinnati Ohio). Infection of lower leaves occurs after any prolonged rainy period during the spring and summer months. During the latter part of the summer infection is usually confined to leaf tips.

Doubtful cases of Ascochyta imperfecta Pk.

Two collections of alfalfa warrant special mention.A form collected by A.H. Gilbert at Lexington Kentucky shows highly variable black, spherical pycnidia on dead stems, containing clear, hyaline, cylindrical spores up to 16μ in length. The presence of quite a number of smaller spores reduces the mean size to 12.7x3.7μ. On account of its saprophytic nature it is difficult to determine exactly the position but apparently it is Ascochyta imperfecta Pk.

A collection from James Weir's herbarium(U.S.D.A.) made by Vernon Simmons at Wendover, Wyoming and listed as determined by Saccardo shows superficial, light, brown-bordered lesions on very coarse stems that are labelled alfalfa, in which many black pycnidia are very prominent. Spores are homogenous as to contents, cylindrical, 12.9x3.9μ and resemble those of A. imperfecta on leaves from Cincinnati material (Plate III fig.

This Wyoming material seems identical with the Spokane material labelled A. pisi var medicaginis(Sacc.(35). The spores are smaller but within the range one might expect. The collection is labelled A. pisi.

While A. imperfecta has been seen only as a leaf spot organism, the writer considers that the Simmons collection is but a form of that species. The spores are not dissimilar and the difference in pycnidial color is directly related to difference in substratum. In this connection it was found thruout the course of inoculation work that the pycnidia produced on stems, especially mature or woody ones, were frequently carbonaceous and pigmented while pycnidia on the leaves from the same inoculum would be partly hyaline (usually basal portion). Now Diedicke considers most of the forms on the stems Diplodina (13) and those on the leaves Ascochyta, making the distinction in all cases chiefly on pycnidial character. It is certain from many inoculations with many hosts and many forms made thruout the course of the work that there is utterly no basis for two genera, that they are all Ascochyta. The genus Diplodina should be removed from literature or at least a new interpretation made of it. Differences in pycnidial character such as Diedicke pointed out are not tenable.

Cultural and inoculation studies are needed to compare Simmon's collections with forms on Medicago, Melilotus and Pisum.

Pleosphaerulina briosiana Pol. was found associated with Ascochyta imperfecta Pk. in one early spring collection near Cincinnati. A darker, discolored (1-3mm.) spot usually marked off the region occupied by the ascigerous form. The writer can see no connection between Ascochyta and this form as was suggested by the somewhat doubtful work of Bubak with European forms on alfalfa (6).

ASCOCHYTA MEDICAGINIS BRES. IN NORTH AMERICA.

An examination of scanty material on black medick(Medicago lupulina L. collected at Oxford Ohio in 1897 by Kellerman showed a few leaf tips infected with A. medicaginis Bres. These spores had a mean spore size of 16.8x 3.9μ(Plate III fig. I). This and Dearness' (Kabat' et Bubak' Imp. Ex. 667) from London , Ontario, indicate that A. medicaginis is at least occasional on this continent. The spore size of Dearness' collection also measured considerably above that for A. imperfecta (plate III fig. J). The whit spots with their prominent pycnidia resemble the Stagonospora spots on alfalfa leaves and are not easily confused with the smaller indefinite often brown stained leaf spot caused by A. imperfecta .

The writer has examined abundant field material of black medick around Cincinnati where it thrives along every roadway but has found no definite case of A. medicaginis Bres. M. lupulina was subject to a variety of obscure leaf spots and blights apparently due to Cercospora, Phyllosticta-like forms and others but locally A. medicaginis was lacking.

There is no reason for not accepting Davis' verdict (9) that Ascochyta imperfecta Pk. and A. medicaginis Bres. are not distinct. Cultural and host range studies would be additional proof for the latter's position.

SPECIES OF ASCOCHYTA AND PHYLLOSTICTA ON PHASEOLUS.

Historical.

Saccardo describes Ascochyta phaseolorum (32) on Phaseolus vulgaris L. from Italy. It causes indefinite tan spots on leaves. Pycnidia are 100 μ in diameter, spores small, 10x2-3μ. Saccardo gives the technical description of A. boltshauseri in an article

by Botlshauser (3) on a leaf spot of beans. The organism described develops comparatively large zonate brown spots (5-20mm) on the first simple leaves of the plant. There is later secondary spotting and defoliation. The pycnidia described were large, 100-150μ and ths pores in comparison to other Ascochytae were huge, 22-28x 7-8μ.

Petrak (26) (page 300) in discussing the well known Phyllosticta phaseolina Sacc. from his garden in Bohemia noted that many spores were two celled, 12-15x 4-6μ. He seemed to think that they were very close to young forms of Ascochyta phaseolorum. He noted what seemed to be the same organism change gradually in September from the non-septate condition to that of a fairly definite Ascochyta. Then during the winter months when saprophytic development set in the fungus could first be seen as a Phoma and later as a Diplodina as interp reted by various European workers.

Diedicke (12) changed A. boltshauseri Sacc. to Stagonosporopsis boltshauseri (Sacc) but Petrak (27) decided that Stagonospora hortensis Sacc. and Malbr. was synonomous. Various collections bear the name Stagonosporopsis hortensis (Sacc. and Malbr.) Petrak. The writer, as was discussed earlier under the broad bean, finds the fungus to be an Ascochyta; and lacking type material of Stagonospora hortensis, he leaves it as Ascochyta boltshauseri. Sacc.

Studies of Phyllosticta phaseolina Sacc.

Introductory.

Typical tan, red-bordered, leaf spots of this organism were gathered on yellow wax and valentine beans (Phaseolus vulgaris L.) near Hazelwood, Ohio and Sayler Park, Ohio during the summer of 1928. One collection of these growing side by side with a

heavy infestation of A. pisi on garden peas was noted.

Pycnidia of the Phyllosticta on beans were deep golden in color, darker around the ostiole. The spores were about 90% non-septate and measured 4-8x1.5-3μ. Their form was cylindrical to ovoid and their contents granular with an occasional oil drop. The septate spores were very slender with pointed ends, measured 9-12x 2-3μ and were scarcely constricted at the septum(plate III fig. K and L.).

Cultural and inoculation studies.

Single spore isolations on potato agar developed first a flat white to cream-colored growth that later darkened in spots to a medium brown. Scattered black semi-stromatic " areas had a few pycnidia on them. In about 14 days after transfer (ice box conditions) a few pycnidia exuded pale pink spore masses. Fluffy aerial growth of a mixture of buff, white and grey overran the culture in a few days and obscured later developing pycnidia.

Inoculations of a considerable range of hosts shows P. phaseolina to be parasitic on most of the beans and other hosts (see table of aerial infections).Most of the injury to inoculated plants occured first in the form of water-soaked red-bordered spots scattered over the leaves and petioles, commonly at the leaf margins and tips. Later these developed into tan to fawn- colored spots with prominent red borders.

Inoculations with A. pisi, M. pinodes, A.pinodella and other forms have shown most of the species of Phaseolus to be singularly immune.Some of the Asiatic beans, chiefly the Mung (Phaseolus aureus Roxb.) and the mat or moth bean(P. aconitifolius Jacq.) are susceptible. On no counts except the septate spores can the writer see any connection between Phyllosticta phaseolina Sacc. and these others. The position of this species will be discussed under the next sub-heading.

In studying herbaria the writer has experienced difficulty,as have others, in separating P. phaseolina from the Phomopsis stage of Diaporthe phaseolorum (C.and E.) Sacc. He has found everything from bacterial spots to composite specimens labelled P. phaseolina . In recording specimens he has given only those numbers that are clearly due to this species and are comparatively free of secondary forms.

W.A. Kellerman, Flora of Kansas 445,1883; Barth. Kans. Fungi , Rooks Co, 1898; G.P. Clinton Conn. 1902;W.M. Gilbert, Cherrydale Va. 1920;A.E. Jenkins Wash. D.C. 1922; R. Sprague(several) Ohio 1928.

Studies of Ascochyta phaseolorum Sacc.

Most phytopathologists do not consider this organism to be present in this country. However two collections labelled A. pisi on Phaseolus lunatus L. from Takoma Park D.C. are very clearly this species. One of the specimens(A. Arnes,U.S.D.A.) collected in August 1908 shows leather-colored spots of a zonate character up to 18mm. in diameter. Golden brown pycnidia are moderately abundant. The spores are chiefly non-septate but some 20% are clearly septate(the false septation in Diaporthe phaseolorum,conidial stage, might confuse). They are short cylindrical and average 7x2.5μ. The second specimen is from the same garden but was collected in October of that year. It is pod material showing a leathery spot somewhat zonate with pycnidia identical with the first(135-180μ diameter). In this specimen the spores showed fully 70% of their number septate. They also are clearly septate, short cylindric and have a mean spore size of 7.1x2.4μ(Plate III fig. M).

A comparison of this material with foreign collections showed the symptomatology and morphology to be the same. The American material has smaller spores and larger pycnidia than most of the foreign, but the short cylindric spores are clearly the same type

in all materials (compare figs. M,N,O,P, of Plate III). They could not be A. pisi as labelled on account of morphology, symptomatology and lack of pathogenicity of A. pisi on the same hosts. Stone (39) considers the form on beans (Phaseolus vulgaris L.) to be distinct because he failed to infect them with Ascochyta from the pea.

The increase in the number of septate spores as shown in the two Takoma Park specimens is in line with the observations made by Petrak(26). The considerably larger size of his early fall spores differs from our findings.

The type of spotting caused by A. phaseolorum and P. phaseolina is, except in smaller spots, so distinctly different; the production of septate spores so much more marked in the former; and the shape and the contents of the spores so different; that the writer does not consider the two related. In cultures P. phaseolina has always remainded non-septate. It is not at all similar in growth habits to any Ascochyta species seen in culture. On inoculated hosts it causes only definite spots and does not tend towards the saprophytic development made by most Ascochyta forms on senile and dead parts.

While it is difficult to decide what percentage of septate spores is allowable in Phyllosticta the writer feels from the evidence disclosed that Phyllosticta phaseolina Sacc. and Ascochyta phaseolorum Sacc. are distinct and rightly named.

The following specimens of A. phaseolorum Sacc. have been examined;

A. Arnes, Takoma Pk. D.C. Aug. and Oct. 1908 on P. lunatus; Petrak Myc. Carpat. 402, on P. lunatus 1925. The collection of this lodged in the mycological herbarium Bureau of Pl. Ind. Washington D.C. is partly A. boltshauseri sacc. J. Jahn Fungi Trans. 1916; Chas. Piper, Phillipines on P. lunatus 1908.

SUMMARY.

1. Confusion due to recent findings makes this research desirable. It is a taxonomic study of Ascochyta on Leguminosae but considers the life histories and physiological traits of the various forms and species as well as merely observing their morphological characteristics.

2. Studies show that most of the Ascochyta collections on Vicia species are forms of A. pisi , differing little morphologically from A. pisi as found on the pea, but being physiologically somewhat specialized on the hosts on which they are found in nature. A form of A. pisi on the broad bean(Vicia faba L.) resembles A. fabae Speg. with its large fruiting bodies and spores. It differs in symptomatology from A. pisi on other vetches but this difference is shown to be due to host reaction and not any great difference in fungus character. Various other names are found to be synonyms of A. pisi. No ascigerous stage was found connected with any forms of A. pisi.

3.Inoculation studies show Mycosphaerella pinodes(Berk. and Blox.) Stone to be pathogenic on a wide range of hosts, a thing that field and herbarium study does not bear out. The writer has found this organism only on Pisum spp., Lathyrus odoratus L., and L. tingitanus L. Stone's material on vetches was not seen, it is suggested that the occurrence of M. pinodes on vetches may be limited to late season.

4. A study of A. pinodella L.K. Jones shows it to be common and widespread on the pea(Pisum sativum L.) and able under greenhouse conditions to attack many hosts on which it has not been found under field conditions.

5. A study of Ascochyta lathyri Trail has shown it to be occasional

on Lathyrus odoratus L. in this country. Connection with Mycosphaerella ontarionensis was not established.

6.A study of Ascochyta imperfecta Pk. proves it to be variable in spore and pycnidial characters but clearly belonging in one species. It is distinct in every way from Ascochyta medicaginis Bres. that is found at times on Medicago lupulina L. in this country.

7.Ascochyta phaseolorum Sacc. and Phyllosticta phaseolina Sacc. are both found in America. They are not closely related.

8.The genus Stagonosporopsis Died. is shown to be invalid and Diplodina West. is of doubtful value. Ascochyta boltshauseri Sacc. is properly placed in Ascochyta and not Stagonosporopsis.

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EXPLANATION OF PLATES.

Pycnospore drawings from water mounts made with the aid of the camera lucida. x 800. Spores with contents plasmolyzed through age or treatment are shown in outline only.

PLATE I.

A.Ascochyta pisi Lib. on Vicia faba L.Leaf spot. Corvallis Oregon.

B.A. pisi from horse bean (V. faba). From leaf spot caused by inoculating plant with culture of pycnospores isolated from broad bean seed of 1918 California crop.

C.A.pisi from broad bean (V. faba) grown on potato-1% dextrose agar.

D.A. pisi on leaf spot on broad bean made by inoculating plant with spores from potato agar culture isolated from Pisum sativum L.

E.Same as fig. D. but inoculum obtained from leaf spot on Vicia villosa Roth. from Pullman Wash.

F.From culture of Ascochyta pisi Lib. on potato-1%dextrose agar isolated from pea(P. sativum L.

G.A. pisi from broad bean inoculated onto Vicia sativa L.

H.A. pisi from leaf spot on V. villosa (Pullman material).

I.Same type as fig. H; from Suecia, collected by Vestergrun.

J. Spores from potato- dextrose culture of isolation from material on V. villosa,Pullman Wash, mentioned in fig. H.

K. Same condition as fig. J. but material from Cincinnati Ohio.

L. Leaf spot material from Valentine Nebr. J.M. Bates, 1897 .A. pisi

on V. villosa Roth.

M. Culture on potato-1% dextrose agar of Cincinnati material of A. pisi on V. villosa.

PLATE II.

A. Ascochyta species on Vicia americana Muhl. from Wisconsin.

B. Type material of Ascochyta ervicola Sydow, referred to A. pisi by the writer.

C. Type material of Ascochyta vicia-lathyroidis Sydow referred to A. pisi by the writer.

D. A. pisi on V. sativa L. Leaf spot from Mississippi.

E. A. pisi on V. sativa from London Ontario(Dearness).

F. Same as fig. E from Russia(Myc. Rossica 3z).

G. A. pisi on V. angustifolia (L.)Reich. from Wisconsin.

H. A. pisi on pod of Vicia hirsuta(L.) S.F. Grey from Maine.

I. A. lathyri Trail on Lathyrus odoratus L. from Minnesota.

J. A. pisi isolated from pea(Pisum sativum L.) inoculated onto Lathyrus odoratus L.

K. Potato- dextrose agar culture of A. lathyri Trail isolated from pod of L. odoratus from Madison Wis.

L. A. pisi(Labelled A. orobi) on Lathyrus vernus Bernh. Germany.

M. A. lathyri Trail on pod of Lathyrus odoratus L. from Madison. Wisconsin.

N. A lathyri on "everlasting pea", New Jersey.

PLATE III.

A. Ascochyta imperfecta Pk. from Medicago sativa L.. Leaf spot, Utah.

B. Same, from Newtown Ohio.

C. Same, from Cincinnati Ohio.

D. Isolation of A. imperfecta on potato agar from Cincinnati O.

E. A. imperfecta . California material(Lee Bonar).

F.Ascochyta pinodella L.K. Jones from pea leaf spot. Utah.

G. A. pinodella on dark, zonate leaf spot,Florida.

H.A. imperfecta Pk. (A. pisi var. medicaginis Sacc.) on stems of alfalfa, Wyoming.

I.A. medicaginis Bres. on Medicago lupulina L. Oxford Ohio.

J.Same as fig. I from London Ontario(Dearness).

K.Phyllosticta phaseolina Sacc. on Phaseolus vulgaris L.Hazelwood Ohio.

L. Same as fig. K, New Haven,Conn.

M.Ascochyta phaseolorum Sacc. on Phaseolus lunatus L. Takoma Park. D.C. Oct. 1908.

N.Same type as fig.M.Petrak, Myc.Carpat. 402.

O. A. phaseolorum sacc. on P. lunatus Phillipines, Piper.

P.A. boltshauseri Sacc. on Phaseolus sp.Albania(Petrak.)

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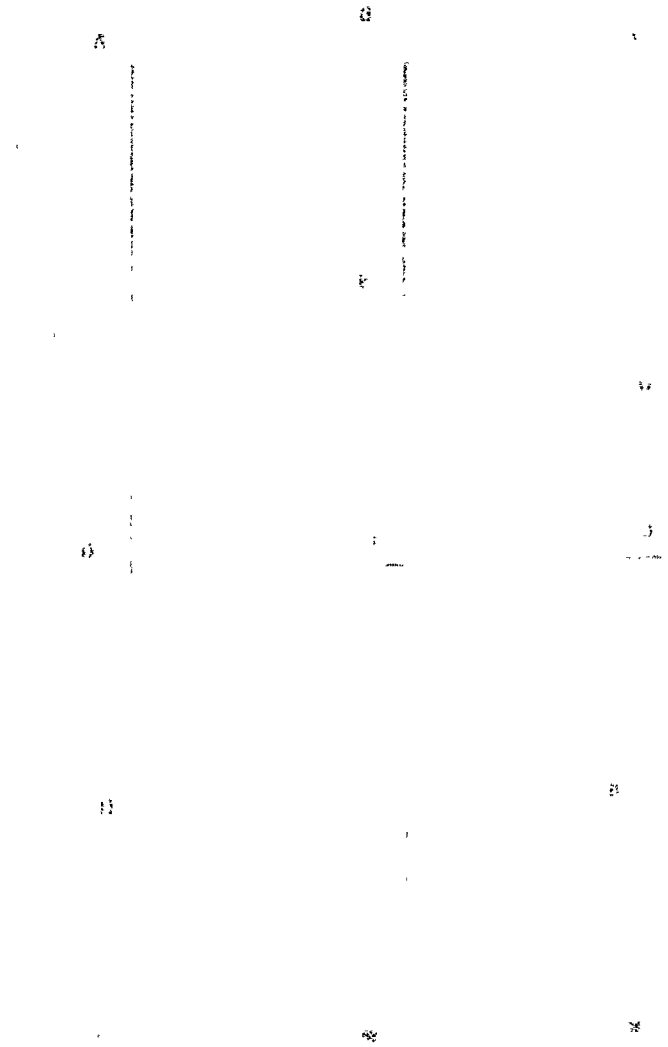

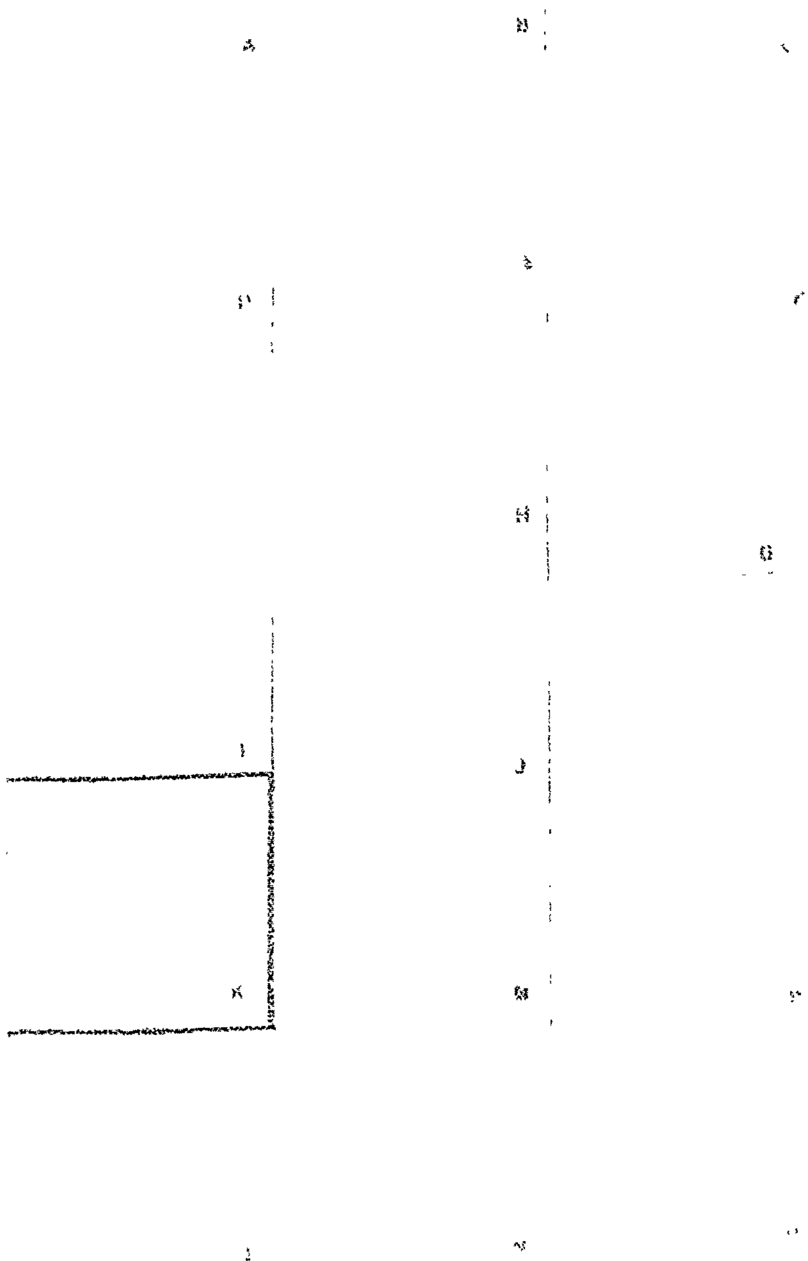

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