A STUDY OF THE BIOLOGY AND CONTROL OF ASCOCHYTA BLIGHTS OF PEAS

by

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I. INTRODUCTION

At the Central Laboratory of the Division of Botany and Plant Pathology Ottawa, tests are run throughout the winter on pea seed samples for the presence of pathogenic and non-pathogenic fungi on them. The seed samples are sent in by growers throughout the Ottawa district.

Ascochyta spp. and Fusarium spp. are the dominant fungi isolated from pea seed originating in the Ottawa district. As shown by Jones in 1927 there are three Ascochyta fungi which cause disease on peas; Ascochyta pisi Lib., Mycosphaerella pinodes (Berk. and Blox.) Stone and Ascochyta pinodella Jones.

The percentage of seed infected with Ascochyta pisi in these samples varies from 0 to 100%. The percentage of seed infected with Ascochyta pinodes and Ascochyta pinodella does not usually run above 5%. Due to the high percentage of A. pisi in the seed and the importance of this fungus in causing reduced stands when infected seed is used for planting, investigations have been undertaken for a number of years in an effort to discover methods of controlling the organism. Most of the work on control at Ottawa is concerned with the effectiveness of seed dusting.

At the suggestion of Dr. W. C. Broadfoot, investigations on the biology of Ascochyta seed-borne infections were undertaken including; longevity of A. pisi in pea seed, shoot infections and its significance, effect of temperature upon growth of A. pisi and infection, comparison of the growth rates of A. pisi, A. pinodella and A. pinodes, and associative effects of A. pisi, A. pinodes and A. pinodella. The importance of soil infestation and seed treatment studies have also been undertaken. The investigations were conducted during the summers of 1945, 1946, 1947 and 1948 at Ottawa and during the winter of 1947 - 1948 at Macdonald College.

II. REVIEW OF LITERATURE

A. Occurrence and Importance of the Blight caused by *Ascochyta pisi* Lib.

Reference to the disease known as pea blight caused by *Ascochyta pisi* Lib. appears quite frequently in the literature. Madame Libert reported the disease for the first time in 1830 although Comes in 1891 stated that the disease had been known centuries before her discovery. Wan Hook reported in 1906 that the pea crop in Ohio was damaged 52% by Ascochyta blight.

During the last twenty-five years Ascochyta blight has been reported from many countries throughout the world. In Europe, Gram and Rostrup in 1922 reported that *A. pisi* completely destroyed the pea crop in a number of gardens in Denmark. Deutelmoser noted the disease occurring in Germany in 1926. Garbawski noted the disease in Poland in 1927. Marchall and Foex in 1931 reported *A. pisi* on peas in France. In 1932 *Ascochyta pisi* was prevalent in the Bristol area causing severe damage. In 1940 *Ascochyta pisi* was reported from Sweden. Reports of its occurrence from Maritius, Russia, South Africa, New South Wales, Cyprus, Kenya, and Western Australia occur in the literature. In North America *A. pisi* has been reported throughout United States and Canada.

Investigations on the diseases of canning peas in United States by Jones and Linford in 1925 showed that Ascochyta blight caused the most serious loss to the industry. Jones claimed in 1927 that the Mycosphaerella blight was more destructive in New York State than Ascochyta blight.

An examination of twenty-nine samples of English pea seed by Hickman in 1940 revealed twenty-six infected with A. pisi, eight to the extent of twenty per cent. Of forty-three foreign varieties examined by him only seventeen were infected none to the extent of twenty per cent and only a few over ten per cent. Western examined pea seed grown in England from 1925 to 1943: he stated that the presence of A. pisi in the seed accounted for the rejection of twenty-three per cent of all pea seed examined. Walker and Hare in 1942 stated that over 40% of all fields examined in Wisconsin showed infection with A. pisi.

The disease is known in Holland and France as anthracnose or ascochytose.

B. Etiology of Ascochyta Blights

An organism associated with the disease known as Ascochyta blight of peas was first described as Ascochyta

pisi by Madame Libert in 1830. The disease however had been known centuries before according to Comes (1891). Link in 1833 and Berkeley (1841 and 1860) gave further reports on the disease. The description of Ascochyta pisi given by Saccardo in 1884 agrees with that of Madame Libert and is regarded as the typical species.

Stone in 1912 and Vaughan in 1913 considered Mycosphaerella pinodes (Berk. and Blox.) to be the ascigerous stage of Ascochyta pisi. Jones 1927 has pointed out that the disease caused by Mycosphaerella pinodes differs markedly from that caused by Ascochyta pisi and that these two organisms have been confused in the literature. Linford and Sprague 1927 substantiate this and give technical descriptions to separate M. pinodes from A. pisi.

Jones (1927) separated Ascochyta blight into three separate diseases and proposes the following terminology: Ascochyta leaf and pod spot Ascochyta pisi Lib., Mycosphaerella blight Mycosphaerella pinodes (Berk. and Blox.) Stone and Ascochyta footrot caused by Ascochyta pinodella Jones.

III. SYMPTOMATOLOGY OF ASCOCHYTA BLIGHTS

The symptoms caused by Ascochyta pisi are definite sunken tan to brown spots circular on leaves and pods and elongate on stems and petioles. A dark brown margin usually surrounds the tan spot. Jones in 1927 stated that stem lesions are quite rare and never noticed below the soil line. In the centre of the old lesions numerous amber to brown pycnidia are developed.

The spots caused by Ascochyta pisi develop on the lower leaves and the young stems. The stems may become completely girdled and plants affected usually die.

The symptoms of the disease caused by M. pinodes develop as irregular purplish-brown spots with no definite margins on the foliage and pods. In the presence of moisture for prolonged periods the lesions become larger, circular and zonate. Small purple spots may enlarge to purple areas or coalesce. On stems, symptoms occur as streaks which enlarge into purple irregular areas on the entire stem.

The symptoms caused by Ascochyta pinodella develop on the leaves and foot of the plant. The spots on the pods are irregular to circular not definitely margined, sometimes zonate and dark brown. On the stems and upper taproot the spots are small to extensive, purplish-brown to dark brown which are especially pronounced on the stems at the nodes.

IV. EXPERIMENTAL WORK

A. Biology of Ascochyta Seed-Borne Infections

1. Longevity of Ascochyta pisi in pea seed

A. pisi in seed of the hairy vetch (Vicia villosa) has been shown by Crosier 1939 to live up to nine years although the average longevity of the organism in the seed is around three years. An effort was made to determine whether A. pisi dies out in infected pea seed before the seed itself loses its viability.

If seed infected with A. pisi could be stored for a number of years and the infection be reduced by storage to 2% A. pisi or less, then the seed could be used for planting providing the germination of the seed was still high. At the present time seed having more than 2% A. pisi is rejected for planting purposes.

Infected seed was secured from the Seed-Borne Disease Laboratory at Ottawa. This seed was sent in by farmers throughout the Ottawa district to be tested for the presence of pathogenic and non-pathogenic fungi. The seed was tested upon arrival and the percentage of A. pisi was noted. The seed was then stored and tested every year for three years to find out if the pathogen was still viable in the seed.

Twenty plates of pea agar, consisting of 20 grams of agar, 120 grams of peas and 1000 grams of water, were used to test each sample. Five peas were planted on each petri plate making a total of 100 peas for each sample. Twelve samples of one, two and three year old seed were tested for the presence of A. pisi in the seed. A test was run on one particular sample for four consecutive years. The plates were held for ten days at room temperature. At the end of this time the seed infected with A. pisi could be determined as the fungus grows out readily from the seed and fruits on the medium.

The results of this experiment are shown in Table 1 and graphically in Figure A. Table 1 shows that the average overall percentage of A. pisi in the seed when it was originally tested was 34 and 48.25 in the two lots.

The average overall percentage of A. pisi in the one year old seed was 17.58 as compared to 34 when the seed was originally tested.

The average overall percentage of A. pisi in the two year old seed was 35.5 as compared to 48.25 when the seed was originally tested.

The average overall percentage of A. pisi in the three year old seed was 29.27 as compared to 48.25 when the seed was originally tested.

TABLE 1

Effect of Storage of Infected Seed on the Viability of *A. pisi*.

Variety	Sample no.	Year of Production	Percentage of seed from which *A. pisi* grew out. 1946	1947	1948
Chancellor	1		27	22	
"	12		27	24	
"	10		32	13	
"	9		33	23	
"	17		37	15	
"	22		34	18	
"	23		35	28	
"	30		34	14	
"	32		49	25	
"	34		35	6	
"	40		33	17	
		Average	34	Average 17.58	
Arthur	271		48	-	27 22
"	270		68	-	51 29
"	250		56	-	25 21
"	268		42	-	32 32
"	823		42	-	39 39
Br. Lion	811		44	-	39 32
Arthur	1031		52	-	25 24
"	1033		60	-	69 53
"	260		41	-	25 36
"	267		38	-	36 28
"	273		44	-	34 18
"	268		44	-	25 23
		Average	48.25		35.5 29.27

Results of this experiment indicate that the percentage of A. pisi in pea seed after three years of storage was 60% of what it was when originally tested.

Storing pea seed heavily infected with A. pisi for a period of three years or less is of no avail as the percentage of the organism in the seed is too high for the seed to be used for planting purposes.

Tests were run on the seed sample number 77 from 1941 when the seed was harvested until 1945 when the seed was four years old for the presence of A. pisi in the seed. From Table 2 it can be seen that the percentage of A. pisi in the seed when first harvested was 54. The next two years this particular sample contained the same percentage of A. pisi. At the end of three years the percentage of A. pisi had dropped to 24.3 and at the end of the fourth year the percentage had dropped to 13.

In most cases holding seed over induced greater germination in the following years. From Table 2 it can be seen that the germination over the three year period from 1941 to 1944 was still at a very high level. The germination dropped however at the end of the fourth year almost 10% over the original germination of 90%.

It would appear from these results that in spite of the large decrease of A. pisi in the seed over the four years, it is not enough to warrant the use of the seed for planting purposes.

TABLE 2

Effect of Storage of Infected Seed on the Viability of *A. pisi*.

Year	Plate Tests	
	% germination	% *A. pisi*
1941	90	54
1942	92	54
1943	92	54
1944	92.5	24.3
1945	80.5	13.0

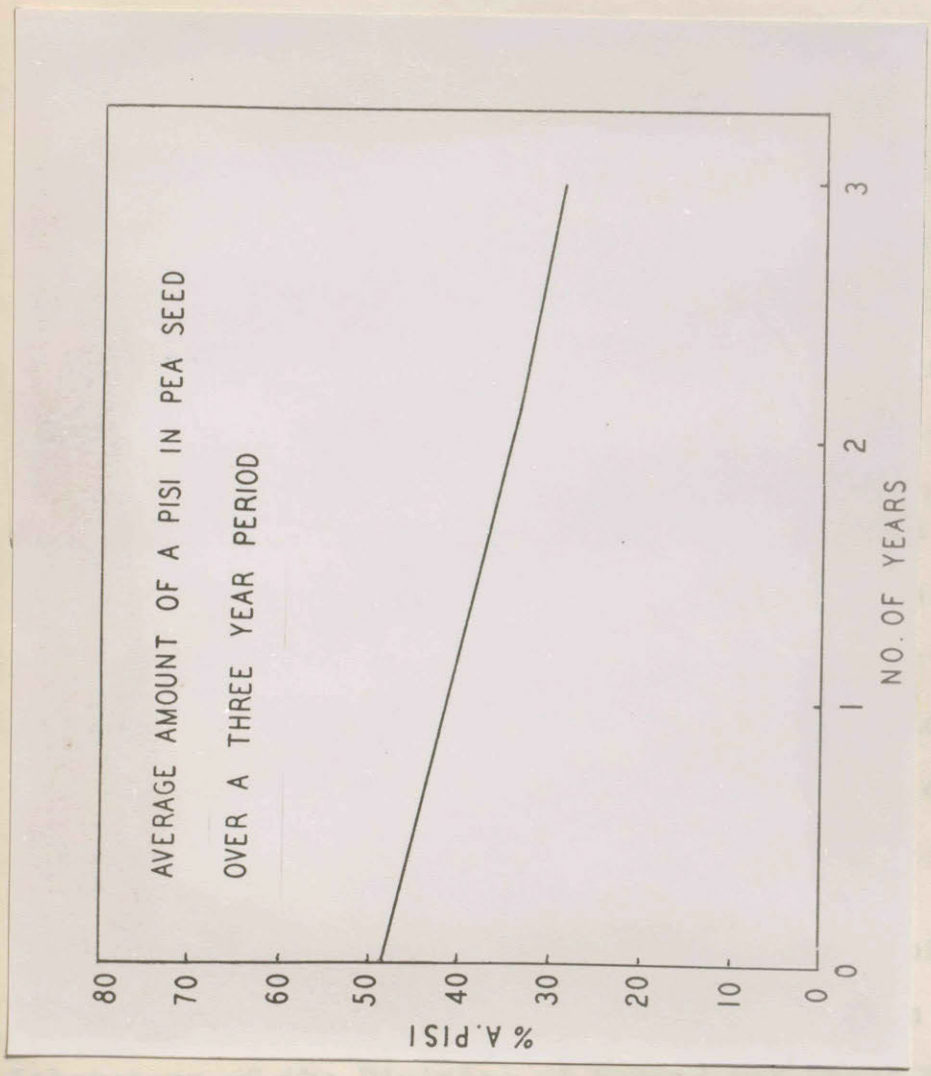


FIGURE A - Graph showing the average percentage of seed from which A. pisi grew out in the year of the production of the seed and after one, two and three years of storage.

2. Shoot infection and its significance

The disease caused by A. pisi is seed-borne and it may cause extensive damage to the young shoots when infected seed is used for planting.

While examining pea seed in the laboratory at Ottawa it has been noticed that the seed infected with A. pisi Lib. might either not germinate at all or on the other hand produce healthy looking plants. (Plate 1) As this plate test ran for a period of ten days only, the healthy looking plants might have developed during their later growth symptoms of the disease caused by A. pisi.

The purpose of this experiment was to find out if the young shoots became infected while still within the seed and carried local infections thus established above the soil line. In addition the test was run to determine the loss in the number of seedlings which died after emergence due to planting infected seed.

In order to attack this problem a sample of pea seed heavily infected with A. pisi was obtained from the Central Laboratory of the Division of Botany and Plant Pathology in Ottawa. This sample was grown in 1947 and was known to contain the following fungi.

Ascochyta pisi Lib. in 49% of the seed.

Ascochyta pinodes (Berk. and Blox.) Stone in 5% of the seed.

Ascochyta pinodella Jones in 5% of the seed.

Alternaria tenuis auct. in 15% of the seed.

Stemphylium botryosum Wallr. in 1% of the seed.

Penicillium sp. in 1% of the seed.

Fusarium spp. in 2% of the seed.

Sterile mycelium in 1% of the seed.

Six flats of steam sterilized soil were taken and two hundred seeds of the above sample were sown in each. The flats were kept watered and all the germinating seeds and seedlings were removed from one flat at the end of three, five, seven, fourteen, twenty-one and twenty-eight days.

The young seeds and seedlings were then washed in sterile water, treated in a 2% solution of chlorine and finally rinsed again in two washes of sterile water. The seedlings were then planted on pea agar in petri plates. These plates were examined after ten days for the number of seedlings from which A. pisi grew out on the media.

The results of this experiment are given in Table 3 and Figure B. From the Table it can be seen that the total number of seedlings declined as the seedlings grew older. When the young seedlings were dug up after three days, there were 180

TABLE 3

The number of seedlings from which A. pisi grew out on pea agar.

Age of Seedlings	No. of Seedlings	No. bearing A. pisi	No. free of A. pisi
3 days	180	38	142
5 days	179	30	149
7 days	152	15	137
14 days	136	17	119
21 days	115	14	101
28 days	108	5	103

out of the 200 seeds originally planted. After twenty-eight days only 108 seedlings could be accounted for from the 200 seeds which were planted. This reduction in the number of emerged seedlings was due to severe lesions due to A. pisi which developed on the stems and leaves. As each infection developed, the lesions became larger resulting in stem girdling and complete dieback of the young plants.

At the same time observations were made with regard to disease development. The symptom expression on diseased seedlings at time of removal from the flats showed much variation; young seedlings infected with A. pisi did not in most cases show lesions in the three- and five-day plantings, however, some of the five- and seven-day old seedlings showed a brownish discoloration of the leaves and a tendency for the leaves to wither and die. Small brown discolorations were noticed on the young stems. The two-, three- and four-week old seedlings showed large sunken elliptical lesions on the stems. Clear, regular zonate lesions, tan to brown in color were noticed on the first true leaves. The seedlings having these lesions when planted on pea agar produced a typical A. pisi growth.

All the lesions on the young leaves and shoots appeared to be localized. This would seem to suggest that these infections had been due to pieces of mycelium being carried above the soil by the growth of the young seedling.

The symptom expression on diseased seed was also observed and an effort was made to correlate this with the health condition of the seedlings produced by infected seed.

Slightly infected seeds showed small brown pin-point lesions on the seed coat but none on the cotyledons and they usually gave rise to healthy plants.

Moderately infected seeds are not readily recognizable when dry but generally show slight to extensive, sunken to flush, firm or spongy, slightly discolored to light brown lesions when wet. These seeds have lesions on the cotyledons and seed coat and appeared to be the ones responsible for infected seedlings. On examining the fleshy part of the cotyledons a reddish ring of diseased tissue is usually noticed. This ring usually extends around the cotyledons and could come in contact with the plumule or stem. In the case of these the young shoot comes in contact with diseased cotyledons. Infection might conceivably occur at any point along the entire length of the shoot. In the cases noticed lesions occurred at all points above the ground level. No lesions were noticed below this point.

Heavily infected seeds showed extensive brown to black lesions on the seed coat and cotyledons which may cover the entire seed. Deep seated infections usually result in the

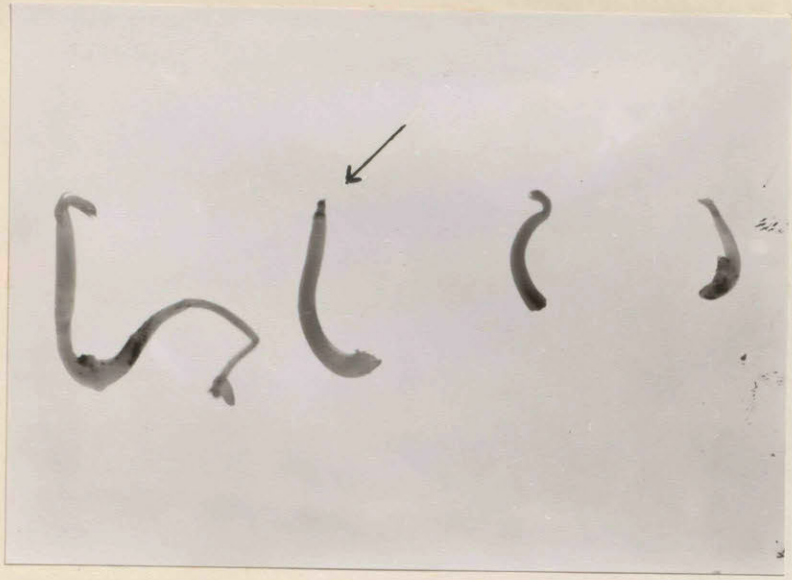
killing of the young shoots and no seedlings emerge from these seeds.

The above observations would seem to justify the following conclusions as to how the fungus gets from the cotyledons into the young shoot. As the young shoot grows within the seed, it may come in contact with a diseased part of the cotyledon. The fungus may grow out of the diseased cotyledonary tissue into the stem or plumule establishing infections on these parts. Infection usually starts while the developing shoot is still within the seed. The sprouts of young seedlings dissected from the seed five days after sowing show small brown to black water soaked spots which later develop into typical Ascochyta spots. The young shoot breaks out of the seed coat and carries the infection with it.

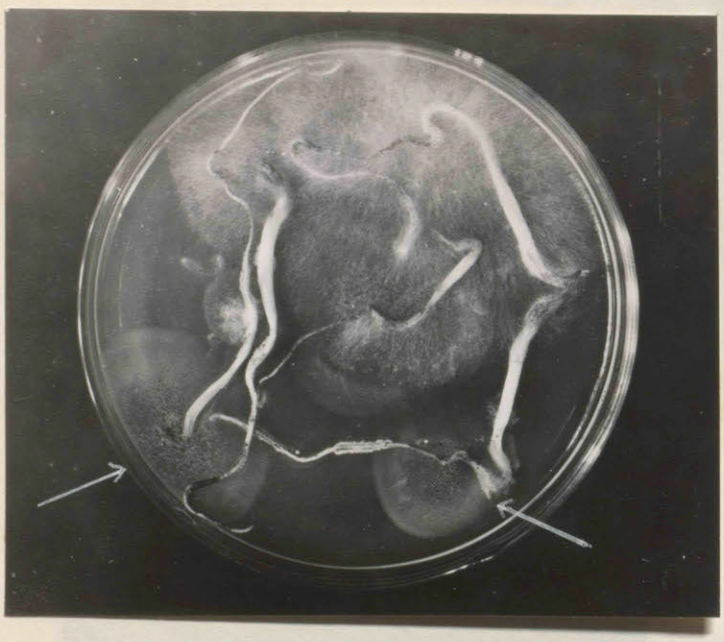
No secondary lesions were produced under greenhouse conditions. However, these primary lesions occurring on the stem and first true leaves would probably constitute the source of inoculum for secondary spread.

A series of photographs were taken showing the course of infection from the diseased seed to a twenty-eight-day-old plant.

PLATE I - This photograph shows A. pisi colonies growing out from infected seeds planted on pea agar. Note the three seeds which have germinated producing healthy looking plants and the one seed which has not produced any seedling at all.

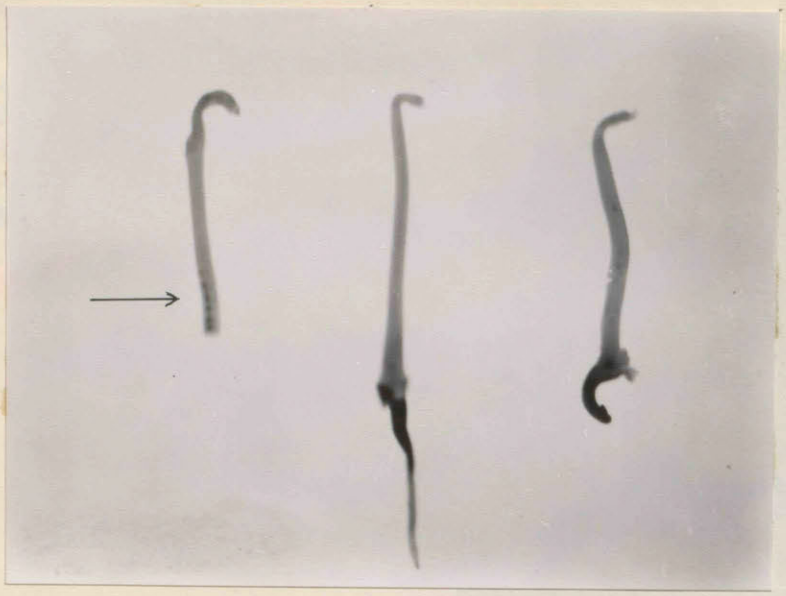


A - Photograph showing seedlings infected with A. pisi which were removed from a flat five days after planting.

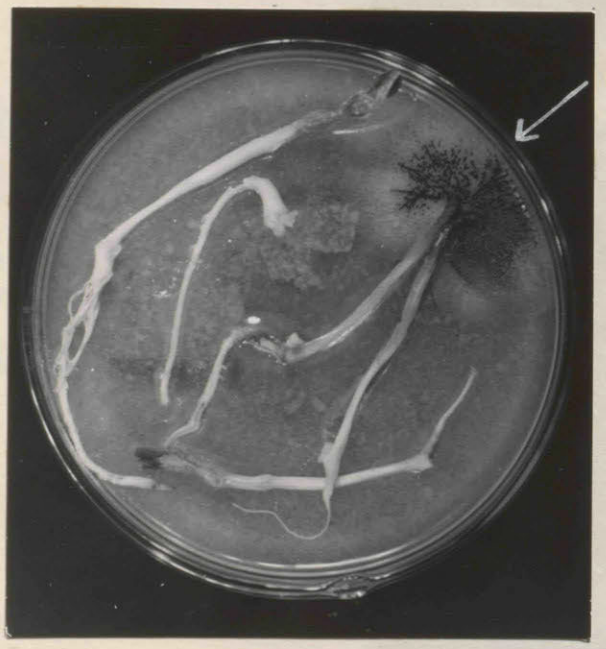


B - Photograph of the same seedlings as above after being planted on pea agar. Note the colony of A. pisi growing out from each of the seedlings.

PLATE II



C - Photograph showing seedlings infected with A. pisi which were removed from a flat seven days after planting.



D - Photograph of the same seedlings as above after being planted on pea agar. Note the colony of A. pisi growing out from two of the seedlings.

PLATE III

E - Photograph showing seedlings infected with A. pisi which were removed from a flat two weeks after planting.

F - Photograph of the seedling in the middle above when planted on pea agar. Note the typical colony of A. pisi growing out from the lesion.

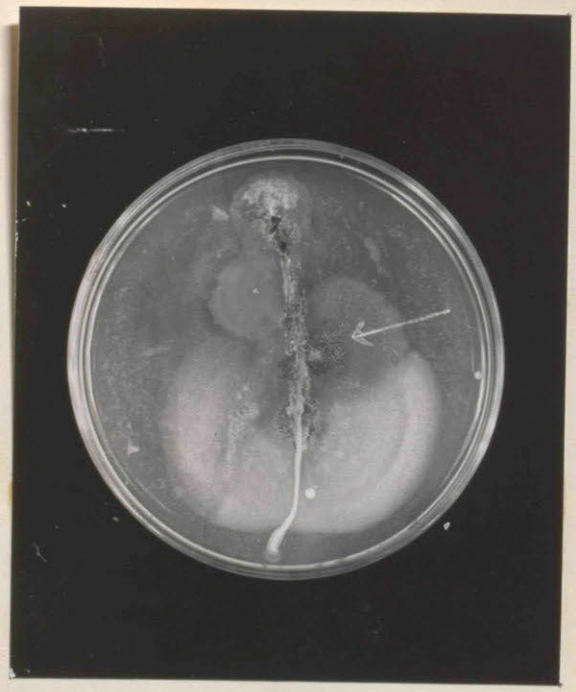


PLATE IV

G - Photograph showing seedlings infected with A. pisi which were removed from a flat three weeks after planting.

H - Photograph of the largest seedling above when planted on pea agar. Note the typical colony of A. pisi growing out from the stem lesion.

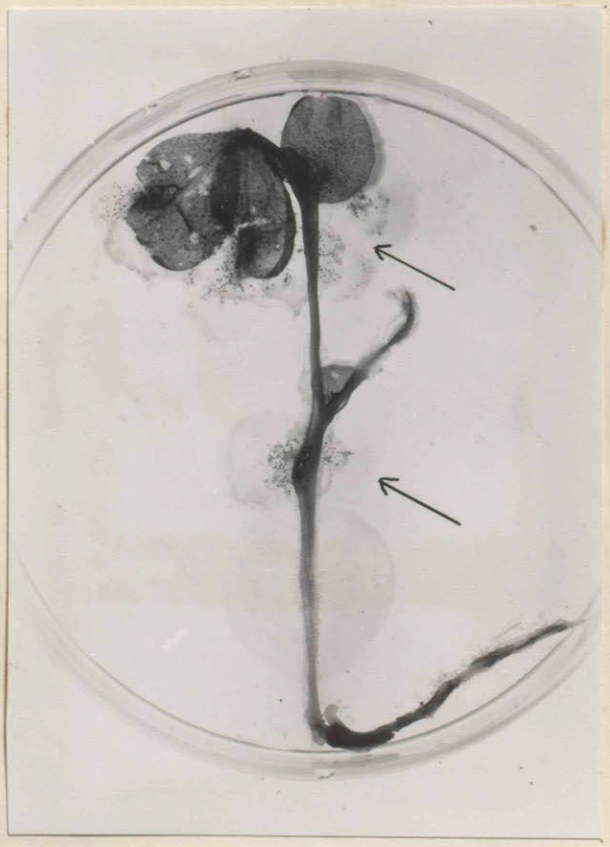
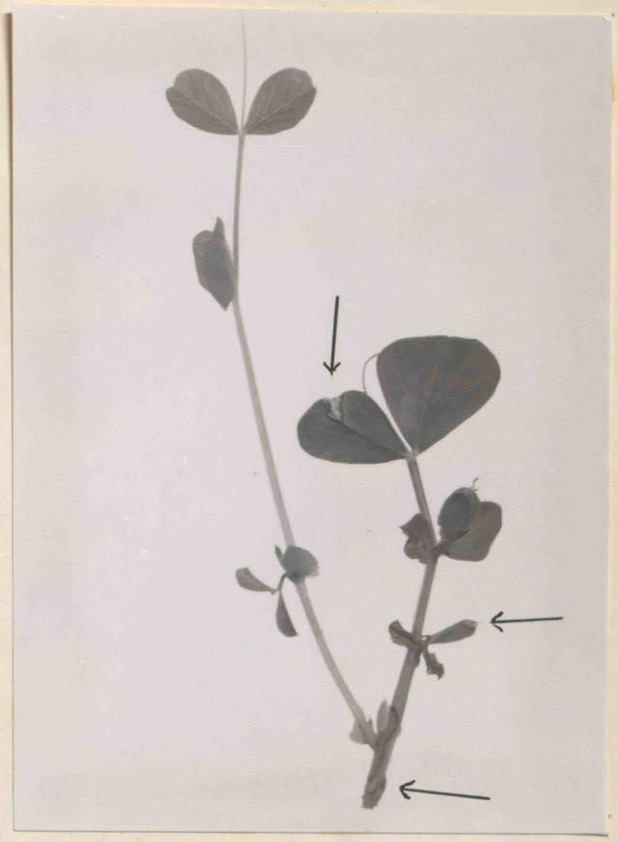


PLATE V

I - Photograph showing seedling infected with A. pisi which were removed from a flat four weeks after planting.



J - Photograph of the seedling above when planted on pea agar. Note the colonies of A. pisi growing out from both leaf and stem lesions.

PLATE VI

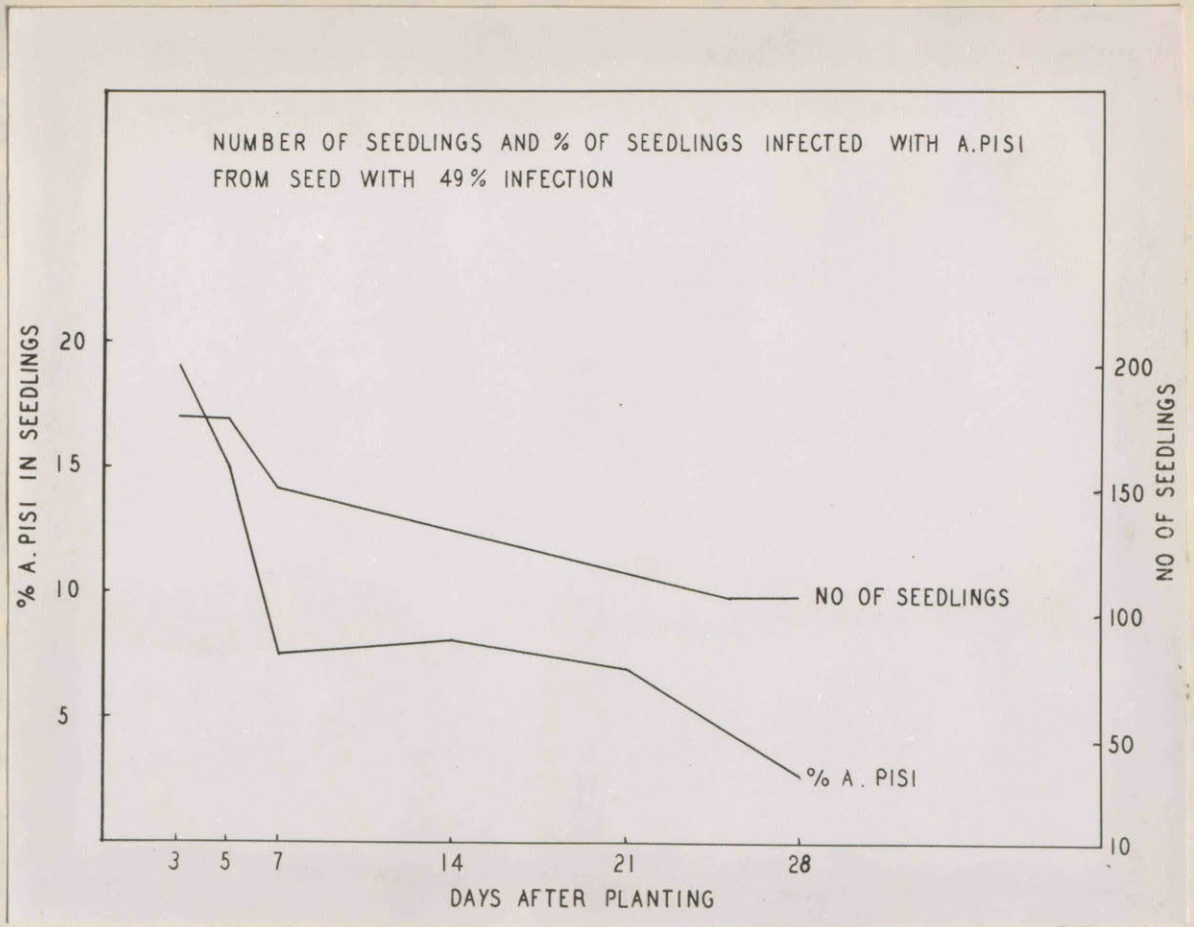


FIGURE B - Number of seedlings at various days after planting two hundred pea seeds 49% of which was infected with A. pisi, and percentage of the seedlings infected with A. pisi.

3. Effect of temperature upon growth of A. pisi and infection

In conjunction with a test determining the influence of temperature upon infection by A. pisi, a similar test was run determining the influence of temperature upon the growth of the fungus.

The fungus was first isolated from diseased pea seed planted on pea agar. The seed was secured from the Central Laboratory of the Division of Botany and Plant Pathology Ottawa, and was known to be heavily infected with A. pisi.

The pea seed was first treated with a 2% solution of chlorine and then fifty seeds were planted on the pea agar described previously, in petri plates, five seeds per plate. A. pisi grew out onto the agar from the seeds infected with it. The fungus was then transferred onto separate petri plates containing pea agar by means of an inoculating needle. The mycelium was then allowed to grow sufficiently to produce enough inoculum for the experiment.

A sterile cork borer with a four mm. bore was used to punch out small discs of mycelium from the petri plate cultures. The cork borer was used in order to punch out discs which would be equal in size. The discs were removed from the petri plate by means of an inoculating needle and then the discs were inverted onto separate petri plates. Acidified pea agar was

used in this case in order to keep down the growth of contaminating bacteria.

The spread of the colonies was measured daily for a ten-day period. Nine cabinets with a temperature range from ten to thirty-six degrees Centigrade were used to hold the plates, four plates to a cabinet.

The results of this experiment are shown in Table 4, Plate VII and Figure C. From these it can be seen that the optimum temperature for the spread of the fungus occurred at twenty-four degrees Centigrade. The minimum temperature for the spread was between ten and fourteen degrees Centigrade, while the maximum temperature for the spread of the fungus occurred between thirty and thirty-six degrees Centigrade. The growth of all the colonies was slower on the acidified agar as can be seen by comparing the growth rate of A. pisi on ordinary pea agar. (Section 4)

At the same time as the above test was being run a similar test was run to determine the influence of temperature on infection by A. pisi on young pea leaves of pea plants growing in the greenhouse.

The young leaves were inoculated by spraying with a pycnidial spore suspension made from cultures of the organism grown on agar slants in test tubes. The spore suspension was

TABLE 4

Diameter in millimeters of colonies of A. pisi in plate culture held at various temperatures over a ten-day period.

Temperature	Plate No.	1	2	3	4	5	6	7	8	9	10
10°C	1	0	0	0	0	0	0	0	0.25	1.00	2.00
	2	0	0	0	0	0	0	0	0.25	2.0	2.25
	3	0	0	0	0	0	0	0	0.25	2.0	2.25
	4	0	0	0	0	0	0	0	0.25	2.0	2.25
14°C	1	0	0	0	0.25	2.0	2.0	4.0	4.5	5.25	6.0
	2	0	0	0	0.25	1.0	2.0	4.0	4.0	4.25	5.5
	3	0	0	0	0.25	1.0	2.0	4.00	4.5	7.5	7.75
	4	0	0	0	0.25	1.5	3.0	3.5	3.75	4.0	4.75
17°C	1	0	0.25	1.0	1.25	3.0	4.0	6.0	6.75	7.5	8.5
	2	0	0.25	1.0	1.25	2.5	3.0	4.0	4.25	4.5	4.5
	3	0	0.25	1.0	1.25	3.0	3.25	4.5	5.0	5.5	6.0
	4	0	0.25	1.0	1.25	2.5	3.5	5.0	6.0	7.25	8.25
19°C	1	0	1.0	2.5	3.0	3.5	4.5	8.0	8.5	10.5	12.5
	2	0	1.0	3.0	3.0	3.75	6.0	8.0	9.0	10.25	11.25
	3	0	0.5	2.5	3.0	4.5	6.0	8.0	10.0	12.00	14.00
	4	0	2.0	2.25	2.25	3.0	5.0	7.5	9.25	12.75	12.75
21°C	1	0	1.5	2.0	3.0	4.0	5.0	7.0	8.75	10.0	11.25
	2	0	1.75	2.0	2.25	2.75	3.5	5.5	7.5	9.0	12.0
	3	0	1.0	2.0	3.5	3.5	7.0	9.0	10.25	12.0	14.0
	4	0	0.75	2.25	3.5	5.0	7.0	9.0	10.75	12.5	14.0
24°C	1	0.25	2.0	3.5	5.5	7.5	10.0	13.0	15.25	17.5	20.75
	2	0.25	2.0	3.0	6.0	6.0	7.5	9.0	11.0	13.0	15.25
	3	0.25	2.0	2.5	6.0	10.0	12.25	14.0	10.25	21.0	23.0
	4	0.25	2.0	3.25	5.25	6.0	7.25	9.5	10.75	12.0	13.5
26°C	1	0.0	2.0	3.5	5.5	7.0	8.75	9.5	10.75	12.25	13.75
	2	0.0	2.0	3.5	4.5	7.25	9.0	10.0	11.5	13.0	15.25
	3	0.0	1.75	3.5	4.0	6.0	8.0	8.5	10.25	12.0	14.0
	4	0.0	1.75	2.5	4.5	5.75	7.5	8.75	11.25	12.5	14.75
30°C	1	0.0	0.25	1.5	1.5	1.25	1.5	1.5	2.0	2.5	3.25
	2	0.0	0.25	0.25	0.25	0.25	1.5	1.5	3.0	4.0	5.25
	3	0.0	0.25	0.25	0.25	0.25	0.25	0.25	1.5	1.5	2.25
	4	0.0	0.25	0.25	0.25	0.25	.50	0.75	1.5	1.75	2.5
36°C	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

prepared by pouring ten ml. of distilled water into each test tube containing the fungus. Then the fungus was scraped from the agar with a sterile needle and mixed with the distilled water. The leaves were then sprayed with the suspension, by means of an atomizer and placed upon filter papers in petri plates which were held in the temperature chambers mentioned above, providing a range of temperatures from ten to thirty-six degrees Centigrade. Although conditions seemed ideal for infection to take place none was obtained.

As an alternative method the leaves were inoculated with mycelium by first injuring the leaf and then implanting the mycelium of the fungus in the leaf. This was done by first rubbing the leaves gently with fine carborundum paper; then the mycelium was transferred from the petri plate cultures prepared in the previous test by means of an inoculating needle to the injured leaf. The mycelium was then moved across the surface of the leaf allowing small pieces of the mycelium to settle on the injured parts of the leaf. The inoculated leaves were then placed on filter papers in petri plates and held in the cabinets used in the previous test.

The results of this test are shown in Table 5 and Plate VIII. From the Table it can be seen that the optimum temperature for the growth of A. pisi in culture media is the same as its optimum temperature for infection, both occurring at 24 degrees Centigrade.

TABLE 5

Time for lesions to show and the size of the lesion developed when held at various temperatures over a ten-day period.

Temperature	Time for lesion to show	Size of lesion
36°C	No definite lesion only slight discoloration	
30°C	No definite lesion only slight discoloration	
26°C	8 days	medium
24°C	6 days	large
21°C	7 days	medium
19°C	7 days	small
17°C	8 days	small
14°C	9 days	small

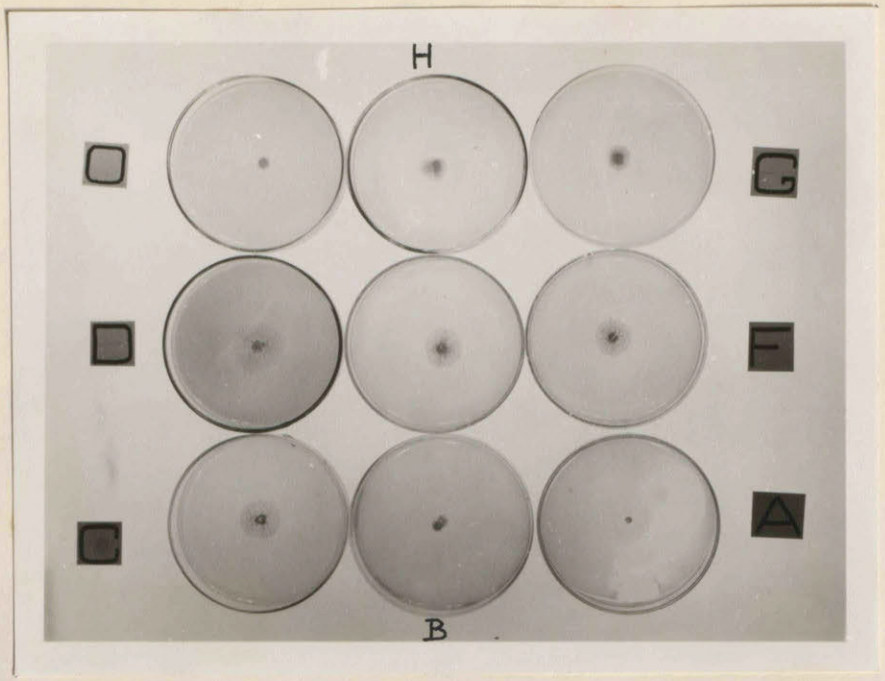


PLATE VII - Growth of A. pisi on acidified pea agar, at different temperatures at the end of ten days.

A - 10 degrees Centigrade	D - 24 degrees Centigrade	G - 17 degrees Centigrade
B - 14 " "	E - 26 " "	H - 30 " "
C - 19 " "	F - 21 " "	O - 36 " "

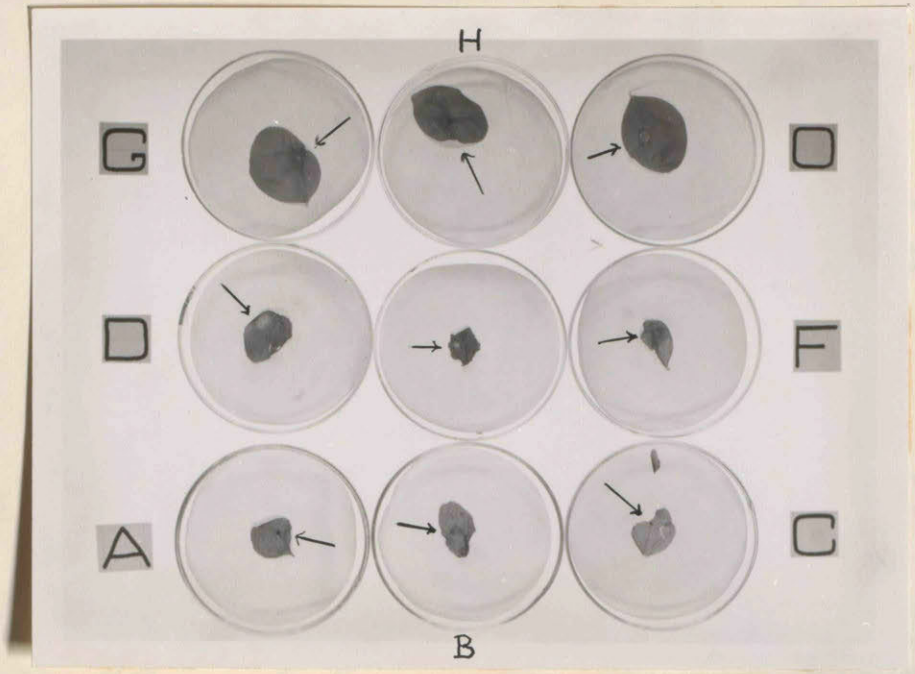


PLATE VIII - Photograph showing the size of leaf lesions produced on pea leaves at various temperatures, ten days after being inoculated with A. pisi.

A - 36 degrees Centigrade	D - 24 degrees Centigrade	G - 17 degrees Centigrade
B - 30 " "	E - 21 " "	H - 14 " "
C - 26 " "	F - 19 " "	O - 10 " "

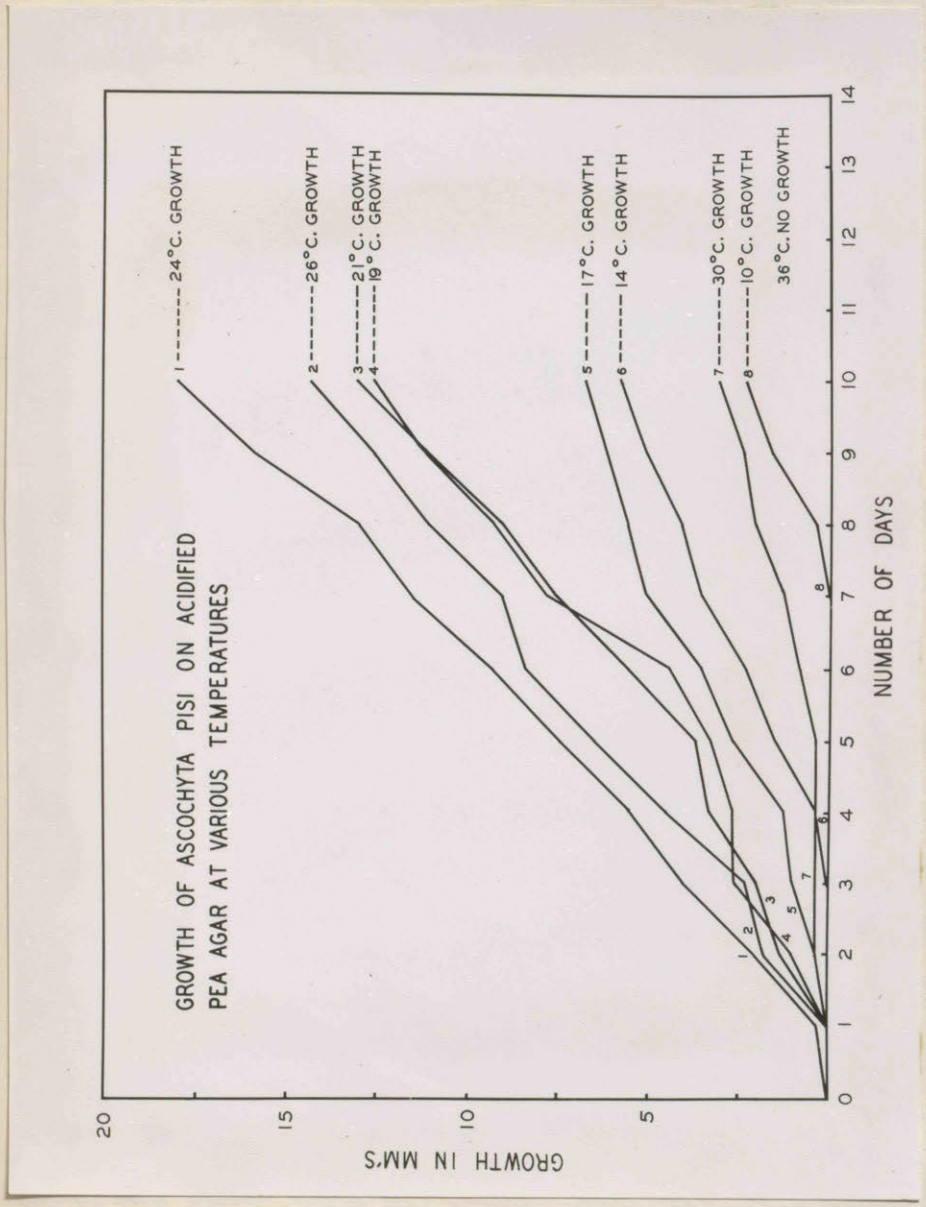


FIGURE C - Diameter of colonies of A. pisi grown on acidified pea agar at various temperatures during a ten-day period.

4. Comparison of growth rates of A. pisi, A. pinodella and A. pinodes.

It has been noticed in tests run on pea seed samples that only in one case have two of the Ascochytas been found in the same seed. In this connection a test was run to determine if the growth rates of the three organisms differed. If the growth rate of one organism is considerably greater than another then the possibility exists that it might be dominant in situations where there is competition between the two. Since the three organisms A. pisi, A. pinodella and A. pinodes all live within the seed, then this competition would likely occur in seeds infected with two or three of the organisms.

Stock cultures of A. pisi, A. pinodella and A. pinodes were secured from the Central Laboratory of the Division of Botany and Plant Pathology at Ottawa. Transfers of the cultures were made onto pea agar in petri plates and after a substantial growth had been obtained a four mm. cork borer was used to punch out small discs of equal size of the three fungi. These discs were then taken by means of an inoculating needle and inverted onto separate petri plates. Four petri plates of each fungus were made up and stored in a constant temperature chamber of 21 degrees Centigrade.

The spread of the colonies on the agar was measured daily for ten days.

The results of this experiment are shown in Table 6, Figure D and Plate IX. As seen from the Table the daily growth rate of each organism was constant. A. pinodella showed the greatest growth over the ten-day period, a spread of over seventy-five millimeters. A. pinodes had a growth rate of over forty-eight millimeters and A. pisi a growth of over forty-one millimeters.

A. pinodella appears to be the fungus with the greatest growth rate and possibly in pea seed infected with all three organisms A. pinodella might dominate over A. pisi and A. pinodes.

TABLE 6

Diameter in mm. of A. pisi, A. pinodes and A. pinodella in plate culture held at 21 degrees Centigrade over a ten-day period.

Organism	Plate No.	Growth per day									
		1	2	3	4	5	6	7	8	9	10
A. pisi	1	0.25	1.25	6.0	12	16.5	20	23.5	26	29.5	32.5
	2	0.25	1.5	5.5	14.5	18.5	26	32.5	37	42	48.5
	3	0.25	1.5	8.0	13.5	19.5	22	28.5	34	38	42.5
	4	0.25	1.5	11.0	15.5	20.5	24	30	35	39.5	43.0
	Average	0.25	1.5	8.5	14.37	18.75	23	28.87	34	37.25	41.62
A. pinodella	1	5.0	13	23	33	39	44	54	61	66.5	74.5
	2	2.5	13.5	19	31	39	46	54	61	69.5	77
	3	3.5	11	24	28	37.5	44	50	56	64	73
	4	0.5	12	24	32	40.5	46	54	61.5	68	76
	Average	2.87	12.37	22.5	31.0	39	45	53	59.37	67	75.25
A. pinodes	1	1.0	7	13	20	25	30.5	37.5	47	47	51
	2	1	6.5	9.5	14	17	22.5	29.5	36	36.5	39.5
	3	1	6.5	11.5	19	24.5	29.5	34	40	45.5	51
	4	2.0	5	12	18	24	29.5	35	42	47	52
	Average	1.25	6.25	11.5	17.75	22.62	28	34	41.25	44	48.37

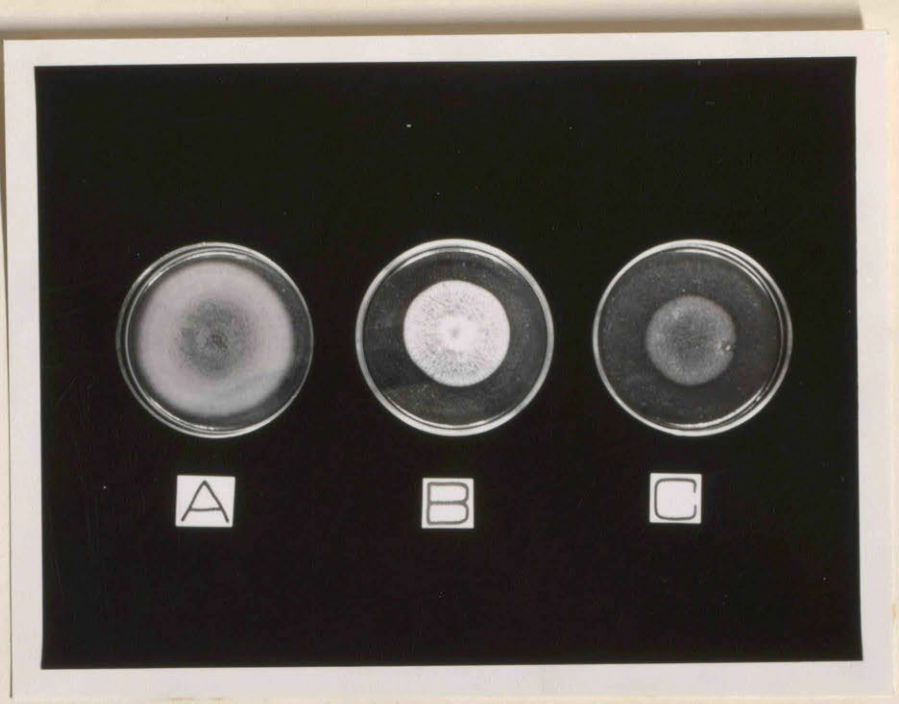


PLATE IX - Photograph showing the spread of colonies of A - A. pinodella, B - A. pinodes and C - A. pisi grown on pea agar and maintained at room temperature 21 - 24 degrees Centigrade for ten days.

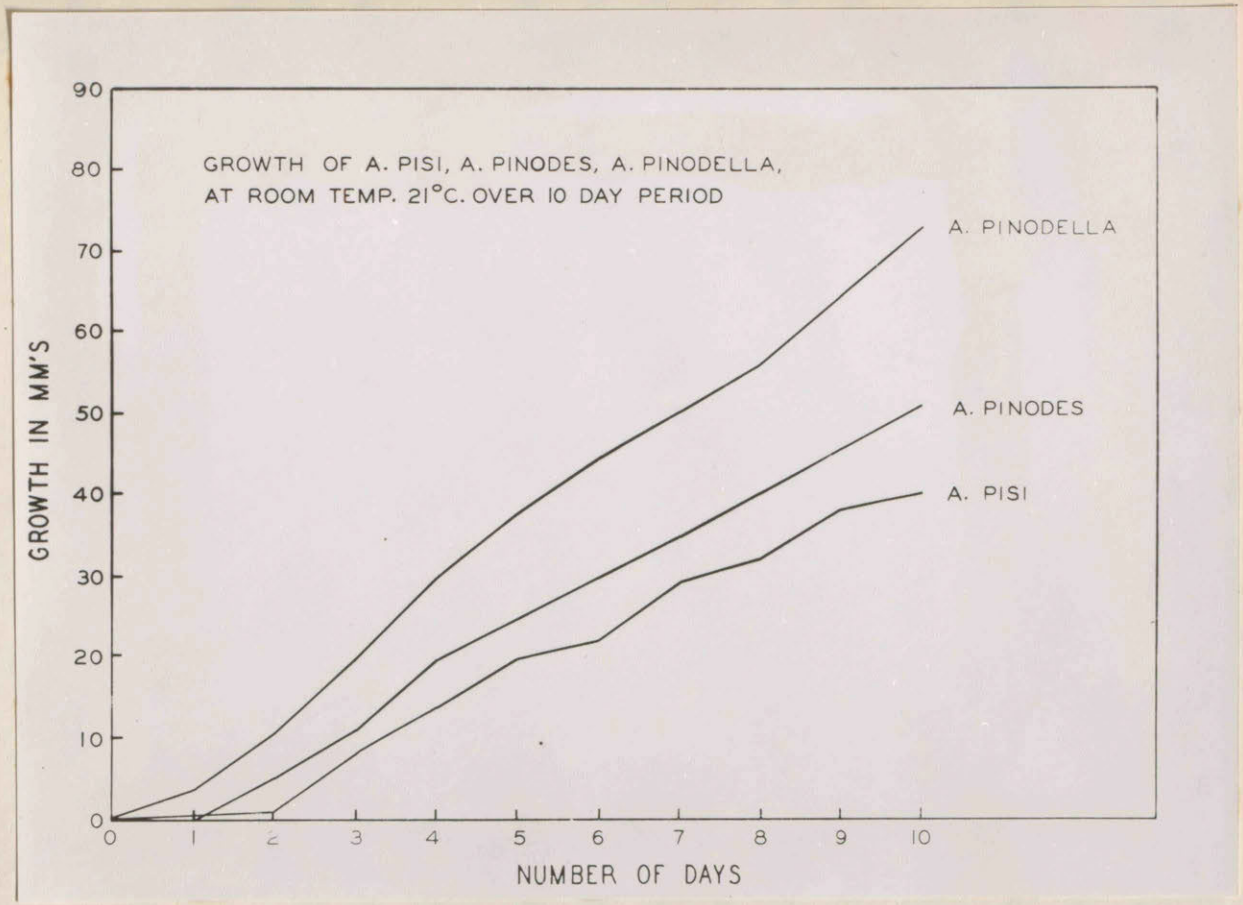


FIGURE D - Spread of colonies of A. pisi, A. pinodes and A. pinodella on pea agar grown at 21°C. over a ten-day period.

5. Associative effects of A. pisi, A. pinodella and A. pinodes

Since Pasteur first profounded in 1863 the principle of microbic association as applied to the coexistence of aerobic and anaerobic organisms in nature, numerous investigations have been made on microbial associations.

The study of the mutualistic influences of microorganisms, usually bacteria and fungi has been carried out largely upon artificial substrates; a pure culture of an organism being inoculated upon an artificial medium, another organism added and the specific effect determined. In the case of fungi, the germination of spores and the development of mycelium were usually used as criteria for the specific action.

Raulin (1869) demonstrated that in a culture during the course of development of a fungus changes are brought about which make conditions more favourable for the growth of the fungus.

Kuster (1909) showed that fungi produce growth promoting as well as growth inhibiting substances.

Fulton (1908) suggested that the tendency of fungal hyphae to turn from the region in which hyphae of the same kind were growing is due to a vegetative reaction to chemical substances

produced by the growing fungus.

De Bary (1879) was the first to emphasize the significance of antagonistic relationships of microorganisms. When two organisms were grown on the same substrate one was found sooner or later to overcome the other and kill it.

Waksman (1931) collected data on the work done by several authors and concluded that the metabolic products of one microorganism may be either beneficial and stimulating, injurious and destructive or indifferent to other organisms. Some of the products of metabolism of one organism may actually be used as nutrients by another, as in the case of nitrites produced in the oxidation of ammonium salts by one bacterium and used as a source of energy by another. Some microorganisms may produce a change in the medium, in the oxygen tension and concentration of nutrients which may be favourable to some organisms and unfavourable to others. A knowledge of the conditions influencing the growth of microorganisms, the rate of growth and changes produced in the substrate, and the mutual interrelations between different organisms will greatly assist in interpreting the role of the numerous organisms in associative processes.

The following experiment was conducted in order to find out if any associative effects exist among the three Ascochytas,

Ascochyta pisi, Ascochyta pinodes and Ascochyta pinodella.

As a greater percentage of A. pisi is found in pea seed than A. pinodes and A. pinodella it is logical to think that A. pisi might be antagonistic toward the other two fungi. However in a previous test (Section 4), it was shown that A. pinodella produced a greater spread on pea agar, in the same time than A. pinodes and A. pisi. From this one might expect higher percentages of A. pinodella than of the other two organisms in the pea seed, but this is not the case.

In order to explore this problem, three sets of petri plate cultures consisting of all possible paired combinations of the three fungi were made up. Each set consisted of four petri plates with fifteen ml. of pea agar in each petri plate.

The plates were inoculated in the following manner. A sterile cork borer with a four mm. bore was used to punch out small discs of mycelium from cultures of the organisms. The discs were removed from the culture plates by means of an inoculating needle. These discs were then inverted onto the petri plates in the proper combinations of the three fungi. The small discs were placed approximately in the centre of the petri plates ten mm. apart.

The plates were observed daily for any antagonistic or synergistic action as the separate colonies grew towards each

other. The production of fruiting bodies, the viability of the spores present in the fruiting bodies and the growth of the colonies were the deciding factors.

The results of this experiment are shown in Tables 7 and 8. A comparison of the data in Table 7 and Table 6 indicates that the spread of the three organisms was approximately the same in association with one another as when they were grown singly. The results given in Table 8 indicate that the three fungi had no effect upon each other so far as fruiting and viability of spores is concerned.

From observation of Plates X, XI and XII, it can be seen that no line of demarcation existed between any of the three combinations of A. pisi, A. pinodes and A. pinodella.

The above results would seem to indicate that in pea seed containing two or all of these organisms A. pisi, A. pinodella, and A. pinodes no synergistic or antagonistic action among these organisms would occur.

TABLE 7

The diameters in mm. of colonies of A. pisi, A. pinodella and A. pinodes, grown in association with one another at 21 - 24 degrees Centigrade over a ten-day period.

Combination	Plate No.	1	2	3	4	5	6	7	8	9	10
A. pisi	1	.25	1.5	6.5	12.5	19.5	23.5	27.5	33	39.5	45
	2	.25	1.5	8.0	13.5	19	22	29	34	38.0	42.5
	3	.25	1.5	5.5	14.5	18	25	33	37	42	48.0
	4	.25	1.5	9.5	15.0	21.5	27	34.5	40	44.5	49.0
	Average	.25	1.5	7.37	13.75	19.5	24.37	30.75	36.0	41	46.12
A. pinodes	1	1.0	7	13.0	20	24.5	30	37.0	47.0	48	51
	2	1.0	6	9.5	14	17.0	23	30	36	39	39.5
	3	1.0	6	12.0	18	25.5	30	34	40	45	51
	4	1.0	5	11.5	19	23.5	29	35	42	46	52
	Average	1.0	6.0	11.5	17.75	22.62	28.0	34.0	41.25	44.5	48.37
A. pisi	1	.25	1.5	6	12	18.5	24.5	30	35	41	46
	2	.25	1.5	6	13	17.5	23.5	29	34	39.5	45
	3	.25	1.5	6	11	18.0	24	31	36	41.5	47
	4	.25	1.5	6	13	21.0	27	33	38.5	43	48
	Average	.25	1.5	6	12.25	18.75	24.75	30.75	35.8	41.25	44.5
A. pinodella	1	5.0	13.0	23.0	33	38	45	52	60.5	67	74
	2	3.5	13.5	19.0	33	40	45	53	61	70	77.5
	3	2.5	11	24.0	29	37.5	44	52	56	64	73
	4	3.5	12	24.0	32	40.5	46	55	62	68	76
	Average	3.63	12.37	22.5	31.75	39.0	45	53	59.37	67	75.25
A. pinodes	1	1.0	6	11	18	23	29	34.5	41	47	51
	2	1.0	5	13	19	25	31	35.5	42	49	53
	3	1.0	7	12	17	24	31	36	41.5	46	52
	4	1.0	5	11.5	17	23	31	36	42.5	50	52
	Average	1.0	6	12.12	17.95	24	30.5	35.5	41.75	48	52
A. pinodella	1	5	13	22	34	42	50.5	57	63.5	69.5	75
	2	5	12	23.5	36	44	52.5	56.5	64.5	70.5	76
	3	4	11	21	32.5	42.5	49.5	55.5	63.0	69	74.5
	4	6	14	24.5	37.5	44.5	54.5	60.0	66.0	72	77.5
	Average	5.0	12.5	22.78	35.0	43.25	51.75	57.75	64.25	76.25	75.75

TABLE 8

The production of fruiting bodies and the germination of spores as influenced by the association of various combinations of *A. pisi*, *A. pinodella* and *A. pinodes* grown over a period of ten days at room temperature, 21 - 24 degrees Centigrade.

Combination	Plate no.	Spore viability	Production of fruiting bodies
A. pisi and *A. pinodes*	1	not affected	not impeded
	2	" "	" "
	3	" "	" "
	4	" "	" "
A. pisi and *A. pinodella*	1	not affected	not impeded
	2	" "	" "
	3	" "	" "
	4	" "	" "
A. pinodes and *A. pinodella*	1	not affected	not impeded
	2	" "	" "
	3	" "	" "
	4	" "	" "

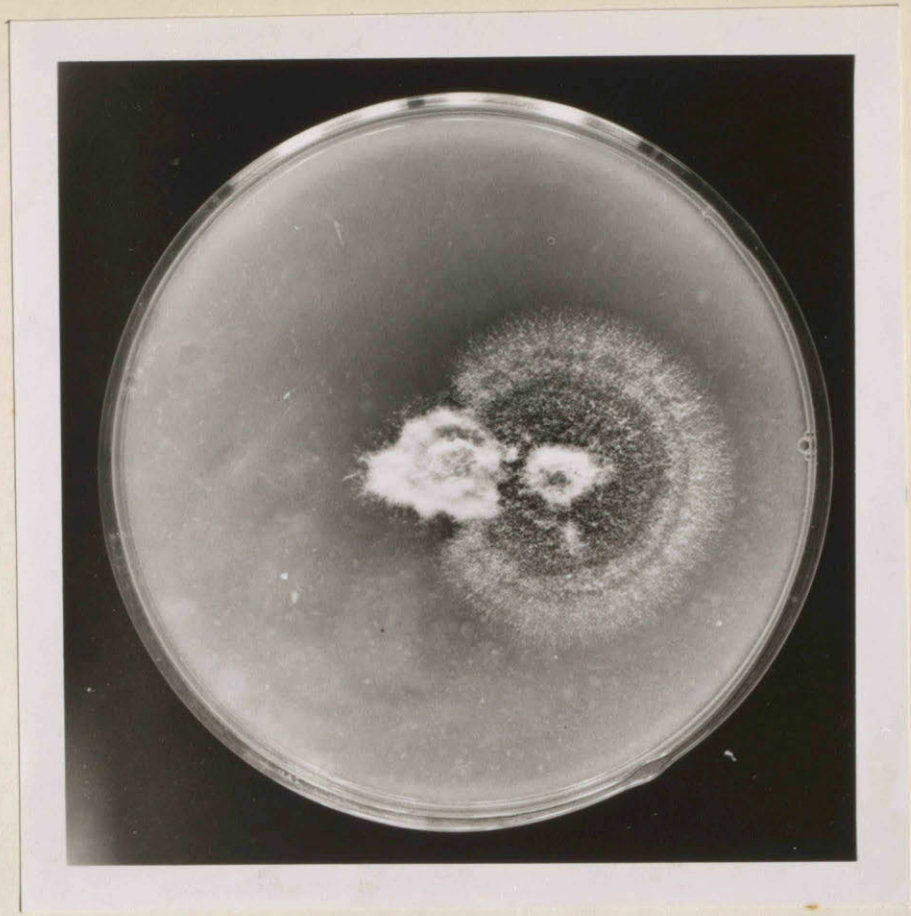


PLATE X - Photograph showing the spread of A. pisi (left) and A. pinodella (right) grown in association with one another on pea agar at room temperature, 21 - 24 degrees Centigrade, for ten days.

PLATE XI - Photograph showing the spread of A. pisi (left) and A. pinodes (right) grown in association with one another on pea agar at room temperature, 21 - 24 degrees Centigrade, for ten days.

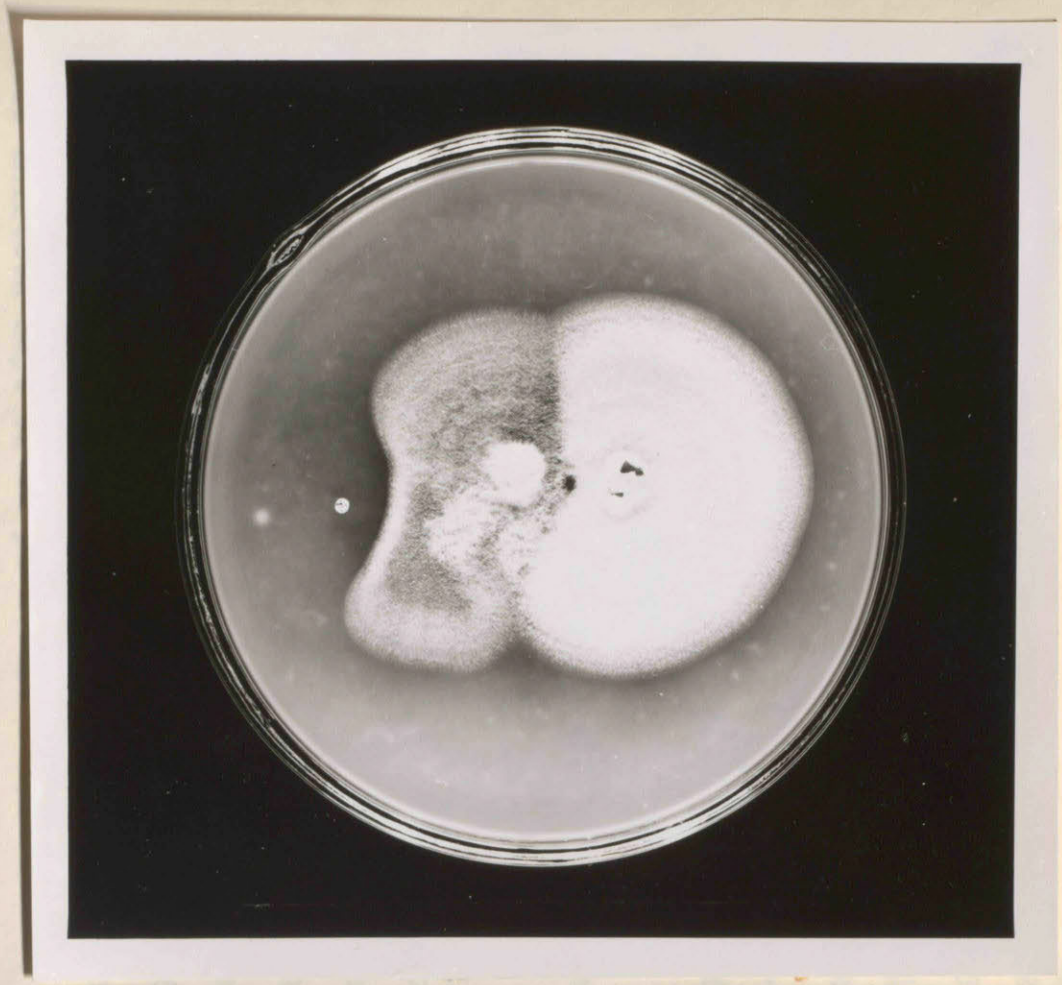


PLATE XII - Photograph showing the spread of A. pinodella (left) and A. pinodes (right) grown in association with one another on pea agar at room temperature, 21 - 24 degrees Centigrade, for ten days. x Note the small actinomycete inhibiting the spread of A. pinodella.

B. Importance of Soil infestation

An attempt was made to find out the importance of soil infestation with A. pisi upon emergence and disease rate using clean seed for planting.

The following experiment was conducted using soil inoculated with a spore suspension of A. pisi. The spore suspension was made by scraping slants with A. pisi on them and adding the spores and mycelium from ten slants to one thousand ml. of water. Two hundred and fifty ml. of this suspension were mixed with the greenhouse soil of each of four flats measuring 14" x 24" x 4". Four other flats were made up with the ordinary greenhouse soil and these flats were used as a check.

The soil in each flat was then watered periodically during a ten-day period. At the end of this time the eight flats were planted with pea seed, variety Arthur which was known to be disease free. The seed was planted at the rate of one hundred and twenty seeds per flat. The eight flats were watered periodically and emergence counts made thirteen days after planting.

From Table 9 it can be seen that the average emergence in the inoculated soil was 93.75 while the average emergence in the non-inoculated soil was 93.25. The closeness of the

TABLE 9

Effect of inoculating soil with *A. pisi* upon emergence and disease rate of seedlings in soil previously inoculated with *A. pisi*.

Flat No.	Soil Treatment	Emergence	No. lesions per plant
1	inoculated	93	0
2	"	99	0
3	"	92	0
4	"	91	0
	Average	93.75	Average 0
5	no treatment	95	0
6	"	96	0
7	"	89	0
8	"	93	0
	Average	93.25	Average 0

emergence counts indicate that the inoculum in the soil did not hinder the emergence of the young plants.

At the same time as emergence counts were made the number of lesions per plant was also recorded. From Table 9 it can be seen that no lesions were noticed during the entire stay of the young plants in the flats.

An attempt was made to isolate the organism from the soil at the same time as emergence counts were made. This was done by taking a small sample of the inoculated soil and sprinkling the soil onto ten petri plates containing fifteen ml. of pea agar. Pea agar was used as this gives better growth than most other media. The plates were incubated for ten days at room temperature and at this time they were examined for the presence of colonies of A. pisi. The examination of the plates did not reveal any trace of A. pisi.

As no lesions could be found on the young plants and as the organism could not be recovered from the soil with the technique used, it seems logical to conclude that the organism did not survive in the soil. The emergence counts in both inoculated and non-inoculated soil would also suggest that the organism was not able to establish itself in the soil, at least long enough to attack. As the attempt to isolate

the fungus was made twenty-three days after planting, it might have been present before this time in the soil. However, if it were present, it must have been lacking in sufficient virulence to attack.

C. Seed Treatment Studies

Most workers have found that treating pea seed with the proper chemical is useful in protecting the seed from seed and soil-borne pathogens and therefore may give rise to healthy plants with greater yields. Clausen in 1923 found while testing Uspulum for its stimulating action, that the yield was increased by 35% by treating pea seed with this material. Scheinpflug 1924 using the same treatment found that Ascochyta pisi was controlled. Nicolaisen reported in 1926 that the development of leaf spot of peas was prevented by immersion in a 0.25% solution of Uspulum. Jones 1931 found that treatment with organic mercury dusts increased the stand of the plants. The amount of this increase was variable being greatest when seed of low vitality was used and when the seed was planted under conditions of high soil moisture and low temperature. He concluded that the treatment of pea seed with organic mercury dusts will usually increase the stand of plants sufficiently to justify their use under the soil conditions that prevail at normal planting time. Ogilvie and Mulligan in 1933 used peas grown from seed heavily infected with A. pisi and M. pinodes. They found that Ceresan and potassium permangenate gave 68% and 62% healthy plants respectively after sixty-two days as compared with 18% healthy plants with untreated seed. The following year seed naturally infected

with A. pisi and M. pinodes gave a crop of one-thousand and seventy-six pounds when treated with Ceresan against one thousand pounds for the control. Hull in 1937 claimed high soil moisture offset the effect of a dust treatment due to the activity of other soil microorganisms and therefore advised late planting when soil moisture was not too high. Hull also found that treatment of seed was better than late planting. Walker (1940) claimed that organisms other than A. pisi and M. pinodes were of little importance in seed rotting. Walker used both copper oxide and 2% Ceresan as dust treatments and found that copper oxide was better. Sharvelle in 1941 determined the value of Spergon as a seed dressing. Spergon was applied at the rate of two ounces per bushel and the seeds planted in seventy, one acre plots. The maximum increase amounted to eight hundred pounds per acre or an overall increase of 14%. He concluded that Spergon showed promise as a seed protectant. In another test Spergon increased the stand of peas 20%. Trials conducted in Minnesota indicated that Spergon was the best protectant tested. The reduction in rootrot amounted to between 40 and 45% in the treated seed. McNew in 1943 used Spergon as a seed treatment on Surprise peas. He found that the treated seed gave an emergence of about 90% while the emergence of untreated seed was only 76%.

The increase in yield amounted to four hundred pounds per acre. Treatment of Wisconsin Early Sweet peas with Spergon produced a stand of 74% compared to 43% for the untreated seed.

McNew stated that the smooth starchy Alaska type of pea is not readily destroyed by soil fungi and does not have to be treated. The sweet types of peas are more susceptible to infection by soil organisms and need seed treatment. He recommended the use of Spergon as a seed treatment of peas over all other treatments tested.

Leach and Smith in 1944 treated seed with Cuproxide, Semesan, Spergon, Ceresan and Arasan. The seed was sown in flats inoculated with Pythium ultimum and Rhizoctonia solani. Results indicated that the highest protective value was given by Semesan and Cuproxide while Spergon, Ceresan and Arasan were only slightly less effective.

Crosier in 1946 on the basis of results from field trials stated that Arasan and Spergon gave the best protection to peas in the soil.

The fungi known to cause decreased emergence and yield in peas have not been thoroughly agreed upon by most pathologists. According to Hull organisms responsible for failure

of emergence were a species of Fusarium and a phycomycetous fungus. Jones and Linford in 1925 stressed the importance of Pythium. Crosier (1936) believed that a Fusarium sp. which causes root rot is not important in depressing the emergence of seedlings. Crosier thought an important cause of damping off was R. solani. Brett et al. (1937) stated that A. pisi and related fungi were responsible for damping off and weak emergence.

1. Seed dusting experiment

The following experiment was carried out in an effort to find out if treating pea seed heavily infected with A. pisi with various recommended dust treatments would improve the emergence and yield of the crop produced from this diseased seed. The experiment was carried on for five years in order to see if holding the treated seed over for one, two, three, four and five years would improve the resulting crop. The seed used in this experiment was grown by George Wickett of Port Hope, Ontario from seed originally imported from Charles Sharp and Co., England, Designation no. 77, variety, Little Marvel.

The percentage germination of the seed and the percentage of the seed infected with A. pisi was determined when the seed was harvested and each year for a four year period as well.

This was done by taking 100 seeds of the sample, surface sterilizing these in a 2% solution of chlorine and planting them on pea agar in petri plates. The plates were incubated at room temperature for ten days at the end of which time they were read for the percentage germination of the 100 seeds and the percentage of A. pisi in the seed.

Four different seed lots in all were made up comprising three lots of treated seed and one lot of untreated seed. The three treated lots were 1% Ceresan, Arasan and Spergon. The seed was treated by placing one-half pound of it in a small metal container with the chemical and mixing the seed thoroughly to allow for full coverage of the seed. Each seed lot was planted in three randomized rows, 100 per row. The rows were seventeen and one half feet long and the seeds were planted approximately two inches apart.

The field in which the seed was planted each year was of a sandy loam and was approximately ten acres in size. One half of the field was planted in potatoes and the other half was planted in peas each year. These two crops were rotated each year during the four year period.

The seed was planted each year at the beginning of June and emergence counts were recorded fourteen days after the seed was planted. Pod yields were taken approximately three

months after the emergence counts were made. When pod yield was taken, a foot at one end of each row was discarded so that in each case a rod row was harvested.

The results of this experiment are shown in Table 10 and graphically in Figures E, F and G. From Table 10 it can be seen that the percentage germination of the pea seed in the plate test remained near 90% during the first three years of storage. The percentage germination in the plate test dropped to 80.5% in the fourth year. The percentage of A. pisi remained at the high level of 54% for two years in the plate test. At the end of the third year the percentage of A. pisi had dropped to 24.3 and at the end of the fourth year the percentage had dropped to 13.

In the field trials the emergence of seedlings from the treated seed was comparatively high in the second year, Ceresan and Arasan treated seed giving an average emergence of 87% and Spergon treated seed an average emergence of 84%. The untreated seed showed an average emergence of 76.6%. The average emergence given by the originally treated seed was somewhat lowered in the third year; however, the percentage emergence did not drop more than 4%. The emergence given by the untreated seed in the third year was 39.6% lower than the second year average emergence. The average emergence in the fourth year varied considerably with the different treatments.

TABLE 10

Plate test showing the per cent germination and the per cent A. pisi infected seed and field tests showing emergence and pod yield of recommended treatments.

Year	Plate tests		Emergence field trials				Pod yield in grams			
	Germ.	A. pisi	Ceresan	Spergon	Arasan	Check	Ceresan	Spergon	Arasan	Check
1941	90	54	-	-	-	-	-	-	-	-
1942	92	54	-	-	-	-	-	-	-	-
1943	92	54	87.0	84.0	87.0	76.6	-	-	-	-
1944	92.5	24.3	83.2	84	84	37	-	-	-	-
1945	80.5	13	35.4	63.2	77.3	23.1	548	803.4	836	336
1946[x]	-	-	1.3	19.6	33.6	0.0	20.26	762.8	933	0

[x] No plate test was run in 1946 because the seed stock had become too depleted.

Ceresan treated seed dropped in emergence from 83.2% to 35.4% a drop of 47.8%. Spergon maintained a higher level than Ceresan treated seed, a drop of from 84 to 63.2% or 20.8%. Arasan maintained the highest level of all treated seed, a drop of from 84 to 77.3 or only 6.7%. The average emergence in the untreated seed was only 23.1, a drop of 13.9. The fifth year of planting showed Ceresan treated seed had an average emergence of only 1.3, Spergon an average emergence of 19.6 and Arasan the highest average emergence of 33.6%. The untreated check gave no emergence.

Pod yields of plants from Arasan and Spergon treated seeds were significantly higher than the untreated check in the last two years when pod yields were taken. This would be expected as the emergence counts were significantly higher in the Spergon and Arasan treated seed plots.

From Table 10 it can be seen that the lower plate germination of 1945 is reflected in a very low emergence in the field trials compared to previous years. In 1946 no plate test was run because the seed stock had become too much depleted. However the very low emergence in the field trials would seem to indicate that the plate test germination would have dropped considerably.

Although the percentage of A. pisi in the seed dropped from 54% in the year of production of the seed to 13% after four years, this did not show any significant difference in infection in the

field trials during the four years of field emergence. The infection was slight and scattered throughout the different treatments with no one treatment having a heavier infection than any of the others.

Arasan and Spergon treated seed gave the highest field emergence over the five-year period and also gave the highest pod yield over the Ceresan and the untreated check, however the low emergence of Spergon and Arasan treated seed of 19.6 and 33.6% respectively does not warrant holding seed which is heavily infected with A. pisi for more than three years as after that time the emergence drops considerably. Holding the seed for three years gave the best results as the percentage of infection due to A. pisi in the seed dropped from 54 to 24.3 and the emergence in the treated seed was still at a high level.

As shown by Figure E the viability of the fungus seems to be maintained for two years and then drops considerably in pea seed heavily infected with A. pisi and stored at room temperature.

Plate germination was maintained at the original level for three years and then dropped slightly as shown by Figure F.

Treated infected seed gave considerably higher emergence than untreated seed. Arasan was the most efficient material with Spergon next followed by Ceresan. (Figure G.)

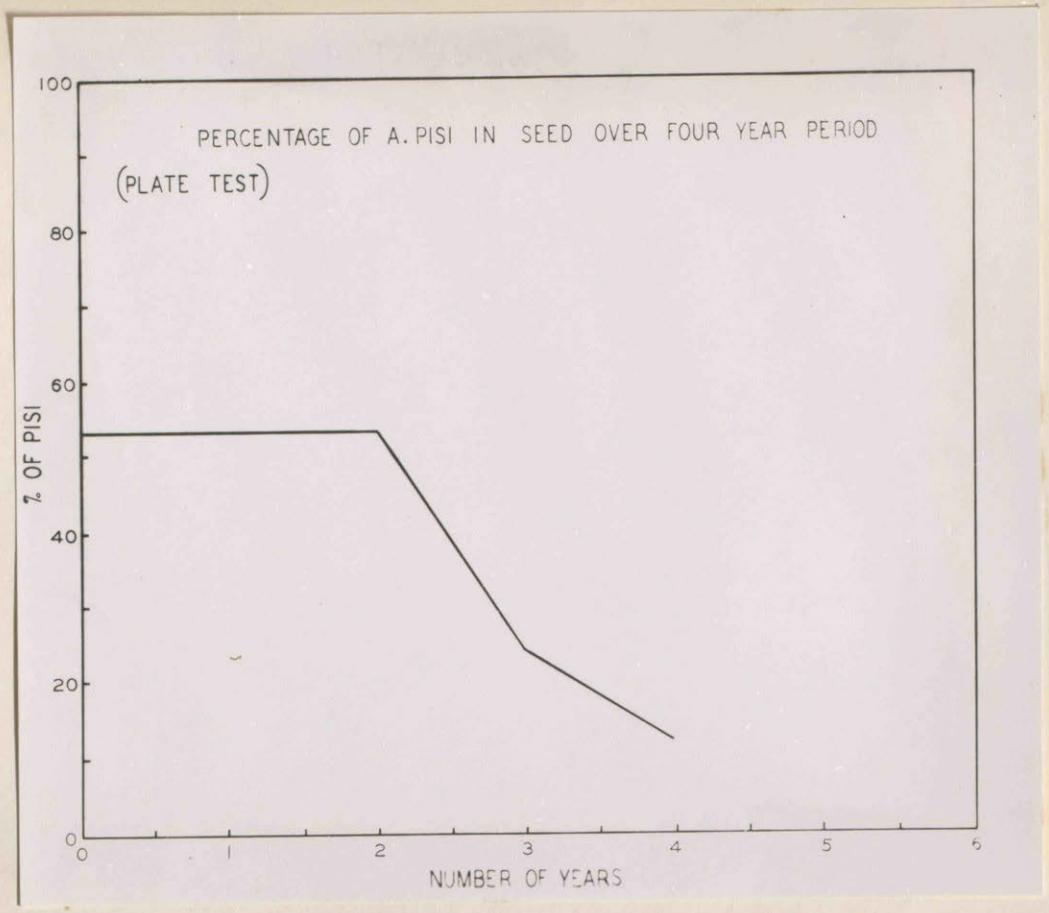


FIGURE E - Percentage of seeds of the same sample from which A. pisi could be isolated during a four year storage period.

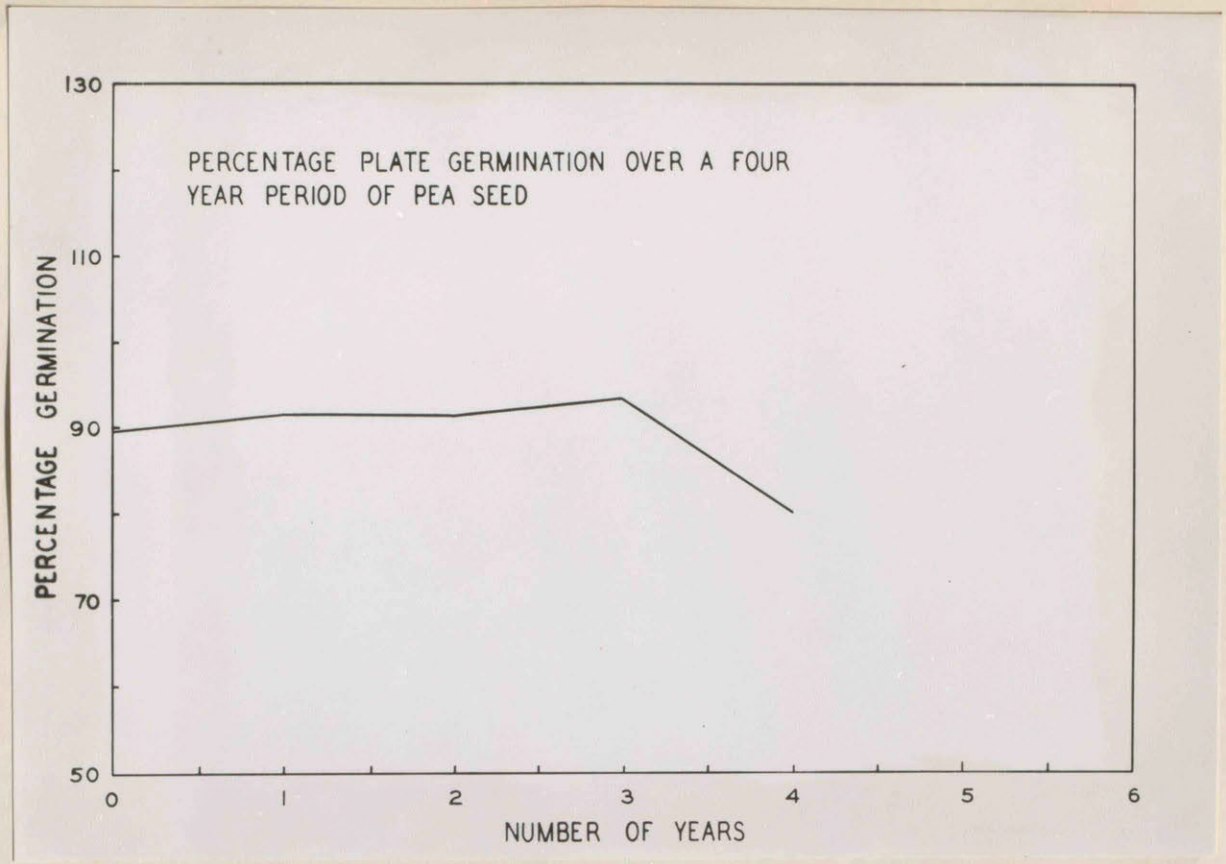


FIGURE F - Percentage germination of seeds of the same sample as in Figure E over a four year period.

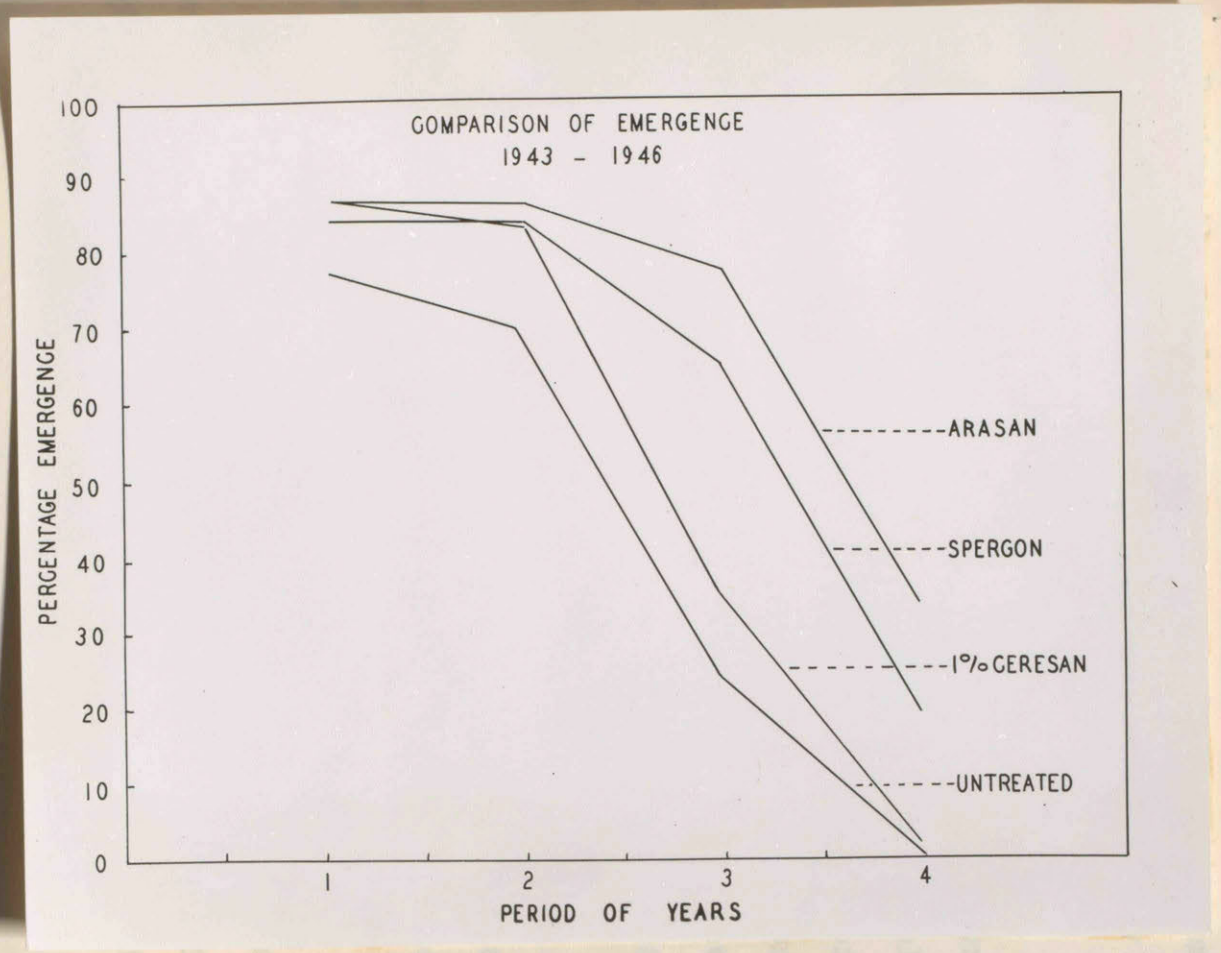


FIGURE G - Percentage emergence over a four year period of seeds of the same sample as in Figure E originally treated with Arasan, Spergon and 1% Ceresan.

2. The effect of various oils and Ceresan upon the emergence, disease rate and yield of peas

During the past few years several cases of human poisoning have been reported from applying mercury dusts to seeds.

The following test was carried out in an effort to see if the application of various oils to the seed would eliminate the dust hazard while treating pea seed. If the oil would act as an adhesive and hold the dust to the seed once it was applied, then free dust would not be present in the air and the poison hazard to the operators of seed treating machines would be lessened.

As a secondary benefit, the oil might act as a protective coating against soil borne organisms and might improve the emergence and yield.

The seed used in this test was the same as used in previous tests containing 49% A. pisi, 5% A. pinodes, 5% A. pinodella, 4% Fusarium poae and 2% Fusarium spp.

The seed dressing applied was Ceresan applied at the rate of one ounce per bushel of seed. The various oils used were; - olive oil, peanut oil, shellsol, soybean oil and mineral oil, applied at the rate of ten ml. of oil to five

hundred grams of seed. The oil was applied to the seed in a flask and then the flask was shaken in order to give the seed a thorough coating with the oil. The seed dressing was then added to the flask and the flask shaken again.

Eleven treatments consisting of eight rod rows of each treatment were sown at the rate of one hundred seeds per row. The treatments were; five lots of seed treated with the various oils mentioned above, five lots of seed treated with the various oils and then an application of Ceresan applied to the oil coated seed, and one lot of seed treated with Ceresan alone. A check series consisting of eight rows of untreated seed was also sown on May 22, 1948.

At the end of one month emergence counts were made and four rows of each lot of seed were dug up and records were made on the number of lesions on the above and below ground portions of each plant.

Readings were taken on the emergence, disease rate and pod yield on the plots planted with the various treatments and are presented in Table 11 from which it can be seen that the Ceresan treated seed gave considerably higher seedling emergences than all other corresponding treatments. The Ceresan and oil treated seed gave an average emergence of 60 plants per row. The combined average emergence of the oil

TABLE 11

Effect of various oils with Ceresan on seedling emergence, disease rate and pod yield.

Treatment	x Emergence	x x No. of plants with lesions				xxx Yield
		Above ground	Below Ground	Both	Clean	
Mineral oil	40	5	33	3	97	1323
Mineral oil and Ceresan	59.75	3	37	7	186	1906
Olive oil	50.25	11	24	3	138	1766
Olive oil and Ceresan	59.75	6	21	0	208	2099
Peanut oil	45.25	7	24	2	152	1487
Peanut oil and Ceresan	58.00	20	16	1	200	1665
Soybean oil	37.75	16	20	3	124	1240
Soybean oil and Ceresan	63.5	10	23	4	222	1622
Shellsol	52.25	9	23	3	128	1255
Shellsol and Ceresan	59.00	17	25	2	168	1456
No treatment	46.25	18	24	6	133	1744
Ceresan alone	59.00	9	31	6	196	1788

x Average emergence of four rows.

xx Total number of lesions on four rows.

xxx Total yield in grams of four rows.

alone treatments was 46.3% which was 13.7% less than the combined average of the oil and Ceresan treated seed. The average emergence of untreated seed was 46.25% while the average emergence of seed treated with Ceresan alone was 59%. Emergence given by the various oil treatments ranged from soybean oil with an average emergence of 37.75% to olive oil with an average emergence of 50.25%. The emergence of all Ceresan treated seed was practically the same.

Lesions on the above and below ground parts of the plants is four rows ranged from olive oil and Ceresan treated seed with a total of twenty-seven lesions, to Shellsol and Ceresan treated seed with a total of forty-two lesions. The untreated seed also had a total of forty-two lesions on the above and below ground portions of the plants in four rows.

The Ceresan oil treated seed gave a higher yield than the untreated seed in two cases. The olive oil and Ceresan treatment gave the highest yield of all treatments, a yield of two thousand and ninety-nine grams. Mineral oil and Ceresan also yielded quite highly, a yield of one thousand and six grams. The seed treated with Ceresan alone gave a yield of one thousand seven hundred and eighty-eight grams as compared to the untreated seed which gave a yield of one thousand seven hundred and forty-four grams. Shellsol and Ceresan, Soybean oil and Ceresan, and peanut oil and Ceresan

all gave reduced yields.

As shown in the previous test the use of Ceresan is beneficial as a seed treatment in increasing emergence and yield over untreated seed. This test indicates that the use of Ceresan in combination with olive oil increases both the emergence and yield of peas as compared to untreated seed and should therefore be beneficial to use as a seed treatment.

From observation, it appeared that the Ceresan dust did adhere better in the seeds coated with oil than on the seed not treated with oil. This would tend to eliminate free dust in the air in seed treating houses and therefore result in less danger from dust poisoning.

V - DISCUSSION OF RESULTS

In 1927 Jones came to the conclusion that the diseased condition of peas, which until that time had been known as the Ascochyta leaf and pod spot or blight was really three distinct diseases caused by three closely related organisms. He came to this conclusion on the basis of symptom expression of the plants and the characteristics of the causal organisms. He named these three diseases as Ascochyta leaf and pod spot caused by Ascochyta pisi Lib., Mycosphaerella blight caused by Mycosphaerella pinodes (Berk. and Blox.) Stone and Ascochyta footrot caused by Ascochyta pinodella Jones.

In the course of my studies I have isolated all these organisms from peas. Ascochyta pisi was the most frequently isolated fungus.

The physiological behaviour of A. pisi, M. pinodes and A. pinodella has been investigated to some extent. In this respect A. pisi has been studied more than the other two.

Tests run each year over a three year period on twelve samples of pea seed heavily infected with A. pisi indicated that A. pisi was still virulent in the seed after this time. Storage of this seed for as much as three years before planting it would not be helpful in producing disease free crops.

An effort was made to determine how the fungus passes from the infected seed to the above ground parts of the seedling. As the young shoot grows within the seed, it may come in contact with a diseased part of a cotyledon. The fungus may grow out of the diseased cotyledonary tissue into the stem establishing infections on it. If the seed is heavily infected the stem may be killed and the seed fail to germinate, while other seeds which have moderate infections on the cotyledons may develop lesions on the young stems which will be carried above the soil line and serve as primary inoculum for new infections. Those seeds which have only slight infections on the seed coat would probably always give rise to healthy seedlings as the fungus is not likely to be able to reach the young shoot in time to infect it.

A test was run to determine the influence of temperature upon the growth of A. pisi together with a test to determine the influence of temperature upon infection by A. pisi. It was noted that the optimum temperature for the growth of A. pisi in culture media was the same as its optimum temperature for infection both occurring at twenty-four degrees Centigrade.

In only one case was there more than one Ascochyta noted occurring on the same seed. This suggests that the three organisms might be antagonistic toward each other but no such antagonism could be found when they were grown in association

with each other on pea agar.

In an attempt to find out the importance of soil infestation with A. pisi on disease development, negative results were obtained. A. pisi could not be reisolated from the inoculated soil nor could any disease due to A. pisi be found on the seedlings which were planted in it. The results of this test would seem to indicate that A. pisi does not survive in the soil or if it did it was lacking in pathogenicity.

Seed treatments with various chemicals used as dusts have been found to increase emergence and yield of plants using seed which was originally 54% infected with A. pisi. The tests were run each year for five years. Of the three dusts used namely Spergon, Arasan and Ceresan, Arasan was slightly better than Spergon and both appeared to be better than Ceresan in increasing the emergence and yield of the diseased seed.

A test was carried out in an effort to see if the application of various oils to the seed coat would eliminate the dust hazard while treating pea seed and also serve as an adhesive in holding the dust to the seed coat. From visual observation of the seed it appeared that the dust did adhere better on the oiled seed and therefore the use of the oil would reduce the amount of free dust in the air. Results

indicated that the application of an oil with the Ceresan did not hinder the effectiveness of the Ceresan.

VI - SUMMARY

1. The longevity of Ascochyta pisi in pea seed has been studied over a period of three years. It has been found that the decrease of the organism in the seed over this period was approximately 40%.

2. Seeds which are moderately infected with Ascochyta pisi usually produce infected seedlings. These seeds have lesions on the seed coat and cotyledons. As the young shoot starts to grow it comes in contact with a diseased part of a cotyledon and in doing this mycelium of Ascochyta pisi is transferred to the young shoot. Stem lesions are produced as the result of infections by the fungus. These stem lesions are believed to be the source of secondary spread. Seeds which are heavily infected have lesions on the seed coat and deep-seated lesions on the cotyledons. These seeds usually do not germinate as the young shoot is usually killed. Slightly infected seeds have lesions on the seed coat only and the seedlings produced from this seed are usually healthy as the fungus is not likely to reach the young shoot in time to infect it.

3. The optimum temperature for the growth of Ascochyta pisi was found to be twenty-four degrees Centigrade. The optimum temperature for infection of the pea plant by

Ascochyta pisi was also found to be twenty-four degrees Centigrade.

4. Ascochyta pinodella was found to have the greatest growth rate of the Ascochytas.

5. No antagonistic or synergistic action appeared among any of the three Ascochytas when grown in all possible paired combinations on pea agar.

6. Emergence of clean seed in soil inoculated with Ascochyta pisi was practically the same as in non-infested soil. No lesions due to Ascochyta pisi appeared on the plants and the organism could not be recovered from the soil into which it had been inoculated.

7. Seed treatment with Arasan and Spergon proved to be beneficial on diseased pea seed in field tests. The emergence of diseased seed was greatly improved with Arasan and Spergon.

8. The use of an adhesive in the form of various oils on pea seed proved to be beneficial in increasing emergence when applied with Ceresan. The oil also held more dust to the seed and therefore would eliminate the dust hazard to the operators of seed treating machines.

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