PARASITISM OF UROMYCES FABAE (PERS.) DE BARY

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STUDIES ON THE PARASITISM OF UROMYCES FABAE (PERS.) DE BARY.

A THESIS

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MASTER OF SCIENCE

by

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STUDIES ON THE PARASITISM OF UROMYCES FABAE (PERS.) DE BARY.

INTRODUCTION

During the past three years a rust of peas has become prominent in Eastern Canada. From a position of obscurity and of purely mycological interest, this rust has rapidly attained a position which is causing considerable concern among growers.

The only pea rust described to date on this Continent is Uromyces fabae (Pers.) de Bary, though the possibility of the European pea rust, Uromyces pisi (Pers.) Wint., having become established in this country is considered herein.

Uromyces fabae is a cosmopolitan species, being reported from Canada, United States of America, Mexico, Central and South America, Europe, North Africa, India, Japan, Asia, Australia and New Zealand. It is of varying importance in these countries, but the paucity of plant pathological literature centred about this fungus would allot it a place, until recent years, of low economic importance. References to the fungus from the above countries have been primarily notes of its occurrence, popular descriptions, or accounts of attempts to determine biologic forms on its several hosts.

The newly acquired economic importance of this rust seemed to warrant further study, particularly in its ecological and physiological relations. In addition, the pea plant offered a suitable medium for observation and growth

of a rust during the winter months, under controlled environmental conditions.

After establishment of the identity of the rust used in these experiments, the work undertaken in connection with this organism, and to be described herein, may, for convenience sake, be divided into two parts: (I) cytological and histological investigations; (II) physiological investigations.

The former section involves studies on the penetration of uredinial germ-tubes and upon the responses of the host cells to fungal invasion, and includes investigations on the nature and role of haustoria. Particular effort is made to determine whether the haustoria of this rust invaginate the peripheral cytoplasm of the host cells or whether haustoria attain an intra-vacuolar environment.

In the latter section cardinal temperatures and humidities for spore germination and infection are determined. Enzymes in diseased and healthy tissues are determined and the fungus, healthy cells, and diseased cells are examined from the point of view of osmotic pressure and cytoplasmic permeability.

Literature pertinent to the different phases of this work will be cited at the beginning of each separate investigation.

ESTABLISHMENT OF THE IDENTITY OF THE FUNGUS

Introduction

The only rust of peas on this continent listed in Arthur's recent manual (1934) is Uromyces fabae (Pers.) de Bary. A European pea rust, U. pisi (Pers.) de Bary, has not yet been reported, but being very similar to the American organism it was considered that the recent virulence of pea rust in Canada was possibly due to the European rust having become established on this Continent. To check this, the method described below was adopted, and efforts made to determine which particular biologic form of the rust was active in this country.

Literature

The earliest mycological descriptions of both Uromyces fabae and U. pisi were made by Persoon in 1794 and 1801, respectively, the former receiving the name Uredo fabae and the latter, Uredo appendiculata var. pisi-sativi. U. fabae has since been given four synonyms (Arthur, 1934) and U. pisi eight (Sydow, 1910).

A recent summary of work on the biological forms of Uromyces fabae is to be found in the publication of E. Gäumann (1934) who describes six distinct forms. The forms are determined according to their host selection, Arthur's (1934) list of hosts including species of the genera Lathyrus, Pisum and Vicia. Gäumann subdivides the species Uromyces fabae as follows:

1. f. sp. viciae-fabae de Bary on Vicia fabae and Pisum sativum.
2. f. sp. pisi sativi Hiratsuka on Pisum sativum.
3. f. sp. craccae Ed. Fischer on V. cracca and P. sativum.
4. f. sp. viciae nipponicae Hiratsuka on V. nipponica var. capitata.
5. f. sp. viciae unijugae Hiratsuka on V. unijuga.
6. f. sp. viciae sepium Gaumann on V. sepium, V. faba and V. monantha.

Determination of Species and the Rust Form

Specimens of rust on peas (Pisum sativum L.) and vetch (Vicia cracca L.) were collected from widely separated districts in Eastern Canada, some of the material having been sent in to the Division of Botany, Central Experimental Farm, Ottawa. Sydow's "Monographia Uredinarum" (1910) was consulted as the morphological authority for the species, and comparison was made with authentic specimens of Uromyces fabae and U. pisi in the herbarium of the Division of Botany, Ottawa. Measurements of large numbers of teliospores were made from the different samples and the results averaged.

In every instance the rust was determined as Uromyces fabae (Pers.) de Bary. The following characters allowed positive determination:

Size of teliospores

Sydow's measurements		Observed measurements
U. fabae	U. pisi	
25 - 38 x 18 - 27μ	20 - 28 x 14 - 22μ	28 - 37 x 21 - 24μ

Depth of truncate apex of teliospore

7 - 11μ	U. pisi has a small hyaline umbo	8 - 11μ

Pedicel

Colour: yellow or brownish	hyaline	brownish
Persistence: persistent	deciduous	persistent
Length: up to 90μ	short	42 - 77μ.

Vetch and pea plants growing in the locality of Macdonald College, Quebec, and bearing natural infections were taken from the field and placed in pots in the greenhouse. Both vetch and peas were then grown from seed and kept in a section of the greenhouse away from the rusted plants. Young specimens of both plants were then cross-inoculated with urediniospores from both hosts, the spores being applied with an atomizer, or with a camel-hair brush. Inoculated plants were then placed in a moist chamber, at saturation pressure and temperature of about 70° F., for 48 hours.

After an incubation period of ten days, following cross inoculation as described above, all inoculated plants produced urediniospores, pustules developing equally freely on either host, irrespective of the host source of the inoculum. This clearly shows the inter-infectivity of the spores from vetch and pea, which indicates that the form used in these experiments

was Uromyces fabae (Pers.) de Bary f. sp. craccae.

It is of interest to note here that during these investigations Vicia cracca was found to be very heavily rusted. Accordingly, the above establishment of a common rust form on pea and vetch at once suggests the probability that the wild vetch (Vicia cracca), which is a very common weed in Quebec, is the source of inoculum leading to the recent epidemics of pea rust. At about the time peas were sown, aecia were found on vetch, indicating that teliospores had already germinated. Since the appearance of rust on peas is uncommon until approaching the time for harvesting green peas, it is considered likely that the principal inoculum for the pea consists of urediniospores from the vetch rather than from overwintering teliospores on debris of pea plants. Prevalent cultural practices would minimize this latter possibility since remains of pea plants are usually covered with soil before spring.

INVESTIGATIONS

Part I. Cytological and Histological Investigations

Introduction

Among the large bulk of literature on rusts already extant no histological treatment of the rust of peas has come to the attention of the writer. Accordingly, preparation of a large number of slides was undertaken, which seems to have been well rewarded. by observation of interesting phenomena which, in part, challenge the conclusions of other workers insofar as penetration of the host cell is concerned. This phase has attracted particular attention because of the interest aroused by Miss Rice's cytological work (1927).

Literature

A great deal of effort and ingenuity has been applied in cytological and histological studies of the rusts. Results of such studies have been published by a great number of workers, of whom pioneers such as de Bary (1853 & 1863) and Marshall Ward (1888, 1890, 1902 & 1903) have presented masterly treatises. Other outstanding contributors are Eriksson (1904), Stakman (1915), Allen (1923, 1924, 1925, 1926, 1927, and 1933), Evans (1907) and Rice (1927). The generally accepted, salient facts drawn from these researches are brought together by Arthur (1929) and Cunningham (1931) in their respective texts on the rusts of North America and New Zealand.

Materials and Methods

Tissues to be imbedded were taken from leaves, petioles, stems and fruit pods of infected pea plants, fixed in Karpechenko's solution or in formalin-alcohol-acetic acid (Rawlins, 1933), dehydrated by the glycerol method (Rawlins), and imbedded in paraffin from chloroform. In some instances, living tissue was examined, in which case the material was cut from the active plant immediately before use. The stains used were thionin-orange G, after the method of Stoughton (1930), iron alum-haematoxylin, and Flemming's triple stain. The last proved most generally satisfactory. Tests for starch were made with iodine-potassium iodide solution, after McCoy(1929). All living tissues were stained in dilute aqueous neutral red. Sections were cut from 3μ to 15μ, and mounted in Canada balsam. Drawings of some of the more interesting microscope fields were made with the aid of a camera lucida. Most material to be imbedded was taken from the field, while tissue to be examined in a living state was taken from potted plants in the green-house, where inoculation had been accomplished by application of urediniospores with a camel hair brush, and the plants kept in a moist chamber for 48 hours at 70° F.

Results

A. Penetration of the Uredinial Germ Tube

In no case was penetration achieved other than through stomata (Plate I, fig. I). The germ tubes travelled in an apparently random fashion, frequently passing directly over a stomatal cavity, and seemed to show no thigmotropic response as suggested by Johnson (1934) in the case of Puccinia graminis on wheat. Formation of a definite appressorium was not noted in every case, though commonly this was so, but the intercellular hyphae invariably began their ramifications from a sub-stomatal vesicle. This is the normal procedure in the penetration of a susceptible host by the uredial germ-tube of a rust, as has been described in full by Ward (1888), Evans (1907), Stakman (1914), and Allen (1921, 1923). Cunningham (1931), however, states that the infection hyphae always take the nearest route to the stomata which was not the case in the material under observation, in which a germ-tube often passed close to, or directly above a stoma before eventually entering the host.

B. Investigation of Haustoria

Following penetration, the intercellular hyphae very soon give rise to haustoria which Rice (1927) and Cunningham (1931) describe as having the primary function of nutrient absorbing organs. Miss Rice (1927) states that rust haustoria are intracellular only with respect to the cell wall. She shows figures to illustrate that the haustoria merely invaginate the

host cytoplasm and do not penetrate into the vacuole, with a consequent rupture of the cytoplasm membranes.

The probability that at least some of the haustoria of Uromyces fabae are intra-vacuolar was envisioned on observation of a haustorium which appeared to have passed through the nucleus of a host cell. Fig. 2, plate I, shows a camera lucida drawing of this cell, of which a very critical microscopic examination by a number of observers, including experienced microscopists, led to the conclusion that either the haustorium had progressed through the nuclear mass or else the nucleus, being in contact with the foreign organ, had surrounded it by its own protoplasm. This particular host cell, being a mature one, contained a large, central vacuole, and the invading organ had penetrated on one side of the cell, passed through the cytoplasmic layer and had moved through the vacuole towards the opposite cell-wall, where it had encountered the nucleus in association with the cytoplasm at this point. This interesting observation, in controversy with the conclusions of Miss Rice, led to further examination of large numbers of sections in which more evidence of the intra-vacuolar state of haustoria was disclosed. Haustoria were frequently found in association with host-cell nuclei, but no other examples of apparent/complete nuclear penetration were found. Fig. 3, plate I, depicts a parenchyma cell in which the haustorium has again made ingress into the vacuole, having passed almost completely across the host cell lumen. Fig. 4, plate I, offers another instance in which the haustorium has emerged from the cytoplasmic layer and has entered the vacuole

while one branch of it has made contact with the host nucleus. Fig. 1, plate II, is very similar, showing a portion of the haustorium free from cytoplasm where a part of the upper cytoplasmic layer of the cell has been removed in sectioning. Fig. 2, plate II, again shows a haustorium within the vacuole, one branch of the haustorium having invaginated or partly penetrated the host nucleus. Fig. 3, plate II, portrays a haustorium which has traversed the vacuole and has almost surrounded the hose cell nucleus. Fig. 4, plate II, once more clearly illustrates intra-vacuolar haustoria among a group of partly plasmolysed parenchyma cells.

Realizing the frequency of artifacts in prepared slides and the difficulties of interpretation when using high magnification, attempts to check the contention that some haustoria of U. fabae are intra-vacuolar, and are not merely invaginated within the cytoplasm, were made with living tissue. It was visualized that, if infected tissue were strongly plasmolysed, the contraction of the vacuole would be sufficient, in some cases, to free at least parts of the larger haustoria from the cytoplasmic layer if, as Rice (1927) states, invagination rather than penetration of the cytoplasm layer had occurred. Accordingly, freehand sections were cut as thinly as possible and plasmolysed in 0.5 molar calcium chloride, to which neutral red had been added. Large numbers of such sections were examined. The vacuoles had contracted to a small proportion of their original volumes, and were stained a bright red. Only cells in which convex plasmolysis had occurred were considered, but, among these, not one instance of a haustorium remaining free

in the plasmolysing solution within the cell walls was to be found. On the other hand, several haustoria were seen inside the contracted vacuoles, plasmolysis having been so severe that the internal presence of the haustorium had distorted the normal spherical form of the strongly plasmolysed cell.

C. Responses of the Cell to Invasion

Cells among which the rust hyphae were ramifying disclosed but little visible difference from cells comprising the neighbouring healthy tissues. In general, the host nuclei, chloroplasts and cytoplasm of the diseased cells all seemed normal except for distortion of some of the nuclei with which haustoria were in close association. Degeneration of cells below the centres of old pustules had occurred. However, one striking difference was noted, in that the tissue interspersed with hyphae contained a much greater proportion of starch grains, than did the healthy tissue. Arthur (1929) indicates this as a common finding, though exceptions are noted by Rice (1927), Tischler (1911) and Mains (1917).

Fig. 1, plate III, shows a dense accumulation of starch grains immediately below an old sorus. Fig. 2 of the same plate, depicts a portion of the same section, on the opposite side of the stem, which is quite devoid of starch.

Figs. 3 and 4, plate III, depict healthy and diseased parts of the same cross-section of stem. The infection is in an earlier stage of development than that in Figs. 1 and 2, the epidermis being not yet displaced. The emphatically greater starch content in the diseased tissue is again apparent.

Figs. 5 and 6, plate III, are drawn from healthy and diseased areas, respectively, in the same cross section of a rusted pea-pod, which is normally rich in starch. The cells below the pustule are gorged with starch; the grains are larger and much more numerous than in the tissue free from mycelium.

Part II. Physiological Investigations

I. Urediniospore Germination

Literature

Johnson (1912), Mains (1915), Hoerner (1921), Doran (1919), Peltier (1922), Hart (1926), and Corneli (1933) have indicated the importance of the cardinal temperatures of rust-spore germination in a consideration of the relationship between meteorological conditions and rust development. Each of the above authors cites figures for cardinal temperatures of economically important rusts, mainly of cereals. Urediniospores of the cereal rusts have a range (Johnson, 1912) of from 2^{0} C. to 31^{0} C., and an optimum of from 12^{0} C. to 17^{0} C.

Doran (1919) states that the optimum temperature range for germination of spores of six rusts is from 10^{0} C. to 16^{0} C. Johnson quotes 12^{0} C to 17^{0} C. for cereal rusts. Howell (1890) lists an optimum range of 11^{0} C. to 16^{0} C. for *Uromyces trifolii*; Weimer (1917), with *Gymnosporangium juniperi-virginianae*, finds a minimum of 7^{0} C., optimum 23^{0} C. to 24^{0} C., and maximum 29^{0} C. Hiratsuka (1934), in a paper published shortly after the initiation of this work, cites a slightly narrower range than this last for *Uromyces fabae*, with an optimum of 16^{0} C. to 22.5^{0} C.

Fewer statements are to hand concerning cardinal humidities. Lauritzen (1919) found that infection of wheat with urediniospores of *Puccinia graminis tritici* could be secured at humidities of 92% to 100%. A film of water on the host leaf was not essential. Doran (1919) states that the aeciospores of

Gymnosporangium clavipes germinate in "moist air". Corneli (1933) states that urediniospores of Puccinia graminis need high humidities for germination. Fromme (1913) finds that a minimum h humidity of 93% is necessary for infection of wheat with Puccinia graminis. Hart (1926) asserts that before germination of spores of Melampsora lini will occur, the spores need to be in contact with free water. Being placed in a saturated atmosphere is not sufficient. Tulasne (1854) germinated teliospores of rusts as effectively in a saturated atmosphere as in a water droplet. Taubenhaus (1911) found the same in the case of Puccinia malvacearum. Weimer (1917) found that teliospores of Gymnosporangium juniperi-virginianae will not germinate until the air becomes super-saturated, so that condensation droplets would be present on the spores. Melhus and Durrell (1920) find that urediniospores of Puccinia coronata will not germinate unless in contact with liquid water.

Materials and Methods

A. The Effect of Temperature on Spore Germination

Urediniospores were collected from active pustules on greenhouse material. Preliminary tests indicated that other organisms, particularly yeasts and bacteria, were apt to make rapid growth in the substrate used for the urediniospores, interfering with germ-tube development. To minimize this effect spores were taken from pustules which had been cleaned of their spores and washed in sterile water twenty-four hours previously.

The apparatus used to obtain the differential temperatures consisted of a long, narrow, copper trough, 3 inches deep, divided into ten compartments. The whole trough could be enveloped by a close fitting wooden cover. Each end of the trough was set into a copper vessel, one vessel being filled with ice, and the other containing water kept at a constant temperature by means of a Dekhotinsky electrical thermo-regulator. The temperature range established was from 10^{0} C. to 32^{0} C. A lower range of from 0.5^{0} C. to 10^{0} C. was obatined by use of a similar apparatus, in which cooling was brought about by an electrically controlled, brine refrigerating system.

Sterile tap water was used as the substrate for the spores, Raeder and Bever (1931) having indicated this medium as the best for this purpose. The spores were germinated in hanging drops in Van Teighem slides with the coverslips raised slightly by the insertion of the edges of two other coverslips to allow aeration. Evaporation of the drops was minimized by placing water in the cavity of the Van Teighem cell. Doran (1919) has shown that the normal arrangement of a hanging drop in a Van Teighem cell does not permit sufficient aeration. The slides were mounted on corks at the centres of the different temperature compartments of the copper trough. The whole apparatus was kept in a small basement room where temperature fluctuation was slight. The lengths of germ-tubes were measured at different time intervals. Germ-tubes of twenty randomly selected spores from each temperature compartment were measured at each time, and the experiment repeated until

about three thousand measurements had been made.

A second method of measuring germination, viz. determination of percentage germination (Doran, 1919) was discarded since it was considered that length of germ-tube gives a better index of power to infect a host than does percentage germination, other things being equal.

B. The Effect of Humidity on Spore Germination

To determine the humidity range for spore germination, fresh urediniospores were scattered over clean glass slides suspended in an enclosed space over solutions of sulphuric acid of concentrations allowing humidities from 50% saturation to 100%, at 20° C. The solutions were prepared according to the tables given by Stevens (1916). The optimum temperature range for spore germination having been found to concur with the prevailing room temperature at the time of the experiment, no temperature control was employed. The criterion set for spore germination was as above.

Results

I. Urediniospore Germination

A. The Effect of Temperature on Spore Germination.

The accompanying graph (Fig. 1) clearly shows the effect of temperature on germination as measured at different time intervals. The ordinates of the curves are the average lengths of the germ-tubes at given temperatures, measured at the specific time intervals after arranging the hanging drop.

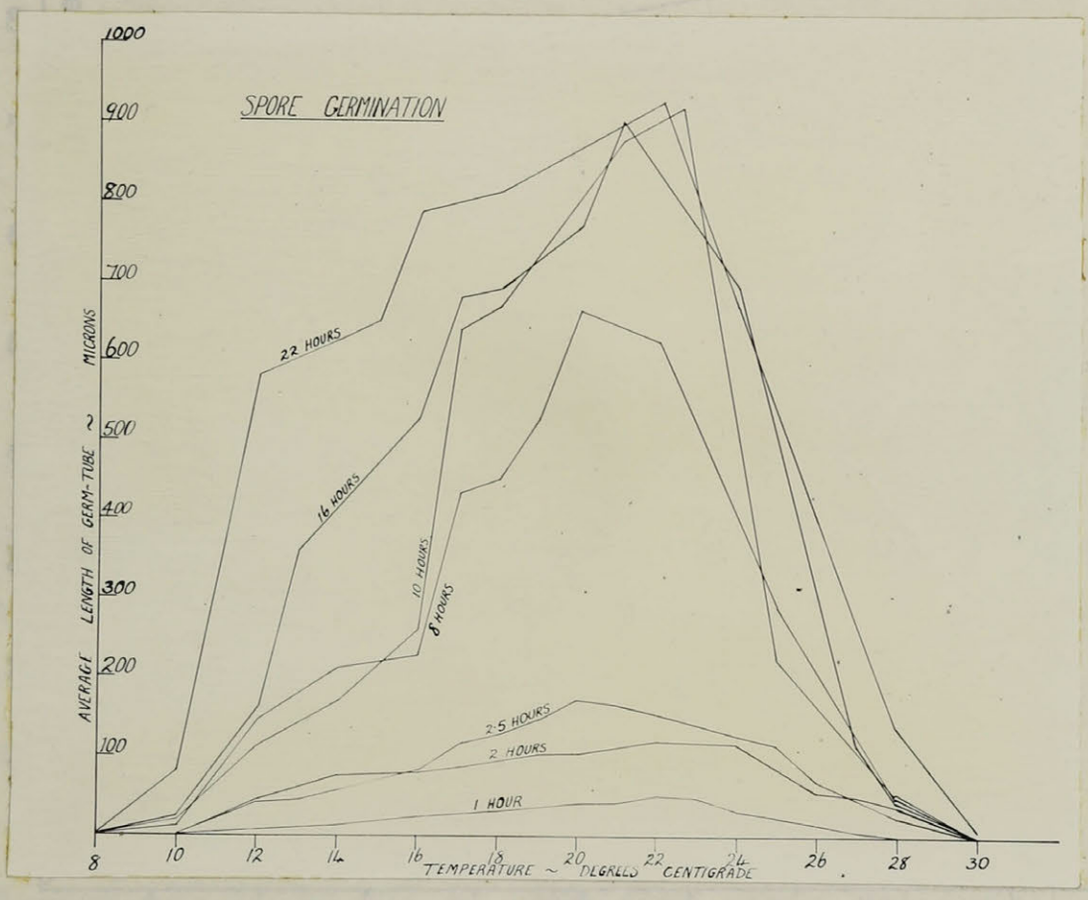

Fig. 1. Spore germination. The average lengths of germ-tubes at different temperatures measured at various time intervals. Compiled from measurement of 3000 spores.

The minimum temperature is 8° C., the maximum 30° C. Germination is poor below 10° C. and above 27° C. The optimum range is from 21° C. to 23° C. Germination begins in about one

hour and the germ-tubes attain their maximum length of approximately 900u in about 16 hours. The period of most vigorous growth is within ten hours as can be seen from the curve (Fig. 2) obtained by plotting the germ-tube lengths at different periods of growth at 22.5° C. (optimum). Growth is very rapid during this period, remaining practically constant from ten hours to twenty-two hours, then dropping quickly to zero at thirty hours.

Fig. 2. Average length of germ-tubes (in hundreds of microns) at different growth periods at optimum temperature (22.5° C.) Taken from Fig. 1 compiled from 3000 measurements.

B. The Effect of Humidity on Spore Germination

In no case would urediniospores germinate when placed on dry slides held in atmospheres of the humidities mentioned. Even in a saturated atmosphere this remained true. Contact with free water was found essential for germination.

The results of the temperature studies are in agreement with those published in connection with other rusts, and with the work of Hiratsuka (1934) on Uromyces fabae, though a much narrower optimum range is indicated here, as compared with the latter work.

2. The Effect of Temperature and Humidity on Infection

Literature

Lauritzen (1919) states, with general reference to fungal parasitism: "never has the influence of temperature and humidity upon infection been studied separately where both have been under control." Though this is no longer true, few references to experiments of this kind in connection with the rusts are to be found. Lauritzen's work discloses that the temperature range for infection of wheat by Puccinia graminis is from 42° F. to 80° F. Humidity range for infection, within the optimum temperature range, is from 95% to 100%.

Hiratsuka (1934), with Uromyces fabae, finds that development of uredosori occurs between the temperatures of 6° C. and 24° C. Statements relative to other rusts are to be found in the literature on spore germination, page 14.

Materials and Methods

Specially equipped chambers were used for housing inoculated pea plants under controlled temperature and humidity.

The chambers consisted of a wooden frame-work with double glass sides and roof. The heating units for each chamber were comprised of four 100 watt lamps, two of which were kept permanently active, and the others connected to a Dekhotinsky thermo-regulator within the chamber. Moisture was supplied by an electrical humidifier standing in a tray of water below the chamber. Water in finely particulate form was blown into the chamber through a tube of one-inch bore reaching about eighteen inches above the floor. The humidity was controlled within 1 - 2% by an electrical humidistat, wired to make and break the connection to the motor of the humidifier. Light was supplied by 100 watt lamps immediately above the glass roof. The chambers were kept in an underground, unheated tunnel, the temperature in which ranged chiefly from 0^{0} - 8^{0} C. during the course of the experiment. The chambers, which proved most satisfactory, were designed and wired by Professor Coulson, Macdonald College.

Temperature and humidity were registered by a thermo-hygrograph.

The variety of peas used was Thomas Laxton, a commonly grown and susceptible variety.

Inoculation was accomplished most satisfactorily by using an atomizer to spray the plants with a suspension of urediniospores after much of the cuticle of the plant had been removed by gently rubbing the surface of the leaves and stem between

the moistened thumb and finger. The droplets of water bearing the inoculum were allowed to dry off, quickly, in a warm, dry section of the greenhouse before being placed in the chambers where humidity relations were being determined. Two series of tests were undertaken; one was maintained at constant humidity while temperature was varied, and the second at constant temperature and varying humidities. The temperature range of the series was from 40° F. to 100° F. (4.5° C. - 38° C); humidity from 75% to 100%.

Three inoculated plants were placed in a chamber at each test, kept under constant conditions for 48 hours, and then removed to a greenhouse of high humidity, and temperature of 65° F. to 70° F. After the necessary incubation period, the number of pustules developing on each plant was counted.

In the discussion of results the term "infection" refers to the formation of uredinial sori after a sufficient incubation period.

Results.

No infection could be induced at any temperature at humidities below 100%, though several tests were made with the humidity fluctuating between 97% and 99%. Accordingly, figures could only be obtained for the different temperatures at a fixed humidity of 100%. At this humidity, condensation particles or water were present on the plants.

These results are presented in graphical form in Fig. 3, page 23, the ordinates of the curve being the total number of pustules developing on twenty plants at each of the temperatures

stated. The minimum temperature at which infection took place was 55° F. (13° C.) At this temperature only a total of five pustules were found on the twenty plants. Optimum infection was obtained at 75° F. (23° C.) The maximum was reached at 80° F. (27° C.), when only 19 pustules matured. From 60° F. (14.5° C.) to 76° F. (24° C.) infection was high, rapidly decreasing beyond these values.

These temperatures approximate the values obtained for spore germination, from which it appears that spore germination is the primary factor in infection of a susceptible host, provided that the humidity is at 100%. It is possible that the higher minimum temperature for infection, as compared with Hiratsuka's results in Japan, is due to the effect on the host of the relatively unfavourable winter greenhouse conditions, or else to the use of different varieties.

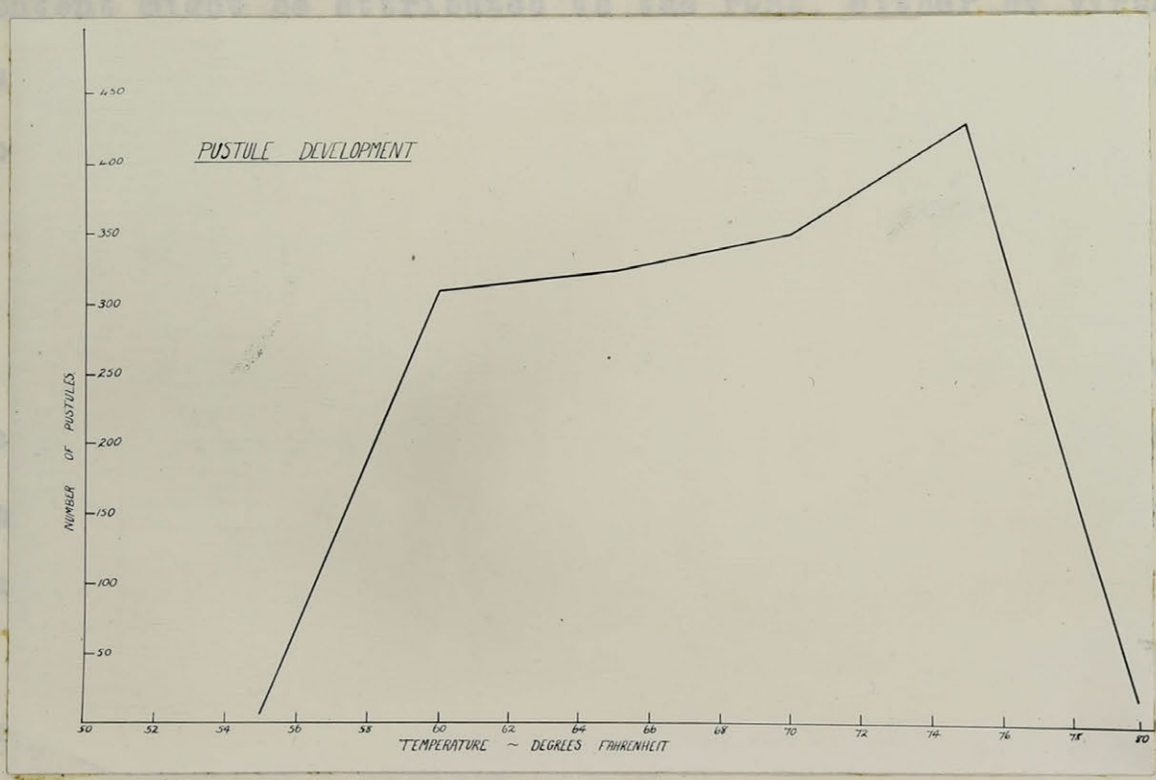

Fig. 3 Pustule Development. The total numbers of pustules developing on twenty plants after being kept for the first 48 hours of their incubation period at the temperatures indicated. Humidity constant at 100%.

3. The Enzymes of Uromyces fabae

Introduction.

The types of compounds used as food by a rust have long been a question of perplexity. It was felt that a new line of approach would be opened, and perhaps some new indication of the rust's nutritional action discovered, if a portion of the enzymic content of the rust cells could be ascertained. No publications on such a study have come to the notice of the writer. A great initial difficulty is immediately manifest in that the rust cannot be grown on an artificial culture medium. Separation of the mycelium from the host tissue being impracticable, the remaining possibility of determination lies in a study of the enzymes of healthy host tissue, and of tissue densely interspersed with rust mycelium. Any difference in content might be attributed to the rust, either by virtue of the rust containing extra enzymes or possessing the power to prevent formation of normal host enzymes.

Literature.

Standard texts consulted in this work, from which much information on methods and reagents was gleaned, were those of Haas and Hill (1921), Waksman and Davidson (1926), Onlsow (1929) and Buchanan and Fulmer (1930).

Menon (1934) and Lanphere (1934), in recent publications on fungus enzymes, give valuable information on technique, which was consulted freely. Lanphere obtained positive tests for seven enzymes from Armillaria mellea, and summarizes the publications

referring to enzymes extracted from this species. Menon (1934) reviews the work on pectinase which has been detected in the extracts from many fungi by several workers including Brown (1915), Harter and Weimer (1921), Muhleman (1925), Willaman and Davison (1927), and Chona (1932).

Materials and Methods.

Infected tissue around uredinial pustules was cut from pea leaves with a cork-borer, throughly ground with fine, washed sand, mixed with a little distilled water, and allowed to stand overnight after the addition of a small quantity of chloroform as a disinfectant. The mixture was then filtered through a Buchner filter and the exzyme fraction precipitated with three volumes of 95% alcohol. This was filtered, and the filter papers dried at room temperature and stored in glass stoppered bottles kept in the dark. For use in tests the filter papers were cut into fine pieces, and distilled water added in such proportion as to make 1 c.c. of the enzyme solution equivalent to one gram of the original tissue. After preparation of the substrates for the enzymes, 2 c.c. of the enzyme solution was added to 10 c.c. of the substrate contained in a test-tube. Addition of 2 c.c. distilled water was added to the check tubes. A small quantity of toluol was included in each tube as a preservative. This is the method outlined by Lanphere (1934).

Tests for the following enzymes were made as indicated:

Diastase

Substrate - Soluble starch
Test - The test for reducing sugars outlined by Schlenker (1933), and Fehling's test.

Invertase
Substrate - 1% sucrose
Test - Schlenker's and Fehling's tests.

Cellulase
Substrate - suspension of pulverized filter-paper
Test - Schlenker's and Fehling's tests.

Carboxylase
Substrate - 10 c.c. M/10 pyruvic acid - 1 c.c. M. K_2HPO_4
Test - Place in fermentation tube in a beaker of water at 37° C, and observe evolution of CO_2.

Oxidase
Substrate - 10% quaiacum gum in alcohol
Test - Add few drops of quaiacum gum solution to 5 c.c. enzyme solution and await blue colour, which indicates presence of oxidase. (Lanphere). The gum solution is prepared from the inner portion of lumps of the gum since the outer layer is oxidised.

Peroxidase
Substrate - As for oxidase
Test - As for oxidase, except that few drops of hydrogen-peroxide are added immediately after the addition of enzyme solution. Await speedy development of a blue colour.

Catalase
Test - Add few drops of hydrogen-peroxide to the enzyme solution. Formation of gas bubbles indicates presence of catalase.

Phenolase
Substrate - Dilute diphenylamine
Test - Indophenol test (a naphthol plus para-phenylenediamine).

Asparaginase
Substrate - 1% asparagine.
Test - (1) Note smell of ammonia after addition of enzyme.
(2) Nessler's reagent.

Inulase
Substrate - 1% inulin.
Test - Schlenker's and Fehling's test, after incubation at 30° C. for 3 days.

Zymase
Substrate - 1% sucrose.
Test - Add enzyme solution to sucrose solution in a fermentation tube. Observe formation of CO_2 after incubation at 25° C.

Sugar splitting enzyme to form pyruvic acid

Substrate - (1) 1% sucrose
(2) 1% dextrose
(3) Starch

Test - Incubate at room temperature for 18 hours, then add a few drops of each of sodium nitro-prusside and 10-20% aqueous pyridine. Note formation of a blood-red, crimson or blue colour.

The whole series of tests was made twice with different extracts of both diseased and healthy tissue.

Results.

The accompanying table (Table 1, page 28) shows the results obtained from enzyme axtracts of both diseased and healthy tissue. The only difference in the two series was the presence of cellulase in diseased tissue and its absence in healthy tissue. This would account for the power the fungus has to penetrate host cell walls. Which of the other enzymes were present in the rust mycelium could not be determined since comparative quantitative measurements were not made. The similarity of the two sets of results would further indicate that invasion by a rust does not greatly modify the metabolism of the host since the normal enzymes remain actively present.

The presence of diastase in the diseased tissue indicates that the accumulation of starch in these tissues is not due to suppression of the starch hydrolysing enzyme.

Table I. The enzymes in Rusted and Healthy Tissues.

	Diseased Tissue		Healthy Tissue		Check	
Enzyme	Result	Incubation Period	Result	Incubation Period	Result	Remarks
Diastase	+	18 hours	+	18 hours	-	
Invertase	+	"	+	"	-	
Cellulase	+	"	-	"	-	
Carboxylase	+	12 hours	+	12 hours	-	
Oxidase	+	5 minutes	+	5 minutes	+	After 24 hours
Peroxidase	+	Immediate	+	Immediate	-	
Catalase	+	"	+	"	-	
Phenolase	+	10 hours	+	10 hours	-	
Asparaginase	-	30 hours	-	30 hours	-	
Inulase	-	3 days	-	3 days	-	
Zymase	+	18 hours	+	18 hours	-	
Pyruvic acid forming	-	"	-	"	-	

Note: The sign + or - indicates that a positive or negative test was returned for the particular enzyme referred to.

4. The Absorption of Nutrients by Uromyces Fabae (Pers.) de Bary

Introduction

The highly specialized parasitic relationship existing between the individual rust fungi and their hosts has been the subject of voluminous comment.

The marked degree of adaptation to a parasitic habitat, frequently analogously referred to the metabolism of mycorrhizal symbionts (Rice, 1927) and indicated by the very slight histological disturbance manifested by a rust-infected plant, is particularly emphasized by the work of the following authors: Ward (1890 and 1902), Sappin-Trouffy (1896), Evans (1907), Fromme (1913), Stakman (1915), Mains (1917), Allen (1923 and 1926), Rice (1927 and 1934), Waters (1928), Dufrenoy (1929), and Forward (1932).

The opinion expressed by Ward (1890) that a rust fungus "taxes" or merely appropriates part of the host's nutritional output, rather than directly injures the cells of its host, is generally accepted. The subsequent results of Fromme (1913), Mains (1917) and Forward (1932) indicate that a rust fungus meets its nutritional requirement by encroachment upon a product dependent upon the active functioning of some phase of the host's metabolism.

The mechanism influential in this delicate withdrawal has received little attention, and, to date, a clear concept of the physiological factors active in the maintenance of this host parasite relationship is not yet established with any degree of certainty.

Accordingly, an attempt was made to obtain data on which an acceptable explanation of food absorption by a rust might be based. The results of this undertaking are described herein.

In living cells, the fundamental processes normally involved in absorption of food materials are those associated with osmotic phenomena and permeability of the cell-membrane. The normal host cell exerts a retentive power on its nutrients by virtue of the properties ascribed to a semi-permeable membrane, in which capacity the cytoplasm of the cell is functioning (Scarth and Lloyd 1931). It is considered likely that the retentive power of the plasma-membrane is impaired, i.e. its permeability increased, when a non-lethal parasite of intercellular type encroaches upon the contents of the attacked cell.

The extensive review of literature on haustoria by Rice (1927) indicates that, by most investigators, the rusts are held to be intercellular parasites in so far as the host-cell membrane is concerned. According to Rice even the rust haustoria are intercellular since she asserts they do not rupture the cell membrane but merely invaginate it. In any case, for a considerable period before extensive haustorial development the rust fungus is essentially intercellular. Accordingly, the effect produced by rust invasion on the permeability of the host-cell membrane, and its corollary, the osmotic relations of the cells involved, were thought to offer a fruitful field for investigation of the physiological effects of rust attack.

In view of the fact that osmotic phenomena are among the fundamental physiological factors involved in food intake by an organism, comparatively little work has been published on this

important phase of host-parasite inter-relationships.

Knowledge bearing on the relative osmotic value of cells of parasite and host, and of diseased and healthy tissue within the host, is largely vague and indecisive, and frequently the result of chance observation rather than direct enquiry. Except for the establishment of a relatively high osmotic value of the rust parasite, close agreement of results within this field has not been achieved.

MacDougall (1911), after studies on angiospermous parasites, indicates the importance of osmotic relationships between host and parasite with the following statement: "The ruling factor (in parasitism) was in all cases the osmotic ratio between the sap of the two plants; one plant may not become parasitic upon another except by the aid of superior osmotic pressure which withdraws solutions from the tissues of the enforced host." The concept offered here is probably a justifiable one, but the latter part of the statement is inadequate since it neglects the fact that the solutes of the host are held within a semi-permeable membrane which precludes the occurrence of purely physical diffusion as inferred in the closing sentence of the quotation.

Tischler (1911), using plasmolytic methods, found that infected cells of pea plants harbouring Uromyces pisi showed a higher osmotic pressure than did normal cells.

Harris (1916) measured the osmotic concentration of extracted sap from many species of Loranthaceae parasitic on various hosts, and found that in general, the osmotic pressure of the parasite was the higher. The osmotic pressure was found to increase progressively from host to primary parasite to secondary parasite.

Weiss (1924) demonstrates that water loss from leaves of a number of plants may be diminished or accelerated by rust infection, suggesting that the effect in the former case is due, collectively, to altered osmotic relations of infected cells, to reduced leaf area, and to structural alteration.

Hursh (1927) found that expressed sap from wheat plants infected with Puccinia graminis tritici had the same freezing point as that expressed from healthy plants, indicating that parasitism had effected no change in osmotic pressure as determined by this method.

Ruttle and Fraser (1927) state that Banner Oats invaded by Puccinia coronata show a heightened turgor among the cells immediately influenced by the rust's attack. This might be interpreted as an effect of an increased concentration of solutes within these cells due to the presence of the parasite. On the other hand, there is no proof that apparent increase of turgidity may not have been consequent upon imbibition of the colloids within the cell, there being a number of factors able to bring about the necessary changes in hydration (Scarth and Lloyd, 1931).

Allen (1923) states that cells in the vicinity of the psrasite are plasmolysed when Mindum wheat is attacked by Puccinia graminis tritici, forms III and XIX. The same writer (1926) reports that the hyphae of Puccinia triticina induce an increased turgor in the adjacent cells of Little Club wheat.

Dufrenoy (1928) states that cells of Arisaema triphyllum infected by Uromyces calladii become plasmolysed in an 8% sugar solution, while healthy cells of the same section remain turgid. Miss Rice, (1934) in studies on the same rust, finds no difference

in degree of plasmolysis of diseased or healthy tissue at this or at higher concentrations.

Fischer and Gaumann (1929) report on modified water relationships associated with plants parasitized by rust fungi, and suggest that alteration of permeability of the cell membrane, together with mechanical injury, bring about a disturbance of osmotic equilibrium responsible for the observed abnormalities.

The following workers have offered closely comparable values for the osmotic pressure of rust spores: Hassebrauk (1932), Rippel (1933), Ronsdorf (1934). Hassebrauk proves statistically that difference in osmotic pressure of urediniospores does not offer a means of distinguishing physiological forms of the same rust, as suggested by Steiner (1930). Rippel (1933) finds that the speed of germination of mature spores of several fungi varies directly as the osmotic pressure of the spores. Such an effect might be expected since water intake, an essential factor in spore germination, is a direct function of osmotic pressure. Attempts to correlate resistance to rust fungi with the osmotic pressure of the host cell-sap have, to date, proved abortive. Pantanelli (1921), working with Uromyces fabae on pea varieties, was unable to demonstrate such a relationship, while experiments of Johnson and Johnson (1934) with wheat rust (Puccinia graminis tritici) lead to the same negative conclusion.

Kuprewicz (1935), following studies on a number of plant diseases, including that caused by Puccinia suaveolens, states that disturbed osmotic pressure is a general effect of disease due to parasites.

Contradiction and incompleteness are apparent in the above results.

Materials and Methods

I. Permeability of the Cell Membrane

In the diffusion of any substance through a membrane from one concentration to another, the amount of substance passing through the membrane is a function of time. Accordingly, the relative time taken for a certain quantity of material to pass through different membranes gives a comparative measure of the permeability of these membranes to the substance in question.

A. Permeability to water

The plasmolytic method demonstrated by Hofler (1930) was used in this work, Hofler having established the relation $K = \frac{1}{tC}$, if cells are plasmolysed to the same degree.

K = permeability constant of the membrane

t = time

C = concentration of plasmolysing solution.

Portions of leaves and petioles of the pea plant (Pisum sativum L.) bearing uredinial sori of Uromyces fabae Pers. were cut in such a manner that a section contained both sporulating areas and areas beyond the extent of the rust mycelium. These materials were sectioned free-hand, as thinly as possible. Transverse sections of the leaf and longitudinal sections of the petiole presented the most convenient orientation of the cells.

The sections were first stained with dilute neutral-red in a solution of calcium chloride, approximately isotonic with the cells, the isotonic strength having been determined previously.

The vacuoles of all living cells became conspicuous through their neutral-red content. Sections were then plasmolysed in a strongly hypertonic solution of calcium chloride, which salt does not enter the vacuole, and were later removed to calcium chloride of lower concentration but still of hypertonic strength. This caused the plasmolysed protoplasts to assume a regular shape which facilitated observation and measurement.

The sections were then removed from this hypertonic solution to a hypotonic solution (both of known strength) and the time elapsing between immersion in this latter solution and the moment at which 50% of the cells were in a condition of incipient plasmolysis was noted. This figure was obtained for cells interlaced with hyphae and for cells beyond the hyphal area. Where possible, a figure for the intermediate zone was obtained from the cells grouped, as closely as could be determined, at the immediate edge of the portion of tissue showing mycelium. In each case, observation of as large a number of cells as possible was made by rapid, successive examination of different fields of the microscope. A number of sections from each portion of tissue were treated in identical manner, and the average times recorded for deplasmolysis in each piece of tissue.

B. Permeability to organic compounds.

Sections were cut and staining in neutral-red accomplished, as described above.

Urea is a compound commonly used to demonstrate permeability of a membrane to certain organic substances, but, with the material used, was found to be so active in penetrating the

membrane that the time taken for deplasmolysis was too short to measure. Use of thio-urea overcame this difficulty.

After staining, the sections were strongly plasmolysed in thio-urea of known hypertonic concentration, and the lapse of time necessary for deplasmolysis determined as before. Deplasmolysis in this case is due to the entry of thio-urea into the vacuole.

C. Permeability to inorganic salts.

Most inorganic salts are only able to penetrate a cell membrane very slowly. (Scarth and Lloyd, 1931). Potassium nitrate is one of the most rapidly penetrating of the non-toxic salts and was, therefore, chosen for this purpose. However, degeneration of the cell began to occur before cells plasmolysed in 0.5 molar potassium nitrate had undergone normal deplasmolysis. This study of membrane permeability was abandoned in consequence.

II. The Osmotic Pressure of Diseased and Healthy Tissue.

Two methods were used for the determination of osmotic pressure. One depended on the estimation of the isotonic value of the cells by examination of sections in different concentrations of calcium chloride (A). Lineal measurements of individual protoplasts plasmolysed in a solution of known strength formed the basis of the second (B).

A. Determination of the isotonic point.

Sections were cut and stained in neutral-red as described in I, A.

Sections from the same portion of leaf or petiole were then placed in a series of solutions of calcium chloride ranging in concentration from 0.1 Molar to 0.3 Molar. After a lapse of 10 minutes (to allow plasmolysis to occur in hypertonic solutions) sections were mounted in solution of the same strength as that from which they were removed and examined under the microscope. As indicated by Huber (1934), Harris and Gortner (1917) and others, osmotic pressure varies among different tissues zones as well as from different organs. Hence, examination was confined to chlorenchyma cells about two to three cell-layers below the epidermis. The solution in which approximately 50% of these cells were showing incipient plasmolysis was considered to be isotonic with the cell sap.

B. Osmotic pressure by cell measurement.

The results of Hofler (1930) and de Haan (1931) show that for cells of regular shape the degree of plasmolysis brought about by a given solution is indicated by the expression $\frac{l-b/3}{h}$, where

l = length of plasmolysed protoplast

b = width of plasmolysed protoplast

h = original length of protoplast before plasmolysis.

By application of Boyle's law (vide Scarth and Lloyd, 1931), the concentration of the cell sap before plasmolysis is given by the product of this quotient and the concentration of the plasmolysing solution. Highly significant values for osmotic pressure are afforded by measuring many cells and by choosing cells of shape as regular as possible. (A rectangular prism is the ideal shape).

Following preliminary staining with neutral-red as above, sections including both diseased and healthy tissue were strongly plasmolysed in calcium chloride solution. The above mathematical relationship is based upon the assumption that the plasmolysed protoplasts are of regular form with hemispherical ends. The desired convex plasmolysis was induced by removing the sections to a second calcium chloride solution of lower concentration than the first, but still decidedly hypertonic to the cells.

Cells as nearly as possible approaching the ideal shape were chosen for measurement, such cells being found in the stem, among the lower chlorenchyma layers in longitudinal section, and in the leaf, among the palisade layer as seen in transverse section. The original length of the cell, the length of the plasmolysed protoplast, and its width were measured for individual cells grouped in the following areas: 1. tissue densely interlaced with hyphae; 2. tissue immediately beyond the observable area of hyphal penetration; 3. healthy tissue removed from mycelial growth. Several hundred cells were measured in all, and their osmotic values calculated according to the above formula.

III. The Osmotic Value of Germinating Spores of Uromyces Fabae.

Efforts to determine the osmotic pressure of germ-tubes of Uromyces fabae by the plasmolytic method yielded unreliable results since the non-continuous vacuoles of the germ-tubes and their small size rendered accurate observation too uncertain even with dark field illumination and high magnification.

The following methods were therefore subsequently adopted.

A. Ronsdorf's Method.

Ronsdorf (1934) germinated rust spores in different solutions of known osmotic value, and considered as isotonic with the spores the solution in which the spores would just germinate.

This method was adopted using calcium chloride solutions kept in moist chambers at room temperature, spores being floated on the colutions contained in Van Tieghem cells. The solution in which about 50% of the spores showed just visible, initial germ-tube protuberances after an exposure of 18 hours was considered isotonic with the spores.

B. Vapour pressure method.

A method was devised based upon the relationship between osmotic pressure and vapour tension. The osmotic pressure of a spore determines its potential power to take up water necessary for germination. Accordingly, fresh spores were scattered over clean glass slides suspended over inclosed sulphuric acid solutions of known vapour tension, the solutions being made up according to directions given by Stevens (1916). It was expected that the solution over which the vapour tension was such as to permit spore-germination, would allow calculation of the osmotic pressure of the spores by substitution in the formula:

$$O.P. = \frac{1000S \times R \times T}{M} \cdot \frac{p_o - p}{p}$$, where

S = specific gravity of the medium (water in this case)

M = molecular weight of the medium

T = absolute temperature

R = Avogadro's gas constant

p_o = saturation pressure

p = vapour pressure of the solution.

C. Modified vapour pressure method.

A modification of the above vapour pressure method was introduced to overcome the initial impermeability of the exospore. Spores were floated on a water film at room temperature until germination had just begun. They were then freed from as much water as possible and transferred to slides above the sulphuric acid solutions described above. The solution above which growth of the germ-tube was allowed to continue, was considered indicative of the osmotic pressure of the spores.

Results.

A. Permeability of the Cell Membrane

i. Permeability to water.

The results as summarized in Table 2, page 43, portray a marked increase in the permeability of diseased tissues in each specimen examined, indicating a decided physical modification in the plasma-membranes of cells in the vicinity of rust mycelium.

ii. Permeability to organic compounds (Thio-urea)

Table 3, page 44, illustrates a large difference in permeability to organic compounds between the diseased and healthy tissues. The diseased cells are much the more permeable, again proving physical modification of the plasma membranes of these cells.

B. Osmotic Values of Diseased and Healthy Tissues.

i. By determination of the isotonic point.

The figures presented in Table 4, page 45, show that a decided difference has been induced in the osmotic pressure of

cells influenced by the rust hyphae. A modification in the osmotic values of different tissues of the same type is noticed. Huber (1934) has shown that this is to be expected. The important feature is that in every sample a reduction in osmotic pressure of diseased cells is manifest.

ii. By lineal measurement.

Table 5, page 46, shows a large number of measurements from which the osmotic pressure of individual cells is computed. Statistical analysis shows a significant difference in the osmotic pressure of cells in diseased and healthy tissues, in agreement with the results expressed in Table 4. Diseased cells are again shown to have a reduced osmotic pressure.

C.. Osmotic Pressure of Urediniospore Germ-tubes.

i. Ronsdorf's method.

The figures in Table 6, page 53 establish the osmotic pressure of urediniospore germ-tubes as lying between the pressures equivalent to that exerted by solutions of from 0.66 Molar to 0.68 M. which is very close to Hassebrauk's (1932) value of 0.65 M. for Puccinia triticina. This is very much above the value for tissues of the host, which, in the case of pea leaves is about 0.16 M.

ii Vapour pressure method.

No readings were obtained by this method since spores failed to germinate unless in a saturated atmosphere. This indicates impermeability of the exospore, the significance of which will be pointed out later.

iii. Modified vapour pressure method.

This method also proved unsatisfactory. Germ-tubes developed irregularly, in scattered instances only, except at saturation pressure.

The following tables indicate clearly: first, that the osmotic value of the invading hyphae is very substantially higher than that of the host cells; secondly, the osmotic pressure of cells in the diseased area is lower than in the healthy cells beyond the area of fungus penetration; and, thirdly, that association of the rust with its host brings about markedly increased permeability of the host plasma-membrane to water and organic material.

PERMEABILITY TO WATER

Tissue	Plasmolysing solution	Deplasmolysing solution	Time for deplasmolysis		
			Healthy	Diseased	
Leaf	0.30M CaCl	0.05M CaCl	4mins.30 secs.	2mins.00secs.	
			5 " 00 "	2 " 00 "	
			3 " 55 "	1 " 45 "	
			4 " 20 "	2 " 00 "	
			4 " 40 "	2 " 00 "	
				1 " 30 "	
				1 " 15 "	
				1 " 45 "	
		Average	4mins.36secs.	1min. 44secs.	
		Difference		2min. 52secs.	
Petiole(1)	0.30M CaCl	0.10M CaCl	12mins.30secs.	2mins.00secs.	N.B. Many diseased cells deplasmolyse too fast to measure.
			10 " 00 "	3 " 00 "	
			10 " 00 "	1 " 45 "	
			15 " 30 "	2 " 00 "	
			14 " 00 "	2 " 00 "	
		Average	12mins.36secs.	2mins.09secs.	
		Difference		10mins.27secs.	
Petiole(2)*	0.30M CaCl	0.10M CaCl	11mins.45secs.	2mins.15secs.	
			10 " 00 "	1 " 45 "	
			10 " 30 "	2 " 00 "	
			11 " 00 "	2 " 00 "	
			9 " 45 "	1 " 30 "	
			12 " 00 "	2 " 15 "	
		Average	10mins.50secs.	1min. 57 secs.	
		Difference		8mins.53 secs	

*Note: An entirely new set of solutions was prepared for this group of tissues.

PERMEABILITY TO THIO-UREA

Tissue	Strength of Thio-urea solution	Time taken to deplasmolyse		
		Healthy	Diseased	Difference
Petiole(1)	1.0Molar	10mins. 00 secs.	2mins.12 secs.	7mins. 48 secs.
		9 " 45secs.	2 " 20 "	7 " 25 "
		9 " 30 "	2 " 17 "	7 " 13 "
		9 " 48 "	2 " 20 "	7 " 28 "
	Average	9mins.46secs.	2mins.17 secs.	7mins. 28 secs.
		Difference---7mins. 29 secs.		
Petiole(2)	0.75 M	15mins.45secs.	7mins.36secs.	8mins. 9secs.
		15 " 20 "	6 " 45 "	8 " 35 "
		--	7 " 45 "	---
		14 " 10 "	4 " 05 "	10 " 05 "
	Average	15 " 05 "	6 " 33 "	8 " 56 "
		Difference---8mins. 32 secs.		

Petiole(3)	0.75M	Healthy	Intermediate	Diseased
		8mins.06secs.	3mins.20secs.	2mins.30secs.
		12 " 05 "	5 " 00 "	2 " 45 "
		13 " 20 "	9 " 20 "	5 " 50 "
		7 " 15 "	3 " 20 "	2 " 25 "
		14 " 25 "	--	2 " 55 "
		12 " 30 "	--	--
	Average	11mins.17secs.	5mins.15secs.	2mins.41secs.

Difference between healthy and diseased--8mins.36secs.

Tissue	Conc. of $CaCl_2$	Degree of Plasmolysis		
		Diseased tissue	Intermediate	Healthy
Petiole	0.25 M	plasmolysed	plasmolysed	plasmolysed
	0.225 M	"	"	"
	0.20 M	"	"	"
	0.185 M	"	"	isotonic
	0.175 M	"	isotonic	not plasmolysed
	0.170 M	isotonic	not plasmolysed	"
Petiole	0.25 M	plasmolysed	plasmolysed	plasmolysed
	0.225 M	"	"	"
	0.20 M	"	"	"
	0.175 M	"	"	"
	0.165 M	"	"	few plasmolysed
	0.160 M	"	isotonic	isotonic
	0.150 M	"	not plasmolysed	not plasmolysed
	0.125 M	"	"	"
	0.120 M	isotonic	"	"
	0.10 M	not plasmolysed	"	"
Leaf	0.25 M	plasmolysed	plasmolysed	plasmolysed
	0.20 M	"	"	"
	0.175 M	"	isotonic	isotonic
	0.15 M	isotonic	not plasmolysed	not plasmolysed
	0.12 M	not plasmolysed	"	"
	0.10 M	"	"	"
Leaf	0.20 M	plasmolysed	plasmolysed	plasmolysed
	0.175 M	"	"	"
	0.165 M	"	"	"
	0.160 M	"	"	isotonic
	0.150 M	"	"	not plasmolysed
	0.125	"	"	"
	0.120 M	isotonic	isotonic	"
	0.100 M	not plasmolysed	not plasmolysed	"

TABLE 5.

OSMOTIC PRESSURE BY LINEAR MEASUREMENTS OF CELLS

Tissue	M Concentration of plasmolysing solution	g Degree of plasmolysis $g = \frac{1-b/3}{h}$	g x M Isotonic conc. in mols of $CaCl_2$	O.P. Calculated osmotic pressure in atmospheres.
Leaf 1				
1. Healthy	0.25 M	0.63	0.15	
		0.61	0.14	
		0.59	0.15	
		0.72	0.18	
		0.72	0.18	
		0.59	0.15	
	0.30 M	0.62	0.18	
		0.58	0.17	
		0.61	0.18	
		0.60	0.18	
		0.54	0.16	
		0.57	0.17	
		Average	0.166	9.5
Diseased	0.35 M	0.28	0.098	
		0.29	0.10	
		0.36	0.13	
		0.25	0.09	
		0.33	0.12	
		0.36	0.13	
		0.34	0.12	
		0.25	0.09	
		0.37	0.13	
		0.39	0.14	
		0.30	0.11	
		0.37	0.13	
		0.30	0.11	
		0.28	0.10	
		0.30	0.10	
		0.26	0.09	
		0.24	0.08	
		0.30	0.10	
		0.35	0.12	
	0.40 M	0.26	0.10	
		0.26	0.10	
		0.26	0.10	
		0.24	0.10	
		0.30	0.12	
		0.26	0.10	
	0.25 M	0.48	0.12	
		Average	0.108	6.74

Table 5 (continued)

Tissue	M	g	g x M	O.P.
3. Intermediate zone	0.30 M	0.45	0.13	
		0.45	0.13	
		0.44	0.13	
		0.43	0.13	
		0.45	0.13	
		0.36	0.11	
		0.49	0.15	
		0.40	0.12	
		0.40	0.12	
		0.38	0.11	
		0.45	0.13	
		0.42	0.13	
		0.48	0.14	
		0.41	0.12	
		0.43	0.13	
		0.44	0.13	
		0.47	0.14	
		0.46	0.14	
		0.37	0.11	
		0.42	0.13	
		0.46	0.14	
		0.40	0.12	
		0.40	0.12	
		0.41	0.12	
		0.46	0.14	
		0.39	0.12	
		0.45	0.13	
		Average	0.128	8.04
Stem				
1. Healthy	0.30 M	0.60	0.18	
		0.60	0.18	
		0.60	0.18	
		0.63	0.19	
		0.65	0.19	
		0.55	0.17	
		Average	0.181	10.26
2. Diseased	0.30 M	0.47	0.14	
		0.52	0.16	
		0.60	0.18	
		0.58	0.17	
		0.56	0.17	
		0.51	0.15	
		0.48	0.14	
		0.35	0.11	
		Average	0.152	8.77

Table 5 (continued)

Tissue	M	g	g x M	O.P.
Petiole 1				
1. Healthy	0.35 M	0.54	0.18	
		0.60	0.20	
		0.56	0.19	
		0.52	0.17	
		0.70	0.23	
		0.63	0.21	
		0.53	0.18	
		0.70	0.23	
		0.54	0.18	
		0.58	0.19	
		0.66	0.22	
		0.54	0.18	
		0.60	0.20	
		0.70	0.23	
		Average	0.196	11.03
2. Diseased	0.35 M	0.33	0.12	
		0.48	0.17	
		0.36	0.13	
		0.33	0.12	
		0.40	0.14	
		0.46	0.16	
		0.42	0.15	
		0.39	0.14	
		0.42	0.15	
	0.30 M	0.55	0.16	
		0.48	0.14	
		0.51	0.15	
		0.50	0.15	
		0.50	0.15	
		Average	0.142	8.25
Petiole 2				
1. Healthy	0.35 M	0.43	0.15	
		0.51	0.18	
		0.47	0.16	
		0.51	0.18	
		0.51	0.18	
		0.48	0.17	
		0.46	0.16	
		0.55	0.19	
		0.42	0.15	
		0.50	0.18	
		0.50	0.17	
		0.57	0.20	
		0.49	0.17	
		0.48	0.17	
		0.49	0.17	
		0.42	0.15	
		0.44	0.15	
		0.47	0.16	
		0.52	0.18	
		0.47	0.16	
		0.56	0.19	
		Average	0.175	10.105

Table 5 (continued)

Tissue	M	g	g x M	O.P.
2. Diseased	0.25 M	0.48	0.12	
		0.52	0.13	
		0.52	0.13	
		0.49	0.12	
		0.53	0.13	
		0.56	0.14	
		0.64	0.16	
		0.61	0.15	
		0.57	0.14	
		0.60	0.15	
		0.57	0.14	
		0.56	0.14	
		0.62	0.16	
		0.45	0.11	
		0.62	0.16	
		0.51	0.13	
		0.54	0.13	
		0.43	0.11	
		0.50	0.13	
		0.35	0.09	
		0.34	0.09	
		0.66	0.16	
		0.64	0.16	
		0.55	0.14	
		0.57	0.14	
		0.49	0.12	
		0.55	0.14	
		0.46	0.12	
		0.52	0.13	
		0.38	0.10	
		0.55	0.14	
		Average	0.133	7.78
Petiole 5				
1. Healthy	0.30 M	0.58	0.17	
		0.60	0.18	
		0.50	0.15	
		0.47	0.14	
		0.46	0.14	
		0.50	0.15	
		0.49	0.15	
		0.52	0.16	
		0.54	0.16	
		0.54	0.16	
		0.58	0.17	
		0.56	0.17	
		Average	0.160	9.18

Table 5 (continued)

Tissue	M	g	g x M	O.P.
2. Diseased	0.30 M	0.47	0.14	
		0.34	0.10	
		0.39	0.12	
		0.46	0.14	
		0.33	0.10	
		0.31	0.09	
		0.25	0.08	
		0.27	0.08	
		0.34	0.10	
		0.48	0.14	
		0.39	0.12	
		0.34	0.12	
		0.40	0.12	
		0.52	0.16	
		0.24	0.07	
		0.33	0.10	
		0.33	0.10	
		0.34	0.10	
		0.44	0.13	
		0.42	0.13	
		0.46	0.14	
		0.38	0.11	
		0.40	0.12	
		0.34	0.11	
		0.45	0.13	
		0.41	0.12	
		0.43	0.13	
		0.33	0.10	
		0.49	0.15	
		0.50	0.15	
		Average	0.117	7.00
3. Inter-mediate zone	0.35 M	0.36	0.12	
		0.43	0.15	
		0.36	0.12	
		0.36	0.12	
		0.38	0.13	
		0.37	0.13	
		0.32	0.11	
		0.34	0.12	
		0.38	0.13	
		0.46	0.16	
		0.41	0.14	
		0.42	0.15	
		0.35	0.12	
		0.37	0.13	
		0.34	0.12	
		0.33	0.12	
		0.41	0.14	
		0.36	0.12	
		0.38	0.13	
		0.43	0.15	

Table 5 (continued)

Tissue	M	g	g x M	O.P.
		0.37	0.13	
		0.43	0.15	
		0.37	0.13	
		0.37	0.13	
		0.34	0.12	
		0.37	0.13	
		0.32	0.12	
		Average	0.131	7.68

Table 5. (continued)

SUMMARY

Average Concentration of Cell Sap.

Tissue	Healthy		Diseased		Intermediate		Difference (Healthy v. Diseased)	Difference (Healthy v. Intermediate)
	Mols.	Atmosphere	Mols.	Atmosph.	Mols.	Atmosph.		
Petiole	0.178	10.105	0.133	7.78			2.32 Atmosph.	
"	0.160	9.18	0.117	7.00	0.131	7.68	2.18	0.68
"	0.196	11.03	0.142	8.25			2.78	
Stem	0.181	10.26	0.152	8.77			1.49	
Leaf	0.166	9.15	0.108	6.74	0.128	8.04	2.76	1.30
						Average	2.31 atmosph. (0.054 mols.)	0.99 atmosph. (0.0335 mols.)

By application of Fisher's formula, the difference between the osmotic pressure of diseased and healthy tissue was proved to be significant. Reference to Fisher's "table of t" indicates that, on the basis P= 0.05, an average difference of 0.030 mols. is necessary for significance. The difference found was 0.054 mols.

Table 6.

OSMOTIC PRESSURE OF GERMINATING UREDINIOSPORES.

	Conc. of CaCl	Germination after 18 hours at 22°C. % germination	Average length of germ tube
(1)	0.60M CaCl	90%	80u
	0.65M "	50%	44u
	0.70M "	30%	25u
	0.75M "	1%	5u
	0.80M "	0	0
(2)	0.65M	57%	64u
	0.67M	42%	slight protrusions
	0.70M	20%	" "
	0.75M "	0%	0
(3)	0.65 "	48%	43u
	0.67	40%	slight protrusions
	0.675M "	28%	" "
	0.70M "	14%	" "
	0.75M "	-	

Average isotonic strength -- 0.66 - 0.68M.

DISCUSSION OF RESULTS

The rust form present in Eastern Canada.

The fact that the same physiologic form of U. fabae attacks both vetch and pea is of economic importance since it designates the almost universal weed, Vicia cracca, as the principal source of inoculum for pea rust. The impracticability of eradication of this weed indicates that control is to be sought among chemical prophylactic measures or the adoption of resistant varieties.

Environment and infection.

The fact that urediniospores need to be in contact with free water before germinating may possibly explain why rust on peas in Canada is not usually severe until the latter part of the growing season. At this time of the year heavy dews, of very frequent occurrence, afford ample opportunity for spore germination, the prevailing temperatures allowing rapid growth of the germ-tubes. The past two seasons have been warm and precipitation frequent (consultation with the Dominion Meteorological Service, Ottawa), which condition not only assures the presence of ample water supply at the time of precipitation, but assures frequent dew formation, at least on foliage near the ground. It is suggested that the marked severity of pea rust during these past seasons has been intimately connected with the occurrence of these conditions.

The danger of repeated epidemics of the rust in Canada is indicated by the fact that frequent occurrence of a set of factors favouring infection is to be expected. The temperature

range for high infection is within the normal summer temperature range for Eastern Canada. Periods of atmospheric saturation, or the presence of free water on the plants, need to be only of short duration since, at the lower temperature limit for infection, germ-tubes reach a length of 70μ in 2 hours, which is considered sufficient length for penetration. Such short periods are likely to occur very often in Eastern Canada.

The absorption of nutrients by U. fabae.

Alignment of the facts divulged by the above studies of osmotic pressure and permeability offers an explanation for the process whereby the rust fungus, U. fabae, is able to extract nutrients from the cells of its host, without killing these cells, and to absorb them into its own hyphae. In addition, light is thrown upon the "green island" phenomenon of rusts, and upon the function of haustoria,

Following initial penetration into the intercellular spaces of the host plant, the rust hyphae, having a higher osmotic pressure than cells of the host, tend to remove water from any of the cells with which they are in contact. (Höfler (1931) has shown decisively that the cellulose plant-cell wall offers negligible impedance to water diffusion). This flow of water, initiated by the fungus, facilitates its growth, while the fungus, in the meantime, brings about an increased permeability of the plasma mambrane which, by 'a priori' reasoning, is due to secretion of some toxin or enzyme by the parasite.

Consequent upon increased permeability of the plasma membrane, the vacuolar solutes, normally retained within the cell by virtue of the properties of the plasmalemma as a semi-permeable membrane, are free to flow outwards, natural diffusion permitting their entry into the fungus, provided such individual substances as are dissolved in the host vacuole are not already at a higher concentration in the fungus cell. It is assumed that this last is not the case.

Analogy is here made to the course of events in attack by a more destructive parasite such as Botrytis, where the balance between host and parasite is of less delicate nature than that found with the Uredineae. The concept of Botrytis attack as depicted by Ward (1890) is of interest in this connection:- "So long as the protoplasm can dispose of small amounts of poison coming in from the hypha, the hypha is barred access to the cells, but immediately the poison succeeds in lessening or destroying the power of the protoplasm to control the cell-sap, the latter exudes through the permeable protoplasm and suffuses the whole tissue with the cell sap, containing just such materials as the fungus flourishes in." The behaviour of the rust fungus is identical in scheme, but the rust, in evolving a more specialized form of parasitism, has developed a non-lethal action which is to this organism's advantage in maintaining continuance of its food supply. The above reasoning suggests an explanation for the phenomenon, first referred to by Stakman (1922), of the formation of "green islands" on the surface of rust infected organs.

Rice (1927) cites a long list of workers who have remarked on this peculiar expression of rust attack, which is most noticeable in a senescent leaf. A rust pustule is frequently centered in an area of bright green, turgid tissue, which appears as an "island" in the rest of the etiolated lamina. At times, an outer circular band of sporulating fungus tissue develops around the initial lesion. The deeper green zone, of healthy appearance, surrounds this outer region of spore production.

Mains (1917) was among the earliest to suggest that this apparent prevalence of normal activity around a rust pustule, while the rest of the leaf is decadent, was due to stimulation of the host cells by the fungus. In addition to this stimulative action, Rice (1927) and others have proposed a probable preservative action upon the chlorophyll of the host cell.

It is here suggested that the primary factor in this prolongation of vigour around a rust pustule is one of water relationship, not necessarily a secreted stimulant or preservative, though such may also be involved. Mains, (1917) proffered such a possibility, but did not support it with data or physiological considerations.

As depicted above, the invading mycelium, by virtue of possessing a higher osmotic pressure than cells of its host, tends to remove water from contiguous host cells. The natural consequence of this would be to initiate a water flow from more remote cells with the focus of flow at the mycelial region. At the inception of normal senescence, etiolation begins at the tip and peripheral regions of the leaf, progressing inwards, following the gradient of water concentration.

The last region to suffer is that around the pustule, due to the fact that water has been converging towards this location. When this region does finally dry and shrivel it does so quickly since it has no further source of water supply. The persistence of green about the pustule, even after death, is due to this rapid drying, just in the same way that dried herbarium specimens retain part of their green colouration. The continuance of green colouring matter about the rust, thus, does not necessarily entail fixation of the chlorophyll while the leaf is in an active state.

Rice (1927) remarked on an instance of excessive rust infection in which the infected leaf was devoid of any expression of the "green island" phenomenon. The writer has made several such observations and offers the explanation that, the rust infection being so heavy, water from uninfected areas of the leaf was not available fast enough, so that, at senescence, no healthy turgid area could be manifest.

It the above postulation relative to the mechanism for nutrient absorption by the rust be considered feasible, then the popular conception of the function of rust haustoria may be open to criticism.

As outlined earlier in this work, the rust haustorium is a specialized absorbing organ according to the most generally accepted idea as expressed by Rice (1927). Miss Rice writes:- "So far as we know haustoria are primarily absorbing organs, although specific evidence as to what and how they absorb is as yet lacking." "The great variety of form found in the absorption sac is evidently an individual working out by the parasites of

a differentiation to effect increased surfaces of contact." The same writer states that rusts are not entirely dependent upon haustoria for their nutrition, since, "....before the development of haustoria the fungus is able to draw some nourishment from the host directly thfough the host cell walls."

The facts and ideas expressed above explain how this last might occur, and at the same time tend to obviate the need for cell-wall penetrating absorbing organs, since host cell-solutes are made available to the fungus in its intercellular condition.

Cytological investigations on Uromyces fabae described earlier in this work show that, in so far as the scope of the present investigation authorizes this statement, the haustoria of this rust are commonly intravacuolar. Since it would appear that such penetration is not essential or, perhaps, even an aid to absorption, it may be doubted as to whether this behaviour has any physiological significance. The fact that the rust does not prey upon the host cytoplasm has already been enlarged upon, and precludes as a possible objective of the haustorium the direct degradatory action on the protoplasmic mass. If the fungus seeks material enclosed within the nuclear membrane one would expect a more general haustorium-nucleus association. That our knowledge on this last relationship is inadequate has been admitted (Rice, 1927) but, in the rust investigated, pathological disturbance of the nucleus was not discerned, though a number of instances of intimate contact between host nucleus and haustorium were observed.

An alternative view-point on the nature of haustoria is that the irregular form of these organs is due to a morphological

distortion of penetrating hyphal threads analogous to the development of involute cell forms in bacterial cultures, noticeably in the development of atypical cell-forms of Rhizobium leguminosarum (Prazmowski, 1890; McCoy, 1929) in tissues of legumes. The observation of irregular, branching or "mycobacterioid" cells from cultures growing under unfavourable conditions due either to adverse external factors, to uncongenial substrata or to biological competition has been observed frequently. The morphology of haustoria closely resembles that of these aberrant cells, the rust haustoria showing all phases of "involution", from an apparently normal hypha to the completely distorted shapes of some of the older haustoria. The above alternative possibility is emphasized by the fact that haustoria-like forms may be induced by germinating rust spores at temperatures approaching their extreme limits for growth. In addition to tardy growth, branching and distortion of the germ-tube are frequent. Similar developments are sometimes apparent if spores germinate in a medium containing a wealth of bacterial or yeast cells. Hence, the irregular contour of a rust haustorium may well be due to direct antibiotic influences of the host cell or to an hypha encountering a physically unfavourable medium. It is the opinion of the writer, resulting from the study of a great many fixed and living sections of Uromyces fabae in pea tissue, that a deformation or lobing of the haustorium is likely to be more pronounced when it is found in close proximity to the host cell nucleus, which suggests a possible antagonistic action on the part of the nucleus against the penetrating rust hyphae. Magnus (1900),

Nemec (1911), Colley (1918), and Allen (1926) offer suggestions that cells of certain plants show a definite active response directed against their respective invading rust fungi. The fact that haustoria in different tissue regions of the same section may have divergent forms tends to suggest the possibility that the specific contour of the haustorium is primarily determined by host action, which again suggests analogy with involute cell forms.

The writer found haustoria in the epidermal cells of pea tissue, but, after scores of attempts, failed to find in this region, any lobed or branched forms characteristic of haustoria in other tissues. Rice (1927) notes a similar observation with respect to corn rust.

That absolute statements on the function and origin of the irregular form of haustoria cannot yet be made is clear, but the above evidence indicates that the popular conception of a rust haustorium being produced specifically for the purpose of absorbing nutrients and for presenting a larger absorbing surface is open to criticism.

SUMMARY

A histological and physiological investigation of pea rust (Uromyces fabae (Pers.) de Bary) is presented. U. fabae f. sp. craccae Fischer is the common and virulent form in Eastern Canada, the major source of inoculum for peas being the wild vetch (Vicia cracca L). Uredinial germ-tubes of this rust penetrate via stomata and resulting hyphae quickly develop haustoria, which are shown to be at least commonly intra-vacuolar, contrary to the opinion of Rice (1927). Stained diseased tissues show slight pathological disturbance except for accumulation of starch, which is not due to suppression of diastase formation.

The temperature range for germination of urediniospores is from 8° C. - 30° C. Contact with free water is shown to be essential for urediniospore germination. The temperature range for infection is 13° C. - 27° C., with an optimum of 23° C. These facts are linked with the prevalence of the disease. A primary factor in natural infection is the presence of water on the surface of the host.

Nine enzymes were detected in an extract from diseased tissue; eight from healthy. The additional one was cellulase, which would enable penetration of the host cell walls by the fungus.

The osmotic value of the fungus was found to be equivalent to 0.66 - 0.68 mols of $CaCl_2$; of the host, 0.16 mols. Permeability of the plasmamembrane of host cells in the vicinity of the rust mycelium is increased with respect to water and organic compounds as compared with the permeability of healthy cells. Cells affected in this way show a decreased osmotic pressure amounting to a

reduction of 0.054 mols (average). Initiation of these changes can be detected in advance of the rust mycelium. Based on this data, the mechanism for absorption of nutrients by the fungus is described, and its possible connection with the "green island" phenomenon of rusts, and with the function of haustoria is discussed.

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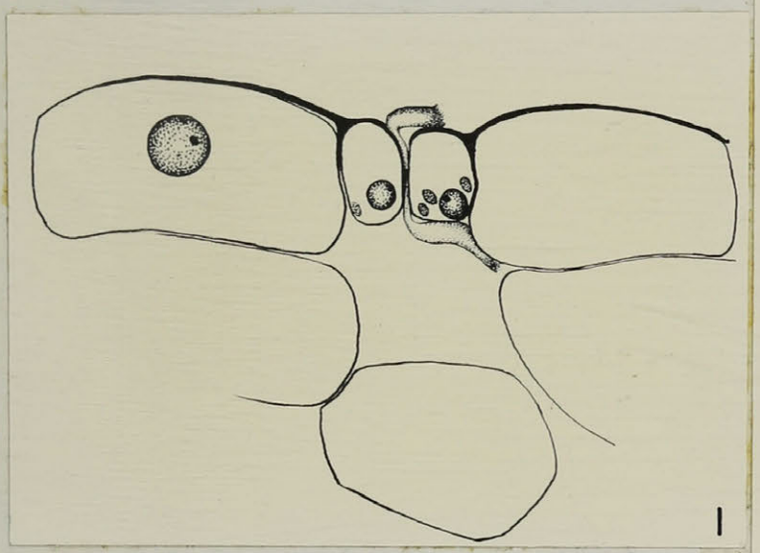
PLATE I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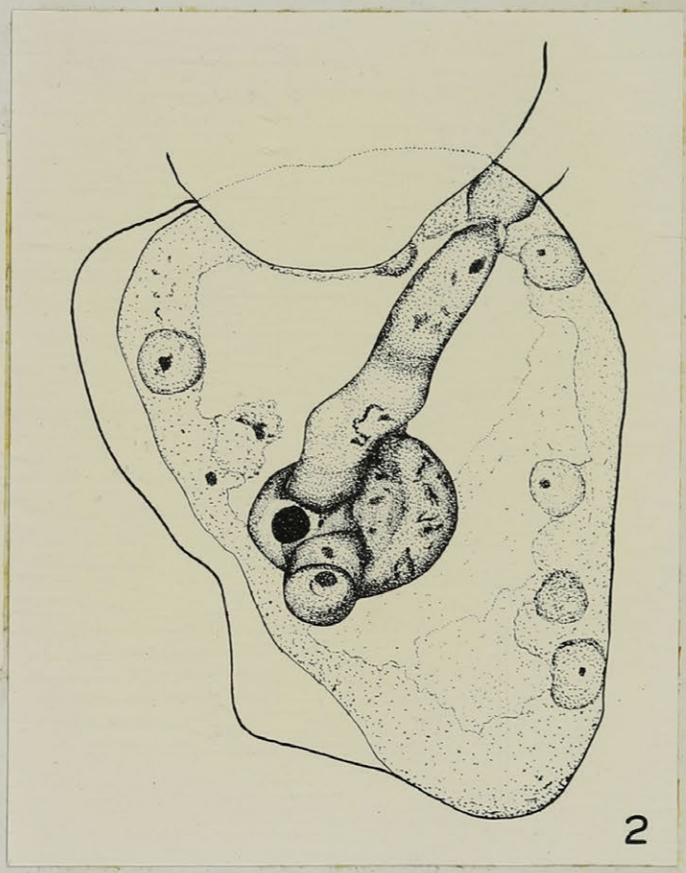
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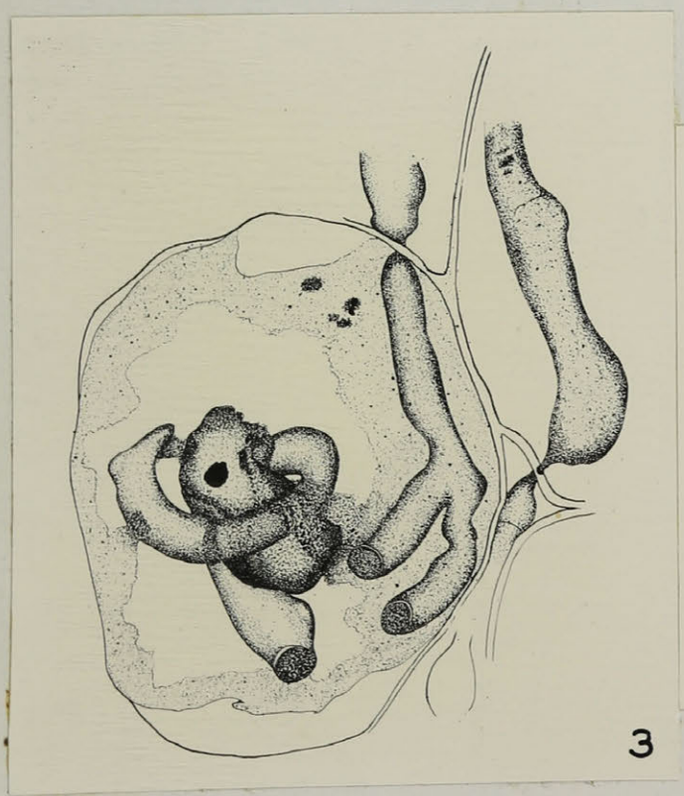
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PLATE II

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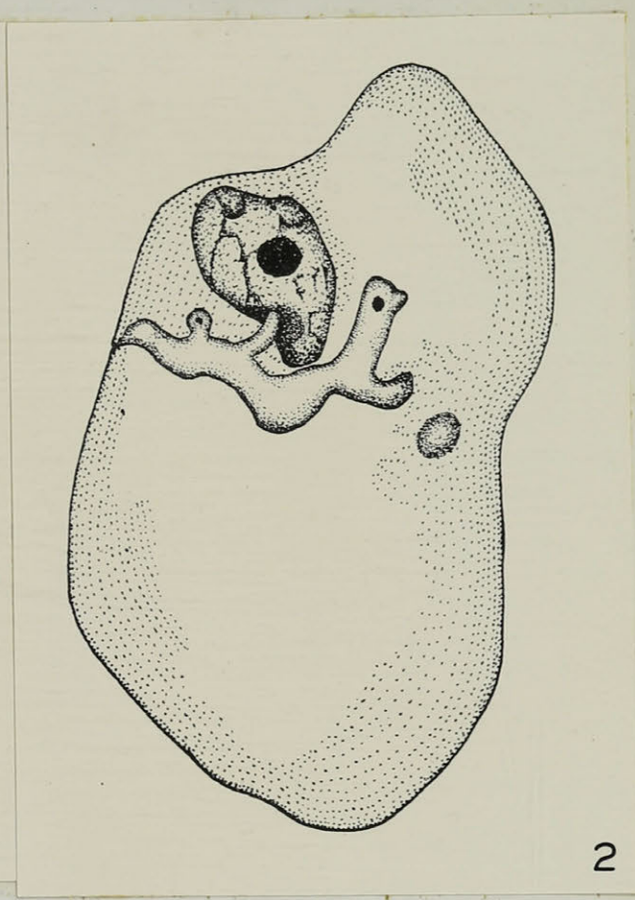

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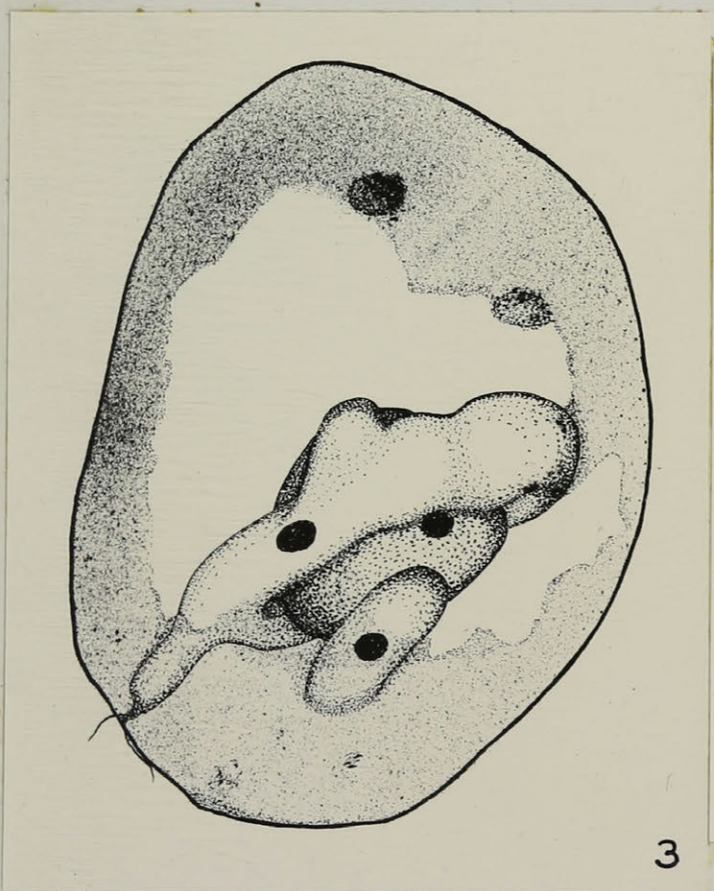

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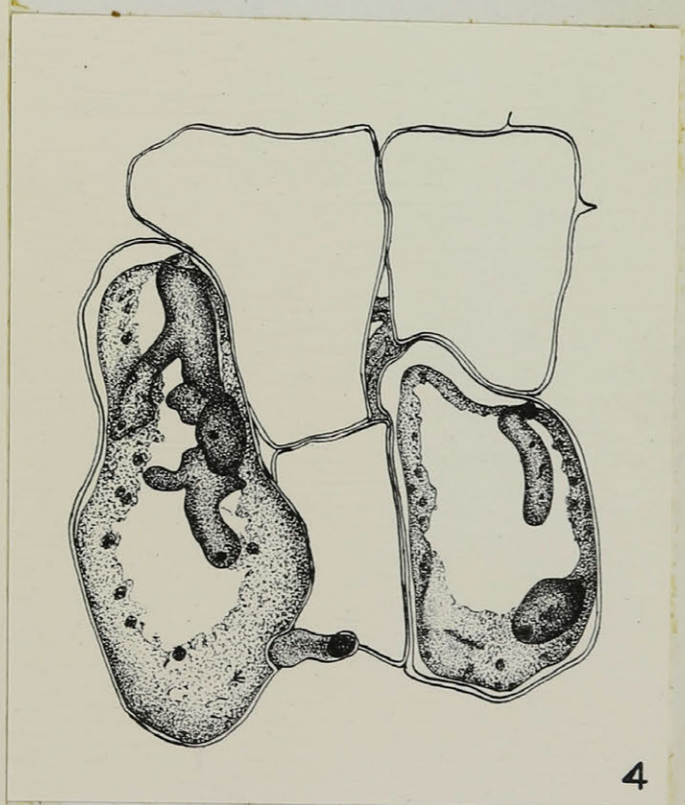

4

DESCRIPTION OF PLATES

PLATE I.

Fig. 1. Penetration of a urediniospore germ-tube through a stomatal opening. X 1000 approx.

Fig. 2. This figure shows a rust haustorium which has penetrated the cytoplasmic layer; has transversed the large central vacuole and has come in contact with the host cell nucleus which is in association with the cytoplasm on the side of the cell opposite the entrance point of the ~~hypha~~ haustorium. The haustorium has either penetrated the nucleus, or the nuclear mass has surrounded the haustorium. X 2400.

Fig. 3. This figure again shows a rust haustorium which has entered the cell at a plane above that of the section; has transversed the vacuole, and has made contact with the host nucleus. X 2400.

Fig. 4. In this section, part of the cytoplasm lining the upper cell wall has been cut away in sectioning, thus disclosing branches of the haustorium, within the vacuole. The lower cytoplasmic surface of the cell was removed entirely, showing clearly the naked outline of the haustorium which has passed completely through the cytoplasmic layer of the host-cell. X 1800.

PLATE II.

Fig. 1. A haustorium is shown within the vacuole. Part of the cytoplasm in the upper plane of the cell has been removed in sectioning showing, in the opening, a portion of the haustorium free from the host cytoplasm. (arrow). X 1200

Fig. 2. In this drawing an observation is made very similar to that in Fig. 1. A branching haustorium is seen within the vacuole, one branch of the haustorium having penetrated or invaginated the host nucleus. X 1800.

Fig. 3. This is an illustration similar to Figs. 1 and 2. Part of the cytoplasm has been removed in sectioning to disclose a haustorium which has transversed the vacuole and has partly surrounded the host nucleus. X 2400.

Fig. 4. This figure depicts a group of parenchyma cells which are partly plasmolysed. Removal of part of the cytoplasm discloses haustorie within vacuoles. That the haustoria have penetrated the peripheral cytoplasm rather than invaginated it, is clearly evident. X 1200.

PLATE III.

Fig. 1. Section of pea stem, immediately below an old pustule, showing starch grains densely scattered among the inner parenchyma cells. X 100.

Fig. 2. This drawing is made from a healthy part of the same stem cross-section as in Fig. 1, and shows complete absence of starch grains. X 100.

Figs. 3 &B4. These are, respectively, healthy and diseased portions of the same cross-section of a stem. The diseased area, below a much younger purtule than Fig. 1, contains large quantities of starch while the healthy tissue shows none. X 100.

Figs. 5 & 6. These are drawn from healthy and diseased parts of the same section of a pea-pod. The tissue below the pustule (Fig. 5) contains many more and larger starch grains than does the healthy tissue (Fig. 6), which is normally rich in starch. X 100.

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