

SUSCEPT-PATHOGEN RELATIONSHIP BETWEEN  
THE ASPARAGUS PLANT AND THE RUST  
FUNGUS, *Puccinia asparagi*

BY

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THE DEGREE OF DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY

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## TABLE OF CONTENTS

I.	Introduction.....	1
II.	Review of Literature.....	2
III.	Methods and Materials.....	3
IV.	Results.....	6
	A. Germination of urediospores.....	6
	B. Formation of appressoria.....	8
	C. Penetration and subsequent development of the substomatal vesicle and primary hypha.....	8
	D. Formation of haustoria.....	12
	E. Sporulation.....	17
	F. Spread and distribution of hyphae in the suscept tissues.....	20
	G. Effect of hyphae on the suscept cells.....	20
V.	Discussion and Conclusions.....	25
VI.	Summary.....	29
VII.	Literature cited.....	30
	Vita.....	32

## I. INTRODUCTION

Smith (18) illustrated and described the penetration of asparagus, Asparagus officinalis L. by the rust pathogen, Puccinia asparagi D. C. He reported that the germ tube of the urediospore penetrated the stoma of the suscept without the formation of an appressorium or a substomatal vesicle.

The findings of Smith as to the mode of penetration of P. asparagi do not agree with the general pattern of uredial infection in the Uredinales which involves the formation of an appressorium over a stoma and the subsequent development of a substomatal vesicle. The writer undertook the present study to investigate the developmental stages of P. asparagi before and after penetration of the suscept. Observations were made on (1) germination of the urediospore, (2) formation of an appressorium, (3) penetration and subsequent development of a substomatal vesicle and primary hyphae, (4) formation of haustoria, (5) spread and distribution of hyphae in the suscept tissue and (6) the effect of hyphae on the invaded suscept cells.

## II. REVIEW OF LITERATURE

Smith (19) investigated the penetration and subsequent development of P. asparagi in the asparagus plant. He illustrated the penetration of the germ tube through the stoma without the formation of an appressorium or a substomatal vesicle in the susceptible tissue. He also mentioned the possibility of direct penetration, although he did not observe it.

Smith described the mycelium as long, narrow, intensively branched, and ramifying between cells in the green parts of the plant, but extending only very slightly into the more "solid portions". Finally, the mycelium formed a thick mass at certain points just under the epidermis and then produced uredia on the surface of the susceptible tissue.

He described the development of haustoria as follows: "They (the filaments) pass between rather than into the cells, forcing them apart, but at numerous points the haustoria develop, which appress themselves closely to the sides of the cells, making an indentation and perhaps breaking through into the interior in some cases."

Regarding the effect of the mycelium on the cells and tissues of the susceptible, Smith mentioned that when the mycelium came into contact with the cells of the susceptible it absorbed nutritive substances from it, thus weakening the susceptible. It also caused the chloroplasts immediately to begin to shrivel, collapse and draw together into the interior of the cell.

### III. METHODS AND MATERIALS

A. Plants. Seed was collected in September, 1955 from plants of the variety Mary Washington, Robert's Strain, growing on the farm of the Rochelle Asparagus Company, Rochelle, Illinois. This seed was used throughout the investigation.

Seed was sown in 5-inch pots containing steam-sterilized soil consisting of four parts loam and one part peat moss. After emergence, the seedlings were thinned to four in each pot. The first seedling was cut back to the soil line three to four days after the second seedling had emerged. The second seedling was used for stem and chladophyll inoculation shortly after it "ferned out."

Shoots from crowns six to eight months old were used in the inoculation of scale leaves shortly after the shoots "ferned out." The scale leaves from these shoots were somewhat larger than those produced by younger crowns and were more suitable for histological studies.

B. Spores. Urediospores used for inoculation were originally collected from infected plants of the variety Mary Washington, Robert's Strain, growing on the Rochelle Company's farm, Rochelle, Illinois. These were increased on plants of the same variety in the greenhouse. Urediospores from heavily-infected plants were collected by means of a cyclone spore-collector or by brushing them off with a camel's hair brush into a small aluminum-foil dish at 3-day intervals after the uredia had ruptured. The urediospores were then sifted through an 80-mesh wire screen to remove all adhering susceptible material. Collections of dry spores were stored until used in closed glass vials in a refrigerator at 39°F.

C. Inoculation. Inoculations were carried out in a greenhouse at the University of Illinois at Urbana, Illinois, during the spring and fall of 1958.

Plants were inoculated at approximately 7:00 P. M. in order to provide a minimum greenhouse temperature suitable for infection.

Two methods of inoculation were used. The first method was similar to the one described by Kahn et al. (14) and was used to obtain single, isolated pustules on the stem for studying the development of the pathogen during and after urediospore production. The inoculation chamber employed was a wooden frame, 4 ft. x 4 ft. x 5 ft. completely covered with clear, heavy, polyethylene plastic film. In addition the outside walls were covered with heavy burlap that was kept wet to provide a cooling effect. Plants were placed in the inoculation chamber and thoroughly wetted with distilled water using a No.15 DeVilbiss atomizer. The plants were then dusted with a 1:200 urediospore-talc mixture. Again distilled water was atomized lightly on the inoculated seedlings for one hour. Following inoculation, the plants were left in the inoculation chamber at temperatures ranging between 70-80 F. for 24 hours. At the end of this period, the plants were removed, placed on the bench, and sprayed with water to remove the talc.

The second method of inoculation was accomplished by wetting a camel's hair brush with a heavy suspension of urediospores and applying them to the stem and the scale leaf. The inoculated plants were treated as described under the first method. This inoculation technique was used to study the penetration of the susceptible by the pathogen and its subsequent development prior to the formation of the uredia.

D. Techniques for killing and fixing of infected susceptible tissue. Inoculated portions of the stem and chlorophyll were cut into segments 5 mm. in length, killed and fixed at intervals of 6, 12, 18, 24, 36, 48 hours and thereafter at 24 hour intervals up to 18 days, and at intervals of 30 and 60 days after inoculation. The killing and fixing solution used was Randolph's Modified Navashin Fluid which gave better results than formalin-aceto-alcohol and formalin-propiono-alcohol (13). The segments were dehydrated and

infiltrated using the tertiary butyl alcohol series and embedded in paraffin (13).

E. Softening of segment tissues. "Joy", a liquid detergent was used as a softening agent (15). Paraffin-embedded segments of stem were fixed on wooden blocks. The paraffin was trimmed to expose one or both ends of the segment tissues. The exposed end of the segment was then immersed in a solution of one per cent "Joy" at room temperature for 2-7 weeks. This method was found to be superior to one employing hydrofluoric acid which damaged the mycelia.

F. Staining of sections. Although several staining methods were tried, the only one that gave satisfactory results was a modification of that employing Safranin O, methyl cellosolve, sodium acetate solution (13) and fast green. The sections were cut 6-8 microns in thickness and placed in Safranin O for 24 hours and then rinsed in a 50, 70, 85, 95, 100 per cent alcohol series instead of going to water, 95 and 100 per cent alcohol before counteracting with fast green.

G. Clearing and staining of whole mounts. The chlorophyll was removed from the scale leaves by placing them in a solution of equal parts of absolute ethyl alcohol and glacial acetic acid (12) for 12-24 hours. Cleared scale leaves were then mounted in lactophenol containing aniline blue. Preparations mounted by this method were excellent for studying the early developmental stages of the asparagus-rust pathogen.

## IV. RESULTS

A. Germination of urediospores. Scale leaves of asparagus were removed at intervals of 1, 2, 4, 6, 8, 10 and 12 hours after inoculation, cleared and stained as already described, and examined microscopically. This whole-mount-method provided a technique whereby observations could be made with respect to the sequential development of the early growth stages of the pathogen, including urediospore germination, germ tube growth, the formation of appressoria, penetration through the stomata, and the formation of sub-stomatal vesicles.

The urediospore begins to germinate on the scale leaf one hour<sup>1</sup> after inoculation by pushing out a germ tube through only one of the four pores in the cell wall of the spore. The protoplasm in the urediospore passes into and along the interior of the germ tube as it grows, leaving very little behind in the older part of the tube. The germ tube reaches a considerable length within two hours after spore germination (Fig. 1). The protoplasmic contents of the germ tube stain deep blue with aniline blue. Approximately three hours after spore germination an appressorium forms at the growing tip of the germ tube. The length of the germ tube is variable. It ranges, on the average, between 25 and 120 microns. The diameter of the germ tube averages 4  $\mu$ , or almost twice the diameter of the intercellular hypha.

Some of the germ tubes became quite long without the formation of appressoria. These were seen to bulge at the tip and disintegrate after 30 to 50 hours. Others tend to form a terminal structure from which a secondary germ tube develops laterally. This elongates and finally disintegrates. Occasionally germ tubes pass over stomata without forming appressoria (Fig. 2).

<sup>1</sup>Spores on glass slides and under the same conditions also begin to germinate after one hour.



Fig. 1. Germinating urediospore on a cleared scale leaf two hours after inoculation. X270.



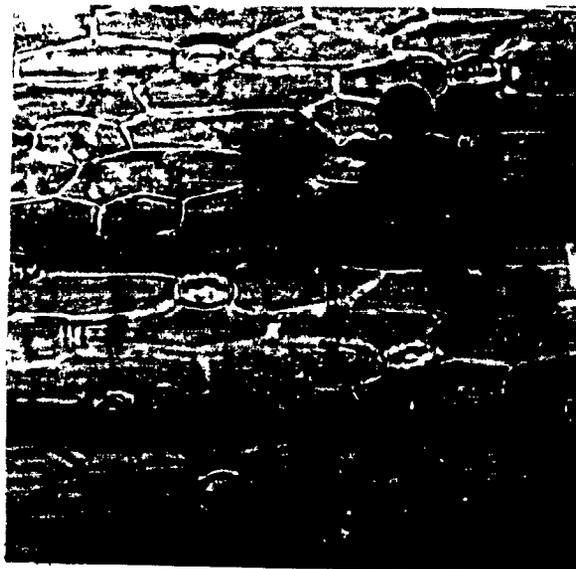
Fig. 2. Germinating urediospores on a cleared scale leaf with spores producing: (A) a straight germ tube; (B) a germ tube that passed over the stoma without the formation of an appressorium; (C) a germ tube that developed a secondary germ tube. X270.

B. Formation of appressoria. The development of appressoria has been observed to occur as soon as three hours after spore germination. The tip of the germ tube over the stoma swells to form the appressorium and nearly all of the protoplasmic material moves into the appressorium. A septum is then formed separating the appressorium from the empty germ tube which soon collapses. When fully formed, the appressorium is at least twice as large as the urediospore from which it came. The appressorium is generally elliptical, wider at the middle and tapering at both ends, and measures on the average about 14  $\mu$  in length and 10  $\mu$  in width (Fig. 3).

The majority of appressoria develop five hours or more after spore germination. Two appressoria have been noted to develop above a single stoma (Fig. 4).

C. Penetration and subsequent development of substomatal vesicles and primary hyphae. Penetration and the subsequent development of substomatal vesicles and primary hyphae have been observed on and in the cleared scale leaves and in paraffin sections.

When the appressorium is fully developed, it produces a penetration peg that enters the stoma. The penetration peg swells at the distal end to form a vesicle within the substomatal cavity. The protoplasmic contents of the appressorium pass into the substomatal vesicle through this peg. This process takes place 5 to 7 hours after spore germination. The movement of the protoplasmic contents out of the appressorium is evidenced by the appearance of empty areas in the appressorium (Fig. 5). Within 8 to 12 hours after spore germination, the entire contents of the appressorium are transferred to the substomatal vesicle (Fig. 6, 7). Although the penetration peg was not actually observed, its presence was evidenced by stained, lens-shaped, protoplasmic material in the aperture of the stoma in the cleared scale leaf. Penetration of susceptible tissue was never observed to take place directly from a germ tube without the formation of an appressorium and the subsequent development of a substomatal vesicle. The vesicle is almost spindle-shaped, or



**Fig. 3.** Elliptical appressorium over the stoma of a clear scale leaf with a septum separating the appressorium from the empty germ tube. X270.



**Fig. 4.** Two appressoria developing over a single stoma of a cleared scale leaf. X270.

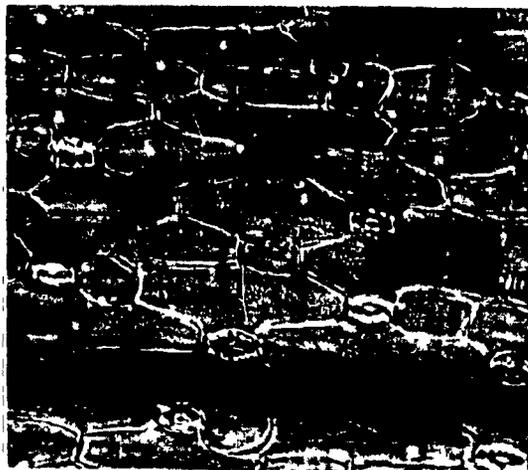


Fig. 5. Partially empty appressorium, indicating the protoplasm has moved through the stoma-cleared scale leaf. X270.



Fig. 6. Completely empty appressorium (arrow) over a stoma of cleared scale leaf. X270.

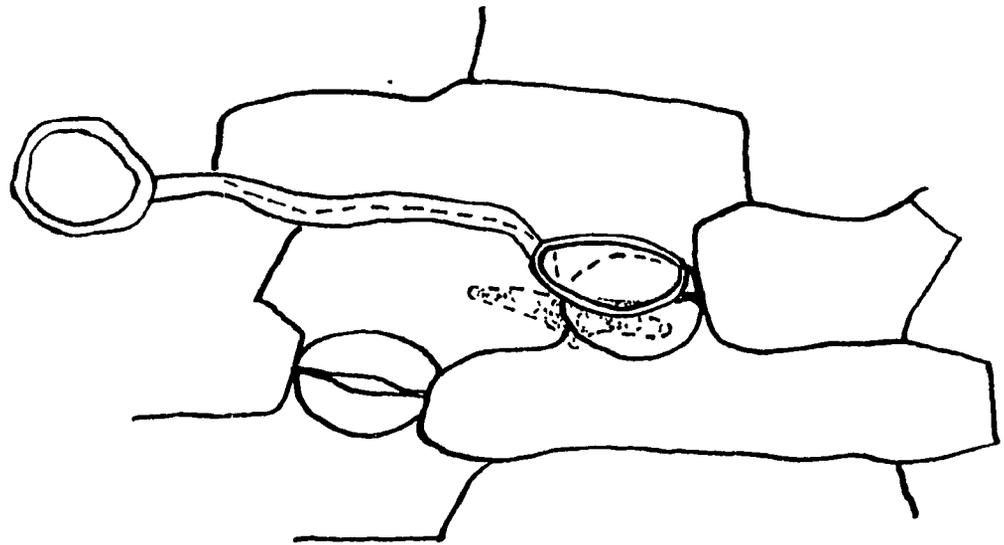


Fig. 7. Contents of appressorium transferred to the substomatal vesicle. Cleared scale leaf. Camera Lucida drawing X 450. (Same appressorium in Fig. 6.)



Fig. 8. Spindle-shaped vesicle in the substomatal cavity 24 hrs. after inoculation. Remnant of an appressorium appears on the stoma. Longitudinal section of stem. X1030.

swollen in the middle and tapering toward both ends with its axis almost parallel to the epidermis (Fig. 8, 9).

Primary hyphae are produced from ends of the vesicle with septum forming between the hyphae and the vesicle (Fig. 10, 11). The primary hyphae grow in the intercellular spaces of the chlorenchymatous layers of the cortical region of the stem and give rise, in turn, to haustorium-mother-cells and secondary or vegetative hyphae.

D. Formation of haustoria. The first haustorium is formed as a result of the tip of the primary hypha coming into contact with a susceptible cell and forming a haustorium-mother-cell. A septum is formed about 8  $\mu$  from the tip of the hypha, cutting off the mother-cell (Fig. 12, 13). This cell is somewhat broader (3.5 $\mu$ ) in diameter than the hypha (2.5 $\mu$ ) and with a relatively broad surface in contact with the susceptible cell. The haustorium-mother-cell gives rise to a fine, peg-like growth which pierces the cell wall of the susceptible cell and enlarges at its distal end to form the haustorium (Fig. 12, 13). Haustoria are variable in shape and are simple or branched (Fig. 14). Recently-formed haustoria and accompanying young hyphae appear quite dense and stain bright-red with safranin and fast green, whereas, older haustoria and hyphae appear empty with the cell walls taking only the green stain. Growth of the hyphae does not cease permanently with the formation of the haustorium-mother-cell and the haustoria. At the time the haustorium is being formed at the tip of the haustorium-mother-cell, the penultimate cell, behind the mother-cell, pushes out a side branch just below the septum, which continues to penetrate the susceptible tissues (Fig. 15).

The period following the formation of the primary hyphae and before the onset of sporulation is one of intense vegetative development. The hyphae branch profusely and form small masses of pseudoparenchyma. These masses fill the intercellular spaces of the chlorenchymatous layers below the stoma through which penetration occurred.

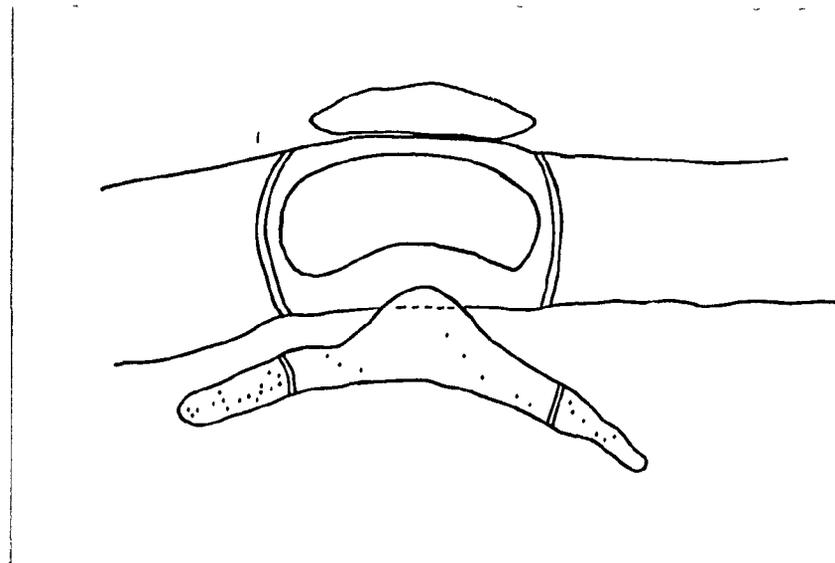


Fig. 9. Substomatal vesicle, 24 hours after inoculation, giving rise at end to primary hyphae. Remnant of an appressorium appears on the stoma. Camera Lucida drawing. X1030.

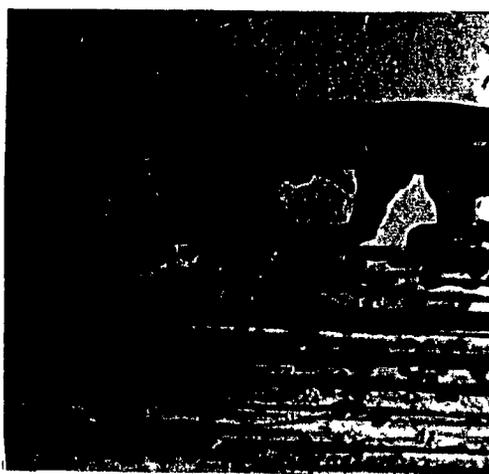


Fig. 10. Substomatal vesicle in the substomatal cavity 24 hours after inoculation. Remnant of an appressorium appears on the stoma. Longitudinal section of a stem. X1030.

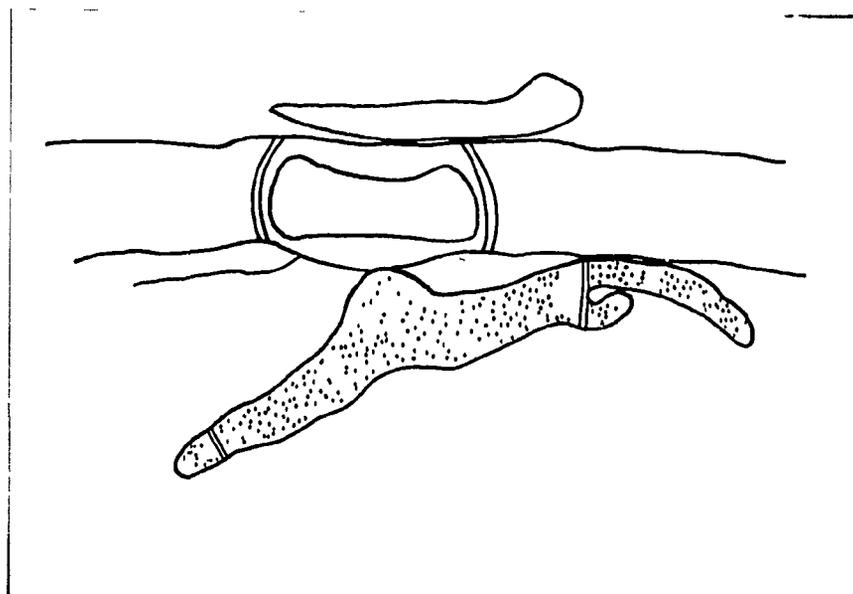


Fig. 11. Substomatal vesicle, 48 hours after inoculation, giving rise at each end to primary hyphae. Remnant of appressorium appears on the stoma. Camera Lucida drawing. X1030.



Fig. 12. Haustorium-mother-cell on the outside of the suscept cell with a peg-like growth and a young haustorium (arrow) inside the suscept cell. Longitudinal section of a stem. X1030.

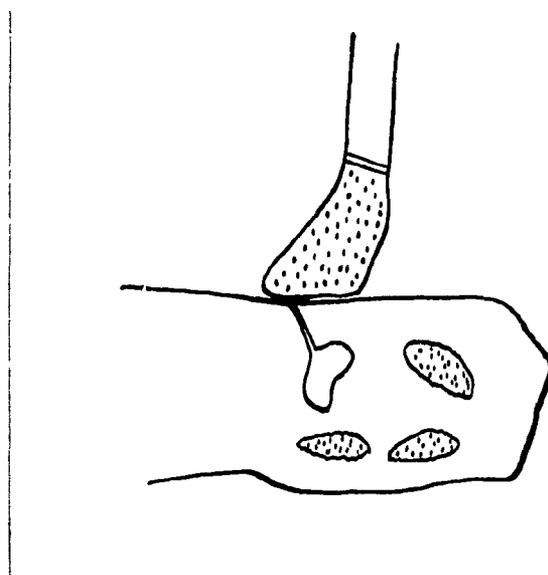


Fig. 13. Haustorium-mother-cell on the outside of the susceptible cell with a peg-like growth and a young haustorium inside the cell. Camera Lucida drawing. X1030. (Same as in Fig. 12).



A



B

Fig. 14. Advanced stages in development of haustoria. (A) Simple haustorium. (arrow) (B) Branched haustorium (arrow). Longitudinal sections of stem. X1030.



Fig. 15. The penultimate cell behind the haustorium mother cell giving rise to the primordium (arrow) of a side branch. X1030.



Fig. 16. Hyphal layer below the stoma separating the epidermis from the cortical region of the stem. (Cross-section view). X450.

E. Sporulation. Five to six days after inoculation, minute yellowish blisters can be detected on the susceptible stem. Sections through these areas show that the vegetative hyphae of the pathogen has become established within the chlorenchymatous layers below the penetrated stoma, and has entered a new phase of growth marked by the initiation of the reproductive and distributive hyphae.

At the onset of the sporulation stage, hyphae begin to aggregate, intertwine, and form a mycelial layer or mat, parenchymatous in nature, between the epidermis and the cortical region (Fig. 16). The pressure resulting from the growth of this layer of mycelia causes the epidermal layer of the cells above it to bulge outward and separate from the chlorenchymatous layers. Here, hyphae become more septate than elsewhere, and may be so closely packed that the individual hypha cannot be easily distinguished. The hyphae nearest the epidermis grow nearly at right angles to the mycelial mat. The tip of each hypha is cut off by a septum, becomes slightly enlarged, assumes a clavate form and becomes filled with dense protoplasm. This tip cell, which is also called sporogenous initial or basal cell, gives rise by budding to one or more bulbous, spore-initial cells which are cut off by septa. The spore-initial cell then divides by a transverse wall into a stalk cell and a young urediospore, both of which are binucleate and filled with protoplasm (Fig. 17).

Young spores formed before the rupture of the epidermis are somewhat flattened due to pressure. Separation of the non-chlorophyll-bearing epidermal cells from the chlorenchymatous cells by the newly-formed urediospores gives a yellowish color to the minute blisters on the exterior of the stem. The pressure exerted by the maturing urediospores and their elongating stalks soon causes the epidermis to rupture at the stoma and to expose the urediospores. Rupture of the epidermis occurs approximately 7 days after



Fig. 17. Binucleate urediospores and stalk cells at different stages of development. Longitudinal section of stem. X1030.



Fig. 18. Teliospore forming in a primary uredium 15 days after inoculation. Longitudinal section of stem. X450.

inoculation. The spores first become well-defined at the center of the sorus under the stoma and development proceeds centrifugally. Occasionally, the first spore to mature comes up through the stoma before the rupture of the epidermis.

Urediospores continue to be formed over a period of several days. Before the first spore has reached maturity, a second spore-initial cell may arise by budding from the basal cell, and, still later, additional spores may develop from the same basal cell. When the first-formed urediospores mature, they become detached from their stalks which wither and disintegrate. As a result of this budding process urediospores are found in almost all stages of development in a young uredium. Very young spores and even spore-initial cells may be seen among the mature spores in a fully-developed uredium (Fig. 17).

At the time the reproductive cells are in the process of differentiation, the protoplasm disappears from the older vegetative hyphae below the uredium and becomes concentrated in the growing tips of the reproductive and distributive hyphae (stolons, runners). The movement of the protoplasm from the older mycelia into the peripheral hyphae at the time urediospore formation is initiated has been noted in other rusts by several investigators (2, 3, 5, 16, 18).

When the pathogen starts to form the primary uredium, a number of distributive hyphae grow out radially from the center of infection. These long, sparsely-septate, branched hyphae grow almost directly across the intercellular spaces instead of closely following the contours of the susceptible cells as do the previously-formed vegetative hyphae. These distributive hyphae do not produce haustoria. However, when they reach fresh tissue they begin to branch and produce haustoria in the new centers of infection under stomata and give rise ultimately to a ring of secondary uredia around the primary uredium. This ring appears approximately eight days after the first appearance of the primary uredia.

About the time the secondary uredia appear, teliospores begin to develop among the

urediospores in the primary uredium (Fig. 18). Distributive hyphae grow unilaterally from the secondary uredia to produce the tertiary uredia. By the time the tertiary uredia appear, the primary uredia are filled with teliospores and the secondary uredia are beginning to produce teliospores. The primary uredium alone gives rise to the secondary ring of uredia, whereas, several uredia in the secondary ring give rise to the tertiary ring.

Tertiary uredia are almost always formed in an incomplete ring in the form of two V-shaped patterns (Fig. 19) on the stem above and below the secondary uredia. The extent of development of rings of tertiary uredia is restricted by the diameter of the stem. The successive production of uredia in a V-shaped pattern followed by the appearance of teliospores may continue for several months or as long as non-infected green tissue is present. The development of secondary and tertiary rings of uredia has been noted in several other rusts (2, 3, 5, 6, 16, 17, 20, 22).

F. Spread and distribution of the hyphae in the susceptible tissues. The hyphae spread and ramify extensively in the intercellular spaces of the chlorenchymatous layers of cells of the stem. The hyphae do not penetrate the non-photosynthetic cells of the cortical region or the lignified fiber ring which surrounds the vascular bundles and the ground tissues of the stem (Fig. 20).

The hyphae in the cladophyll also remain limited to the palisade region and do not penetrate the vascular bundles or the ground tissues although, there is no lignified fiber ring in the young cladophyll (Fig. 21).

G. Effect of hyphae on the susceptible cells. The most prominent effect of the hyphae is on the chloroplasts. In a healthy cell, the chloroplasts are plump, rounded and distributed uniformly around the inner periphery of the cell (Fig. 22). Prior to the onset of sporulation, the cells in the invaded area show no visible abnormalities in either cell walls or contents. However, at the beginning of sporulation the chloroplasts of the cells beneath



Fig. 19. Successive V-shaped rings of uredia and telia on asparagus stems.



Fig. 20. Hyphae localized in the chlorenchyma with no penetration of the non-photosynthetic layers of the cortex of the stem nor the immediately internal lignified fiber ring. Sixty days after inoculation. (Cross-section view). X270.



Fig. 21. Hyphae restricted in the cladophyll to the palisade region (deeply stained). (Cross-section view.) X270.



Fig. 22. Healthy cells with chloroplasts distributed uniformly around the inner periphery of the cell. Longitudinal section. X450.

the uredia begin to aggregate in clumps around the nucleus (Fig. 23). Subsequently the chloroplasts lose their shape, become granular in appearance and then disintegrate. In the later stages of infection, the majority of the susceptible cells appear devoid of protoplasmic content with the cell walls undamaged and surrounded by the massed hyphae of the pathogen (Fig. 24). As a result of the intercellular hyphae growth, some of the outer chlorenchymatous cells become isolated from the rest of the cortical region and then collapse.



Fig. 23. Invaded chlorenchymatous cells with chloroplasts aggregating around the nucleus. Longitudinal section of stem. X1030.

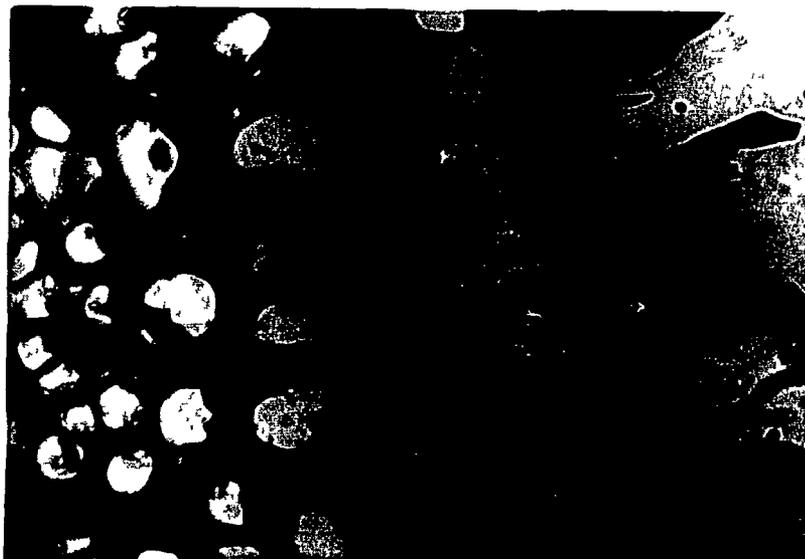


Fig. 24. Empty palisade cells surrounded by masses of hyphae in a cladophyll. Longitudinal section. X450.

## V. DISCUSSION AND CONCLUSIONS

The urediospore of P. asparagi germinates by giving rise to a germ tube through only one of the four pores in the cell walls. This kind of germination is common in several species of rust pathogens. However, in at least two species, namely, Puccinia graminis tritici Eriks. & Henn. (1) and Puccinia graminis phlei-pratensis Eriks & Henn. Stakman & Pieme (17), two germ tubes may arise from the urediospore although one usually develops more vigorously than the other.

The cause of the variability in the length of the germ tubes of P. asparagi is not known. It may be that the length of the germ tube is governed by genetic or environmental factors. The fact that on occasion germ tubes may pass over a stoma without forming appressoria needs further study.

It was found in this investigation that the germ tube forms a well-defined appressorium over the stoma with the appressorium in turn producing a penetration peg and a vesicle in the substomatal cavity. This is in contrast to the claims of Smith that the germ tube penetrates the stomata without the formation of an appressorium or a substomatal vesicle. He also mentioned the possibility of direct penetration of the germ tube from the urediospore through the epidermis, although he did not observe it. No evidence of direct penetration was noted in the present investigation nor has direct penetration by the germinating urediospore been reported for other rust pathogens. Urediospore germination and penetration of the susceptible asparagus-rust pathogen follows the pattern for other rusts as described by Pole-Evan (17), Stakman (20, 21), Allen (1, 3, 5), Caldwell & Stone (8) and Rothman (17).

Following inoculation formation of appressoria and subsequent penetration occurred on the scale leaves of plants kept in the dark for 24 hours, but not on plants subjected to

natural or artificial illumination during this period. Although stomatal behavior in the asparagus plant in relation to light was not studied, it is possible that the stoma must be closed for ingress of the pathogen since penetration did not take place in plants held under natural or artificial light. There is a possibility that light may have a direct adverse effect on the formation of appressoria. Cochrane (9) reported that under artificial light of 1,250 foot candles both the germination rate and final germination of urediospores of rose rust, Phragmidium disciflorum (Tode) James, were depressed.

The observation that more pustules are produced on asparagus shoots 7-9 days old at time of inoculation than on younger or older plants confirm those of Kahn et al. (14), Beraha (7) and Hepler et al. (11). This phenomenon does not seem to be related to resistance because once infection takes place secondary and tertiary rings of uredia and telia may continue to develop for several months or as long as non-infected green tissue is present. It is possible that some of the stomata are not yet fully developed in shoots less than 7 days old and become non-functional in shoots more than 9 days old.

The tip of the primary hyphae gives rise to the haustorium mother-cell from which a peg develops and penetrates the cell. The distal end of the peg in the cell becomes swollen and forms the haustorium. This is in disagreement with Smith who stated that at numerous points on the filaments, the haustoria develop and "appress themselves closely to the sides of the cells, making an indentation and perhaps breaking through into the interior in some cases." It is not known whether the penetration of the haustorium peg into the cell is purely mechanical or is facilitated by the secretion of an enzyme, a possibility mentioned by Allen (1) in the case of stem rust of wheat. The presence of haustoria only in the chlorenchymatous cells would indicate that the peg is stimulated in some fashion to penetrate only the cells that manufacture food.

The concentration of the protoplasm in the growing tips of the hyphae and its absence in the older parts of the hyphae suggest that the protoplasm actually moves along the hyphae to the tip. Allen (2) working with stem rust of wheat postulated that the older hyphae which appeared empty might have been killed by a substance secreted by the suspect. In the present investigation examination of stained germ tubes and appressoria at different stages of development strongly suggests that there is progressive movement of protoplasm toward the tip of the germ tube into the appressorium and into the substomatal vesicle. It appears unlikely to the writer that the protoplasm of the pathogen is killed progressively by any secretion from the suspect.

The hyphae are limited to the chlorenchymatous layers of the cortical region of the stem and the palisade layers of the cladophyll and do not extend into the lignified fibral ring. However, Smith stated that the hyphae extend very slightly into the more "solid portions" of the plant. By the "solid portions" he apparently was referring to the lignified fibral ring. Actually, the anatomical details of the asparagus plant were not worked out until 1935 or about 30 years after Smith published his paper.

As a result of invasion of the chlorenchymatous layers by the pathogen, the protoplasmic contents of the cells gradually disappear, although the cell walls apparently remain undamaged for a long time. Because of the destruction of the chloroplasts there is a reduction in the amount of food manufactured by the plant and the plant becomes weakened.

Since the chlorenchymatous cells of the stem are the only tissues invaded by the hyphae, the vascular bundles and the ground tissues inside the lignified fibral ring continue to function. It has been observed many times that the upper portion of the stem may remain green and continue to grow for several months, even though the outer tissues of the lower portion of the stem have been severely damaged by the pathogen.

It is obvious that the cyclic production of secondary and subsequent rings of uredia results from internal spread of the pathogen from a single, original locus of infection. In this investigation the plants after the original inoculation were maintained under environmental conditions that were decidedly unfavorable for urediospore germination. In addition, appressoria or substomatal vesicles were never found at the site of the secondary or tertiary uredia.

At the time secondary uredia are being developed, teliospores begin to form in the primary uredia from the same hyphae. However, it was not determined whether urediospores and teliospores arise from the same reproductive cells. These cells are so compact that it is exceedingly difficult to delineate individual cells. In addition it is impossible to distinguish between very young urediospores and teliospores.

The exact reason for the cessation of urediospore production and the subsequent development of teliospores in the uredia is not known. The production of teliospores in *P. asparagi* is not stimulated by maturation of susceptible tissue per se as in the case of the cereal rusts (10). It is possible that this phenomenon is due to a growth substance in the susceptible or the pathogen.

## VI. SUMMARY

The urediospore of P. asparagi germinates by pushing out a germ tube through one of the four pores in the cell wall of the spore. The tip of the germ tube over the stoma swells to form an appressorium which is separated from the germ tube by a septum. The appressorium produces a penetration peg which enters the stoma and swells at the distal end to form a vesicle within the substomatal cavity. The protoplasmic contents of the spore appear to move into the appressorium and thence into the penetration peg and the vesicle. Primary hyphae are produced at each end of the vesicle with septa forming between the hyphae and the vesicle. The primary hyphae grow in the intercellular spaces of the chlorenchymatous layers of the stem and produce, in turn, the haustorium-mother-cell and secondary or vegetative hyphae. The haustorium-mother-cell gives rise to a fine, peg-like growth which penetrates the cell wall of the suscept and enlarges at its distal end to form the haustorium. Urediospores are produced in the primary uredia approximately 7 days after inoculation. Teliospores then develop in these primary uredia about 15 days after inoculation, or at the time the secondary ring of uredia appears. Hyphae are intercellular and are restricted to the chlorenchymatous layers of the cortical region of the stem and in the palisade cells of the cladophyll. Suscept cells in the invaded area lose all of their protoplasmic contents and some of these cells become isolated.

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## VITA

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