SCANNING ELECTRON MICROSCOPE EXAMINATION OF SUGARBEET FLOWERS AND FRUITS INFECTED WITH PHOMA BETAE

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ABSTRACT

There are three natural openings in a mature sugarbeet fruit which serve as avenues of entry for microorganisms: 1) the basal pore which contains dried parenchyma and vascular tissue and is the point where the flower was connected to the stalk; 2) the apical pore where the style was inserted; and 3) the peripheral zone of dehiscence where the operculum separates from the fruit cavity wall during germination. The apical pore was first described in this study.

Scanning electron microscopy of naturally infected fruits showed, for the first time, hyphal penetration through both the basal pore and the peripheral zone.

Examination of sugarbeet flowers artificially infected with Phoma betae also showed fungal penetration through the apical pore. Dense hyphal growth was associated with stigmal lobes and ungerminated pollen grains. Fungal growth apparently was stimulated by excretions from the stigma.

Penetration of the fruit cavity wall and the operculum would render the fungus inaccessible to protectant fungicides and explains why the most successful seed treatments for P. betae have included volatile mercury fungicides or seed soak in thiram. Such treatment allows direct contact between the toxin and the pathogen.

APPROVAL OF THESIS

This thesis is approved by:

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Hossien M. El-Nashar

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INTRODUCTION

Sugarbeet (Beta vulgaris L.) flowers have been described by Artschwager (1,2) as sessile flowers usually in clusters of two or three which are attached to the inflorescence axis or secondary branches of the latter. There are five stamens inserted at the base of the calyx lobes. The stamens are introrse, bilocular and open by longitudinal fissures. The three carpellary ovaries are sunk in a fleshy disk enclosing a compylotropous ovule which is attached laterally to the ovary wall by a short funiculus. The style is very short, terminating in a 3-5 lobed stigma (Fig. 11,12) which persists in the fruit. Artschwager also included a full description of the anatomy of the sugarbeet flower. He described the basal vascular connection between the flower and the inflorescence. In addition, he described how the pollen grain germinated from a single pore and how the pollen tube grew on the surface of the stigma between the loosely connected epidermal papillae and passed through a short styler canal.

Since 1915 Phoma betae (Oud.) Fr. [= Pleospora bjoerlingii, Byford] has been known as the only important seed-borne pathogen of sugarbeet (10,11). Once P. betae attacks sugarbeet roots in the field or in the storage pile, the pathogen can survive as a saprophyte on decaying plant tissue. However, P. betae survived in soil for 26 months after seed were planted (4). Such results proved that P. betae could cause a serious problem as a soil borne pathogen. Although the fungus persists in the soil on organic material, it may develop on a high percentage of beet seed produced in regions with summer rainfall (3).

Infected or contaminated seed, germinating in cool damp soil, may produce infected seedlings that fail to emerge or that die following emergence. Such disease is known as damping off. Infected seedlings that survive are stunted and retarded in growth until warm weather permits recovery (3).

Phoma betae attacks almost every part of the sugarbeet, causing seedling black-leg symptoms (7,10,11), leaf spot (19), root rot and crown rot in storage and sometimes in the field (6,8,10,20).

This fungus is found wherever the sugarbeet crop is planted on a commercial scale. Damage caused by P. betae is variable worldwide. One of the most serious problems caused by Phoma occurs once the fungus is within the fruit of the sugarbeet. Seed transmission is a method by which the fungus can spread over long distances from regions of seed production to regions where sugar production takes place. Transmission of P. betae from Europe to U.S.A. on imported sugarbeet seed has occurred for many years.

Phoma betae naturally infects flowers and causes serious problems in the seed lots, e.g., an infection rate of 99% occurred in one 1977 Oregon seed lot. This pathogen reduces the quality and the quantity of the sugar extracted from the sugarbeet roots. Little is known about the mechanisms of sugarbeet seed infection by Phoma spp., thus the present ultrastructural study was undertaken. The objectives of the study were to determine how P. betae enters the fruit and remains in a resting stage, subsequently to cause seedling and root diseases.

MATERIALS AND METHODS

The structure usually called a seed in monogerm sugarbeet is technically a fruit composed of a single, true, lentil-like seed, lying within dead corky tissue (pericarp or the fruit wall). Throughout this study the seed (the dark brown, shiny, lentil-like structure) surrounded by dried suberized corky tissue will be termed the fruit. The seed occurs in a horizontal position and is curved in such a manner that its lower part is covered with the receptacle. This portion of the pericarp will be called the fruit cavity wall. The structure covering the upper part of the seed will be called the operculum. The pericarp with the dried floral parts still attached, will be termed the florocarp.

Sugarbeet fruit of cultivar US H20 were harvested from a seed production field near Salem, Oregon in the fall of 1977. They were found to be 99% naturally infected with P. betae.

Sugarbeet roots that had been stored for at least 80 days at 5° C, were planted in autoclaved soil and placed in a growth chamber, with 8 hr. night at 16° C ± 4° C and 16 hr. day at 21° C ± 4° C. Ten to 15 days before maturation of the fruits, the inflorescences were removed. The cut ends of the latter were placed into sterile distilled water in flasks and placed in a plastic box moist chamber at room temperature with 8 hr. night and 16 hr day under sterile conditions. The flowers were inoculated in the moist chamber by spraying a conidial suspension of P. betae. Flowers were harvested after 24 or 48 hr. and 5 days after inoculation. Samples then were prepared for scanning electron

microscope (SEM) examination.

Naturally infected fruits were superficially treated with 90% ethanol for 1½-2 min., soaked in running sterile distilled water for ½-1 hr., dried on sterilized filter paper, then immersed in a fixative solution of 5% gluteraldehyde in Millonig's phosphate buffer at pH 7.4 for 5 to 6 hr. Fruits then were washed thoroughly in phosphate buffer at pH 7.4 for 1 hr., and post fixed overnight in 2% osmium tetraoxide. Subsequently the specimens were washed in a phosphate buffer at pH 7.4. The fruit wall was separated from the seed while in the buffer. Pericarps (operculum and the fruit cavity walls) were dehydrated in a graded ethanol series and cryofractured in liquid nitrogen by using a razor blade that had been precooled in liquid nitrogen. Samples were dried in a Tousimis critical point dryer. Dried specimens were mounted on metal stubs using silver adhesive paint and then coated with gold under vacuum in a Hummer II sputter coater. Specimens were examined in a JEOL JSM35 scanning electron microscope. Images of secondary electrons were recorded on Polaroid film, type 55 positive-negative.

Artificially infected flowers, were prepared following the same procedures. Inflorescences with black lesions caused by P. betae also were prepared for SEM examination following the same methods.

RESULTS

Isolation from naturally infected sugarbeet fruits using water agar (15) and selective medium (5) methods, have shown that the fruits were highly contaminated with P. betae. The pathogen was prevalent in the pericarp, and very scarce but could be isolated in pure culture from the seed coat and the cotyledon.

Examination by light microscopy (17) and scanning electron microscopy showed that the sugarbeet true seed is covered with impervious layer of sclerenchyma cells. There are three openings to the seed: 1) an apical pore in the upper part of the pericarp (operculum); 2) an eccentrically orientated pore at the base of the lower part of the pericarp (the fruit cavity wall) previously described as the basal pore (9,12,17); and 3) the peripheral zone (9) of dehiscence, between the upper and lower part of the pericarp.

The pericarp (the fruit wall and the operculum) is composed of roughly isodiametric closely packed sclereids with markedly stratified thickening, with frequent converging pits and small lumens with occasional crystal inclusions (9,17). Sclereid cells were thicker in the lower than in the upper part of the pericarp.

The basal pore in the pericarp wall is located close to the radicle of the embryo. The radicle is surrounded by the perisperm. The basal pore is filled with loose cells characteristic of dead, dried parenchyma and conducting tissue (17). The apical pore is densely clothed with

papillate (1) dried cells. The basal pore is almost 10 times larger than the apical pore, but even so some apical pores were seen by the naked eye.

Naturally infected fruits were so heavily infected that the hyphae of P. betae covered all the inner surface of the pericarp (Fig. 5,7). Hyphae on the outer surface of the pericarp was observed mostly near the basal pore (Fig. 3,4). Phoma betae and other saprophytes colonized the fruit cavity wall (Fig. 7,8) and the inner surface of the operculum (Fig. 5,6). Some of the stigmatic lobes persisted on the operculum even after the fruit had been processed (Fig. 1,2). Hyphae grew over the funiculus (Fig. 8) and passed through the dried parenchyma cells near the bascular tissue (Fig. 8a). Figure 9 shows a hypha penetrating the outer perianth into the fruit cavity through the peripheral zone. Septate flattened hyphae characterized the resting hyphae located between the seed coat and the pericarp (Fig. 10).

Sugarbeet flowers, artificially inoculated with P. betae, were infected at several sites on the flower. Anthers and stigmal lobes were infected (Fig. 11,12). Stigmal papilli were completely invaded and surrounded with hyphae (Fig. 13). Spores on the surface of the flower germinated (Fig. 14) and penetrated the exocarp (Fig. 18).

Figures 15 and 16 show an immature sugarbeet fruit, composed of embryo, seed coat, fruit cavity, endocarp, mesocarp and exocarp. The exocarp is a unicellular layer of parenchyma cells which dries during the maturation of the fruit. The mesocarp consists of several layers of parenchyma cells which are usually dried by the time the fruits have completed their maturation. The endocarp darkens and becomes hard and dries up before complete maturation of the fruit. Both the exocarp and

the mesocarp are rubbed-off with the other exterior floral parts during seed processing, leaving the seed enclosed in the endocarp which is known as the pericarp.

Hyphae covered the outer surface of the flower and penetrated the exocarp. They were intercellular as well as intracellular (Fig. 17,18). Figure 18 shows a hypha that penetrated one of the mesocarpic parenchyma cells vertically and passed from one corner to another. Phoma hyphae penetrated mesocarpic cells, expanded, branched, and passed from one cell to another in a progressive invasion as shown in Figures 17, 17a and 17b. Figure 19 shows a branched hypha with internal cytoplasm that has emerged from the inner side of the apical pore.

Examination of the black lesions that formed on the seed stalks after inoculation showed hyphae extending over the outer surface of the stalk (Fig. 20,21). Ungerminated spores (Fig. 20) and hyphae mixed with pollen grains were associated with the black lesions (Fig. 21). In a cross section of the artificially infected stalks, hyphae appeared to penetrate the epidermal layers directly and continue penetration inside the cortical parenchyma cells (Fig. 22). No appressoria or haustoria were seen.

DISCUSSION

Not much was known about the location of Phoma betae in sugarbeet fruits, until Leach and MacDonald (14) demonstrated that the removal of cortical tissue covering the pericarp during seed processing reduced the percentage of infected fruits. However, in some highly infected seed lots the infection was still high even after seed processing (14).

Leach and MacDonald (14), also demonstrated that the presence of the pathogen in the sugarbeet florocarp ranged from superficial to deeply embedded. They categorized the seed lots infected with P. betae as follows: 1) Type A, little or no P. betae (< 5%); 2) Type B, P. betae mostly superficial (5-20%); 3) Type C, moderate to severe infection (30-60%); and 4) Type D, seeds heavily infected (>60%). The naturally infected fruits used in my study fall into the fourth group or type D.

Isolation methods and light microscopic examination of heavily infected fruits showed that P. betae was associated with the inner fruit wall much more frequently than were other common saprophytes. Isolation from the seed coat gave a very low frequency of recovery of P. betae and other fungi were not present. This indicated that P. betae invaded first followed by other saprophytes. Attempts to isolate P. betae from the cotyledons also gave a very low frequency of recovery. Isolation from the cotyledons that were successful could have been due to contamination of the cotyledons by P. betae in the seed coat during the delicate procedure of separating these two

tissues. Observations showed that P. betae colonized the fruit cavity and remained there and did not penetrate the highly cutinized seed coat.

Rye pollen mixed with the conidia of P. betae resulted in expansion of sugarbeet leaf spots (21). Anthers and stigma lobes were heavily infected with P. betae (Fig. 11,12). Hyphae grew over the stigma papillae (Fig. 13), and pollen grains were present with the hyphae on the outer surface of the flower and stalks (Fig. 14,16,17,18,21). Nectary excretions and pollen grains probably played an important role in the heavy amount of hyphae on the stigma lobes. They also could have stimulated spore germination.

Physiological studies on the effects of water, hydrochloric acid and other chemical agents showed that loosening the operculum or removing it improved seed germination in both monogerm and multigerm fruits (12,18). It was also demonstrated that water passed through the basal pore and effected seed germination. Perry and Harrison (17) claimed that the only passageway for water through the pericarp was the basal pore. Coumans (9) also added that water reached the seed through the basal pore as well as the peripheral zone and affected the seed germination. No one has shown the important role of the apical pore as an avenue of entry for air or fungi. The apical pore must be considered as one of the possible ways of entry for fungi, air and also water.

Therefore, there are three sites at which a fungus can penetrate the fruit without encountering a mechanical barrier: 1) the apical pore; 2) the basal pore; and 3) the peripheral zone. The microscopic examination described herein shows that this does occur. Direct penetration also occurs. Infection hyphae penetrated the exocarpic

unicellular layer (Fig. 18) and became inter- and intracellular within the mesocarpic parenchyma cell layers (Fig. 17,17a,18). The hyphae were not seen to penetrate the highly suberized sclerenchyma cell layers of the endocarp.

Hyphae were seen in the dried cells of the funiculus (Fig. 8,8a) and at the basal pore of the naturally infected fruits (Fig. 3,4). Stem lesions of P. betae developed near flowers after inoculation. These observations suggest that the fruit could become infected through the basal pore by hyphae progressing from the stem lesions. Also this possibility of infection through the basal pore could increase when the seed stalks are cut and wind-rowed to dry in the field. This late infection could be serious if the fungus is in the soil and moisture is adequate.

Hyphal penetration through the peripheral zone of the sugarbeet fruit was not seen after artificial inoculation, whereas such penetration was observed in the naturally infected fruits (Fig. 9). Flowers used in artificial inoculations were not mature enough for the peripheral zone to have developed.

The stigma unfold about 7 hr. after the flowers open (2). Infection might happen any time after the flowers are opened or even after the flowers are mature. Infection through the apical pore may occur as early as pollination. Hypha may penetrate and colonize the fruit cavity before the embryo has matured and the ovule has reached full size. When the embryo continues to enlarge, it would then press the hyphae against the pericarp. This could account for the unique and peculiar flattened hyphae found between the seed coat and the fruit wall in the naturally infected fruits (Fig. 10).

If infection happened during embryo maturation, hypha could reach the fruit cavity either by direct penetration through the exocarp and mesocarp or through the apical pore. Penetration through the apical pore may develop from lateral growth of hyphae that have penetrated the exocarp and/or the mesocarp (Fig. 23).

Ingress through natural openings (basal pore and peripheral zone) obviously cannot occur until some late stage in fruit maturation, when the openings develop during dehydration of the fruit. However, infection of the florocarp could occur early. This infection could be reduced when the florocarp parts are removed in seed processing (14). If the environmental conditions particularly moisture, remain adequate then the fungus could continue to penetrate the florocarp and progress to reach the fruit (Fig. 23). Once the inoculum is inside the fruit cavity, the fruit would be classified as deeply infected or type D.

Leach and MacDonald (14) found that the most effective fungicides for seed treatment are thiram, maneb or mancozeb. In some areas captan also could be used. These fungicides gave satisfactory control with processed seed carrying the B or C type infection (14), but were only partially effective with fruits carrying the D type of P. betae infection or with unprocessed seed carrying C type of infection (14).

Once the fungus has penetrated deeply inside, there is no way to reduce the amount of infected fruits to a satisfactory level by either seed processing alone or insoluble fungicidal seed treatment. The location of P. betae in the fruit cavity of type D infected seed explains why volatile mercury or thiram seed soak (16) are the most effective sugarbeet treatments. Direct contact of the fungus with a non systemic fungicide can only be accomplished in this way. Without

the natural openings previously mentioned, such methods of seed treatment would be ineffective.

Further ultrastructural studies are needed in order to obtain information about how primary infection occurs in different stages of flower ontogeny.

Invasion through the basal pore suggest that seed infection might occur by systemic activity of the fungus. Koch (13) recently claimed systemic infection of seed stalks and seed by P. betae. This must be confirmed and if true, an ultrastructural examination of this infection should be pursued.

Plate One:

Fig. 1-4. Outer (exterior) surface of a sugarbeet fruit.

Fig. 1. Operculum of the pericarp with a central apical pore (AP). Note the stigmal lobes (StL) persist at the apical pore. (38 X).

Fig. 2. Enlargement of the apical pore (AP) and stigmal lobe (StL). Note the hyphae (H) at the edge of the apical pore. (260 X).

Fig. 3. Basal part of the pericarp, with eccentric basal pore (BP); (H) hyphae. (40 X).

Fig. 4. Enlargement of the basal pore (BP), showing hyphae (H) associated with the pore. (78 X).

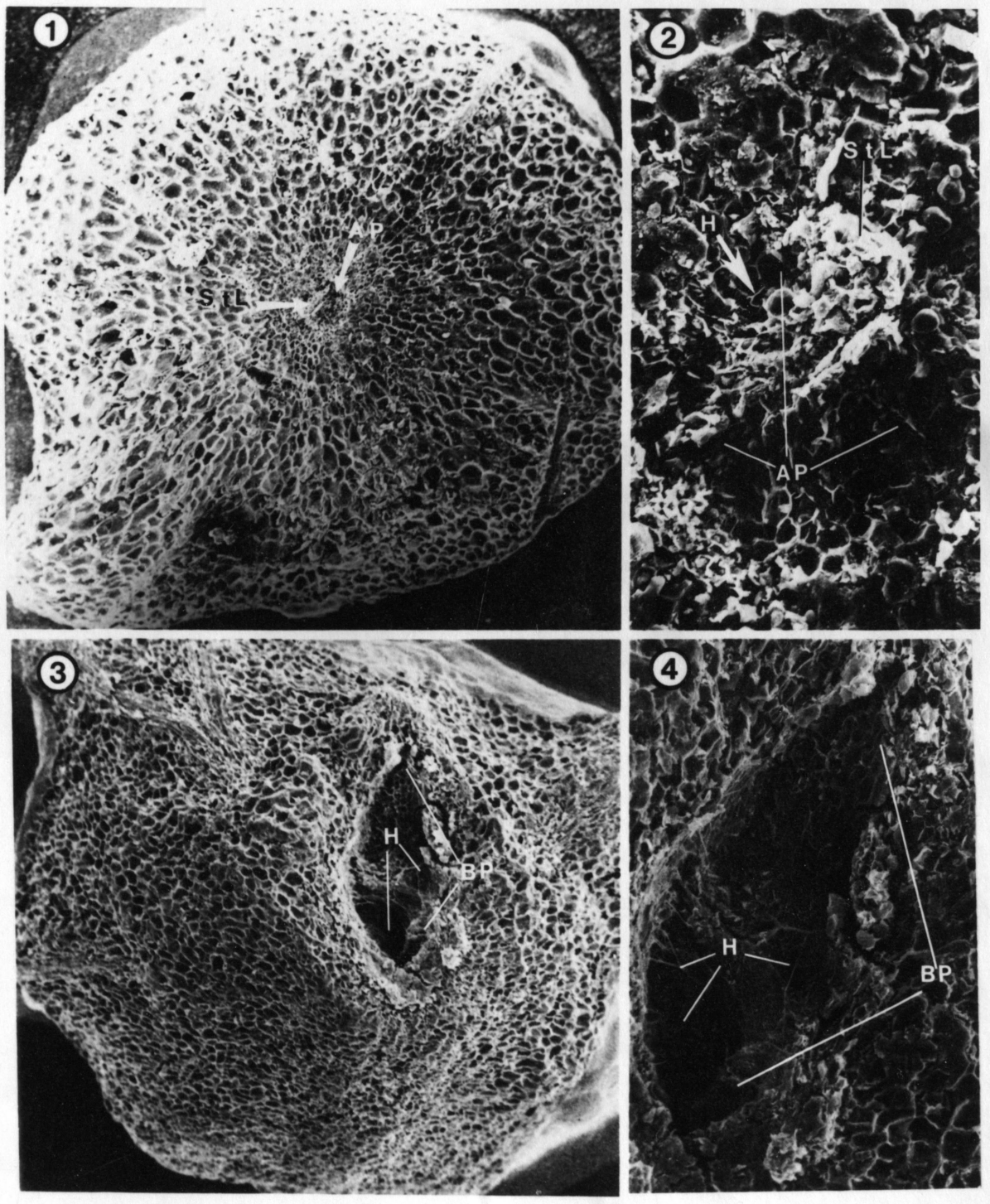

1
AP
StL
2
StL
H
AP
3
H
BP
4
H
BP

Plate Two:

Fig. 5-10. Interior (surface) of the pericarp of a sugarbeet fruit naturally infected with P. betae.

Fig. 5. Operculum of the pericarp with a central apical pore (AP). Hyphae (H) cover the entire inner surface of the operculum. (38 X).

Fig. 6. Enlargement of the apical pore (AP) in Fig. 5, heavily invaded with hyphae (H). (225 X).

Fig. 7. Inner surface of the fruit cavity wall (FrW) covered with hyphae (H). (BP), basal pore; (F), funiculus. (30 X).

Fig. 8. Enlargement of the basal pore (BP) and the funiculus (F) shown in Fig. 7. Note hyphae (H) growing on these tissues. (180 X).

Fig. 8a. Enlargement of the funiculus, showing a hypha (H) passing through dried vascular tissue. (1690 X).

Fig. 9. Perianth remnant attached to the fruit cavity and thin hypha (H) passing through the fruit wall. (FrW), fruit wall ; (Pr), prianth. (120 X).

Fig. 10. Flattened hyphae (FlH) characteristic of the mycelium growing between the seed coat and the pericarp. (720 X).

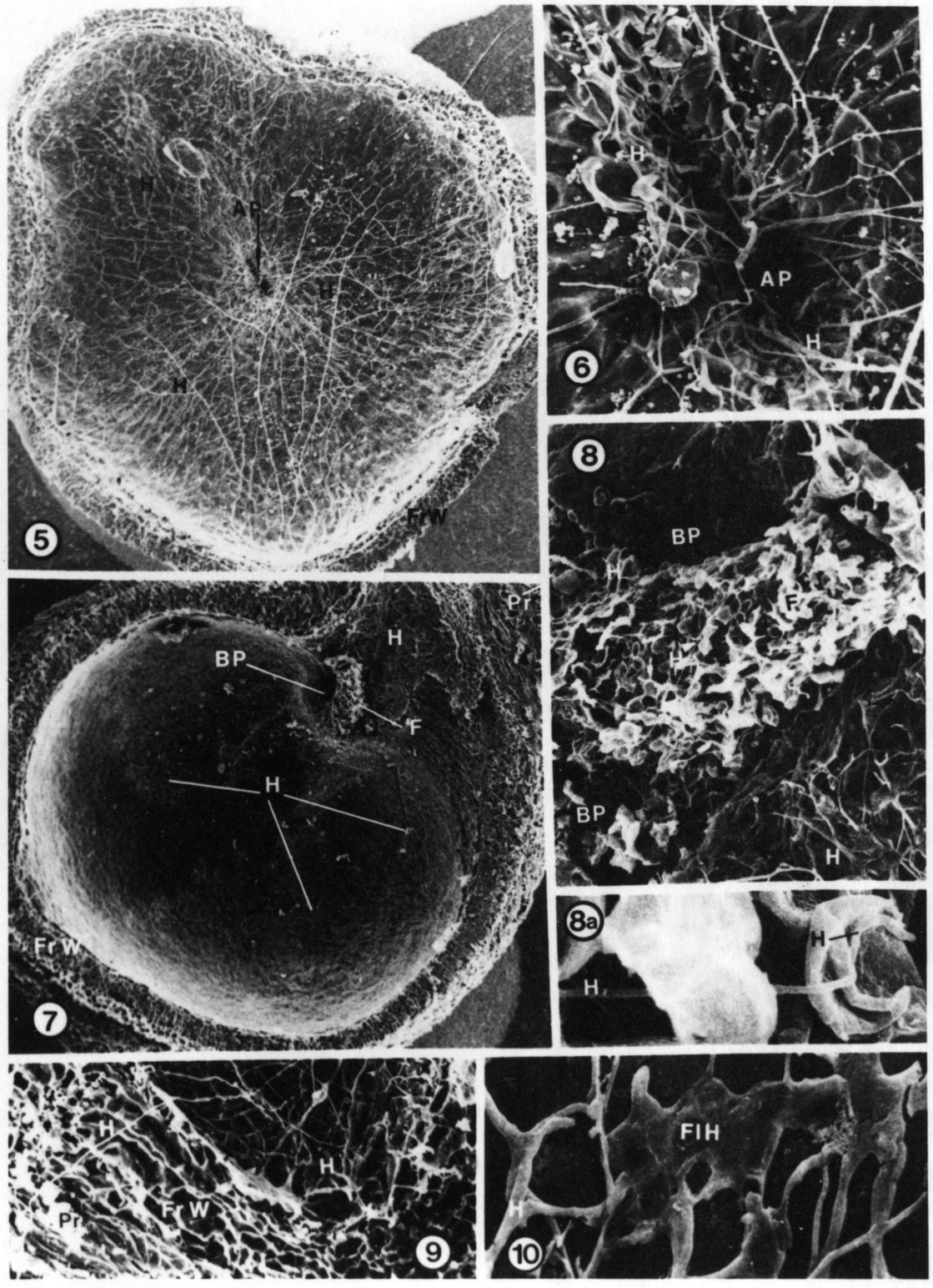

H
AP
H
H
FrW
5
H
H
AP
H
6
8
BP
H
F
H
BP
H
8a
H
H
7
BP
H
F
H
Pr
FrW
H
H
Pr
FrW
9
FIH
H
10

Plate Three:

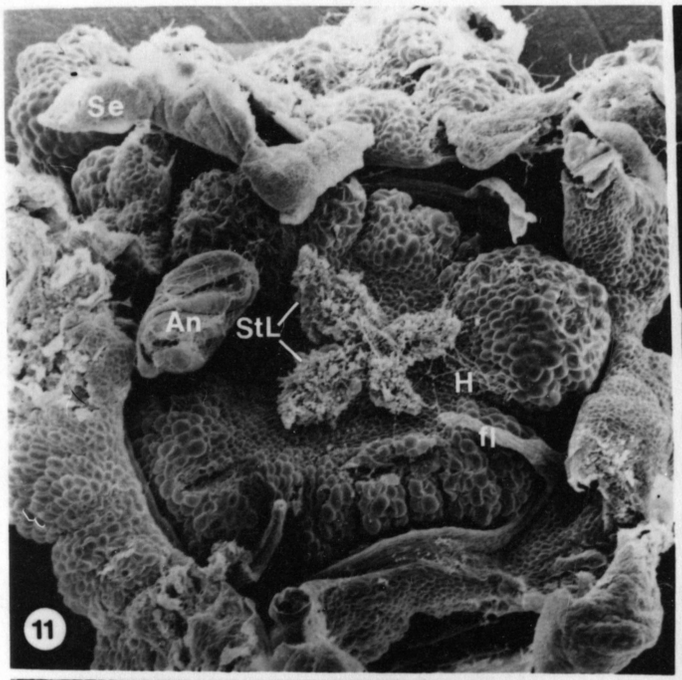
Fig. 11-14. Infection of a flower by P. betae after artificial inoculation.

Fig. 11. Flower parts include, sepals (Se), anther (An), filament (fI) and stigmatic lobes (StL). (20 X).

Fig. 12. Ungerminated pollen grains (Po) and hyphae (H) on the surface of a sugarbeet flower near the stigmatic lobes (StL) and apical pore (AP). (45 X).

Fig. 13. Enlargement of one of the stigmatic lobes, showing hyphae (H) that have penetrated the stigma papilla (StPa). (480 X).

Fig. 14. Ungerminated P. betae spores (Sp), germinated spores (gSp) and hyphae (H) growing on the surface of the flower, near ungerminated pollen grains (Po). (900 X).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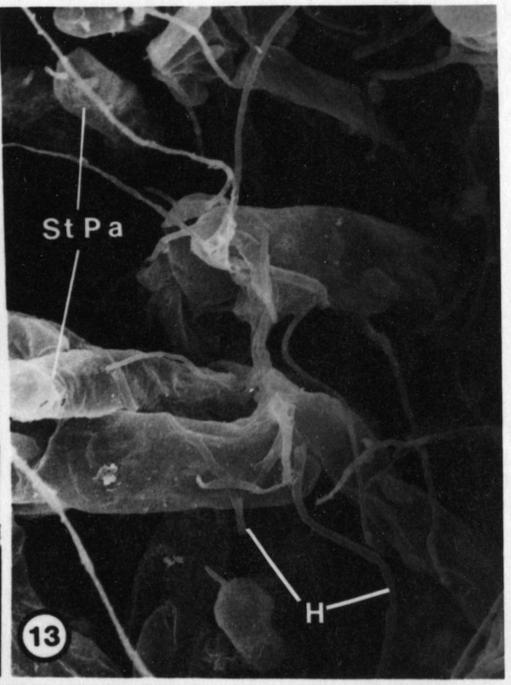
11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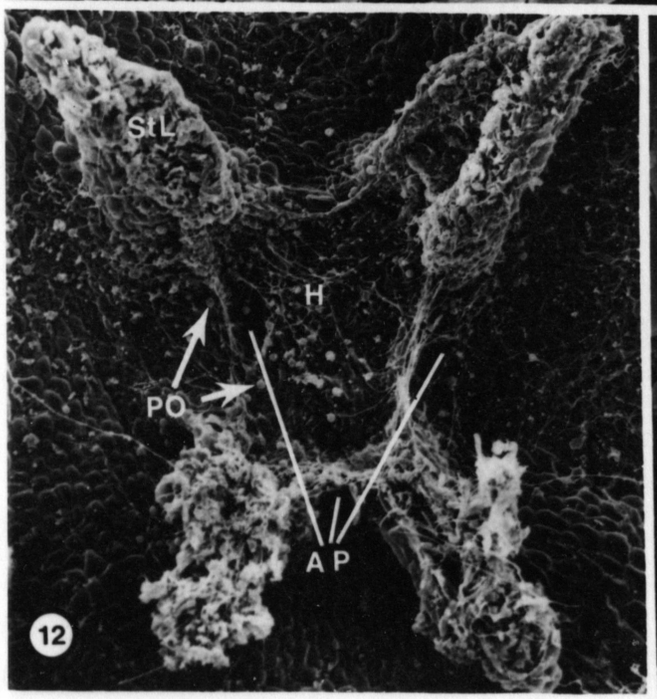
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A P
12

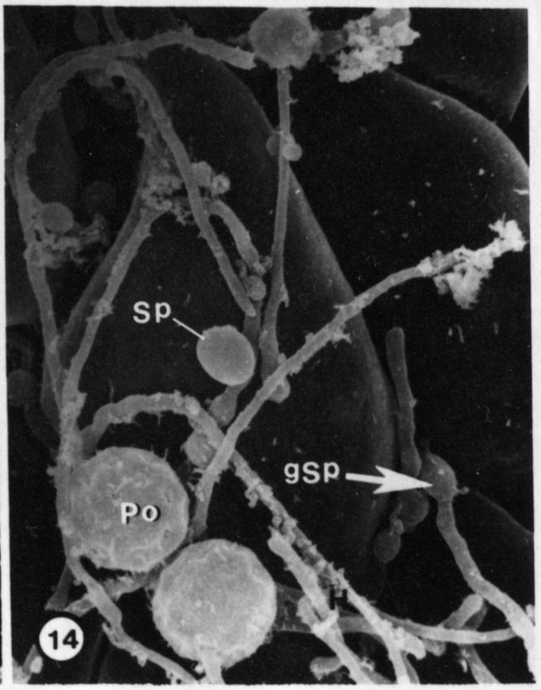

Sp
gSp
Po
H
14

Plate Four:

Fig. 15-18. Scanning electron microscope view of the inner side of the sugarbeet flower infected with P. betae.

Fig. 15. Cross section of immature flower showing, embryo (Em), seed coat (SeC), fruit cavity (FrCa), endocarp (EnCr), mesocarp (MeCr), exocarp (ExCr) and sepals (Se). Note hyphae (H). (30 X).

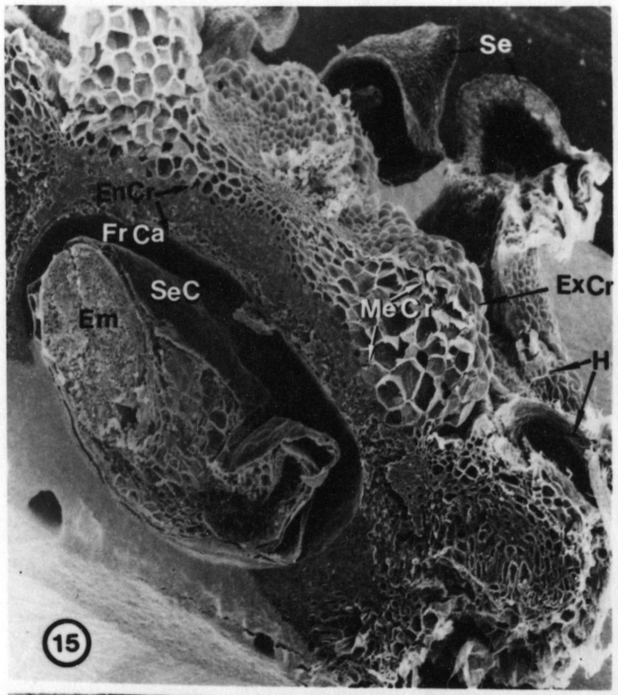
Fig. 16. Flower parts in cross section showing embryo (Em), seed coat (SeC), fruit cavity (FrCa), peripheral zone (Pe).
Note ungerminated pollen (Po) and intercellular and intracellular hyphae (arrows) in both the exocarp and the mesocarp. (45 X).

Fig. 17. Enlargement of the florocarp in cross section 5 days after inoculation, showing that intercellular hyphae (IH) have penetrated the exocarp (ExCr) and the mesocarp (MeCr). (300 X).

Fig. 17a. Enlargement of mesocarp cells, showing that intercellular hyphae have penetrated the exocarp (unicellular epidermal layer) and invaded the mesocarp intra- and intercellular. (540 X).

Fig. 17b. Intracellular hyphae (IH) growing from cell to cell in the mesocarp. No hyphae were found in the endocarp cells (EnCr). (580 X).

Fig. 18. Cross section in the exocarp and mesocarp 48 hr. after inoculation, showing that hyphae (H) grew on the surface of the flower and penetrated the exocarp and mesocarp. Note the ungerminated pollen grains (Po) laying on the surface of the flower. (IH), intracellular hyphae. (220 X).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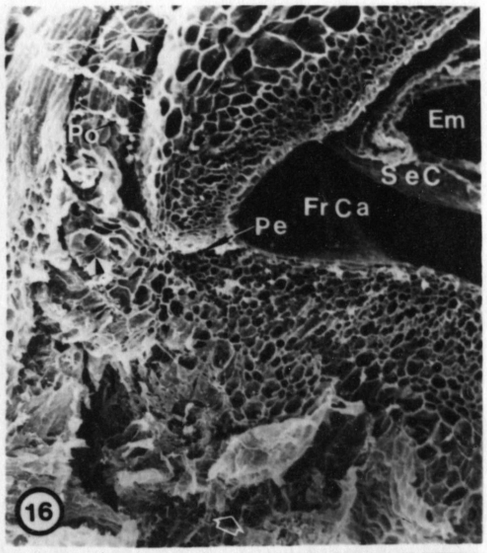
Em
MeCr
ExCr
H
15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FrCa
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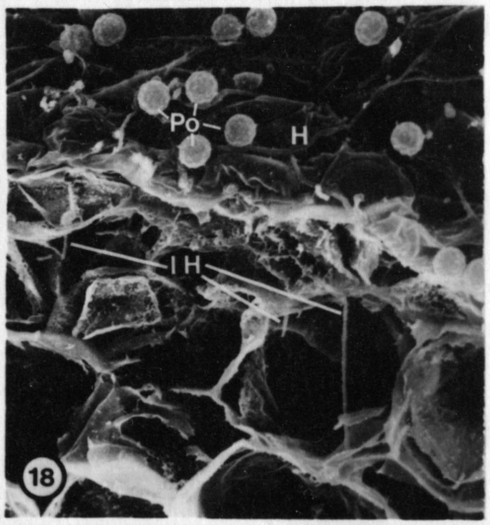
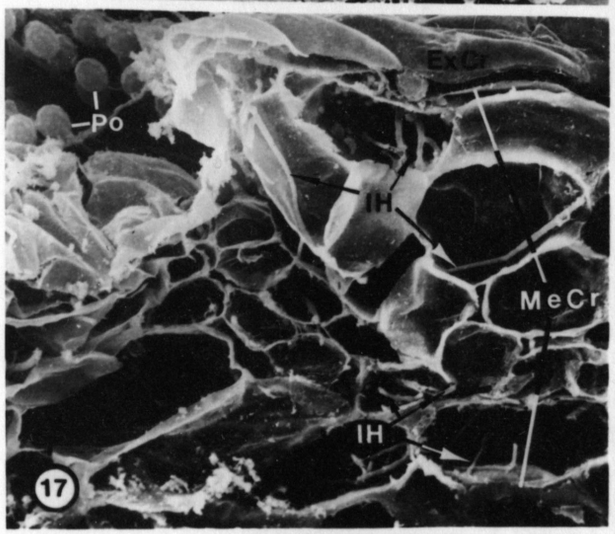
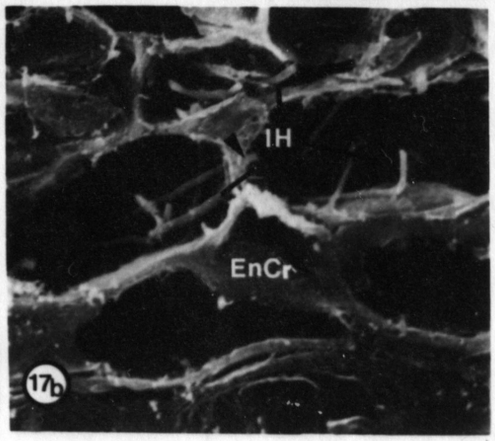

Po
H
IH
18


ExCr
Po
IH
MeCr
IH
17


IH
EnCr
17b

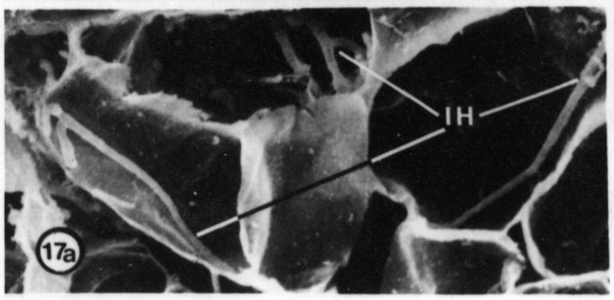

IH
17a

Plate Five:

Fig. 19. Infected flower 5 days after inoculation, showing the apical pore (AP) and forked hyphae (H) which penetrated the apical pore (AP) into the fruit cavity. (Cy), cytoplasm of hypha. (6000 X).

Fig. 20-22. Scanning electron microscope view of the seed stalk infected with P. betae containing black lesions, 5 days after inoculation.

Fig. 20. Ungerminated P. betae spores (SP) and hyphae (H) on the surface of the seed stalk. (190 X).

Fig. 21. Ungerminated pollen (Po) and branched hyphae (H). (360 X).

Fig. 22. Hyphae (H) grew on the surface of the seed stalk shown in Fig. 21 penetrated the epidermis (EP), infected the host cells and became intracellular hypha (IH). (1100 X).

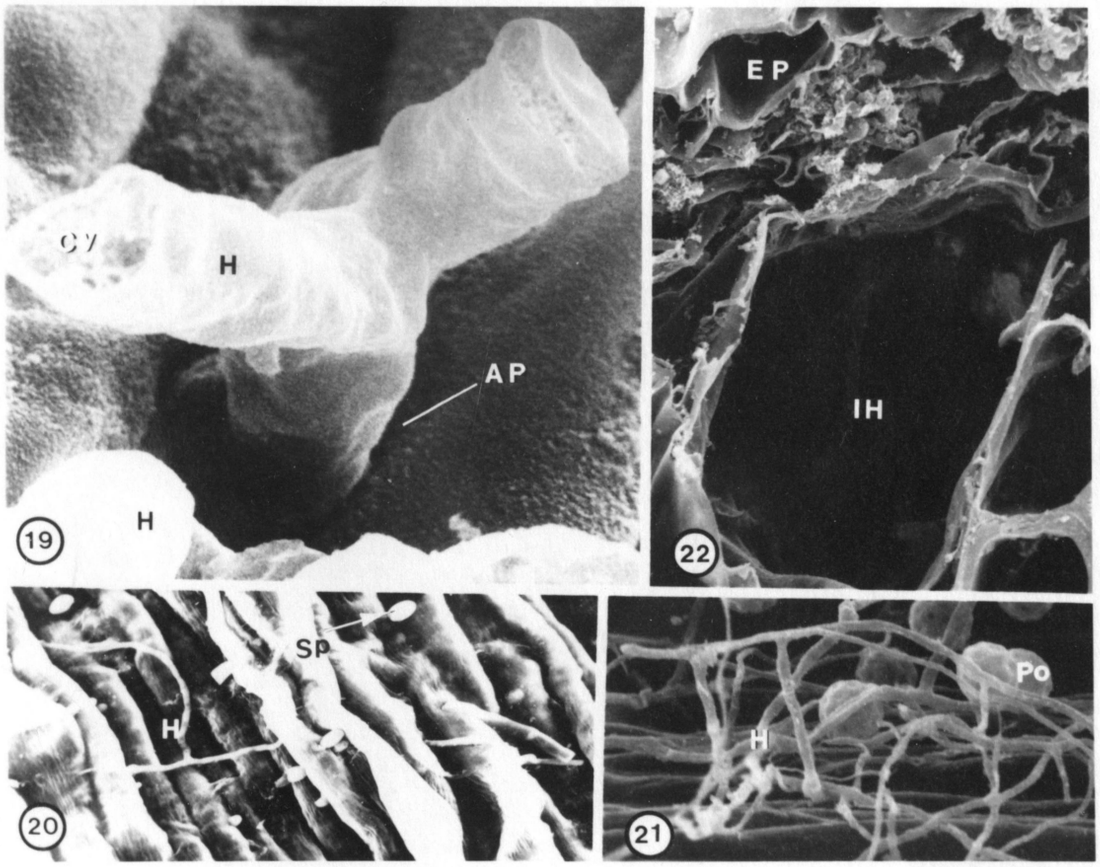
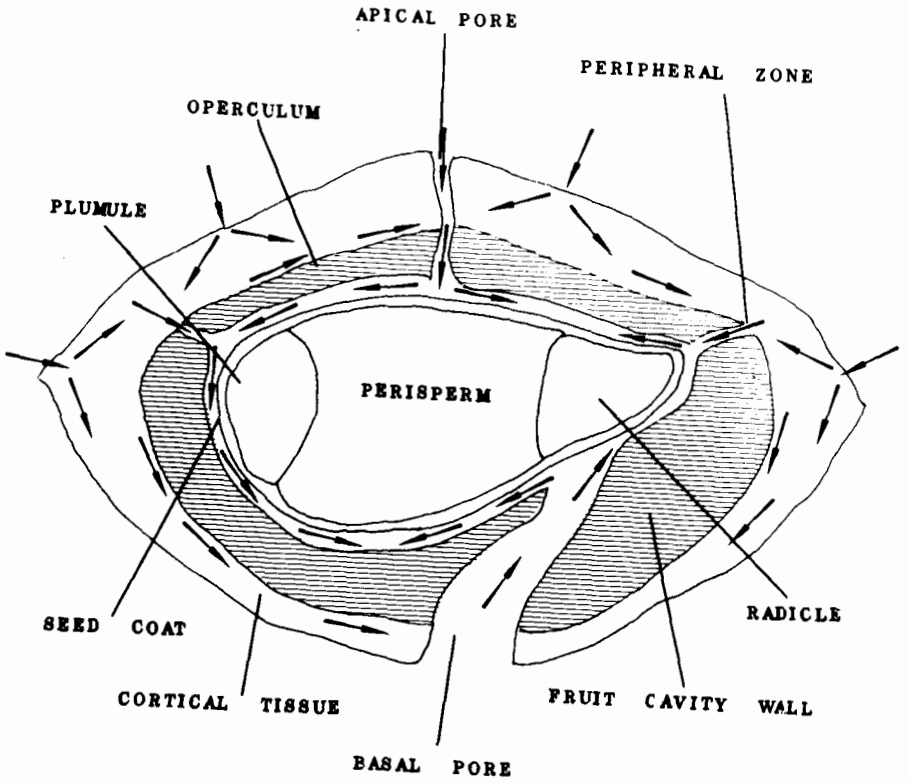

cy
H
AP
H
19
EP
IH
22
SP
H
20
Po
H
21

Plate Six:

Fig. 23. Diagram of a mature sugarbeet seed enclosed in the florocarp, showing the possibilities of P. betae penetration through the different layers and openings of the pericarp.


APICAL PORE
PERIPHERAL ZONE
OPERCULUM
PLUMULE
PERISPERM
SEED COAT
RADICLE
CORTICAL TISSUE
FRUIT CAVITY WALL
BASAL PORE

LITERATURE CITED

1. Artschwager, E. 1927. Development of flowers and seed in the sugarbeet. J. Agr. Res. Vol. 33. (1):1-25.

2. Artschwager, E., and R. C. Starrett. 1933. The time factor in fertilization and embryo development in the sugarbeet. Vol. 47. (11):823-843.

3. Bennett, C. W., and L. D. Leach. 1971. Sugarbeet diseases and their control. Advances in sugarbeet production: principles and practices. The Iowa State University Press, Ames, Iowa. Chap. 9.

4. Bugbee, W. M., and O. C. Soine. 1974. Survival of Phoma betae in soil. Phytopathology 64:1258-1260.

5. Bugbee, W. M. 1974. A selective medium for the enumeration and isolation of Phoma betae from soil and seed. Amer. Phytopathology Soc. 64 (5):706-708.

6. Bugbee, W. M. 1975. Dispersal of Phoma betae in sugarbeet storage yards. Plant Dis. Report. 59:396-397.

7. Byford, W. J. 1972. The incidence of sugarbeet seedling disease and effects of seed treatment in England. Plant Pathol. 21:16-19.

8. Cormack, M. W., and J. E. Moffatt. 1961. Factors influencing storage decay of sugarbeets Phoma betae and other fungi. Phytopathology 51:3-5.

9. Coumano, M. 1978. Role du pore basal dans la germination du glomerule de Betterave scriere. Biologia Slaniarum Vol. 20 (2):114-118.

10. Edson, H. A. 1915. Seedling disease of sugarbeet and their relation to root-rot and crown-rot. J. Agric. Res. 4:135-168.

11. Edson, H. A. 1915. Histological relation of sugarbeet seedling and Phoma betae. J. Agric. Res. (5):55-58.

12. Heydecker, W., R. S. Chetram, and J C. Heydecker. 1971. Water relations of beet root seed germination, effects of the ovary cap and of the endogenous inhibitors. Ann. Bot. 35:31-42.

13. Koch, F., and M. Panagiotaku. 1979. Untersuchungen zur infektion und pathogenese bei Befall der Zuckerrube durch Phoma betae (Pleospora betae Bjorl). Zuckerindustrie (in German) Vol. 104 (7):611-617.

14. Leach, L. D., and J. D. MacDonald. 1976. Seed-borne Phoma betae as influenced by area of sugarbeet production, seed processing and fungicidal seed treatment. J. Amer. Soc. Sugarbeet Technol. Vol. 19 (1):4-15.

15. Mangan, A. 1971. A new method for the detection of Pleospora bjorlingii infection of sugarbeet seed. Trans. Brit. Mycol. Soc. 57 (1):169-172.

16. Maude, R. B., A. S. Vizor, and C. G. Shuring. 1969. The control of fungal seed borne disease by means of a thiram seed soak. Ann. App. Biol. 64:245-257.

17. Perry, D. A., and J. G. Harrison. 1974. Studies on the sensitivity of the monogerm sugarbeet germination to water. Ann. App. Biol. 77:51-60.

18. Peto, F. H. 1964. Methods of loosening tight seed caps in monogerm seed to improve germination. J. Amer. Soc. Sugarbeet. Technol. Vol. 13 (3):281-286.

19. Pool, V. W., and M. B. McKay. 1915. Phoma betae on the leaves of the sugarbeet. J. Agri. Res. 4:169-174.

20. Tompkins, C. M., and S. B. Nuckols. 1928. Development of storage disease in sugarbeets. Phytopathology 18:939-941.

21. Warren, R. C. 1972. Attempts to define and mimic the effects of pollen on the development of lesion caused by Phoma betae inoculated onto sugar beet leaves. Ann. Appl. Biol. 71:193-200.