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INFECTION OF ORIENTAL MUSTARD BY NEMATOSPORA: A FLUORESCENCE AND SCANNING ELECTRON MICROSCOPE STUDY

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Abstract

Fluorescence light microscopy and scanning electron microscopy were used to study penetration by the yeast Nematospora coryli through the seed coat and into the embryonic tissues of oriental mustard seed (Brassica juncea).

Infection of the seed was associated with its physical injury; however, it was evident that the yeast was capable of successfully invading healthy plant cells. The pathological process was followed in parallel using both the above types of microscopy. Foci of yeast infection on the seed coat outer surface were characterized by swelling of the infected epidermal cells. Nematospora hyphae were seen in the lumina of the seed coat palisade cells and spread laterally when the hyaline layer between the seed coat and embryo was reached. Sites of infection at the surface of cotyledon cells appeared as zones of localized erosion. Asci and spores were visible, embedded in disorganized and disintegrating plant tissue.

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Introduction

It is not uncommon to find that spices are naturally contaminated with bacteria and fungi (Chandra et al. 1981, Ayres et al. 1980), to such an extent that in many countries their sterilization by treatment with ethylene oxide is routinely carried out. This is done mainly to reduce the risk of pathogenesis as well as early food spoilage by the introduction of large numbers of microorganisms during seasoning. Under normal circumstances most of the organisms present in spice seeds (e.g., anise, caraway, celery, coriander, cumin, nutmeg, dill, fennel, mustard, poppy, pepper, sesame) are on the surface of the seeds (Cowlen and Marshall 1982, Leistner et al. 1981, Pivnick 1980). Indeed, Leistner found that below the palisade layer of the peppercorn testa, the seed was essentially sterile. It should be noted that Chandra et al. (1981) found about 25% of seeds to be still infected following surface disinfection, although this may reflect storage at higher than normal humidity (Schans et al. 1982).

In spices several compounds, but, in particular, essential oils (e.g., isothiocyanates), are potent antimicrobial agents (Virtanen 1962, Pivnick 1980). It is believed that this is also true in oriental mustard with respect to Nematospora coryli (Holley and Timbers 1983). However, despite the natural toxicity of the seed to the infecting Nematospora it became of some interest to examine the development of yeast penetration into the seed, especially since the latter occurred spontaneously in the field.

Nematospora coryli is an internationally important plant pathogen capable of causing devastating damage to many crops in different parts of the world. Phytopathogenic Nematosporaceae are more frequently found in warmer parts of the world (Batra 1973) but recently were reported to occur in oriental mustard grown in a restricted area of western Canada (Burgess et al. 1983). Although at present the outbreak has abated, it has become important to examine the development of yeast penetration into the seed, especially in view of the expanding role mustard crops will probably play in Canadian agriculture. Concern is expressed that crop quantities larger than 80,000 hectares planted to mustard in Canada in 1982 may be at risk. In addition, should mustard serve as a

reservoir for infection of other susceptible crops, the potential for damage would be significant.

Materials and Methods

Electron microscopy

Oriental mustard seed (Brassica juncea) for these studies was obtained from a pocket of infestation in a field in southwestern Saskatchewan. In order to ensure that individual seeds for microscopic examination were infected with Nematospora, they were dissected approximately in half with a scalpel. One half was stored for later microscopic examination, the other was crushed in a sterile mortar with a pestle. The crushed seed was mixed with molten 50°C plate count agar (Difco) containing 50 ppm each of tetracycline and chloramphenicol to retard bacterial development. These additions of antibiotics, lower than the 100 ppm recommended (Speck 1976), were used because Nematospora growth was retarded by higher concentrations. The seed-agar mixture was allowed to solidify in a petri dish and incubated at 35°C under oxygen-free nitrogen for 4 to 5 days to further reduce competition from bacteria and filamentous fungi. The presence of typical subsurface cream-coloured and star-shaped colonies in the agar was taken to be indicative of Nematospora and this was confirmed by phase contrast light microscopy. Approximately 20% of seeds examined from this source were contaminated. Visual inspection and separation of mechanically damaged seeds was not an efficient or productive method for isolation of infected seeds. For comparative purposes, our original isolate of Nematospora coryli (Holley and Timbers 1983) which had been lyophilized, was used as a reference in pure culture work.

When it was determined from incubation of the halves of suspect seeds in agar that seeds were infected with Nematospora, the other half of the seed was bisected. Seed samples were fixed for 24 h at 4°C in 2.8% glutaraldehyde. Fixed seed was rinsed three times in distilled water, frozen in melting freon, transferred to liquid nitrogen, freeze-fractured, and then freeze-dried at -80°C (Speedivac-Pearce Tissue Dryer Model 1). Fractured seeds were mounted on aluminum stubs with silver cement, and coated with carbon and gold (20 nm) in a coating unit (Speedivac Model 12E6/1258, Edwards High Vacuum Limited, Crawley, Sussex, England). Specimens were examined in a Cambridge Stereoscan Mark 2A scanning electron microscope (SEM) at 20 kV.

Pure cultures of Nematospora grown on plate count agar (Difco) at 35°C for 3 to 7 days were removed from the agar surface and fixed in 2.8% glutaraldehyde for 4 h at 4°C in a test tube. The fixed culture, now a suspension, was transferred to freshly cleaved mica sheets (5 x 13 mm) which had been pretreated with 0.1% aqueous poly-L-lysine hydrobromide for 20 min and rinsed with water. Samples were applied before the mica dried. After 15 min exposure on the mica support, samples were rinsed in distilled water three times, frozen in melting freon, transferred to liquid nitrogen, and freeze-dried. Samples were mounted on SEM stubs and treated as described previously for SEM examination of seed.

Fluorescence microscopy

Mustard seeds were fixed and embedded in glycol methacrylate (GMA)(Eastman Kodak Co., Rochester, NY) using the method described by Yiu et al. (1982). Briefly, seed tissues were fixed in 3% glutaraldehyde in 0.025 M potassium phosphate buffer, pH 7.2, at 4°C for 48h, dehydrated through methyl cellosolve, ethanol, n-propanol and n-butanol, and infiltrated with GMA for 3 to 5 days prior to polymerization at 60°C in gelatin capsules. Sections were cut 3 to 7 µm thick using glass knives and affixed to glass slides for examination.

Mustard seed sections were stained with 0.05% (w/v) aqueous Aniline Blue (C.I. 42755, Polysciences Inc., Warrington, PA) in 0.07 M K_3PO_4 for 1 min and/or 0.001% (w/v) aqueous Calcofluor White M2R (American Cyanamide Co., Bound Brook, NJ) for 1 to 2 min. After a rinse in water they were air-dried, mounted in immersion oil, and examined for fluorescence using an exciter/barrier filter set with maximum transmission at 365 nm/>418 nm (FC I) or at 450-490 nm/>520 nm (FC II). Alternately, seed sections were stained 2 to 5 min in 0.01% (w/v) aqueous Congo Red (C.I. 22120, Fisher Scientific Co., Fairlawn, NJ). They were then rinsed in water, air-dried, and mounted in non-fluorescent immersion oil for fluorescence examination using either FC II or an exciter/barrier filter set with maximum transmission at 546 nm/>590 nm (FC III).

All sections were examined with a Zeiss Universal Research Microscope (Carl Zeiss Ltd., Montreal, Quebec) equipped with a III RS epi-illuminating condenser combined with an HBO 200 W mercury-arc illuminator for fluorescence analysis. The III RS condenser contained all three fluorescence filter combinations of FC I, II, and III. Photomicrographs were obtained using 35-mm Kodak Ektachrome 400 ASA daylight film.

Results

Scanning electron microscopy

Seed coat Normal uninfected mustard seed as viewed from the outer surface looked very much like a golf ball. The surface contained a semi-regular array of ridges which compartmented the seed coat to form a network that gave an almost honeycomb pattern (Figs. 1 and 2) with little apparent debris and no significant interruption of the surface pattern. Rarely, an ascospore could be seen on the outside surface of a normally appearing seed coat of an infected seed (Fig. 3).

Examination of Nematospora pure cultures by SEM revealed that as the culture aged beyond a week, hyphae and spindle-shaped ascospores predominated (Fig. 4). These latter ones were seen together with elliptical vegetative cells in infected seed tissue.

When seeds known to be infected by the yeast were examined further, most of the seed surface appeared to have the normal grid-like pattern; however, areas where this pattern was interrupted were visible at intervals on the seed coat (Fig. 5). Interruptions consisted of raised, somewhat smooth areas which were "pebbled" and appeared as if they were areas of seed epidermis swollen by the growth of an underlying yeast microcolony.

Globose and elliptical vegetative yeast cells were visible in the vicinity of these affected areas on the seed surface (Fig. 6). The contents of the raised areas were not amorphous as would be expected if it were mucilage.

Further examination revealed that these raised areas contained a tightly packed array of elliptical and globose yeast cells (Fig. 7).

It was usual to find sites of what appeared to be physical injury near where swollen yeast-infected tissue was seen at the surface of the seed coat (Fig.6).

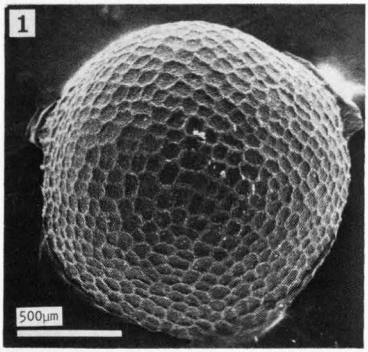
Visible lesions were not clearly defined on the inside surface of the seed coat since this surface did not fracture cleanly. Often amorphous stringy debris would adhere to the exposed surface. Most spectacularly and in association with many infected cells, the hyaline layer was filled with vegetative yeast cells (Fig. 8). Occasionally mature asci and ascospores were also visible.

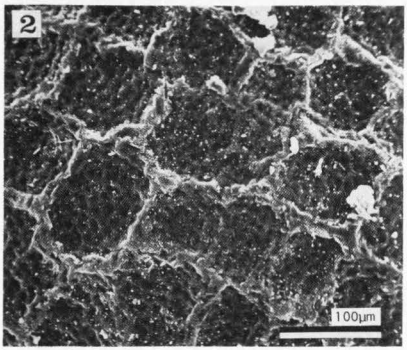
Cotyledon surface Cotyledon tissue was also affected by the development of the pathogenic Nematospora. Zones of eroded or partially digested tissue were evident in isolated areas across this surface and occurred only where the infecting yeast was present (Fig. 9). Frequently, evidence of extensive tissue damage due possibly to physical injury was present in the areas of the lesion (Fig. 10). Apparently undamaged cotyledon cells were also infected by the invading yeast. The presence of spores in these cells and the development toward disorganization of seed cell structure was also seen (Fig. 11).

Fluorescence microscopy

When a smear containing a 6-day old broth culture of Nematospora was prepared on a glass slide and was dried and stained with Aniline Blue, the results obtained were as shown in Fig. 12. Globose and elliptical cells were seen to have folds in their cell walls which fluoresced a pale green colour. The ascospores were also stained fluorescent, but with intensity at both the tip of the anterior (acuminate) end and the entire posterior half of the spore. No fluorescence was noted in the mid- to anterior region of the spore. It has been reported that Aniline Blue dye is specific for β-(1-3)-D-glucans (Fulcher 1982) which almost certainly occur in the vegetative cell walls and those of spores. However, the possibility that the dye may have affinity for other chemical groups cannot be ruled out. Certainly, the result obtained here reflects a difference between the anterior and posterior halves of the spores. Differential staining of these spores has been reported by others, with the anterior portion being refractile to staining with Acid Fast and cytoplasmic stains (Carmo-Sousa 1970, Batra 1973).

An examination of GMA-embedded serial sections of infected seed halves showed results similar to those found during SEM. Yeast on the





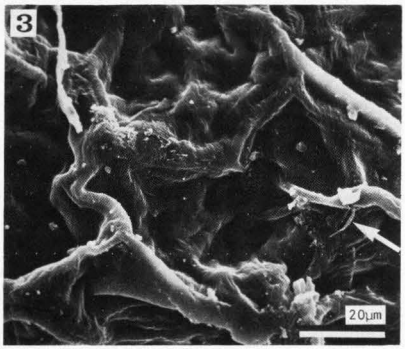
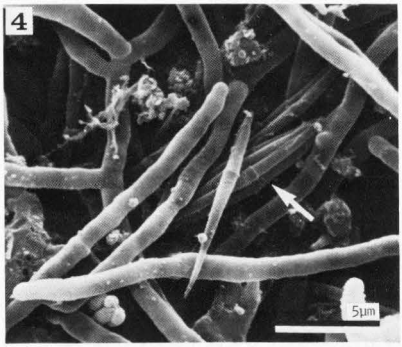
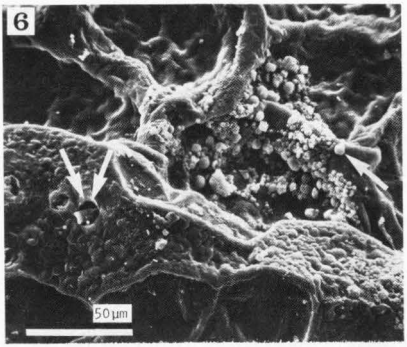


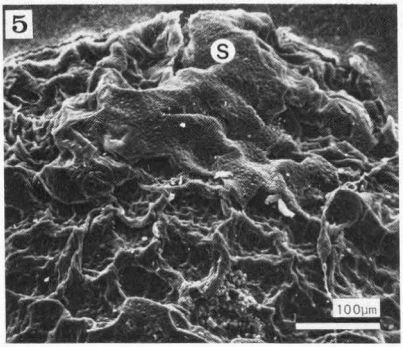
Fig. 1. Surface view of an uninfected oriental mustard seed by SEM.

Fig. 2. Seed coat of oriental mustard by SEM, showing semiregular network of surface ridges.

Fig. 3. Surface view of the seed coat from an infected mustard seed showing a single ascospore (arrow) of Nematospora.







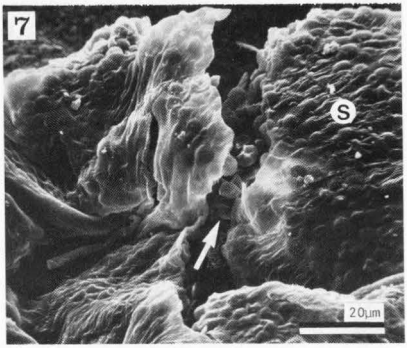


Fig. 4. Hyphae and pseudomycelium of Nematospora coryli also showing ascospores (arrow).

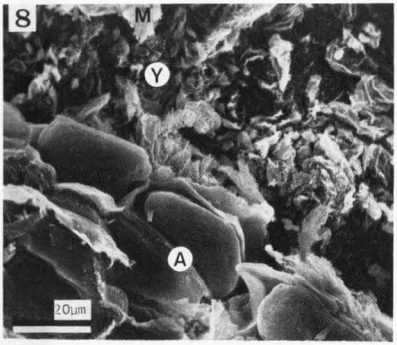
Fig. 5. Surface view of the seed coat from an infected mustard seed showing interruptions (S) in normal surface pattern which are believed to be areas of Nematospora involvement.

Fig. 6. Seed coat surface of an infected seed showing exposed yeast (arrow) and a swollen area of the seed surface where physical injury (double arrow) may have been inflicted.

Fig. 7. Seed coat surface of an infected seed showing an area where subsurface yeast growth has caused swelling (S). Vegetative yeast cells can be seen below the seed coat surface (arrow).

outside of the seed was integrated among cells of the mucilagenous epidermal layer (Fig. 13). This growth by the yeast mycelial form was observed to penetrate through the subepidermis into the palisade cells where yeast were visible in the lumina of the palisade cells (Fig. 14). A zone of eroded tissue or a site of physical injury was also visible. A cross sectional view of the seed coat is shown in Fig. 15. Fungal hyphae are visible in the mucilagenous epidermal tissue. Extensive damage can be seen in the pigment layer above the aleurone cells as well as in the latter tissue.

A cross-section of the seed coat below the epidermis is shown in Fig. 16. Significant damage was seen beneath the aleurone cells while the aleurone layer appeared largely unaffected. Yeast spores were seen to spread laterally throughout the hyaline layer and in some preparations actually circled the entire embryo. Tissue damage was also visible in the peripheral cells of the cotyledon (Fig. 17) and yeast spores with their characteristic arrangement in packets of eight were seen in cross-section inside some of the infected cotyledonous cells (Fig. 18). No infection was detected beyond the periphery of the cotyledonous tissue.



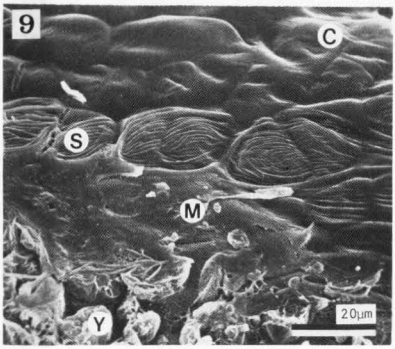
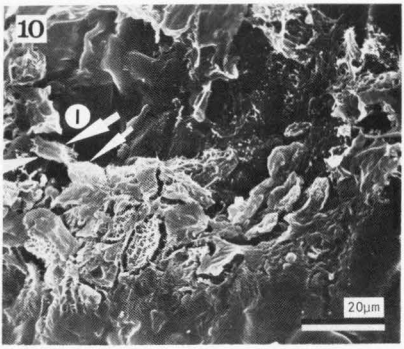


Fig. 8. A fracture of an infected oriental mustard seed tagential through the aleurone layer of the seed coat (A) through the hyaline layer showing vegetative yeast cells (Y) in a matrix (M) of amorphos material.

Fig. 9. View of the cotyledon surface of an infected mustard seed showing the progression of Nematospora infection of healthy tissue. Normal healthy cells (C), stressed cells (S), amorphous materia (M), and yeast cells (Y) are visible.



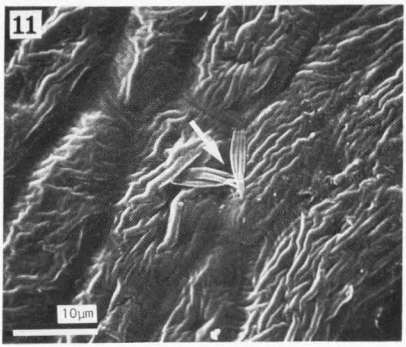


Fig. 10. Cotyledon surface from an infected seed illustrating tissue disorganization resulting from Nematospora infection from a site of physical injury (I). Yeast asci (arrows) are visible in necrotic tissue.

Fig. 11. Yeast ascospores (arrow) "exuding" from a cell of the surface of cotyledon tissue in an infected seed. Other adjacent cells appear distorted perhaps due to the yeast infection.

Discussion

Growth of Nematospora in pathological lesions of the seed or in laboratory culture resulted in the same diversity of cellular morphology. The distinct character of vegetative cells, mycelium, and ascospores was maintained in the two environments and equivalent yeast forms were observed in each milieu. The most striking morphological feature of the yeast was its spindle-shaped ascospores which possessed spiral ridges on the pointed or posterior end which resembled an auger (Fig. 4). In all probability this pattern on the surface of the spores was nothing more than the decoration that has been reported before on fungal spores (Martinez et al. 1982). The ridges may serve to some extent in the process of spore dissemination.

Seed coat - external

When seed rinse and surface sterilization procedures (Chandra et al. 1981) using 2% sodium hypochlorite were used, little evidence was obtained for the presence of contaminating Nematospora on the seed surface. An examination of contaminated seed by SEM and fluorescent light microscopy did result in the observation of surface contamination on the seed coat, usually, but not always, adjacent to foci of epidermal infection (Figs. 3, 6, and 13). The proportion of organisms on the seed surface easily removed by surface rinsing was small in relation to the total numbers of organisms present in infected seed (approx. 1%), although for surface-contaminated seed rinse-soak methods are recommended to routinely quantify microorganisms (Cowlen and Marshall 1982).

Oriental mustard seed surface topography (Figs. 1 and 2) resembled in a very general way images of Brassica napus published elsewhere (von Hofsten 1974), Brassica nigra (Vaughan et al. 1976), and also black pepper (Leistner et al. 1981) with differences noted in the following discussion. The predominating feature of the seed coat appearance was an informally arranged network of interconnecting ridges (Fig. 1). There were fewer ridges on the seed coat of black pepper than on oriental mustard. On B. napus surface ridges were closer together and valleys in between were deeper. With B. nigra the same pattern was evident but the ridges were not as conspicuous (Vaughan et al. 1976) and the surface was more like that of oriental mustard as seen by fluorescence microscopy (Fig. 13).

Surface contamination of oriental mustard by Nematospora was visible by both SEM (Figs. 3 and 6) and fluorescence microscopy (Fig. 13), and occurred mainly in areas where physical damage of the epidermis was visible (Fig. 6). If the latter were "puncture" damage, in all probability this physical injury was due to the feeding activity of insects like the false chinch bug (Nysius ericae) and others which have been implicated as vectors in disease transmission (Burgess et al. 1983, Heinrichs et al. 1976, Batra 1973). Prior physical injury is considered to be an important prerequisite for the establishment of infection in spice seeds (Leistner et al. 1981).

Foci of Nematospora infection on the outer seed coat surface appeared as elevated or swollen areas and interrupted the normal pattern of surface ridges (Fig. 5). These elevated areas had a "pebbled" appearance due to the underlying masses of globose and elliptical vegetative cells (Fig. 7). Engorged areas probably developed as a result of rapid yeast growth prior to seed maturation and desiccation in the seed pod. This hypothesis is consistent with the result obtained by Burgess et al. (1983) during laboratory infection of oriental mustard by infected insects. It is unlikely that swelling was due to hydration of mucilage since the underlying material contained structures resembling vegetative yeast cells (Fig. 7).

Seed coat penetration

Evidence for the spread of yeast infection through the seed coat was taken largely from results obtained using fluorescence microscopy techniques (Fulcher 1982, Yiu et al. 1982).

Images obtained in cross sections of the seed coat (Figs. 15 and 16) were identical in outline to those previously published for B. juncea (Aoba 1972, Vaughan et al. 1963) by light microscopy and were similar to those published for yellow mustard (Vaughan et al. 1976) and for rapeseed using fluorescence microscopy (Fulcher 1982, Schans et al. 1982, Yiu et al. 1982). In cross section, three major layers of cells were evident in the oriental mustard seed coat: the outer epidermal, underlying palisade layer, and inner aleurone cells (Fig. 15). The hyaline layer between the seed coat and cotyledon cells was also visible (Fig. 16) but detail of parenchymal tissue overlying the aleurone cells was not clear in infected specimens.

Yeast and mycelia were present on the outer epidermal layer (Figs. 6 and 13) and were believed to penetrate into and through the lumina of the palisade cells (Fig. 14), the aleurone layer (Fig. 15) and then to the underlying hyaline layers where lateral spread and multiplication of organisms occurred (Fig. 16). Shown in Fig. 8 is a comparable view by SEM, tangential to the hyaline layer through the aleurone layer. Vegetative cells of Nematospora were visible in large numbers. Often by both SEM and fluorescence microscopy the hyaline layer was seen to be filled with both vegetative cells (SEM) and spores (fluorescence).

Cotyledon penetration

Cotyledon tissue was heavily infected in some peripheral areas with localized foci of eroded and apparently necrotic tissue often in association with vegetative yeast cells (Fig. 9). In contrast with the swollen areas on the seed coat surface, these erosion zones (Fig. 10) resembled erosion troughs around the bacterium Alteromonas putrefaciens on pork skin (Butler et al. 1980). Results were interpreted to mean that foci of yeast infection developed in areas that had suffered physical injury, although the yeast appeared to be an invasive parasite. For example, amorphous tissue was often found in an area adjacent to a focus of infection. As one moved farther from the focus of infection, intact cotyledon cells could be seen which contained structures resembling maturing ascospores (Fig. 9). Thus, Nematospora appeared capable of infecting otherwise normal tissue - an observation made by Heinrichs et al. (1976) during a study of inoculated soybeans.

The pattern of oriental mustard seed infection by Nematospora seen here at each layer of tissue seemed to be associated with physical injury, and was likely caused by an insect vector (Burgess et al. 1983). There appears to be a consensus that physical injury, probably through insect feeding with consequent Nematospora inoculation of the damaged seed, is a necessary prerequisite (Burgess et al. 1983, Heinrichs et al. 1976, Batra 1973). On the other hand, at artificially high temperature and moisture, successful invasion of rapeseed with concomitant destruction of cotyledon cells by Aspergillus, Penicillium, and Verticillium was accomplished without prior physical damage to the seed (Schans et al. 1982). It is very probable that oriental mustard would be attacked by many fungi in a similar successful manner under the same abusive storage conditions without physical injury (Holley and Timbers 1983).

Schans et al. (1982) traced the invasion route and found that the inoculated fungi crossed the seed coat tissue and entered the rapeseed cotyledon without apparent difficulty. Once below the palisade cells of the seed coat, the fungi went laterally among the crushed parenchyma. In our study of infected oriental mustard, some lateral movement of the yeast hyphae may have taken place above the aleurone cells, but major lateral growth occurred below the aleurone layer and almost filled the entire hyaline layer. Intra- and extracellular growth of the fungi and yeast in cotyledonous cells was similar in both kinds of seed.

In contrast to results obtained by Heinrichs et al. (1976), who used soybeans inoculated with Nematospora, oriental mustard cotyledon tissue was not deeply penetrated by invading Nematospora. Substantial growth by the yeast occurred in the hyaline layer between the seed coat and embryo. It is possible that myrosine granules in cotyledon cells may play a role in the natural seed defence system to prevent deep penetration by microorganisms into cotyledon tissue. Work on the autotoxicity of mustard seed to the yeast Nematospora is continuing.

Conclusion

Support was obtained for the hypothesis that the infection process in the seed was initiated by physical injury. This injury was probably caused during the feeding activity of contaminated insects with piercing-sucking mouth parts. Areas of apparent physical injury were found adjacent to sites of yeast infection on the surface of the seed coat and on the cotyledon surface. Vegetative yeast cells and hyphae were seen to penetrate through the seed coat and to grow laterally at the hyaline layer between the seed coat and cotyledons. All morphological forms of the yeast were found both inter- and intracellularly with respect to the cotyledon cells.

Acknowledgments

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Discussion with Reviewers

D. N. Holcomb: Could you give more detail of the fluorescence microscopy technology and provide some warnings as to artefacts with this technique?
Authors: The formation and recognition of artefacts are important aspects of this and other techniques in microscopy. Substantial additional information on the applications and limitations of fluorescence microscopy are provided in the paper by Fulcher (1982) cited in the bibliography.

L. van Caeseele: In view of the differential color obtained by Schans et al. (1982) using Acridine Orange and Malachite Green, did you try combinations of stains such as this?
Authors: The major part of our work was done before the latter was published and thus the dyes mentioned were not used. In view of the success achieved by Schans et al. (1982) with rapeseed, they may be quite appropriate for use with mustard as well.

L. van Caeseele: Fig. 6 shows puncture marks (arrows). In the discussion you speculate that these may be caused by the false chinch bug. If this were so, would you expect smooth edges on the puncture hole? Would the holes vary in diameter? Is the diameter of the false chinch bug proboscis known?
Authors: Insects, which could be responsible for inflicting puncture damage upon these crops, vary in size and thus the lesions they cause also vary in their dimensions. Indeed,the male false chinch bug is significantly smaller than the female. The proboscis of the false chinch bug (the most likely insect to be involved) female measures approximately 50-80 µm in diameter. This includes an outer sheath which does not penetrate. Inside the sheath are 4 stilets, two of which cut the hole. The diameters of the "holes" in Fig. 6 are within the size range of those which would be produced by these insects (10-20 µm). The edge of these puncture wounds would initially be ragged, but as the seed matured and dried, one would expect changes to occur in the perimeter of these lesions.

J. G. Vaughan: Are the authors interested in carrying out a controlled experiment on yeast with healthy B. juncea seed?
Authors: Yes, and we would be especially interested in studying the progress of yeast infection during seed maturation. It is an interesting contradiction that the host seed is quite toxic toward the yeast parasite.

S. H. Humphreys: Could the folds shown in the vegetative yeast cells (Fig. 12) be artefacts of drying?
Authors: Undoubtedly this is true. Although less clearly resolved, irregular surfaces of vegetative yeast cells are also visible in the seed lesion viewed by SEM in Fig. 8.

Reviewer V: Is a reference strain of the infecting organism available?
Authors: Yes, the Nematospora culture studied has been deposited in the collection of the Centraal Bureau voor Schimmelcultures, Baarn, the Netherlands and has been assigned CBS#8199. The culture is also preserved at the National Mycological Herbarium, Ottawa, Ontario, Canada K1A OC6, where it is given the number DAOM 187446.

Fig. 12. Aniline Blue-stained culture smear showing yeast cells and spores (arrow). Photographed using FC I.

Fig. 13. Congo Red-stained GMA-embedded paradermal section of infected mustard seed showing the epidermal mucilage (M) and the yeast (Y) structures. Photographed using FC III.

Fig. 14. Congo Red-stained GMA-embedded paradermal section of the palisade layer (P) of infected mustard seed penetrated by yeasts (Y) in an area of seed tissue showing signs of disorganization. Photographed using FC II.

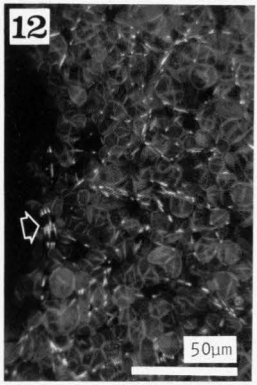
Fig. 15. Aniline Blue - Calcofluor White-stained GMA section of infected mustard seed coat showing epidermis (E), palisade (P), pigment (arrow), aleurone (A), and hyaline (H) layers. Photographed using FC I.

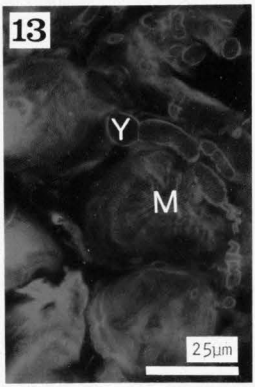
Fig. 16. Congo Red-stained GMA section of infected mustard seed showing yeast spores (arrows) spreading throughout the hyaline (H), palisade (P), and aleurone (A) layers. Photographed using FC II.

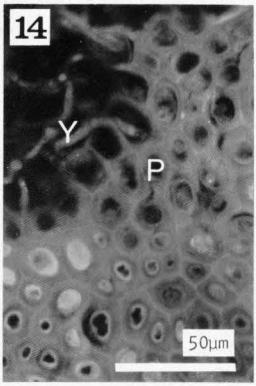
Fig. 17. Congo Red-stained GMA section of infected mustard seed showing yeast cells (Y) at the periphery of the damaged cotyledonous cells (*). Photographed using FC II.

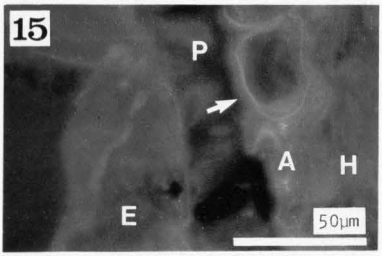
Fig. 18. Congo Red-stained GMA section of infected mustard seed showing yeast spores (arrows) inside an infected cotyledonous cell (*). Photographed using FC III.

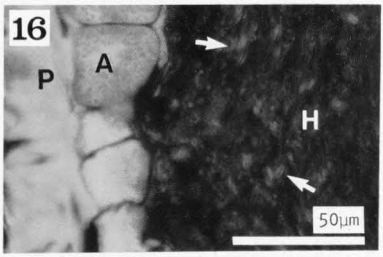
Errata: The correct marker on Fig. 18 is 20 µm.

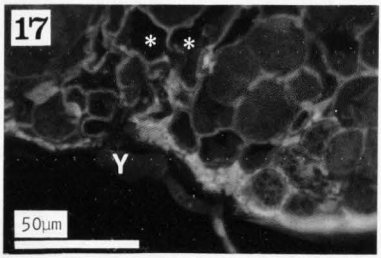

12
50µm

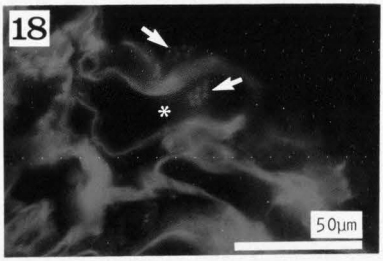

13
Y
M
25µm


14
Y
P
50µm


15
P
A
H
E
50µm


16
P
A
H
50µm


17
Y
50µm


18
50µm