Some aspects of the host-parasite relations between *Sclerotinia sclerotiorum* (Lib.) de Bary and rapeseed.

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INTRODUCTION

De Bary (1886 - 1887) in his classical work studied host-parasite relationship of diseases caused by *Sclerotinia sclerotiorum* (Lib.)de Bary. His work includes the description of the formation of appressoria from germinating ascospores and the dependence of the invading mycelium on its pre-penetration nutritional status. Purdy (1958) confirmed the dependence of appressoria formation on nutrients and a firm surface. Survey of rapeseed (*Brassica napus* and *B. compestris)* fields in France have revealed the incidence of stem rot by *S. sclerotiorum* during flowering period. The role of dead flower parts in infection of various hosts has been demonstrated by various workers (Abawi et al., 1975; Krüger, 1975 and Natti, 1971). Scanning electron microscopy (SEM) was used to follow pathogenesis by *Sclerotinia minor* in comparison to *S. sclerotiorum* in bean hypocotyls tissues by directly infecting with infested oat kernels (Lumsden and Wergin, 1980). This study was undertaken to examine the role of flower parts in initiating aerial infection by ascospores in rapeseed. Pairing discs technique developed by authors was employed to observe ascospores germination simultaneously on leaf and petal tissues and their subsequent pathway of infection using SEM.

MATERIALS and METHODS

Apothecia were produced from initiated Sclerotia of sunflower isolate as described by Lamarque, 1980. Leaflets were obtained from the third node of twenty days old seedlings of rape variety "Jet neuf" and mustard variety "picra" raised under sterilized condition on modified culture medium of Murashige and Skoog, in 25 x 4 cm test tubes, in a growth chamber at 20°C. Petals under study were excised from the flower budswhich were on the way of blooming in a glass house.

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Discs of convenient sizes of 5 mm and 10 mm of petals and leaves were cut for subsequent observations. The pairing of petal and leaf discs was done using kaoline as an adhesive agent. paired discs were carefully transferred one by one avoiding any physical injury on to the previously U.V.sterilized cellophane mounted on the small (10.00 x 15.00 mm) glass tubings. In order to avoid desiccation of samples, these tubings were kept in a humid chamber before deposition of ascospores. These paired discs were inoculated by inverting the 20 days old mature apothecia for 5 minutes at room temperature. These inoculated paired discs alongwith cellophane mounted tubings were replaced in humid chamber and were incubated at 20°C.For observations of host surfaces with SEM, infected paired discs were removed at various intervals during infection period. The specimens were examined under JEOL - JSM - 35 scanning electron microscop operating at 25 K.V using cryoscan device.

RESULTS

The SEM comparisons of healthy petals of rapeseed and mustard revealed no significant morphological differences for such type of study (Fig. 1). Moreover, their colonisation after ascosporic deposition was observed to be same. However, mustard leaves (Fig. 3) were considered better for SEM observations over rapeseed leaves (Fig.2)because of less epicuticular substances like wax etc. The observations, however, were confirmed on both the hosts.

Initiation of germtube was observed after 9 - 10 hours of ascospore deposition on petals. Most of the ascospores germinated by giving rise to one germ tube and some of them were also observed to germinate by two each produced at distal ends (Fig. 4 & 5). Each germinated ascospore was found to loose its morphological identity after 24-30 hours of germination. The petal cells were lysed before loosing morphological identity of germinated ascospores or well in advance of infection (Fig. 6 & 7). After 7 - 8 hours of germination, each hypha produced on the petal surface develops dichotomous branching perpendicular to each other whose tips were generally rounded (Fig. 8 & 9). Successful germination of ascospores on petal surface and projection of penetrating hyphae towards leaf surface is well evident in fig.10.

On contrary ascospores deposited on leaf surface exhibited signs of desiccation after 6 - 7 hours of deposition (Fig. 11) and failed to germinate even after 17 hours of placement (Fig. 10,11 & 13). Non germinated ascospores, however, were found to produce lesion around stomata on leaf surface (Fig. 12). Long, tappered penetrating hypha tightly appressed against leaf surface is visible in fig. 13.

Appressoria formation were observed at the distal contact point of tappered penetrating hyphae and leaf surface, after 19-21 hours of ascospore germination (Fig. 14 & 15). Small hyphae emerging through the host surface via deformed stomata (Fig. 16 & 17) were evident after six days of inocultation.

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DISCUSSION

The incidence of *S. sclerotiorum* in rapeseed and mustard fields in France was noticed during mid flowering season. Therefore, an attempt was made to study some aspects of host-parasite relationships and the role of young flowering parts in initiating infection. Emphasis was laid to examine the sequence of pathogenesis on petal and leaf surfaces using SEM.

Germination of ascospores on petals and their failure to do so on leaves (Fig. 10) revealed that petals serve an important intermediatory role to initiate infection. After their germination, infection cushions were produced on petals by dichotomous branching of hyphae (Fig. 14 - 15). Long tappered penetrating hyphae were observed originating from these infection cushions (Fig. 13). These specialized hyphae were attracted by leaf surface where they formed appressoria in response to thigmotropism (Fig. 14 & 15). Lumsden and Wergin (1980) were of the openion that these specialized hyphae act as a stabilizers during penetration, however, in the present study these were found to satisfy pre-penetration phase of pathogenesis. It is postulated that promotion of infection process at petal level is probably due to easy nutritional access of pathogen leading to its aggressiveness and formation of appressoria. However, appressoria formation was not observed on petals. According to Van Burgh soft tissues are penetrated most quickly without the aid of observable appressoria. Efforts to locate hyphae penetrating through stomata were invain although hyphae emerging from the host through stomata were evident (Fig 16 & 17) which was in agreement with the findings of Lumsden and Wergin (1980). Failure of ascospores germination on leaf surface may be due to metabolites released by it which needs investigation. The lesion production on the leaf tissue at the site of non-germinated ascospores might be due release of certain metabolites, cell wall degrading enzyme during the process of constriction of these spores. Lysis of petal cells by germinating spores in advance of infection indicate that pathogen is capable of synthesizing enzymatic metabolites essential for softening the tissues *S. sclerotiorum* in diseased tissue produces abundant endopolygalacturonase (Hancock, 1966; Lumsden and Dow, 1970).

From the foregoing discussion it was concluded that flower petals in the mid flowering period served an intermediatory energy source to initiate further aerial infection of rapeseed and mustard in France.

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