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Infection Processes of *Colletotrichum lagenarium* on Watermelon

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ABSTRACT

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The rate and process of penetration and infection on watermelon leaves by *Colletotrichum lagenarium* were similar to those on fruit rinds based on histological observations. The fungus formed appressoria on epidermal cells after conidial germination and most of penetrations were complete within 24 hr after inoculation. Destruction of epidermal cells beneath appressoria was usually accompanied with penetration. The hyphae from infection peg grew rapidly and ramified into mesophyll cells. More hyphae were found in the phytosynthetic cells than in non-phytosynthetic cells. Invaded cells as well as surrounding cells were soon disintegrated or collapsed. Water-soaked lesions showed up 4 days after inoculation and then acervuli of the fungus were found in subepidermal cells 5 days after inoculation. There were no significant difference in percent of germination, percent of appressorial formation and penetration of the fungus on intact fruit rinds or on excised fruit rinds, on harvested or on unharvesed fruit rinds, and on different growth stages of fruit rinds.

Key words: *Colletotrichum lagenarium*, histology, infection process, watermelon anthracnose.

INTRODUCTION

Anthracnose disease of watermelon caused by *Colletotrichum lagenarium* (Pass.) Ellis & Halster is common in humid cultivation areas in the world. Taiwan locates at subtropic with long monsoon during spring and high relative humidities and high temperature during summer which make anthracnose a serious problem on some susceptible watermelon cultivars (5). Busch and Walker (2) found that penetration of the fungus was equally well in both resistant and susceptible leaves, but that the growth of the hyphae was much slower in the resistant tissue. Anderson and Walker (1), in their histological studies, found that reactions of susceptible and resistant varieties to anthracnose pathogen were different at stage after infection. The objectives of this investigation were to study anthracnose symptom development on susceptible cultivar at different growth stages, to compare behaviors of the fungus during the period of its germination and penetration on excised and intact fruit rinds and to examine whether it was possible to use this excised rind inoculation technique for ecological studies.

MATERIALS AND METHODS

Preparation of inoculum

The culture of *C. lagenarium* used throughout this work was isolated from infected watermelon fruits in Taichung area. The cultures were maintained on PDA slants and single-spore isolation was performed regularly to maintain them in a heavily sporulation condition. *C. lagenarium* grown on PDA slants for one week under 24-25 C with 12-hr illumination was used as inoculum. Spore suspension was prepared by adding sterile distilled water with 0.1% Tween 20 into slants and the number of conidia was adjusted to the concentration approximately 5.5 x 10^5/ml by using a hemocytometer for counting.

Preparation of inoculated tissues for histological observations

Watermelon cultivar Shaw-yi was the most susceptible cultivar to anthracnose in Taiwan and was used in this study. Fully mature watermelon fruit rind was cut into 3.5 cm^2 with about 5 mm thick. The center of cut rind was

marked a circle of 2 cm diameter using nail polish and a drop of conidial suspension was pipetted on the circle. The inoculated cut rind was placed on moisted tissue paper in a petri dish and incubated at 24 C. The third or 4th true leaf of watermelon seedlings grown in a pot (15-cm diam.) was detached and inoculated as excised rind. These inoculated excised rinds and detached leaves were sampled at fixed intervals, *i.e.* 24 hr, for following cytological observations.

Scanning electron Microscopical observations

Inoculated detached leaf samples were cut into 0.5 cm^2 and prefixed in 3% glutaldehyde for 2 hr. After rinsing for three times with 0.1 M phosphate buffer (pH 6.8), the samples were post-fixed with 2% Osmium tetraoxide at 4 C for 2 hr. A series of different concentrations of ethyl alcohol started from 50% to 100% was used to dehydrate the tissues. The rest procedures were as described by Glauert (3). Finally, the samples were observed under SEM (Bausch & Lomb Nanolab 2100).

Light microscopical observations

Inoculated detached leaves or cut rinds were sampled and cut into 0.5 cm^2 at 24 hr intervals for 7 days. After being fixed in FAA, the samples were embedded in TissueTek'O.C.T. compound, frozen at−20 C and thin-sections of 15–20 μm made by Yamato Kohki Ma 101 microtome. The thin-sections were stained by safranin and observed under a light microscope.

Determination of infection

The method used was as that described for rice blast fungus (4). It is based on that when appressoria are fully with cytoplasma before penetration and can be deeply stained by cotton blue. After penetration, cytoplasm in appressoria is transferred into infection peg in host cells. The empty appressoria showed light blue color after stained with cotton blue.

RESULTS

Infection processes on leaves

As the size of conidia was so small that they tended to settle in the depressions between leaf epidermal cells after inoculation and appressoria were formed above the vertical cross-walls. The conidia which did not settle on the wall between cells usually germinated and grew toward to cross-walls prior appressorial formation (Fig. 1). No penetration through stomata was observed. An infection peg was formed from appressorium about 16 hr after inoculation (Fig. 2). There was a swelling of cell wall below appressorium prior penetration. As a result of destruction of nucleus and chloroplast, the cell was deeply stained by safranin to become deep red color (Fig. 3). A swelling infection hypha was observed in the epidermal cell beneath an appressorium 48 hr after inoculation (Fig. 4). The

Fig. 1. Conidia of *Colletotrichum lagenarium* formed appressorium on the surface of epidermal cells of watermelon leaf (X1320).
Fig. 2. Penetration hypha from an appressorium penetrated between epidermal cells of watermelon leaf (X1320).

hyphae grew from penetration peg (Fig. 5). and passed rapidly into palisade and spongy mesophyll cells (Fig. 6). More number of hyphae ramified in the phytosynthetic cells than in non-phytosynthetic cells. Many cells were disintegrated before the hyphae reached. After 5 days of inoculation, the hyphae grew to lower epidermal cells (Fig. 7), where acervulus with masses of conidia was formed subepidermally (Fig. 8).

Infection processes on fruit rinds

The rate and process of infection on fruit rinds were very similar to those on leaves. However, there was no obvious junctures between fruit rind epidermal cells, inoculated conidia were randomly distributed on the

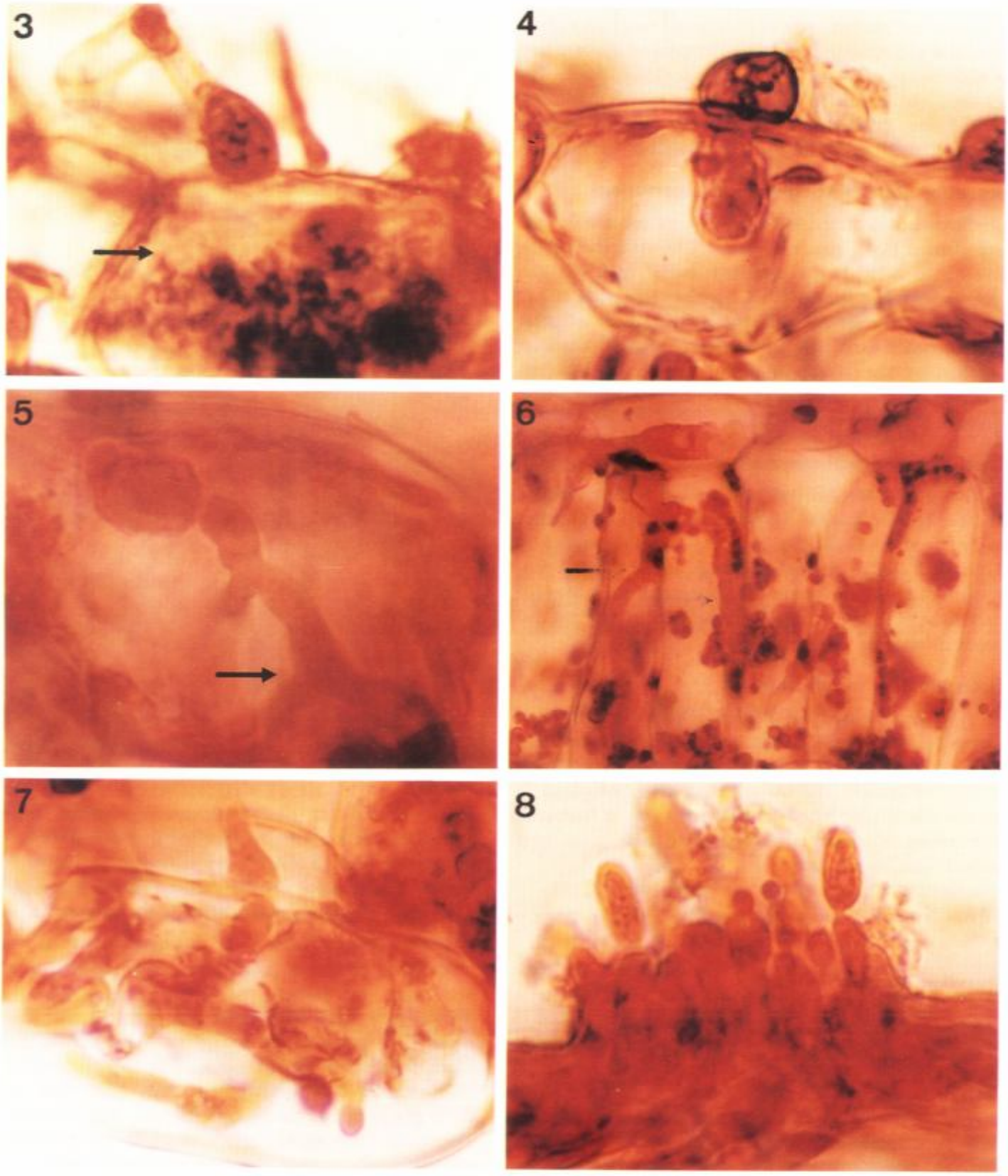

Fig. 3. A collapsed epidermal cell of watermelon leaf stained deep red with safranin (X1665).
Fig. 4. A swelling penetration hypha in an epidermal cell of watermelon leaf (X1665).
Fig. 5. Infection hyphae grew through epidermal cell into mesophyll cell (X1665).
Fig. 6. Infection hyphae ramified in mesophyll cells (X666).
Fig. 7. Infection hyphae grew into lower epidermal cell of watermelon leaf (X1665).
Fig. 8. Acervulus with conidial mass formed subepidermally in watermelon leaf (X1665).

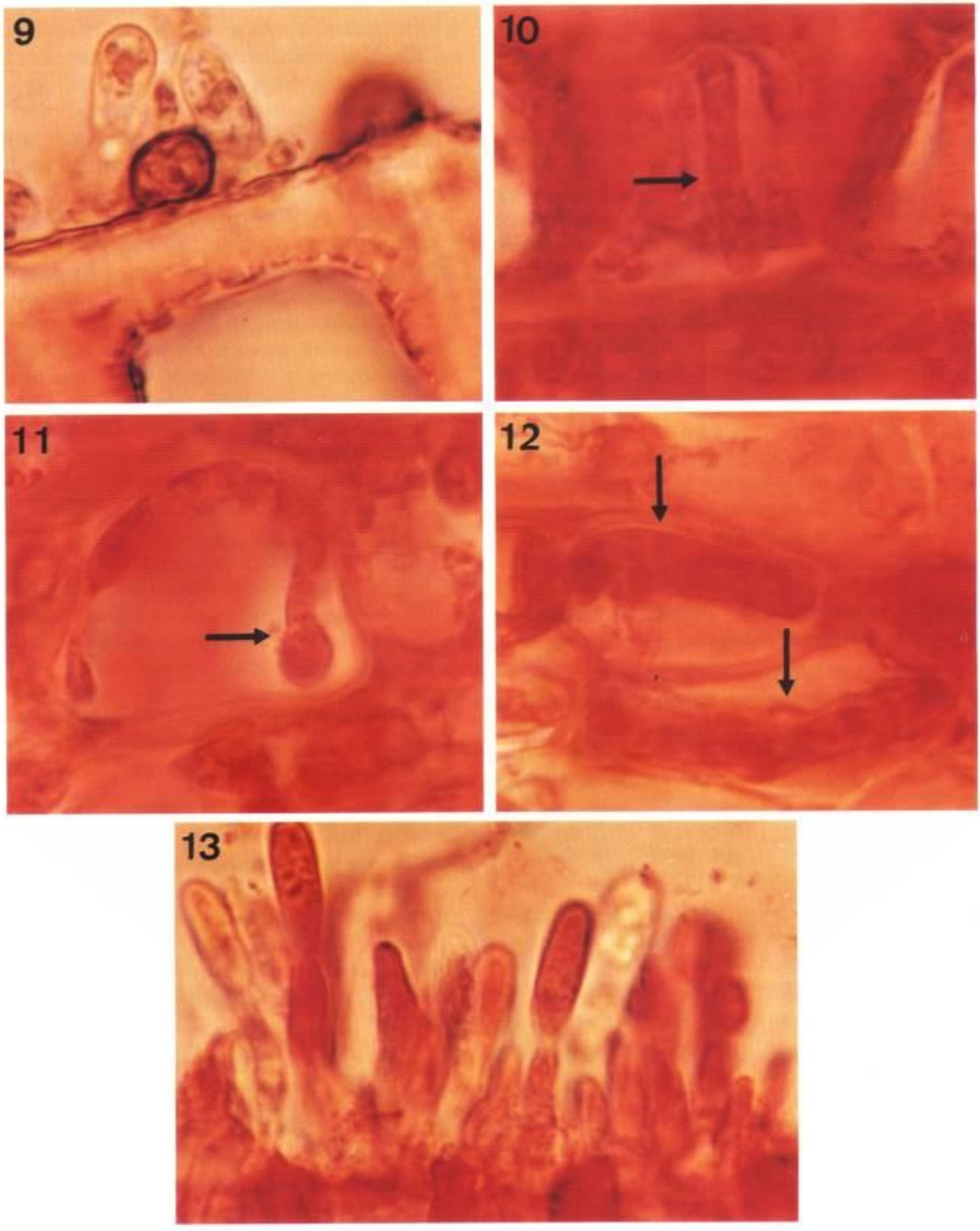

Fig. 9. A conidium with an appressorium on surface of watermelon fruit (X1665).
Fig. 10. A penetration hypha from a appressorium penetrated into epidermal cells of watermelon fruit (X1665).
Fig. 11. Infection hyphae grew through epidermal cells into fruit cell (X1665).
Fig. 12. Infection hyphae ramified in fruit cells (X1665).
Fig. 13. An acervulus with conidial mass formed subepidermally in watermelon fruit (X1665).

surface of the fruits. As on leaf most of germinated conidia formed appressoria on waxy fruit surface 24 hr after inoculation (Fig. 9). The penetration peg grew from appressorium and a swelling hypha was observed in the infected epidermic cell 48 hr after inoculation (Fig. 10). These hyphae grew continuously and penetrated into sclerified parenchyma cells 72 hr after inoculation (Fig. 11). After penetration, the hyphae ramified between the epidermis and the sclerified parenchyma layer, and most of infected cells were disintegrated 96 hr after inoculation (Fig. 12). At this stage water-soaked spots could be seen on surface of fruit rinds. Some of hyphae formed knots in subepidermal cells. These hyphal knots generated into stroma and eventually acervulus was formed and protruding through the epidermis 120 hr after inoculation (Fig. 13).

Comparing behaviors of the fungus on excised and intact rinds of harvested fruits

There were no much difference in rates of conidial germination, appressorial formation and penetration of the fungus on excised and those on intact fruit rinds (Table 1). About 95% of conidia germinated on excised and intact fruit rinds and more than 75% of germinated conidia formed appressoria. Approximately 35% of appressoria had completed their penetration 24 hr after inoculation.

Comparing behaviors of the fuagus on excised and intact rinds of unharvested fruits

As the last experiment, the behaviors of the fungus on excised and intact rinds of unharvested fruits were no significantly different (Table 2). However, rates of conidial germination and appressorial formation were slightly lower on unharvested fruits as compared to those on harvested fruits.

Comparing behaviors of the fungus on different growth stages of watermelon fruits

The rinds of watermelon fruits at different growth stages, *i.e.* 5, 15, 25, and 35 days after setting were excised and used to test the behaviors of the fungus. It was obvious that the rates of conidial germination on these excised rinds were 95.6–97.5% and were not signifcantly different among different fruits with different ages (Table 3). No differences were also observed on appressorial formation and penetration of the fungus on these excised rinds.

TABLE 1. Comparison of spore germination, appressorial formation and penetration of *Colletotrichum lagenarium* on excised and intact rinds of harvested watermelon[1]

Sources of rinds	Germination(%)	Appressorial formation (%)	Penetration(%)
Excised rinds	96.7 a	77.0 a	35.9 a[2]
Intact rinds	95.2 a	75.8 a	34.3 a

1. Spore suspension pipetted onto excised or intact rinds and kept in moist chamber at 24 C and examined 24 hr after inoculation.
2. Means (n=10) in the same column follow by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

TABLE 2. Comparison of spore germination, appressorial formation and penetration of *Colletotrichum lagenarium* on excised and intact rinds of unharvested watermelon[1]

Sources of rinds	Germination(%)	Appressorial formation (%)	Penetration(%)
Excised rinds	91.9 a	53.1 a	34.5 a[2]
Intact rinds	90.4 a	51.7 a	31.8 a

1. Spore suspension pipetted onto excised or intact rinds and kept in moist chamber at 20–25 C and examined 24 hr after inoculation.
2. Means (n=10) in the same column follow by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

TABLE 3. The spore germination, appressorial formation and penetration of *Collectotricum lagenarium* on fruits of different growth stages

Fruit growth stage (days)	Germination(%)	Appressorial formation (%)	Penetration(%)
5	97.5 a	63.9 a	28.1 a[1]
15	96.9 a	59.0 a	23.3 a
25	97.2 a	62.9 a	23.0 a
35	95.6 a	61.1 a	25.1 a

1. Means (n=10) in the same column follow by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

DISCUSSION

As most pathogenic fungi, conidia of *C. lagenarium* were able to finish germination, formation of appressoria and penetration into host tissues within a short period of time which was usually less than 24 hr. Under normal condition, percent of conidial germination of this fungus on host plants was mostly more than 90%. The percent of appressorial formation from germinated conidia was about 50–70%. Approximately 30% of appressoria initiated penetrations into host tissues. Direct penetration was observed on all cases and no penetration through stomata had been found on leaves. Physical contact stimulus may be required for direct penetration, and stomata would not be

provided this requirement. Since there are no stomata on watermelon fruits, the penetration of this fungus was all by direct penetration from appressoria. Cotton blue staining was a good simple method to differentiate those appressoria already initiated penetration from those not yet initiated penetration (4). Appressoria containing cytoplasma were stained deep blue to indicate penetration was not yet started and those stained light blue containing no cytoplasma indicating cytoplasma had passed through penetration peg and penetration had finished.

The rate and process of infection on watermelon leaves by *C. lagenarium* were very similar to those on fruit rinds as evaluated histologically by time courses of each step from conidial germination, appressorial formation, penetration, infection and sporulation on the same host plant. From our studies as well as early publications (1,2), it was clear that the behaviors of the fungus on excised fruit rinds from conidial germination to penetration were the same on different susceptibilities of the host plants. Resistant was expressed only after penetration by restricting the growth of invading hyphae. In addition, the source of fruit rinds was not critical, the fruit rinds from different varieties or from different growth stages as long as there was not fungicide residue could be used as materials for pre-infection studies. This excised rind inoculation technique would be ideal tool applicable to study some ecological factors affecting the conidial germination, appressorial formation and penetration. Furthermore, it also can be used to make components analysis of the infection cycle of the fungus (6) and to make preliminary evaluation of the effective of the fungicides.

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摘　　要

余立文、謝式坤鈺．1992．西瓜炭疽病菌侵染寄主組織之過程．植病會刊1:124-129．(台中市國立中興大學植物病理學研究所)

炭疽病菌侵染西瓜葉片之方式和速率與侵染果實者相似，當分生孢子發芽於寄主表皮細胞後即形成附著器與直接侵入；接種後24小時，附著器下方之表皮細胞遭破壞而崩潰；接種後48小時，於表皮細胞內可見膨大之侵入菌絲，菌絲繼續穿越表皮細胞，侵入葉肉及果肉細胞行細胞穿生，菌絲偏向於侵入行光合作用較非行光合作用之細胞爲多，被侵入細胞迅速崩潰，周圍細胞之葉綠體也會崩潰；接種後96小時，侵入菌絲蔓延於葉肉及果肉細胞間，且寄主表面出現肉眼可見之水浸狀病斑；接種後120小時，表皮細胞下形成胞子盤（Acervuli）並產生大量分生孢子。病菌在寄主上之發芽率、附著器形成率及侵入率，無論在切開果皮、未切開果皮、不同生長期果皮或收穫及未收穫果皮上皆無明顯之差別。

關鍵字：組織學、感染過程、西瓜炭疽病、*Colletotrichum lagenarium*。