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Light and scanning electron microscopy studies on the infection of oriental lily leaves by *Botrytis elliptica*

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Abstract

Light, scanning electron and fluorescent microscopy were used to observe the infection process of *Botrytis elliptica* on leaves of oriental lily (cv. Star Gazer). At 20 °C and 100% relative humidity, conidia germinated on both adaxial and abaxial foliar surfaces, but germ tubes failed to invade epidermal cells on the adaxial surface. On abaxial surfaces, short (<20 μm) swollen germ tube appressoria penetrated through stomatal openings (19%), through the epidermis near guard cells (52%), or directly through epidermal cells (29%). Esterase activity was detected on germ tubes and conidia after 6 h of incubation, and deformation of the cuticle on abaxial surfaces of lily was observed surrounding infection sites. By 3 h after inoculation, almost 70% of the conidia had germinated, but no penetration was observed. At 6 h after inoculation, almost one-third of germinated conidia had penetrated epidermal cells, and water-soaked lesions were associated with 20% of the penetrations. By 9 h after inoculation, approximately 60% of the germinated conidia had penetrated plant tissues, and water-soaked lesions were associated with 60% of the infections. Fluorescent microscopy with a specific fungal stain allowed assessment of successful infection and visualization of sub-epidermal hyphae. We conclude that penetration of abaxial foliar surfaces of oriental lilies by *B. elliptica* occurs via short swollen germ tube appressoria mostly near stomata.

Introduction

Leaf blight caused by *Botrytis elliptica* (Berk.) Cooke is one of the most important foliar diseases of lilies (*Lilium* spp.) (Doss et al., 1988a; Hsieh and Tu, 1993; McRae, 1987; Wright, 1928), and it has become a limiting factor for the production of ornamental lilies in Taiwan in recent years (Hsieh and Huang, 1998). *B. elliptica* attacks all aboveground parts of lilies including stem, leaf and flower tissues (Hsieh and Tu, 1993). Leaf spot symptoms are often observed on oriental lilies (mostly hybrids of *Lilium auratum* and *Lilium speciosum*), and leaf blight symptoms often appear on Asiatic (*Lilium asiatic* hybrids) and Easter lilies (*L. longiflorum*).

Botrytis species are known to penetrate host tissues by hyphae or germ tubes infecting through epidermal cells, stomata or wounds (Verhoeff, 1980). Chemical and mechanical processes are usually involved in direct penetration by *Botrytis* species (Cole et al., 1998a,b; Fourie and Holz, 1995; Rijkenberg et al., 1980; Verhoeff, 1980; Williamson et al., 1995). Germ tubes, appressoria and infection cushions of *B. cinerea* were observed on leaves of French bean (Van den Heuvel and Waterreus, 1983). Doss et al. (1988b) speculated that hydrolytic enzymes play an important role in the infection process of *B. elliptica* on Easter lilies. Simple lobed or digitate appressoria of *B. elliptica* formed at the end of long germ tubes 16 h after inoculation (Doss et al., 1988b). With higher levels of inoculum,

fungal hyphae were visible inside the leaf after 16 h, and the epidermis was damaged 24 h after inoculation (Doss et al., 1988b). However, Hsieh and Huang (1998) found that *B. elliptica* penetrated foliar tissues of oriental lilies and water-soaked symptoms appeared on detached leaves within 12 h after inoculation with incubation at 20 °C and high relative humidity.

Previous research has demonstrated that other *Botrytis* species can infect hosts by short germ tubes (Blackman and Welsford, 1916; Cole et al., 1996; Elad, 1988; Fourie and Holz, 1995; McKeen, 1974; Rijkenberg et al., 1980; Salinas and Verhoeff, 1995; Verhoeff, 1980; Williamson et al., 1995). Therefore, the objective of this paper was to further elucidate the interaction of *B. elliptica* and oriental lily leaves by means of light, fluorescent, and scanning electron microscopy.

Materials and methods

Inoculum preparation

Isolate B066 of *B. elliptica* from a diseased oriental lily (cv. Star Gazer) at Hsin-She in central Taiwan was used in this study. A single-spore isolate was stored on potato dextrose agar (Difco PDA) at 4 °C. To maintain pathogenicity of the isolate, conidia were periodically inoculated onto surface-disinfested lily leaves, and then re-isolated by harvesting freshly-formed conidia from diseased tissues incubated under near-UV light (Black Light Blue, F10T8BLB, Sankyo Denki, Japan) (Doss et al., 1984).

To prepare fungal inoculum (Hsieh and Huang, 1999), *B. elliptica* isolate B066 was grown on autoclaved lily leaves or petals for 7–10 days at 20 °C under near-UV light. Conidia were harvested from fungal colonies by lightly scrapping with sterile forceps, which were then dipped into 5 ml sterile water in a centrifuge tube. The spore suspension was centrifuged at 3000 × *g* for 10 min, the supernatant discarded, and 5 ml sterile water added to the tube. The collection of conidia from these leaves or petals was repeated three more times for each round of inoculation. Conidia were then counted using a hemacytometer, and conidial concentration was adjusted to desired levels. Conidial suspensions were stored for less than 2 h at 4 °C before inoculations.

Conidial germination on glass slides

Aliquots (25 µl) of a suspension containing 10^5 conidia ml^{-1} were pipetted onto glass slides. They were incubated at 20 °C inside Petri dishes containing distilled water to maintain 100% relative humidity (RH), and the plates were sealed with parafilm to prevent desiccation. The slides were examined with a compound microscope every hour in the first 8 h, and afterwards at 2-h intervals up to 30 h for germination and appressoria formation. At least 100 conidia were examined at each interval. This experiment was carried out twice.

Esterase activity

Esterase activity was assessed by a method in which indoxyl acetate served as the substrate of nonspecific carboxylic acid esterases (Barnett and Seligman, 1951). Substrate hydrolysis results in the accumulation of pigmented crystals of indigo blue at the site of hydrolysis, which are visible as a dark color reaction under light microscopy. A 25 µl aliquot of a 10^5 ml^{-1} conidial suspension of *B. elliptica* B066 was placed onto a cover glass and incubated inside a Petri dish at 20 °C. After 6 h incubation, 25 µl of indoxyl acetate solution (25 ml 2 M sodium chloride, 10 ml 0.1 M phosphate buffer at pH 7.8, 0.25 g calcium chloride in 14 ml distilled water, 20 mg indoxyl acetate dissolved in 1 ml acetone) were added. The droplet was then examined directly under light microscopy (BX50, Olympus Optical Co. Ltd., Tokyo, Japan) at 12 V/100 W. At least 100 spores and germ tubes were observed. Esterase activity was rated using an Esterase Index (Hsieh and Huang, 1999) on a scale of 0–4, where 0 = no blue crystal materials observed in germ tubes, 1 = 1–10% areas of germ tubes with blue crystal materials, 2 = 11–25% areas of germ tubes with blue crystal materials, 3 = 26–50% areas of germ tubes with blue crystal materials, and 4 = over 50% areas of germ tubes with blue crystal materials. This experiment was carried out twice.

Inoculation of lily leaves by B. elliptica

Oriental lily (cv. Star Gazer) plants grown from bulbs in a greenhouse for 1.5–2 months were used to prepare leaf disks. The fifth to tenth leaves below the apex were surface-disinfested by dipping entire leaves in 0.1% sodium hypochlorite for 3 min and rinsing in three changes of sterile water. Leaf disks (15-mm-diameter) were cut from surface-disinfested leaves with a cork borer, and eight disks per 90-mm-diameter petri plate were placed abaxial side up on a piece of 60-mm-diameter autoclaved filter paper. A 2 ml aliquot

of autoclaved water was added to each piece of filter paper to maintain moisture. A conidial suspension adjusted to 10^5 spores ml^{-1} was sprayed (Sigma spray unit, Sigma Chemical Co., St. Louis, MO, USA) over the disks to form inoculum droplets of up to 0.3 mm in diameter. Approximately 400 droplets formed on each disk (Hsieh and Huang, 1999). The leaf disks were then incubated at 20 °C under 100% RH.

Scanning electron microscopy

To observe the infection process of *B. elliptica* on lily leaves, adaxial and abaxial surfaces of leaf disks were inoculated with 25 µl of a 10^5 ml^{-1} conidial suspension and incubated in 100% RH at 20 °C. For scanning electron microscopy (Hayat, 1986), samples of leaf disks 16 h after inoculation were pre-fixed in 2% glutaraldehyde-p-formaldehyde in 0.05 M sodium cacodylate buffer at pH 7.2 for 2 h at room temperature. The samples were then washed three times with a 0.05 M sodium cacodylate buffer solution for 10 min. Samples were post-fixed in 1% osmium tetroxide in the same buffer for 1 h. Samples were washed again in the buffer solution and dehydrated using a graded series of ethanol. After dehydration, samples were critical-point dried (HCP-2, Hitachi Koki Co. Ltd., Tokyo, Japan) with liquid carbon dioxide as a transitional fluid. The dried materials were adhered onto aluminum specimen mounts with colloidal silver paste, and then sputter-coated (ion coater, IB-2, Giko Engineering Co. Ltd., Japan) with gold (approximately 15 nm thickness). The specimens were examined and photographed on a Hitachi S-570 scanning electron microscope (Hitachi, Co. Ltd., Tokyo, Japan) at 15 kV. At least 50 leaf disks were observed in this experiment.

Fluorescent staining and light microscopy

To examine the infection process, a fluorescent stain procedure was used (Hood and Shew, 1996). Leaf disks were inoculated as above, and at 3-h intervals up to 18 h after inoculation, at least 40 disks per treatment were examined. The disks were placed into a 1 M KOH solution for 30 min, and then autoclaved in this solution for 15 min at 121 °C to clear the leaf tissues. After three rinses in deionized water, cleared samples were mounted in stain solution (0.05% aniline blue in 0.067 M K_2HPO_4 at pH 9.0). Percentage of conidial germination and infection were assessed with light and fluorescent microscopy (BX50 + BX-FLA, Olympus Optical Co. Ltd., Tokyo, Japan) at 19 V/100 W.

In addition, for 30 disks, the infection structures above the leaf surface were removed by sonication (Transsonic Digitals, Elma-Hans Schmidbauer GmbH & Co. KG, D-78224 Singen, Germany) 16 h after inoculation. Before staining, inoculated leaf disks were put in water and placed into the sonicator at room temperature for 5–10 min at 100% ultrasound power to remove the spores, and then stained and viewed with both light and fluorescent microscopy.

Results

Conidial germination on glass slides

Nearly 100% germination of *B. elliptica* conidia on glass slides occurred within 8 h in sterile water at 20 °C (Figure 1). Conidia usually produced one or two germ tubes, and two types of germ tubes, a swollen type and a slender type, were observed (Figure 2A) sometimes on the same conidium (Figure 2B). The swollen germ tubes were on average twice as thick (Table 1) as the slender germ tubes which were typical of normal hyphal growth. On glass slides, the growth from most swollen germ tubes became thinner and resembled the slender germ tubes (Figure 2B–D). Conidial germination with either type of germ tube was approximately 27% at 2 h, 58% at 3 h, 79% at 6 h, and over 90% at 8 h. Simple lobed or digitate appressoria formed initially at the tips of elongated slender germ tubes by 8 h (Figure 2C). By 20 h, 20% of the long germ tubes

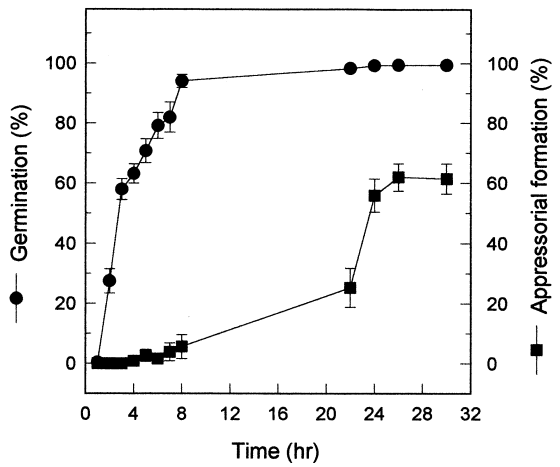

Figure 1. Time course for conidial germination and digitate appressoria formation of *B. elliptica* in droplets (25 µl) of a conidial suspension (10^5 spores ml^{-1}) on glass slides at 20 °C under 100% RH.

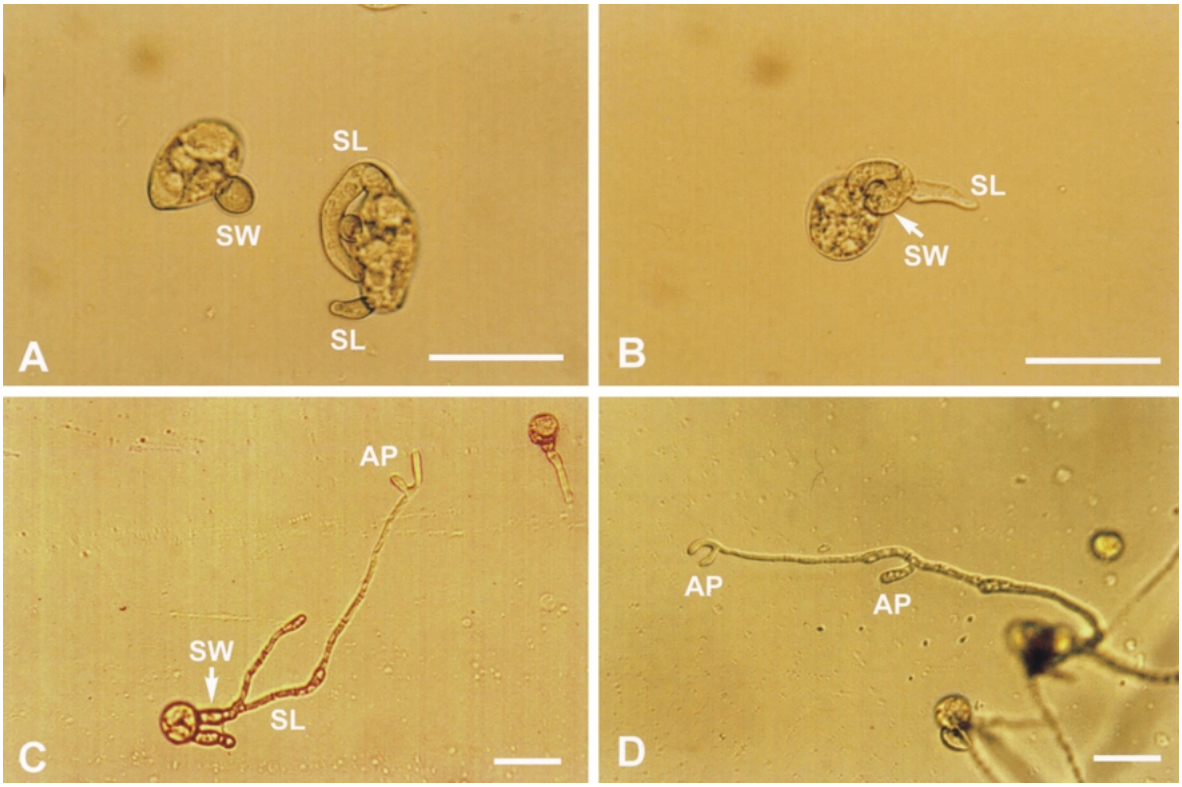

Figure 2. Conidial germination and appressoria formation of *B. elliptica* in water droplets on glass slides. A: Conidia with swollen (SW) and slender (SL) types of germ tubes were often observed. B: Growth from swollen germ tubes (SW) became thinner and resembled the slender germ tubes after growth and elongation. C: Single-lobed or digitate appressoria (AP) formed at the tips of elongated slender germ tubes initially after 8 h incubation. D: Single-lobed or digitate appressoria (AP) could continue to grow and then produce secondary germ tubes which could also form appressoria. Magnification bar = 50 μm.

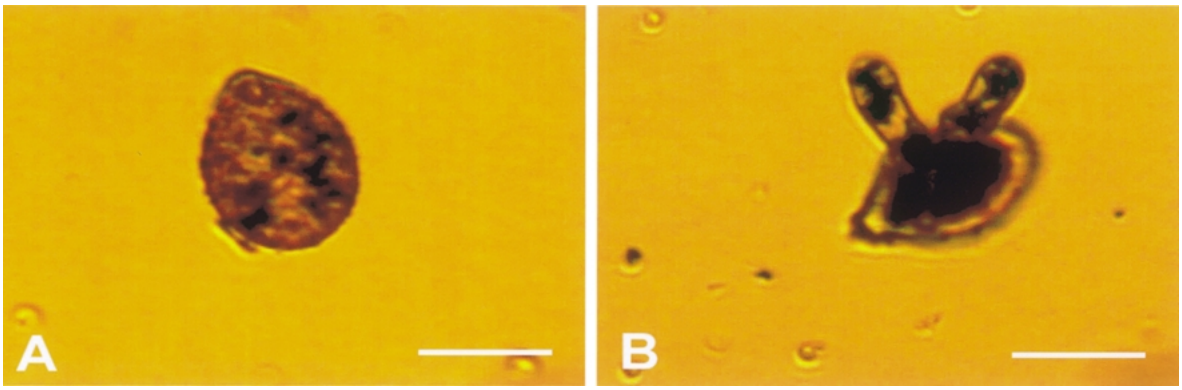

Figure 3. Esterase activity of conidia and germ tubes of *B. elliptica* indicated by the dark color reaction. A: Esterase activity in conidia was minor before germination. B: After 6 h incubation on a cover slip, high esterase activity in conidia and on germ tubes was observed. Magnification bar = 25 μm.

Table 1. Germination and penetration by conidia of *B. elliptica* on the abaxial foliar surface of oriental lily (cv. Star Gazer) between 9 and 18 h after inoculation with incubation at 20 °C and high relative humidity

Type of germ tube	Width[1] (μm)	Frequency[2] (%)	Successful penetrations[3] (%)	Site of penetration[4]		
				Stomata	Near guard cells	Epidermal cells
Swollen	10.2	90	62	19	52	29
Slender	5.2	7	0	0	0	0

[1]Average width of germ tubes based on 100 observations.
[2]Ten leaf disks were examined and at least 50 conidia per leaf disk were observed.
[3]Fluorescent microscopy was used to assess successful penetration into leaf tissue.
[4]Percentage of successful penetrations at different sites: Stomata = stomatal opening; Near guard Cells = accessory cells around the guard cells with infrequent penetrations directly into guard cells; and Epidermal cells = regular cells of the epidermis.

had formed appressoria, and by 24 h, 56% had appressoria (Figure 1). Appressoria continued to grow and produce secondary germ tubes. These secondary germ tubes also formed appressoria (Figure 2D).

Esterase activity

Esterase activity in conidia incubated in a water droplet on a glass cover slip at 20 °C was negligible before germination (Figure 3A). After 6 h, esterase activity was high in conidia and germ tubes (Esterase index [EI] = 2.1 ± 0.3), but decreased by 16 h of incubation (EI = 0.5 ± 0.2) and remained low to 24 h (EI = 0.3 ± 0.2) (Figure 3B).

Scanning electron microscopy

Conidia germinated on either adaxial or abaxial surfaces, but germ tubes failed to penetrate the adaxial surfaces which lacked stomata. On the abaxial surfaces, conidia usually produced one or two germ tubes (Figure 4A,C–F), but conidia with three germ tubes were also observed (Figure 4B). Two types of germ tubes, a swollen type and a slender type, were often observed on a single conidium at the same time (Figure 4B). On abaxial surfaces, slender germ tubes penetrated plant tissues by growth toward and into natural openings such as stomata (Figure 4A), but this infection process was infrequently observed (<1%). Direct penetration by short (<20 μm) swollen germ tube appressoria into epidermis and cells near stomata was most commonly observed for infection of leaf tissue of oriental lily by *B. elliptica* (Figure 4C,D). A mucilaginous material was visible around the swollen germ tube appressoria (Figure 4B,F). Under scanning electron microscopy, an indentation on the leaf surface around the point of penetration was visible (Figure 4E,F), and this was interpreted as possible cuticular degradation.

Fluorescent staining and light microscopy

Examination of the infection process of oriental lilies by *B. elliptica* under light microscopy revealed swollen germ tube appressoria on abaxial surfaces. However, the infection hyphae within epidermal cells were difficult to see (Figure 5A,C). With a fluorescent stain, it was much easier to evaluate whether penetration had occurred (Figure 5B,D) and to visualize infection hyphae inside the plant (Figure 5D,F). When infection structures above the leaf surface were removed by sonication, penetration points were visible (arrow in Figure 5E).

At 3-h intervals between 0 and 15 h after inoculation, the time course of infection was evaluated (Figure 6A–F). Within 3 h after inoculation, conidia germinated, but no penetration was observed (Figure 6A,B). With fluorescence staining, hyphae were observed inside the leaf tissues as early as 6 h post-inoculation (Figure 6C). Subsequently, penetration hyphae expanded under the epidermal cells (Figure 6D–F). Water-soaked lesions appeared as early as 9 h after inoculation (Figure 6G). Fluorescence was also observed in tissues near the site of infection (Figure 6H), which indicated successful infection by *B. elliptica*.

Germination of *B. elliptica* conidia and water-soaked symptoms of leaf blight disease were assessed after conidial inoculation. Approximately 70% of the conidia germinated on abaxial surface of lily leaves 3 h after inoculation, however no conidial penetration was

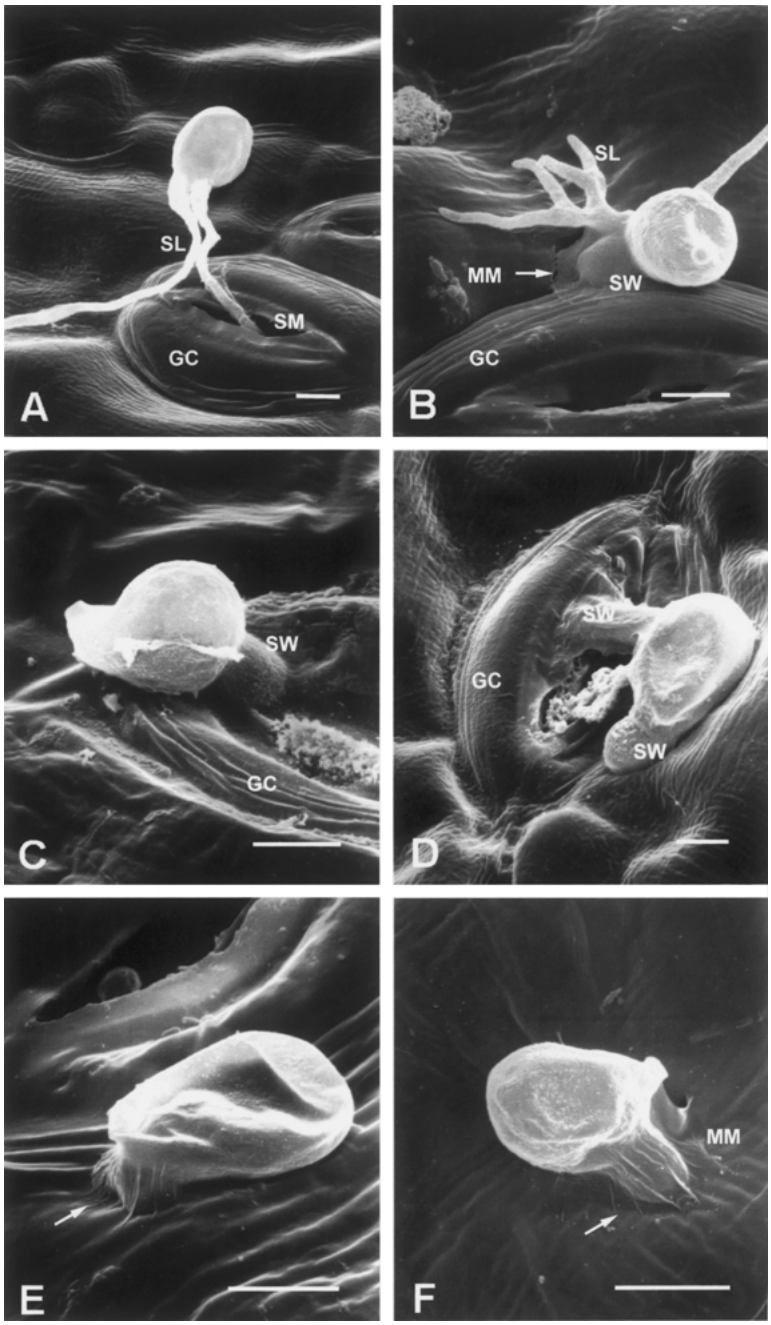

Figure 4. Scanning electron micrographs of the abaxial foliar surface of oriental lily (cv. Star Gazer) inoculated with *B. elliptica*. A: Slender germ tubes (SL) penetrating host through an open stomate (SM). This was very infrequently observed. B: Conidium producing two types of germ tubes: slender germ tube (SL) and swollen germ tube (SW) which acted as an appressorium with mucilagenous material (MM) around it. C: Swollen germ tube appressorium directly penetrating through guard cells (GC). D: Two swollen germ tube appressoria (SW) arising from a conidium and penetrating host via guard cell (GC) and epidermal cell. E: Swollen germ tube appressorium of *B. elliptica* directly penetrating epidermal cell (arrow). F: Swollen appressoria directly penetrating epidermal cell. An indentation on the cuticle surface (arrow) around the penetration site was visible, as well as mucilagenous material (MM). Magnification bar = 10 μm.

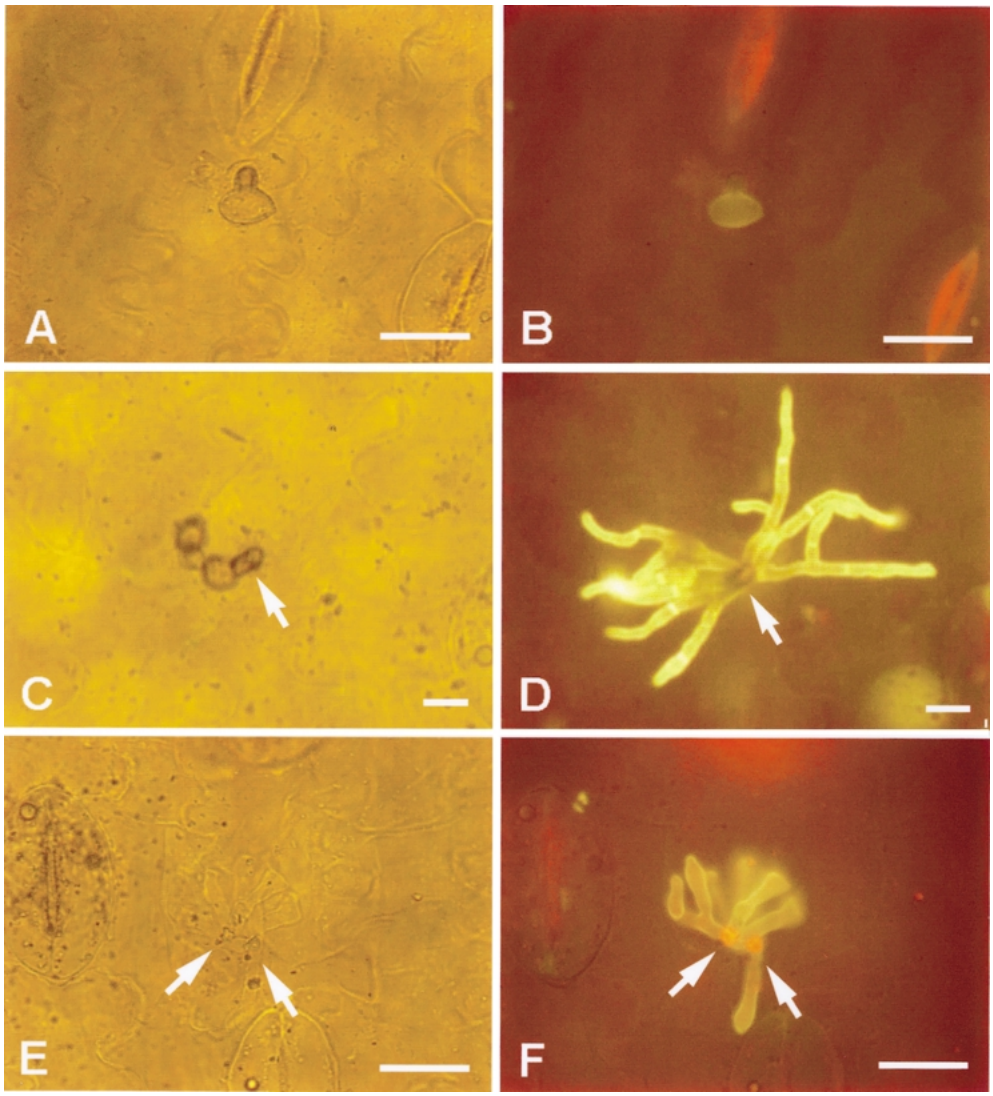

Figure 5. Light (A, C, E) and fluorescence micrographs (B, D, F) of abaxial surfaces of lily leaves. (A and C) Swollen germ tube appressoria. With light microscopy it was difficult to assess whether penetration had occurred, but with a fluorescent stain (B and D), successful penetration (D arrow) was easy to assess. Infection hyphae that penetrated into cuticle and epidermal cells appeared clearly under fluorescent microscopy. When infection structures above the leaf surface were removed, penetration points (arrows) were visible under light (E) or fluorescent (F) microscopy. Magnification bar = 50 μm.

observed within 3 h of inoculation (Figures 5A,B and 6B). At 6 h after inoculation, 89% of conidia had germinated, and of these 32% penetrated into plant tissues, and 14% caused water-soaked lesion symptoms. At 9 h after inoculation, 95% of conidia had germinated, and 60% of germinated conidia penetrated plant tissues. Of the successful penetrations, over 65% caused water-soaked symptoms after 9 h of inoculation (Figure 7).

The rate of penetration through stomata, around guard cells, and into regular epidermal cells was evaluated with microscopy. Only swollen germ tube appressoria were successful in penetration. Among these, 19% penetrated through stomatal openings, 52% through the epidermis near guard cells, and 29% directly through epidermal cells (Table 1). Infection of oriental lily leaves by *B. elliptica* was through swollen appressoria penetration of abaxial foliar surfaces. No

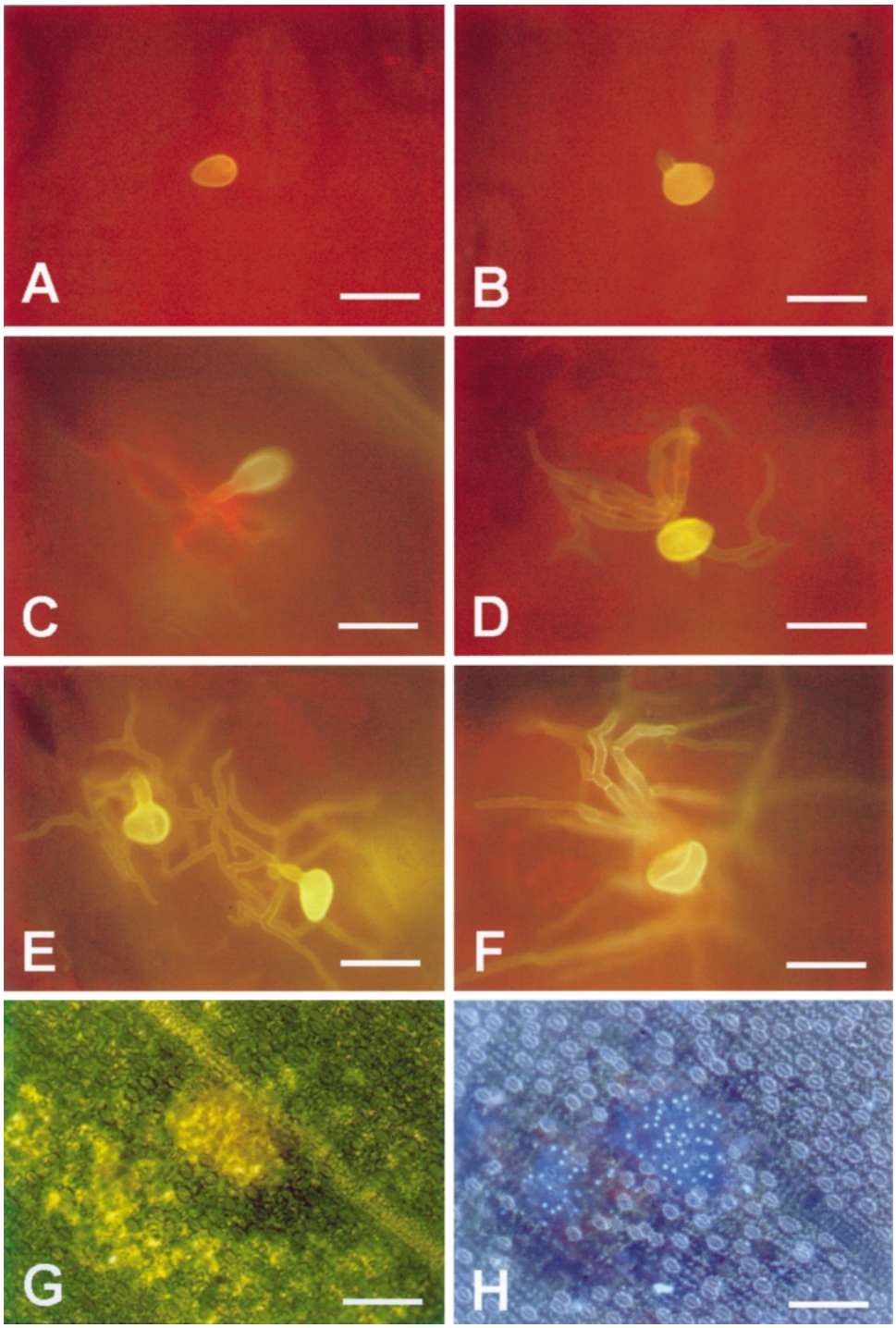

Figure 6. Time course of infection by conidia of *B. elliptica* on abaxial foliar surfaces of oriental lilies at 20°C under fluorescent microscopy. A: Ungerminated conidium right after inoculation. B: Swollen germ tube visible but no penetration into leaf tissues 3 h after inoculation. C: Penetration by swollen germ tube appressorium was observed and penetration hyphae were visible in epidermal cells 6 h after inoculation. D: Branching penetration hyphae were visible and expanding in leaf tissues 9 h after inoculation. E: Penetration hyphae continuously expanded, and fluorescence was apparent around infected cells 12 h after inoculation. F: Penetration hyphae expanding in leaf tissues 15 h after inoculation. Magnification bar = 50 μm. (G and H): Symptomatic area observed with light and fluorescence microscopy, respectively (Bar = 200 μm).

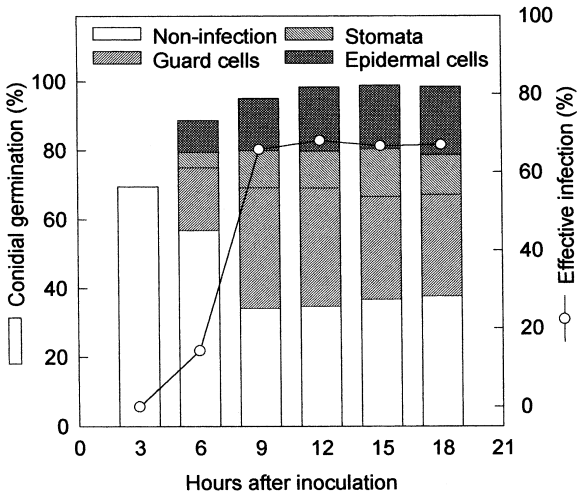

Figure 7. Time course of conidial germination on abaxial foliar surfaces of oriental lily cv. Star Gazer, and effective infection (successful penetration by germinated conidia) into guard cells, stomata or epidermal cells respectively, as assessed by lesion formation with incubation at 20 °C and 100% RH.

penetration by single lobed or digitate appressoria was observed during 24 h of incubation on leaf surfaces.

Discussion

There are few previous studies on the infection process of *B. elliptica* on lily. Ward (1888) used light microscopy to describe infection of Madonna lily (*L. candidum*) by *B. elliptica*, while Doss et al. (1988b) used both light and scanning electron microscopy to study *B. elliptica* infection of Easter lily. We used light microscopy to examine conidial germination and appressoria formation on glass slides, and a combination of light, scanning electron and fluorescent microscopy to examine infection of oriental lily by *B. elliptica*. Some of our results differed from those of Ward (1888) and Doss et al. (1988b), with respect to timing of penetration and symptom expression, and in particular, the type of appressoria formation.

Emmet and Parbery (1975) described three types of simple appressoria: swollen germ tubes adhering to host surface and penetrating directly; swellings at tips of germ tubes which are usually delimited by septa; and dark appressoria characteristic of *Colletotrichum* spp., and *Magnaporthe grisea* (Howard et al., 1991). The first two types of appressoria formation have been described previously for *Botrytis* species. 'Often penetration occurs directly from the distal end of the germ tube, but sometimes a typical digitate appressorium forms first' (Jarvis, 1977 p. 64). McKeen (1974) found both types of appressoria formation for *B. cinerea* on *Vicia faba* leaves, although most produced the short germ tube which penetrated directly into the epidermis. Other researchers have also found that *Botrytis cinerea* infect hosts by short germ tubes less than 20 μm in length (Blackman and Welsford, 1916; Cole et al., 1996; Elad, 1988; Fourie and Holz, 1995; McKeen, 1974; Rijkenberg et al., 1980; Salinas and Verhoeff, 1995; Verhoeff, 1980; Williamson et al., 1995).

In some fungal species, appressoria formation is morphologically constant, with specific types arising from germ tubes or hyphae, or from conidia or ascospores (Emmet and Parbery, 1975). Variation in appressorial morphology can be caused by physical factors such as temperature and water availability (Emmet and Parbery, 1975), but host species may also affect appressoria formation. Doss et al. (1988b) reported long germ tubes with terminal single-lobed or digitate appressoria for *B. elliptica* on Easter lily, but did not mention short swollen germ tube appressoria which we described here for *B. elliptica* on oriental lily.

One difference between these lily species is stomatal density. Counts of stomata based on 60 mm^2 abaxial surface areas gave an average of 48.0 stomata/mm^2 for oriental lily cv. Star Gazer, and an average of 33.2 stomata/mm^2 for Easter lily cv. Snow Queen. On Easter lily, with a greater average distance between stomata, conidia produced appressoria at the tip of long germ tubes (Doss et al., 1988b), while on oriental lily, short swollen germ tube appressoria were observed. In both of these studies, only a single isolate was used, and a greater number of isolates on a range of host species and cultivars would be required to determine the frequency of appressorial types produced by *B. elliptica*. Although penetration mediated by long germ tubes with terminal appressoria was not observed, our isolate was capable of forming such structures on glass slides.

On glass slides, formation of appressoria at tips of slender hyphae was optimal at approximately 60% 24 h after inoculation, and this followed a similar time course as that of Doss et al. (1988b), where they found 70% of conidia on plant tissue had terminal appressoria by 24 h. However, on plant tissues, 60% of the germinated conidia had penetrated plant tissues via swollen germ tube appressoria at 9 h after inoculation. As a

result of these different types of appressoria formation, the timing of symptom expression differed between our study and that of Doss et al. (1988b). On inoculated oriental lily tissues, water-soaked symptoms were observed within 9 h, which is much earlier than Doss et al. (1988b), who found widespread damage to epidermal cell walls within 24 h after inoculation of Easter lily leaves.

Ikata and Hitomi (1933, cited in Jarvis, 1977) found that *B. elliptica* infected lily through the leaf cuticle. Ward (1888) noted the penetration of germ tubes via regular epidermal cells of leaves. Doss et al. (1988b) observed infection by appressoria of *B. elliptica* via stomata, guard cells, or regular epidermal cells after inoculation of the abaxial surface of Easter lilies. Among penetration sites that could be discerned, most appressoria (60%) were associated with the stomatal apparatus, and the rest with regular epidermal cells (Doss et al., 1988b). Similarly, infection of oriental lilies by swollen germ tube appressoria of *B. elliptica* favored stomatal cells, with 71% of penetration sites near guard cells, with the rest on regular epidermal cells.

Ward (1888) concluded that chemical processes were involved in penetration by *B. elliptica* after observing germ tubes dissolving a pathway into the host tissue. Other researchers have reported that during penetration by *B. cinerea* into host plants, the cuticle is ruptured mechanically by pressure of the tip of the germ tubes, or the epidermal cell walls are dissolved enzymatically (Blackman and Welsford, 1916; Brown, 1916; Cole et al., 1998a). McKeen (1974) and Rijkenberg et al. (1980) observed small holes with sharp and noncurling edges in the cuticle of leaves of *Vicia faba* and tomato fruits at the point of penetration by *B. cinerea* which implied chemical degradation.

Esterase activity was present in the tip of germ tubes of *B. cinerea* at the time of penetration (McKeen, 1974), and esterase activity, especially cutinase, has been found in the infection processes of some other pathogens (Deising et al., 1992; Francis et al., 1996; Guo et al., 1996; Kolattukudy et al., 1995; Koller, 1991; McKeen, 1974; Pascholati et al., 1993). Doss et al. (1988b) speculated that enzymes were involved in penetration of Easter lily foliar tissue by *B. elliptica* based on indentations at the points of penetration and the use of filter-sterilized germination fluid to cause damage. We also observed cuticular degeneration on foliar surfaces around penetration germ tubes, and we measured high esterase activity in germ tubes of *B. elliptica* on glass cover slips within 6 h of incubation. This is similar to that observed by McKeen (1974) in the infection process of *B. cinerea* on leaves of *Vicia faba*. We conclude that penetration of abaxial foliar surfaces of oriental lilies by *B. elliptica* occurs via short swollen germ tube appressoria formed mostly near stomata, and postulate that esterases may facilitate this penetration.

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