Scanning Electron Microscopy of Flyspeck of Apple, Pear, Japanese Persimmon, Plum, Chinese Quince, and Pawpaw

H. NASU, Plant Pathologist, Okayama Prefectural Experiment Station, Sanyo-cho, Okayama, 709-08, Japan, and H. KUNOH, Associate Professor, Laboratory of Plant Pathology, Faculty of Agriculture, Mie University, Tsu-city, 514, Japan

ABSTRACT

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Flyspeck of apple, pear, Japanese persimmon, plum, Chinese quince, and pawpaw was observed by scanning electron microscopy. Fungal structures formed and behaved similarly on all plant surfaces examined. Hyphae from germinated conidia grew on waxy bloom of the respective plants, degenerated bloom crystals around them, and aggregated to form sclerotiumlike structures on plant surfaces. Waxy bloom crystals disappeared along the hyphae. Hyphae and sclerotiumlike structures were covered with a thin film.

In Japan, flyspeck disease occurs on grape, Japanese persimmon, and pear. The causal fungus had been identified as *Leptothyrum pomi* (Montague et Fries) Saccardo. However, our recent investigation (7) revealed that the causal fungus of flyspeck in Japan as well as that of leaf speckle of banana (6), greasy blotch of carnation (2), and flyspeck of apple (4,5) is *Zygophiala jamaicensis* Mason.

Although the pathogen has been reported to occur on at least 78 species in 36 families of flowering plants over much of the temperate and tropical world (2), the only reports on detailed observation of the pathogen on host plant surfaces are of carnation greasy blotch (2) and flyspeck of apple (2) and grape (8,9). Our recent investigation of grape flyspeck with light (8) and a scanning electron microscope (9) revealed that the causal fungus was an ectoparasite growing on berry surfaces and probably using waxy bloom crystals as its sole source of external nutrition without penetration into epicarp tissues. Hyphae growing on berry surfaces were covered with a thin film probably composed of degenerating product(s) of bloom crystals. The sclerotiumlike structures with a raised, thick center and accompanying thin margin also were entirely covered with a similar thin film. In this paper, flyspeck of apple, pear, Japanese persimmon, plum, Chinese quince, and pawpaw was studied by scanning electron microscopy.

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MATERIALS AND METHODS

Fresh, infected fruits of apple (*Malus pumila* Mill. var. *domestica* Schneid. 'Fuji'), pear (*Pyrus serotina* L. 'Taiheiyou'), Japanese persimmon (*Diospyros kaki* L. 'Fuyu'), Chinese

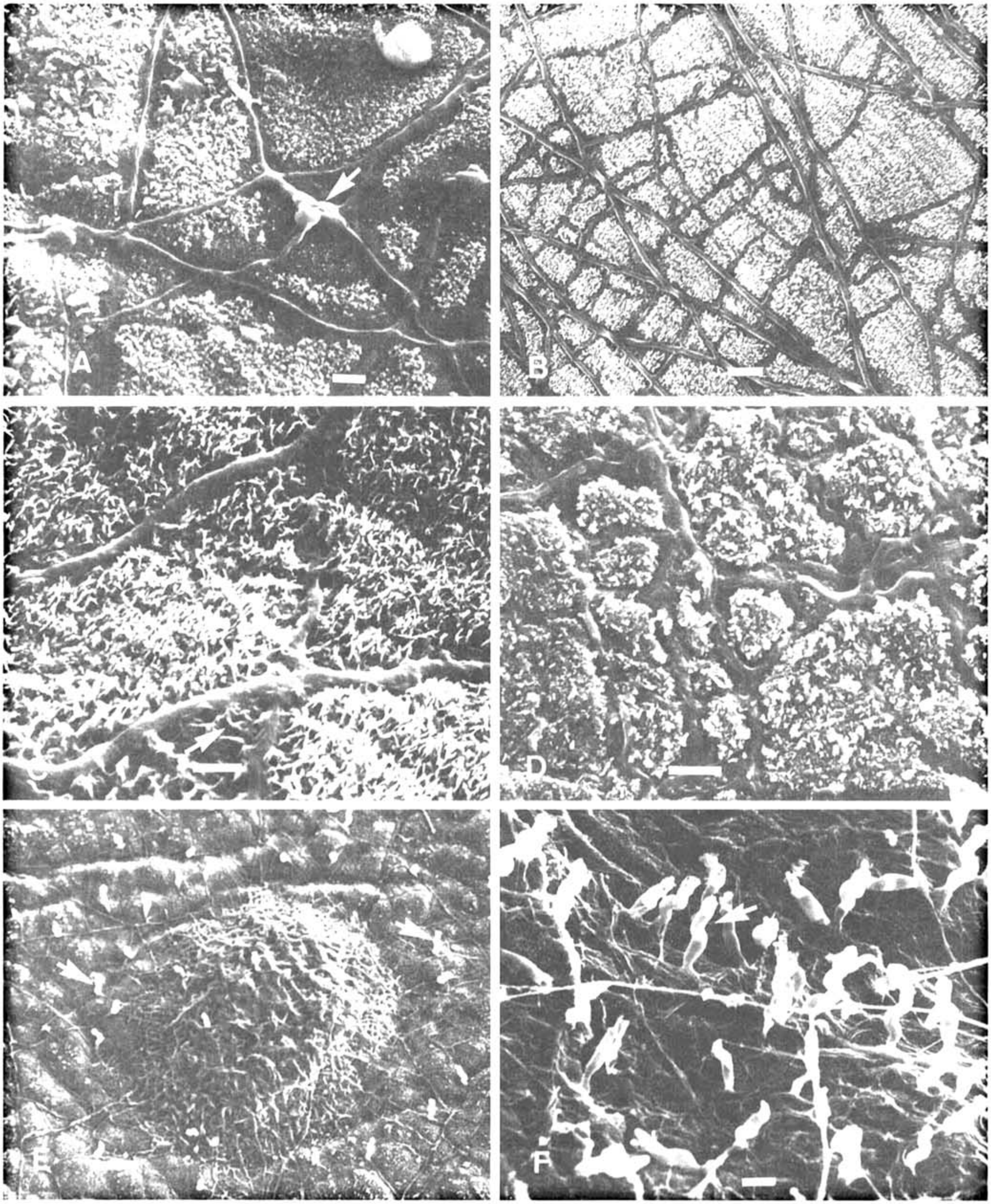

Fig. 1. (A) Areas free of bloom crystals along hyphae arising from paired, germinated conidia (arrow) on a plum fruit. Scale bar = 10 μm. **(B)** Areas free of bloom crystals along many branching hyphae growing on a Japanese persimmon fruit. Scale bar = 20 μm. **(C)** Enlarged view of one of the hyphae shown in B. Arrow indicates a thin film probably composed of degenerating product(s) of bloom that covers the hypha. Scale bar = 5 μm. **(D)** Hyphae growing on surface of a pawpaw fruit. Waxy bloom crystals have disappeared along the hyphae. Scale bar = 10 μm. **(E)** Mature sclerotiumlike structure produced on a plum fruit. Arrows indicate conidiophores at the marginal region of the structure. Scale bar = 50 μm. **(F)** Conidiophores (arrow) arising from hyphae that have grown on surface area free of waxy bloom crystals on a pear fruit. Scale bar = 10 μm.

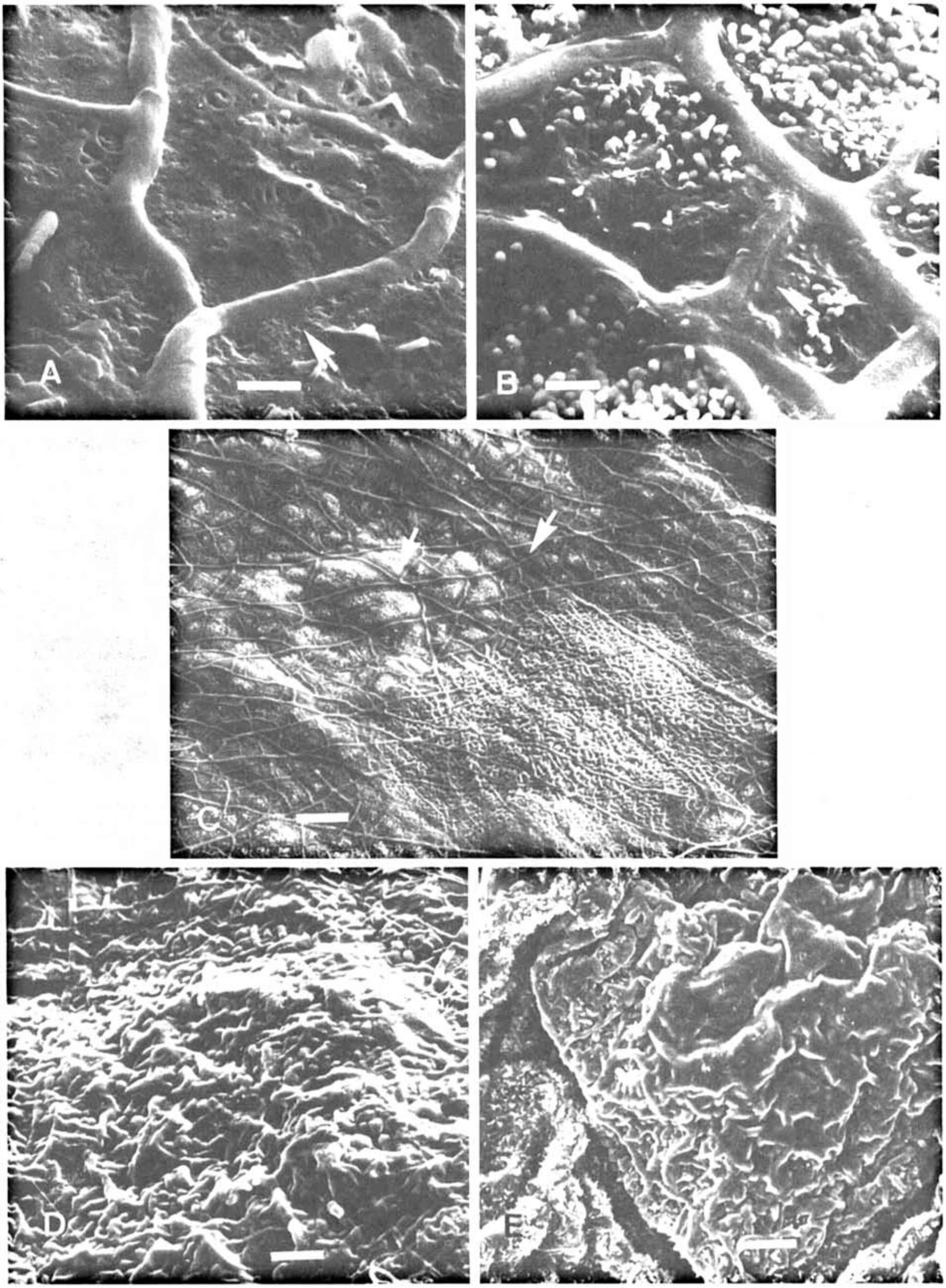

Fig. 2. (A) Hyphae covered with a thin film probably consisting of degenerating product(s) of bloom of apple. Arrow indicates the most prominent portion of this phenomenon. Scale bar = 5 μm. **(B)** Hyphae covered with a thin film, similar (arrow) to that of A, on a Chinese quince. Scale bar = 5 μm. **(C)** Two relatively young sclerotiumlike structures formed on a Japanese persimmon fruit. Arrows indicate hyphae growing in waxy bloom crystals. Scale bar = 50 μm. **(D)** Enlarged view of a mature sclerotiumlike structure produced on a pear fruit that has been covered with a thin film, thereby giving indistinct contours of individual hyphae. Scale bar = 20 μm. **(E)** Enlarged view of a mature sclerotiumlike structure produced on a pawpaw fruit whose entire surface has been covered with a continuous film, thereby making individual hyphae invisible. Scale bar = 20 μm.

quince (*Chaenomeles sinensis* Koehne), plum (*Prunus salicina* Lindle. 'Ohishi-nakate'), and pawpaw (*Asimina triloba* Dun.) were harvested from commercial orchards in Okayama Prefecture, Japan, in 1981 and 1982. Epicarps were peeled off the fruits, cut into small pieces, attached bloom-side up on glass slides, and kept in a desiccator for dryness. The specimens were further cut into pieces about 5×5 mm^2, attached bloom-side up on specimen stubs, coated with gold about 25 nm thick in an ion-coater, and observed with a Hitachi scanning electron microscope HHS-2X at 15–20 kV accelerating voltage.

RESULTS AND DISCUSSION

De Bary (3) emphasized that epicuticular wax patterns on plant surfaces varied from species to species and proposed a classification for types of wax coating as follows: 1) single layers of granules, 2) small rodlets perpendicular to the cuticle, 3) several layers of very small needles or granules, and 4) membranelike layers or incrustations. As far as the present materials were observed, bloom patterns of apple corresponded to type 1 of De Bary's classification, those of Chinese quince and pawpaw to type 2, and those of pear, Japanese persimmon, and plum to type 3.

On all plants observed, conidia were usually paired and each of them produced two germ tubes elongating toward different directions so as not to cross each other (Fig. 1A). This observation was consistent with the cases of carnation greasy blotch (2), banana leaf speckle (6), and grape flyspeck (8). Bloom crystals around germinated conidia and along hyphae arising from them degenerated on all fruit surfaces (Fig. 1A–D), as in carnation greasy blotch and apple (2) and grape flyspeck (8,9). Such an area free of original bloom crystals was wide near the conidia (Fig. 1A) and tapered toward the hyphal apex. A higher magnification of electron micrographs indicated that hyphae growing on the fruit surface were covered with a thin film probably composed of degenerating product(s) of bloom crystals, although some of the hyphae appeared to grow on the degenerating product(s) (Figs. 1C and 2A,B). Such a pattern of hyphal growth had previously been observed with grape flyspeck (8,9) and was observed commonly on all plants examined in this study. Assuming that the film consists of the degenerating product(s) of bloom crystals, it might be in a gellike state after degeneration, and thus the hyphae could grow underneath it. Otherwise, it is hard to account for such hyphal growth.

When networks of growing hyphae expanded on fruit surfaces, almost all bloom crystals disappeared from the surfaces and a great number of conidiophores with conidia arose from the hyphae (Fig. 1E,F). The conidiophores showed a unique S shape similar to those illustrated by Baker et al (2) and Martyn (6). At the cleared surface area free of bloom, mycelia gathered to form a thick sclerotiumlike structure with a raised center and a thin margin (Figs. 1E and 2C–E). Figure 2C shows a relatively early stage of formation of the structure where individual hyphae were distinguishable from each other, whereas Figure 2D,E shows a mature stage of the structure where hyphae were tightly intermingled, making features of individual hyphae indistinguishable. A higher magnification of such a structure (Fig. 2E) revealed that the entire surface appeared to be covered with a thin layer similar to that covering growing hyphae shown in Figure 2A,B. A similar structure was produced in cleared surface areas free of bloom of all plants examined. As shown in Figure 1E, many conidiophores were visible in the bloomfree area surrounding the structure, although several also were produced on the structure. Baker et al (2) reported that the hyphal structure similar to the one we observed formed ascospores in culture and on apple fruit. He called this structure a pseudothecium. In contrast, Atkinson (1) regarded it as a sclerotium. We are not aware of a description of ascospore formation on such a structure in literature published in Japan. Moreover, we have never seen ascospore formation on various plants in fields in this country. Therefore, we tentatively term this a sclerotiumlike structure until ascospore formation is confirmed, although we believe that the description of Baker's group may be correct. Our recent investigation by micromanipulation of the scanning electron microscopic level (10) demonstrated that within this structure, tightly intermingled hyphae were embedded in a somewhat amorphous material probably composed of degenerating waxy bloom product(s). In this sense, the structure we observed is unique and differs from the typical sclerotium or infection cushion, which consists solely of hyphae and their associated structures.

The structures of the causal fungus behaved very similarly on all plants examined, although morphology of epicuticular bloom crystals varied among the plants. Even though there is no indication that the cuticle underneath the bloom is penetrated by the fungus (10), its ability to alter bloom structure may have serious physiological consequences for fruits of various infected host plants.

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