

**Greasy Blotch of Carnation and Flyspeck of Apple:
Diseases Caused by *Zygomphala jamaicensis***

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ABSTRACT

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Greasy blotch of carnation and flyspeck of apple are caused by the fungus, *Zygomphala jamaicensis* (perfect state, *Schizothyrium pomi*). Inoculations with single-conidia and single-ascospore isolates produced both stages on apple fruit. The pathogen occurs on at least 78 species in 36 families of flowering plants over much of the temperate and tropical world. The fungus usually removes the waxy bloom from the cuticle and forms characteristic clusters of pseudothecia ("flyspecks") in the colony. It can utilize carnation wax or paraffin as a carbon source in vitro. Utilization of the wax on carnation foliage by the mycelium produces a greasy appearance that also may develop on the upper stem, reducing flower marketability. Conidia are forcibly

discharged and airborne. Since pseudothecia rarely or perhaps never mature on carnation, conidia are important in spread in glasshouses, where there is no need of the pseudothecial stage. Mature pseudothecia develop on apple and other plants outdoors, and ascospores discharged in the spring facilitate distant dissemination; conidia apparently are an intensification mechanism, important because each infection is restricted to about 2 cm in diameter. Control on glasshouse carnations is by maintaining relative humidity below 85%. In leaky, poorly ventilated glasshouses fungicidal sprays may be necessary. Destruction of carryover inoculum, plus fungicidal sprays, will control flyspeck on apple.

Additional key words: *Leptothyrium pomi*, *Microthyriella rubi*, *Dianthus*, *Malus*.

A fungus inadvertently may be listed in the literature under different binomials on its several hosts, particularly if its spore stages and the symptoms produced vary with the host. Recognition that the pathogens are identical then may be delayed because an investigator seldom studies a sufficiently wide range of hosts. Such was the case for *Zygomphala jamaicensis* Mason, the fungus that causes flyspeck of apple fruit, greasy blotch of carnation leaves, and also parasitizes a wide range of other plants.

During the winter of 1952-53 a new disease was recognized on glasshouse carnations in California (5); it was especially severe in Alameda and Contra Costa counties. The injury was so slight in some glasshouses that growers were unaware of the presence of the pathogen or the disease. In other glasshouses, however, the injury was so severe that the yield of marketable blooms was greatly reduced. This disease, which is called greasy blotch, was investigated by the first two authors at Los Angeles (5). Flyspeck of apple was being studied simultaneously and independently by the last two authors at Berkeley (14). When Durbin transferred to the Los Angeles campus, cultures and specimens were compared, and it was discovered that the fungi causing flyspeck and greasy blotch were identical (16).

The taxonomy, ecology, and pathology of the fungus, *Zygomphala jamaicensis* (*Schizothyrium pomi*), on carnation, apple, and other hosts are presented in this paper.

RESULTS

Symptoms.—The usual symptom on leaves is the loss of the waxy bloom in small patches in which tiny, apparently superficial, black, shiny pseudothecia ("flyspecks") may be formed.

The most distinctive symptom in carnation, which is best seen with a hand lens and on young advancing spots, is loss of the glaucous waxy bloom in a radiating spider-web pattern (Fig. 1-E, G). This pattern is the result of degradation of the wax along the branching mycelium of the pathogen, an effect also produced by *Sporobolomyces roseus* on needles of *Larix* (39). As the spots enlarge, they become dense in the center, owing to coalescence of traces formed by hyphal branches, and develop shiny green or greasy-appearing blotches with radiately fibrillose margins (Fig. 1-E, F, G). The appearance of spots produced by dried drops of spray residue or water differ from those caused by the fungus in being uniformly round and with smooth margins (Fig. 1-B). Since a single fungus colony attains a maximum size of only 1.0 cm, thousands of infections are necessary to

produce severe plant injury. As adjacent blotches coalesce, the entire leaf, particularly at the base of the plant, becomes shiny and has the appearance of being coated with a film of oil, hence the name greasy blotch (Fig. 1-E, H).

Raised pimples with yellow halos or centers may occur

at stomata either within or outside of the infected areas (Fig. 1-C, D). These pimples, and the incurling of petals and closing of flowers ("sleepiness") that sometimes occurred with greasy blotch, gave rise to the early notion that the trouble was caused by atmospheric pollutants. Several factors probably are able to induce these pimples.

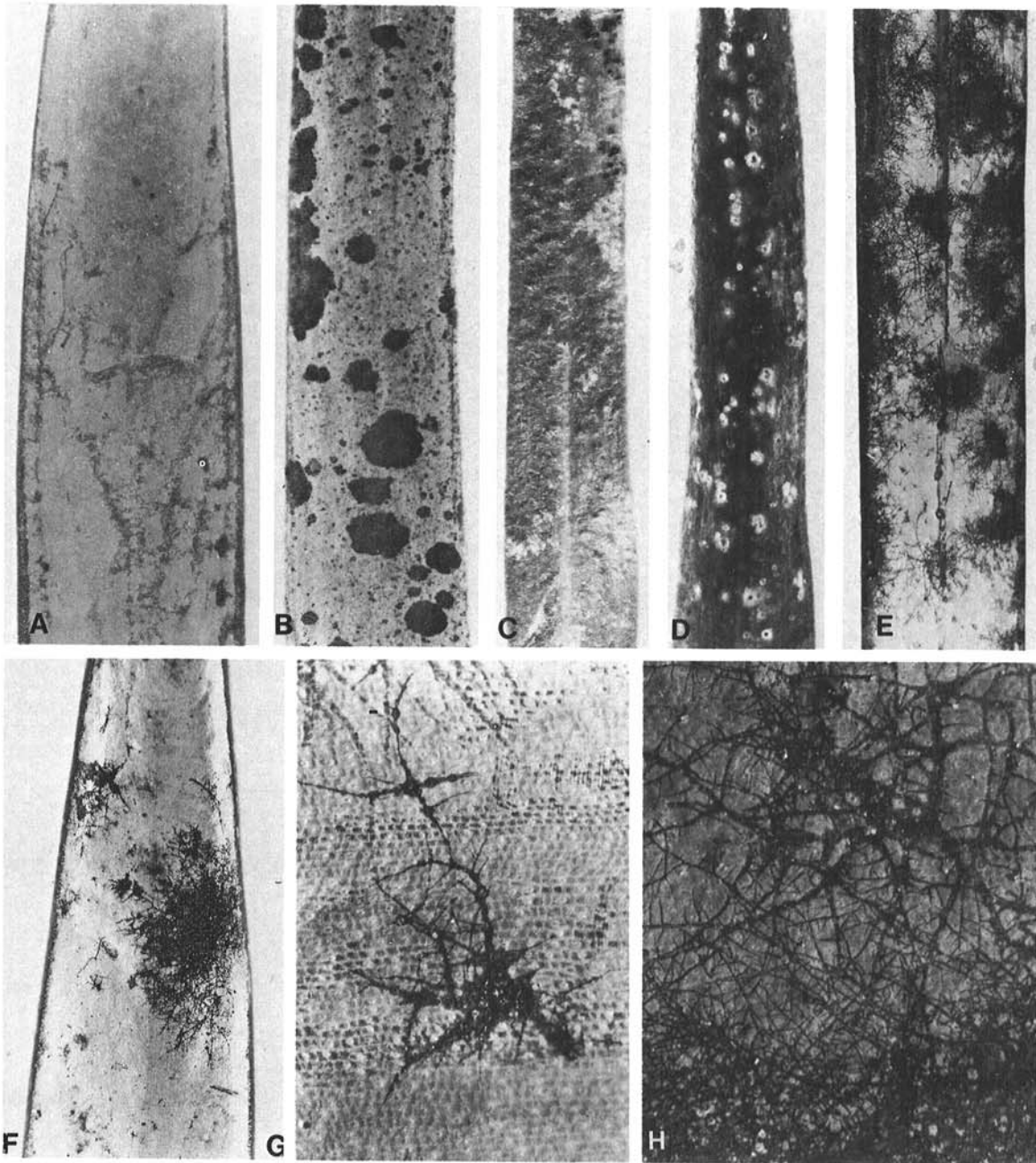


Fig. 1-(A to H). Various symptoms of wax disturbance on carnation leaves (A to F magnification $\times 2.5$). A) Mechanical abrasions commonly observed in commercial glasshouses. B) Wax displacement by drops of spray or water. C-D) Pimples caused by air pollutants or insect feeding, shown in reflected (C) and transmitted (D) light. E-H) Blotches of wax degradation caused by *Zygothiala jamaicensis*. Young radiate colony from near plant apex (G), $\times 40$; larger blotch colonies from near center of plant (E, F); advanced, nearly wax-free colony from a leaf at the base of the plant (H), $\times 200$.

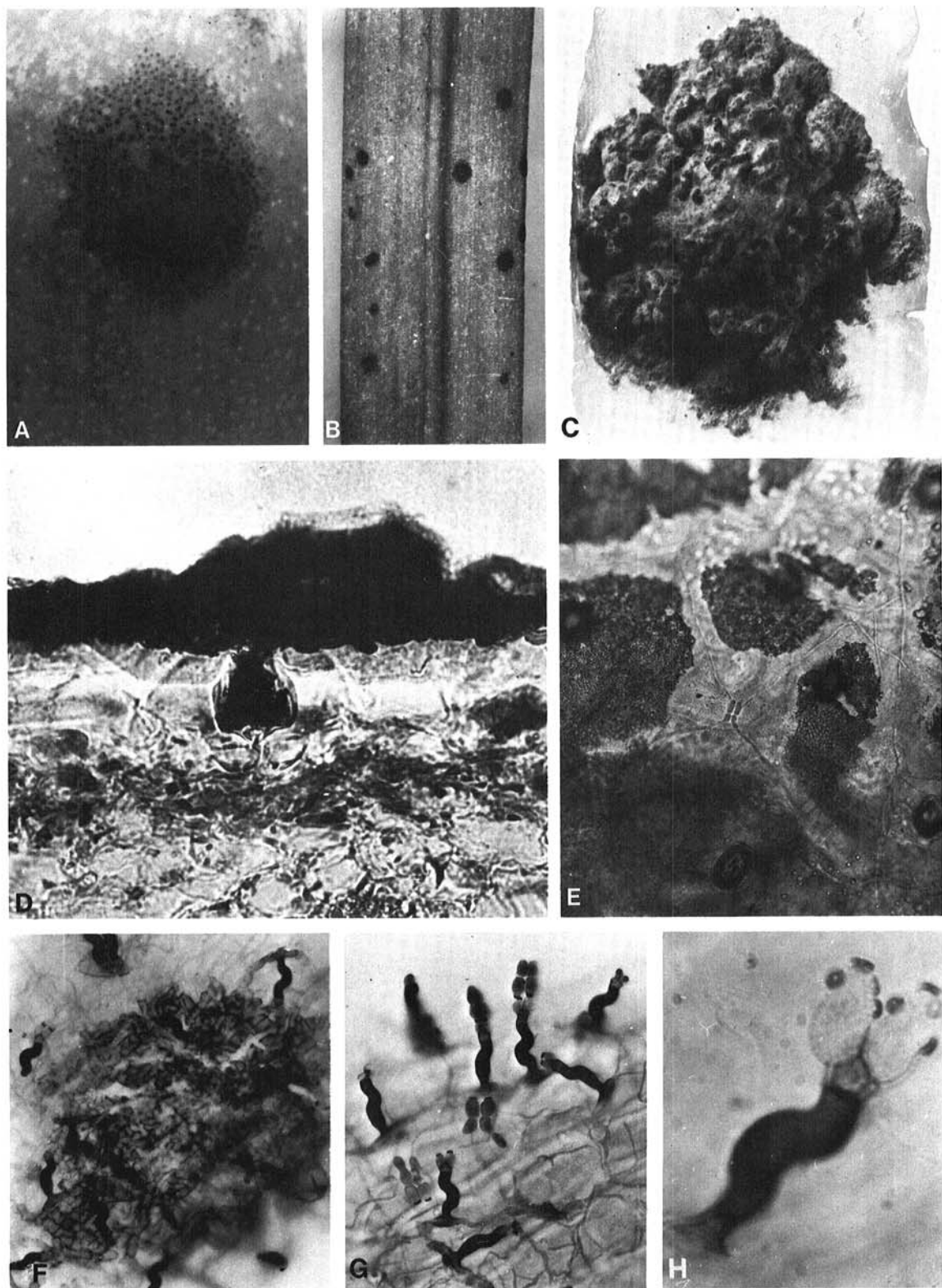


Fig. 2-(A to H). Morphological features of *Zygophiala jamaicensis* (*Schizothyrium pomi*). **A)** Cluster of mature pseudothecia ("flyspecks") on apple inoculated with ascospores from *Rhus parviflorus*. **B)** Isolated immature pseudothecia on carnation leaf, $\times 5$. **C)** Six-week-old culture on PDA, carnation isolate, $\times 2.5$. **D)** Cross section of immature pseudothecium on carnation leaf, showing a basal peg formed in the deeply sunken stomatal pit, $\times 300$. **E)** Quadripolar germination of pair of two-celled conidia on carnation leaf, showing area free of wax along the hyphae, $\times 250$. **F)** Surface view of immature pseudothecium and conidiophores on carnation leaf, $\times 325$. **G)** Conidiophores and conidia on carnation leaf, $\times 375$. **H)** Conidiogenous cells from conidiophores on carnation leaf, showing conidial scars, $\times 1500$.

Detailed microscopic examination of them indicated that some, which were not centered on stomata, may have resulted from aphid feeding. *Zygothiala jamaicensis* does not appear to induce them, as inferred earlier (5). However, the extremely fine fungus mycelium was observed in microscopic sections to penetrate the cuticle of the lateral walls of cells that formed the stomatal pit, and to be present within these cells (5, 23, 55). Mycelium occasionally was observed in the walls of cells of the upper epidermis. Basal pegs from pseudothecia project into the deeply sunken carnation stomatal pits (9, 54), holding the pseudothecia in place (Fig. 2-D).

The disease may progress upward from the basal carnation leaves; the height and severity of injury is influenced by environmental conditions. Severely affected basal leaves become yellow and brittle, and may die prematurely. The middle leaves on the stems show smaller, distinct, greasy blotches with radiately fibrillose margins (Fig. 1-E), and upper leaves may have small, fibrillate, wax-cleared areas formed by germinating spores (Fig. 1-G). Growers report that severely affected cuttings root slowly and poorly (29). When greasy-appearing leaves develop high up on the flower stems, marketability is destroyed. Pseudothecia (flyspecks) are formed much less commonly on carnation than on apple fruit, but may be found, particularly at stem nodes of plants growing under very humid conditions (Fig. 2-B). They develop well on stems held in a moist chamber. The *Zygothiala* stage is commonly formed in affected areas; the sparse conidiophores are readily seen under $\times 72$ magnification with critical lateral illumination (Fig. 2-F, G). The mycelium is seen only under higher magnification.

The "greasy mutant" of carnation has partial loss of waxy bloom from stem and leaves (26) resembling greasy blotch; the loss of wax is general over the entire plant, rather than in spots, as in greasy-blotch disease.

The symptoms on apple fruit are well described by the name flyspeck. Six to 50 minute, shiny black, round structures that appear to be superficial on the cuticle develop in well-defined groups in areas 1-2 cm in diameter (Fig. 2-A). This disease was discovered in California in 1951 (14), and the fungus was cultured directly from the flyspecks (15, 16). Single-ascospore cultures were obtained from apples from California and Virginia. Both produced the *Zygothiala* state in cultures that were identical in appearance.

The stromatic mycelial structures and the tortuous dark brown conidiophores commonly occur on leaves, stems, and fruit of most hosts. For example, bananas obtained from the Los Angeles market usually had the conidiophores, especially around the stem end. It was not possible to culture the fungus from these bananas, even when obtained prior to the customary fumigation when fruit is landed at an American port.

Host range.—The flyspeck fungus has an extensive host range on plants occurring in cool temperate to hot tropical areas. The following list includes 78 species in 36 families of flowering plants [letters indicate: a = fungus identification based on morphology of perfect state; b = identification based on morphology of imperfect state; c = inoculation successful; d = pathogen isolated from specimen; numbers = references or Commonwealth

Mycological Institute (C.M.I., I.M.I.) herbarium numbers]: *Acer macrophyllum* Pursh (a, c, 15), *A. negundo* L. (a, 13), *A. saccharum* Marsh (a, d, 4, 21); *Annona squamosa* L. (a, I.M.I. 126231a); *Antiaris africana* Engl. (b, 28); *Arbutus menziesii* Pursh (a, c, 15); *Aristolochia* sp. (a, 59), *A. siphon* L'Hérit (a, 51); *Asimina triloba* Dunal (d, 21); *Barbacenia purpurea* Hook. (a, 17); *Benzoin aestivale* Nees (d, 21); *Brassocattleya* sp. (a, 8); *Brassolaeliocattleya* sp. (a, 8); *Calycophyllum candidissimum* DC. (a, 17); *Cattleya* sp. (a, 8); *Celastrus scandens* L. (a, 21); *Cercis canadensis* L. (a, 21); *Cinnamomum camphora* Nees & Eberm. (a, 36); *Citrus grandis* (L.) Osbeck (a, 56), *C. paradisi* Macf. (a, 18), *C. sinensis* (L.) Osbeck (a, 1); *Cladium mariscus* R. Br. (a, 7); *Cornus alternifolia* L. (d, 21), *C. rugosa* Lam. (a, 56); *Crataegus* sp. (d, 21), *C. oxycantha* L. (a, c, d, 31, 44); *Dendrobium* sp. (a, 8); *Desmodium canum* (Gmel.) Schimz & Thell. (a, I.M.I. 124325); *Dianthus caryophyllus* L. (a, b, c, d, 5); *Dirca palustris* L. (d, 21); *Epidendrum* sp. (a, 8); *Euonymus europaea* L. (a, d, 31),

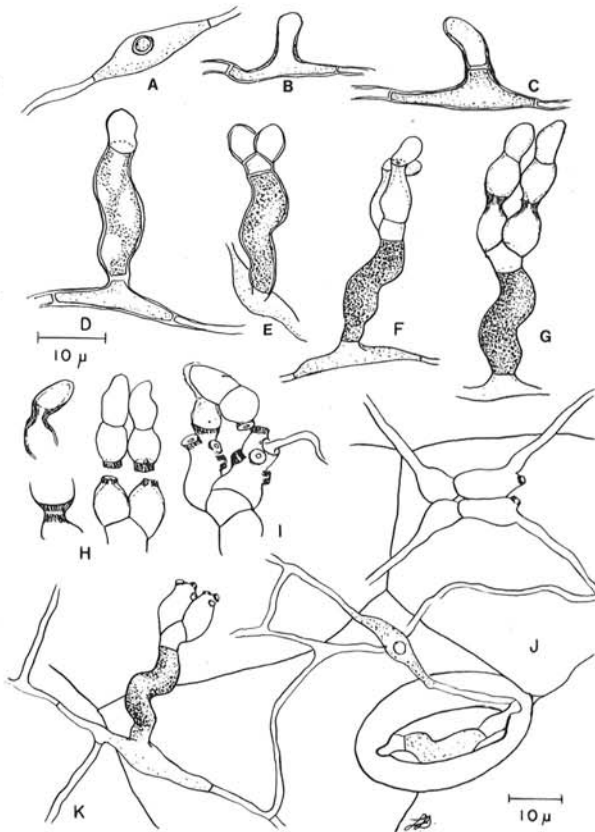


Fig. 3-(A to K). Formation, abscission, germination, and infection by conidia of *Zygothiala jamaicensis*. A-G) Stages in development of conidiophore and conidia. H) Detail of point of attachment of conidia. I) Conidial scars on sporogenous cells, showing connecting disks that split during abscission. Mycelium proliferating through central pore of one disk. Material from culture. J) Germination of a pair of two-celled spores on carnation leaf, showing mycelial invasion of a sunken stomatal pit. K) Conidiophore, showing basal cell, dark "stipe," terminal cell, and sporogenous cells with conidial scars.

E. obovatus Nutt. (a, 21); *Ficus elastica* Roxb. (b, authors); *Fraxinus americana* L. (a, c, d, 21), *F. excelsior* L. (a, d, 31); *Gaultheria procumbens* L. (a, 35); *Gleditsia triacanthos* L. (a, 4); *Gymnocladus dioica* Koch (a, 56); *Hymenocardia acida* Tul. (a, I.M.I. 105980g); *Juglans regia* L. (a, 10); *Ligustrum vulgare* L. (a, 31); *Liriodendron tulipifera* L. (d, 21); *Lonicera* sp. (b, 4), *L. hispidula* Dougl. (a, c, 15), *L. japonica* Thunb. 'Halliana' Nichols (c, authors); *Mahonia aquifolium* Nutt. (a, 51); *Malus sylvestris* Mill. (a, b, c, d, 15, 42); *Musa paradisiaca* L. var. *sapientum* Kuntze (b, 38); *Persica armeniaca* L. (a, 31); *Platanus occidentalis* L. (a, c, d, 21); *Polygonum sachalinense* F. Schmidt (a, 58); *Populus tremula* L. (a, d, 31); *Prunus* sp. (a, d, 31), *P. divaricata* Led. (a, 10), *P. domestica* L. (a, 62), *P. persica* Batsch. (a, d, 31); *Psidium guajava* L. (a, I.M.I. 116901); *Pyrus communis* L. (a, 43); *Quercus agrifolia* Née (a, 47), *Q. alba* L. (a, d, 4), *Q. robur* L. (a, d, 31); *Rhamnus californica* Esch. (a, b, 15); *Rhus glabra* L. (a, 4); *Rosa* sp. (a, 31); *Rubus* sp. (a, d, 31), *R. allegheniensis* Porter (a, d, 4), *R. argutus* Link (a, 53), *R. idaeus* L. (a, 43, 46), *R. parviflorus* Nutt. (a, 15), *R. vitifolius* Cham. & Schlecht. (a, 15); *Salix* sp. (d, 21), *S. caprea* L. (a, 31), *S. nigra* Marsh (a, d, 4); *Sambucus caerulea* Raf. (a, 15); *Sassafras variifolium* Kuntze (a, d, 4); *Smilax hispida* Muhl. (a, d, 4, 21); *Staphylea trifolia* L. (a, d, 4); *Tilia vulgaris* Hayne (a, d, 31); *Tinospora caffra* (Miers) Troup. (a, I.M.I. 107399b); *Ulmus fulva* Michx. (a, 56); *Vanda* sp. (a, 8); *Vitis* sp. (a, 6), *V. cordifolia* Lam. (d, 21); *Zanthoxylum americanum* Mill. (a, d, 4, 21). Because of the diversity of known hosts, the large pathogen synonymy, the extensive geographical distribution, and the limited studies made, it is certain that the host list is much larger than presented here.

Carnation seems to be an excellent host, and all cultivars are susceptible. Cultivars Pink Sims, White Sims, Fisher's Pink, Venus, Vulcan, Midas, Saturn, and Orion commonly were infected in California. Red Sims was moderately resistant.

Geographical distribution.—The fungus is widely distributed in Europe (Austria, Bulgaria, Czechoslovakia, England, France, Germany, Italy, Norway, Poland) (56), widespread in the United States and Canada, and occurs in Argentina (20), Australia (2), the Bahamas (60), Brazil (6, 55), Brunei (I.M.I. 150108), Chile (1), Cuba (17), Ecuador (1), Ghana (28), India (30), Iran (57), Israel (48), Jamaica (38), Mozambique (12), New Zealand (3), South Africa (61), Southern Rhodesia (27), Tanzania (17), and in Costa Rica and Honduras (authors).

The pathogen has been reported on carnation in California (5), Massachusetts (22), Pennsylvania (52), Holland (45), Denmark (23), and England (29), but undoubtedly is much more widely distributed.

Taxonomy of the pathogen.—The fungus was first described by Montagne (42) in 1834 as *Labrella pomi* Mont. mss. (Fr. in litt.) in the Fungi Imperfecti; globose spores were reported without indicating how they were borne. Their specimen No. 847 was examined by Colby (11), who verified that the fungus was the one causing flyspeck rather than that causing sooty blotch. Von Thümen (60) reported in 1879 that spores of *L. pomi* were solitary, sparse, subglobose, light gray, and 7 μ m in diameter, but did not indicate how they were borne.

Colby (11) also confirmed that von Thümen's No. 1483 was the flyspeck fungus. There were no further reports of asexual spores in the life cycle of the fungus until Durbin et al. (16) showed in 1953 that *Zygophiala jamaicensis* Mason (38), described in 1945, was that stage.

Desmazieres (13) transferred *Labrella pomi* to *Microsticta pomi* (Mont.) Desm. in 1849 because the flyspecks were considered to be sterile structures. Saccardo (49) tentatively transferred *Labrella pomi* to *Leptothyrium ? pomi* (Mont. & Fr.) Sacc. in 1880, probably mistaking immature pseudothecia for pycnidia; no spores were reported. He removed the ? in 1884 (50). This binomial has been widely and erroneously used for the flyspeck fungus.

No fungus of the *Leptothyrium pomi* type has ever been demonstrated to be part of the life cycle of the flyspeck fungus, nor has it been cultured or inoculated on a host (16). No pycnidial stage was ever observed in material examined in our studies. The binomial, *Leptothyrium pomi*, was based on a misconception, and should be set aside pending demonstration of a definite relationship to *Schizothyrium pomi*.

The literature prior to Colby's work (11) in 1920 also is confusing because many workers considered the fungus that causes sooty blotch of apple to be identical with that causing flyspeck. He showed that they were distinct: sooty blotch being caused by *Gloeodes pomigena* (Schw.) Colby, and flyspeck erroneously attributed to *Leptothyrium pomi* (Mont. & Fr.) Sacc. However, he did not observe a perfect stage of either fungus. Baines (4) confirmed the distinction between these two fungi and verified the perfect state of flyspeck as *Microthyriella rubi*. This binomial had been erected in 1923 by Petrak (46) for an Ascomycete found to be abundant on *Rubus idaeus* L. It is still the name most commonly used for the flyspeck fungus.

The ascomycetous genus *Schizothyrium* was erected by Desmazieres (13) in 1849, and *S. acerinum* Desm. was described on *Acer negundo* L. This fungus was considered to be identical to the flyspeck fungus by von Arx (59), who took Montagne's 1834 *pomi* for the specific name. He also transferred *M. rubi* Petrak to *S. perexiguum* (Rab.) v. Höhn., and the apple flyspeck fungus to *S. pomi* (Mont. & Fr.) v. Arx on the basis of pseudothecial wall structure and opening. Many literature references to *M. rubi* actually refer to *S. pomi*, but Petrak's specimen apparently was a different fungus.

Ascospores of the flyspeck fungus perhaps were observed first by Desmazieres in 1849. Baines (4), however, in 1930 first clearly showed that the flyspecks were pseudothecia, and conducted the first inoculation tests. Immature apples were inoculated with mycelium from single-ascospore cultures isolated from *Acer*, *Quercus*, *Rubus*, *Sassafras*, and *Salix* spp.; typical symptoms and pseudothecia were produced.

The unique S-shaped conidiophores of this fungus on *Lonicera* were illustrated by Viégas (55) in 1946. The terminal conidiogenous cells and conidia were not observed, and the fungus tentatively was named *Helminthosporium ? loniceræ* Viégas. Lafon and Messiaen (31) in 1954 mentioned and roughly illustrated small, brown, irregular appendages on the extremely fine mycelium of the apple flyspeck fungus growing on

cellophane on prune agar. The structures were thought not to be spores because they were not deciduous and rarely germinated. Neither of these workers observed the transitory pair of thin-walled hyaline conidia usually present on the dark conidiophores that they illustrated.

Asteroma dianthi Cke. & Harkn. was collected in 1889 at San Rafael, California, on leaves of *Dianthus* (47). This was thought possibly to be an early collection of the pathogen on carnation in California. However, examination of specimen No. 1451X, kindly loaned by the California Academy of Sciences, San Francisco, had no *Zygophiala* or *Schizothyrium* stages present.

The present correct binomials for the pathogen are:

Perfect state: *Schizothyrium pomi* (Mont. & Fr.) v. Arx; Proc. Koninkl. Nederl. Akad. Wetensch., ser. C, 62:336-337, 1959.

Imperfect state: *Zygophiala jamaicensis* Mason; Commonw. Mycol. Inst., Mycol. Pap. 13:5, 1945.

The synonymy is given by von Arx (59) except that the following should be added:

Helminthosporium ? *loniceræ* Viégas; *Bragantia* 6: 380-381, 1940.

Life history of the pathogen.—We isolated the pathogen from pseudothecia on nine hosts, Baines (4) cultured it from seven hosts, and Lafon and Messiaen (31) from six. We isolated the pathogen by removing pseudothecia from the host, treating with sodium hypochlorite (0.5% available chlorine) for 2 minutes, and plating on water agar plus oat straw; mycelial tips were removed and transferred to potato-dextrose agar (PDA) as they appeared. Bits of leaf tissue with pseudothecia were also taped inside the lid of a petri dish of PDA, so that the ascospores discharged onto the medium; the minute colonies formed then were transferred. The colonies on PDA are slow-growing [about 1 cm in diameter in 21-30 days from a spore "tetrad" (actually a pair of two-celled spores) at laboratory temperatures (about 21 C)], dark gray-green, compact, furrowed, with irregular fimbriate margins and raised centers (Fig. 2-C). The *Zygophiala* state develops in culture, and the pseudothecia form and occasionally mature there. There were only slight differences in cultural appearance among the isolates from different hosts. Pieces of carnation stem, sterilized with propylene oxide and placed on water agar, provide a good medium for sporulation of the fungus.

Inoculations with mycelium from conidial isolates from carnation were successful on leaves of *Lonicera japonica* 'Halliana', *Rubus parviflorus*, and *Dianthus caryophyllus*, on leaves and fruits of *Musa paradisiaca* var. *sapientum*, and on immature fruits of *Malus sylvestris* in moist chambers at 21 C. Both the *Zygophiala* and *Schizothyrium* states were formed in each instance; the pseudothecia on carnation, however, did not form ascospores. When mycelia from single-ascospore isolates from apple and seven wild hosts were inoculated on immature apple fruit, both the imperfect and perfect states were produced. A stem of *R. parviflorus* bearing mature pseudothecia was suspended over immature apple fruit in a moist chamber outdoors at Berkeley in September; typical flyspeck symptoms, *Zygophiala* conidiophores, and mature pseudothecia were produced on the apple (Fig. 2-A).

The unique conidiophores are sparse, and consist of

four parts: a modified mycelial basal cell that is subhyaline and $16-20 \times 4 \mu\text{m}$ (Fig. 3-A to C); a conspicuous, tortuous, smooth, thick-walled, dark-brown sector, $16-18 \times 4-5 \mu\text{m}$ (Fig. 3-D to G); an angular, subhyaline, terminal cell $4-6 \times 3-4 \mu\text{m}$ (Fig. 3-E, H) which bears on its apical sides two divergent, hyaline, ovate to ampulliform conidiogenous cells, $6-15 \times 4-6 \mu\text{m}$, that bear few to numerous, thickened, circular, prominent, dark conidial scars (Fig. 2-H, 3-I, K). The thickened disk between conidiophore and conidium splits so that half of it remains on the spore and half forms the scar of the conidiogenous cell (Fig. 3-H, I). The conidiogenous cell may proliferate through the pore in the center of the scar in culture to form mycelium (Fig. 3-I). The conidiogenous cells are sensitive to drying, may shrivel, and even be forcibly discharged (41). The conidia are two-celled, elliptical to obovate, constricted at the septum, dry, smooth, hyaline, thin-walled, and $13-20 \times 4-6 \mu\text{m}$ (Fig. 3-G). A conidium is borne on each conidiogenous cell, and may be forcibly discharged, as shown by the new colonies formed on agar plates from spores some distance from sporulating plant tissue. Meredith (40, 41) showed that exposure of the moist turgid conidial apparatus to dry air caused vertical contraction of the dark sector ("stipe") of the conidiophore, followed by sudden elongation when a gas bubble formed in the stipe, forcibly discharging the conidia. As a result the spores often are shed in pairs (Fig. 2-E, G). The pair of two-celled spore "tetrads" are a distinctive feature adhering on the surface of affected plants (Fig. 2-E, G, 3-J). The terminal cell of each may germinate before discharge; the basal cells germinate only after spore release.

The individual conidia may be carried for short distances by air currents and splashed water. When they land on a waxy surface they germinate and initiate growth under moist conditions (Figs. 1-G, 2-G, 3-J). The rate of spread of the greasy-blotch disease in a carnation glasshouse and on an individual plant is quite slow. The principal spread of the fungus on carnation is by conidia, since mature pseudothecia are rare or lacking on that host. Compared to glasshouse carnations, the *Zygophiala* state may be relatively less important on apple and other hosts outdoors, since mature pseudothecia commonly are formed on these hosts. However, conidia undoubtedly account for many secondary infections after ascospore discharge ceases.

Mycelium in an infection on a carnation leaf is scanty, inconspicuous, appressed, hyaline, septate, and hyphae are about $2 \mu\text{m}$ in diameter. A cleared area free of wax is produced along the sides of each hypha, narrowest toward the hyphal tip, and progressively wider toward the base (Fig. 2-E). Mycelium may form superficial, thickened, shiny black, circular knots or flyspecks, the incipient pseudothecia, in random clusters (Fig. 2-A, B). These structures are $150-375 \mu\text{m}$ in diameter and $30-50 \mu\text{m}$ in height, with irregular margins (Fig. 2-D, F). The top of the ascogenous tissue consists of dark interwoven mycelium that merges into the stromatic plate at the margins. At maturity, the elevated center of the cover separates in an irregular crack, exposing the asci below. The asci are spherical to oval, apically thickened, bitunicate, $19-44 \times 6-10.5 \mu\text{m}$, and are imbedded singly in centrum tissue. Each ascus contains eight two-celled,

hyaline ascospores $10-14 \times 3-5 \mu\text{m}$, with the upper cell shorter, wider, and less pointed than the lower. Pseudothecia viewed from above after ascospores are discharged have the appearance of small brown rings. Mature pseudothecia and asci have been illustrated (7, 31, 34, 59).

Since mature pseudothecia with ascospores sometimes were formed in culture and on apple fruit from single ascospore isolates, the fungus is homothallic and would be expected to exhibit relatively slight variability.

The ascospores are discharged in April in coastal California (15), in late May to early June in France (31), in June in Indiana (4), and at least by July to early August in Pennsylvania (24) and West Virginia (25), under field conditions. These spores are airborne for considerable distances and initiate primary infections on newly developed plant parts. An ascospore gives rise to a fungus colony that may form many pseudothecia, rather than a single one, as suggested by Tehon (53). About 3 weeks are required for symptoms to develop on apple under cool moist conditions (21). Under moist outdoor conditions in coastal California the cycle on apple fruit from inoculation with ascospores to production of the *Zygothiala* state and mature pseudothecia may occur in about 1 month. Flyspecks form on carnation in about 3 weeks in a moist chamber at laboratory temperatures (21 C).

The pathogen may overwinter outdoors in California on fallen apples in the orchard, or on stems and leaves of adjacent *Rubus*, *Lonicera*, *Acer*, *Arbutus*, *Rhamnus*, or *Sambucus* (15). It also overwinters on plants adjacent to apple orchards in France (31). Growth of the pathogen is continuous throughout the year on glasshouse-grown carnations.

The fungus can grow on a cellophane membrane placed on prune agar in a petri dish, forming mycelium and the *Zygothiala* stage (31). Since the fungus developed on nutrients diffused through the membrane, it seems probable that it can develop on plant surfaces by utilizing exosmosed nutrients present there (37). In addition, the restricted penetration of carnation stomata by hyphae could supply nutrients.

Growth of the pathogen on wax and paraffin.—Because we observed that *Zygothiala jamaicensis* degrades the minute, simple, granular wax particles (23, 33) on the surface of carnation leaves, tests were conducted to determine whether it can use lipids as a carbon source, as has been shown for other fungi (19).

Melted embedding paraffin was poured in a thin film on washed, sterile, glass microscope slides. The slides were held in petri dish moist chambers and inoculated with small mycelial bits cut from the raised centers of in vitro colonies. Eight days later the fungus was growing and sporulating vigorously, and had formed small pseudothecia.

The cuticular wax was dissolved from *Dianthus* and *Lonicera* leaves by immersion in vials of diethyl ether for 5-10 minutes. This solution was pipetted onto washed, sterile, microscope slides and warmed to evaporate the solvent. The wax residue on some of the slides then was melted to form a smooth surface. A comparable series was made with embedding paraffin. Drops of suspensions of conidial tetrads in water or 0.1% calcium nitrate were

pipetted onto the surface of the medium.

There was good germination within 17 hours in a water suspension of the spores on *Dianthus* wax and on paraffin, but not on *Lonicera japonica* 'Halliana' wax; growth was greater in the calcium nitrate than in the water suspensions. Germination and growth were poor in all series and times on *Lonicera* wax for undetermined reasons. There were no conspicuous differences in this or in subsequent examinations between nonmelted and melted films of wax or paraffin. Growth was slight after 4.5 days in the water series on *Dianthus* wax and on paraffin, with conidiophore formation beginning. The calcium nitrate series had more vigorous growth and conidiophore formation. Conidiophores, but not conidia, were common after 9 days on *Dianthus* wax and on paraffin, particularly on the calcium nitrate slides. Small immature pseudothecia had formed on the calcium nitrate-paraffin slides. After 28 days, conidiophores, but not conidia, were abundant on the *Dianthus* wax slides with water and with calcium nitrate. Conidiophores and some spores formed on paraffin with water and with calcium nitrate, and immature pseudothecia without ascospores formed on both.

It is clear that the fungus can sporulate and develop pseudothecia on *Dianthus* wax and on paraffin without a nitrogen supplement, but that development is improved by calcium nitrate amendment. The requisite nitrogen apparently was supplied on leaves and stems by exosmosis from mesophyll or cortical tissue and by mycelial penetration of stomatal cells.

Ecology of the pathogen.—The pathogen is favored by humid conditions outdoors (14, 15, 23, 24, 31, 40, 41) and in the glasshouse (5, 23). Growth of the fungus in culture was best at 15-24 C, but extended from 5-27 C (4). The pathogen has a wide pH tolerance on oatmeal agar, growing from 1.8 to 8.2 (4).

Meredith (40, 41) found that the fungus required moist conditions for conidial formation. In dry weather in Jamaica, conidia matured during the humid nights, and were discharged with the decreasing humidity between 0600 and 0900 hours, but very few were discharged thereafter. It was shown by spore trapping that 32 conidia per cubic meter were present in the air of banana plantations, and this comprised about 0.12% of the total air spora.

The flyspeck disease of apple in California occurs in the coastal fog belt, and on carnation in glasshouses that are maintained at an excessively high (95 to 100%) relative humidity. The temperatures maintained in carnation glasshouses (ranging from 10 to 26 C) are favorable to development of the fungus. The disease is most prevalent and severe there in winter months, on second-year plants, and on the lower parts of the plants where the leaves are most dense, and relative humidity is highest.

Control of the disease.—The disease is relatively easy to control on carnation in glasshouses by maintaining relative humidity of <85% by providing adequate ventilation and, if necessary, with supplemental heat. It is more difficult to control the disease in old, leaky, poorly ventilated glasshouses, and fungicidal sprays may be necessary. Spray applications at 7- to 10-day intervals of zineb (zinc ethylenebisdithiocarbamate), captan (N-trichloromethylmercaptan-4-cyclohexene-1,2-dicarbox-

imide), or ferbam (ferric dimethyl dithiocarbamate) at 2.4 g/liter (2 lb per 100 gal) water have proved satisfactory (22).

Control of the disease on apple is made easier by sanitation within and around the orchard. Removal and burial or burning of fallen fruit, and removal of alternative hosts from around the orchard will reduce the amount of inoculum reaching the fruit. Fungicides applied for control of other diseases will, in most areas, provide adequate control of flyspeck. For example, Lewis and Hickey (32) obtained satisfactory control of flyspeck under epiphytotic conditions with sprays of ferbam, zineb, glyodin (2-heptadecylglyoxalidine acetate), or lead arsenate, but captan and thiram (tetramethylthiuramdisulfide) did not provide adequate control.

DISCUSSION

The pathogen that causes greasy blotch of carnation and flyspeck of apple has been listed in the literature under many binomials, only a few of which have been carefully studied. Paradoxically, many of these names referred to an imperfect state unrelated to the causal fungus (42, 60), to misinterpreted immature pseudothecia (flyspecks) (11, 13, 49, 50), or to a fragment of the asexual state (55). Despite its common occurrence and conspicuous features, the imperfect state, *Zygothrips jamaicensis*, was not described until 1945 (38), and its affinity with the perfect state of the causal fungus was not recognized until 1953 (16). The perfect state has had an equally confusing treatment. The original binomial, *Microthyriella rubi*, which was erected in 1923 and was the one commonly used for many years, was considered in 1959 to refer to a different fungus, and the presently accepted binomial, *Schizothyrium pomi*, was substituted (59). Binomials for fungi on numerous hosts not listed here also probably refer to one of the states of this fungus. The pathogen synonymy, the host range, and the geographical distribution are, therefore, certainly larger than presented here.

The limited cross-inoculation studies have so far given no evidence for host specialization, but because of the known differences in composition of wax of different plants (37), more work is needed.

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