Etiology of Necrotic Flecks on *Dendrobium* Blossoms

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ABSTRACT

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Dark necrotic flecks are common on field-cultivated *Dendrobium* flowers in Hawaii and are a significant factor in quality reduction. Microscopic examinations revealed one or more amerospores, dictyospores, or phragmospores near the center of 41.7% of the flecks. Among the many fungi isolated from the flecks, only *Botrytis cinerea, Alternaria alternata, Bipolaris setariae, B. urochloae, B. sorokiniana, Exserohilum rostratum,* and *Stemphylium* sp. incited flecks by artificial inoculation. *A. alternata* was the most frequently isolated organism. The recovery rate of *A. alternata* from flecks was 40.5% 3 days and 4.1% 6 days after laboratory inoculation. The low isolation rate of fungi from field-collected flecks and the rapid loss of viability of *A. alternata* after artificial inoculation are consistent with the proposition that flecks are predominantly aborted infections.

Additional key words: Bipolaris hawaiiensis.

Blossom blights and rots caused by *Botrytis cinerea* Pers. ex Fr. (1,8), *Botrytis* spp. (1,7,8), *Alternaria* spp., and *Gloeosporium* spp. (7) create serious problems in orchid production. Numerous restricted necrotic lesions or flecks (less than 0.5 mm in diameter) also occur on *Dendrobium* flowers. In a previous study (8), isolations from field-collected flecks did not yield *Botrytis* or reveal any consistent fungal associations. Artificial inoculation with *B. cinerea* and *Botrytis* spp., however, induced flecks on *Dendrobium* blossoms.

The persistent occurrence of flecks on *Dendrobium* blossoms, particularly in outdoor cultivation and often without *Botrytis* outbreaks, suggests there are other fleck incitants. Even though functional damage to the flowers is insignificant, flecking reduces market acceptability and causes considerable economic losses. The present study was undertaken to resolve the etiology of flecks on *Dendrobium* blossoms.

MATERIALS AND METHODS

During the 6 mo from November 1976 through April 1977, flecked flowers of cultivars O-580 and UH-44 of artificial hybrid *Dendrobium* Jacquelyn Thomas, cultivar Y-975 of *D.* Neo Hawaii, and *D.* Tomie were harvested for microscopic examination and fungal isolation. Small pieces (5 × 15 mm) of petal or sepal tissue with necrotic flecks (0.2–1 mm diam) were fixed and cleared in a Formalin-alcohol-acetic acid solution (5 ml each of 37% Formalin and glacial acetic acid added to 100 ml of 50% ethyl alcohol in distilled water). The fixed materials were mounted on glass slides and examined microscopically for fungal propagules. The incidence of amerospores, dictyospores, and phragmospores associated with necrotic flecks was recorded. Single flecks were dissected out, surface-sterilized by momentarily dipping into 0.5% sodium hypochlorite in 1:2,000 Tween 20 solution, and plated on water agar. As fungal colonies developed, mycelial or conidial transfers were made to vegetable juice agar (VJA) (100 ml of V8 juice, 2 g of $CaCO_3$, 17 g of agar, and 900 ml of deionized water). Cultures were exposed to continuous cool-white fluorescent irradiation (about 2,200 lux at the level of colonies) at 22–26 C to induce sporulation. Fungi were identified, and the incidence was recorded in relation to the numbers of flecks cultured.

Cultivars O-580 (*D.* Jacquelyn Thomas) and Y-975 (*D.* Neo Hawaii) were used in inoculation studies. Spikes for inoculation had a minimum of five open flowers and one bud. One isolate of each of the following fungi from *Dendrobium* blossom flecks was used: *Alternaria alternata* (Fr.) Keissler, *Bipolaris setariae* (Saw.) Shoemaker, *B. urochloae* (Putterill) Shoemaker, *B. sorokiniana* (Sacc. ex Sorok.) Shoemaker, *B. hawaiiensis* (Bugnicourt ex M. B. Ellis) Uchida & Aragaki comb. nov., *Exserohilum rostratum* (Drechsl.) Leonard & Suggs, *Curvularia* sp., *Stemphylium* sp., *Phyllostictina* sp., *Nigrospora* sp., and *Pestalotia* sp. [*Bipolaris hawaiiensis* is transferred from *Drechslera* because its conidial morphology and bipolar germination are consistent with Shoemaker's (11) description of *Bipolaris*. Thus:
Bipolaris hawaiiensis (Bugnicourt ex M. B. Ellis) Uchida & Aragaki comb. nov.
= *Drechslera hawaiiensis* (Bugnicourt) Subramanian & Jain ex M. B. Ellis, 1971, Dematiaceous Hyphomycetes, Commonw. Mycol. Inst., Kew, Surrey, England, p. 415
= *Drechslera hawaiiense* (Bugnicourt) Subramanian & Jain, 1966, Curr. Sci. 35:354
= *Helminthosporium hawaiiense* Bugnicourt, 1955, Rev. Gen. Bot. 62:238.]

Inoculations also were made with single isolates of *B. setariae* from leaf spots of paragrass (*Brachiaria mutica* [Forsk.] Stapf) and kikuyugrass (*Panicum clandestinum* Hochst. ex Chiov.) and from blighted macadamia (*Macadamia integrifolia* Maiden & Betche) racemes and with an isolate of *B. urochloae* from molassesgrass (*Melinis minutiflora* Beauv.). In addition, inoculations were made on both cultivars with six isolates of *Colletotrichum gloeosporioides* Penz.—three from *Dendrobium* flower flecks and one each from *Dendrobium* leaf spot, papaya anthracnose, and anthurium anthracnose. With the exception of the isolates noted above and the isolate of *B. sorokiniana* (from a fleck on a yellow *Dendrobium* hybrid flower), all fungi were obtained from the four cultivars listed in Tables 1 and 2. Inoculum for each fungus was prepared by growing cultures for 4–7 days under light as described and suspending resulting conidia in 1:2,000 Tween 20 solution. Conidial suspensions, adjusted to 10^4–10^5 conidia per milliliter, were sprayed onto the *Dendrobium* spikes until runoff. Inoculated spikes were incubated under moisture saturation for 24 hr at 22–24 C, then placed on the laboratory bench for 6 days to allow symptom development. Reisolations were made from induced flecks after this period. Control spikes were sprayed with 1:2,000 Tween 20 and incubated as described.

A single conidial isolate of *A. alternata* (688) was used to determine recoverability of the fungus in necrotic flecks. Cultures for inoculum were grown on VJA for 5 days under continuous

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fluorescent illumination as described. Conidial suspensions were adjusted to 2.5×10^4 spores per milliliter in 1:2,000 Tween 20. Spikes of cultivar Y-975 with at least one bud and averaging 15 open flowers were inoculated by spraying thoroughly until runoff, followed by moisture saturation for 24 hr at 22–24 C. Flecks were processed 3, 6, 10, 14, and 18 days after inoculation. Flecks (63–122 per time period) were dissected out, surface-sterilized with 0.5% sodium hypochlorite, and plated on water agar. The incidence of *A. alternata* recovery for each of the days was recorded. The test was repeated with a higher (5.0×10^4 spores per milliliter) inoculum level, and 105–112 flecks were dissected out for each time period.

RESULTS AND DISCUSSION

Fungal propagules were found in most naturally formed flecks on *Dendrobium* flowers (Table 1). An amerospore, dictyospore, or phragmospore was at or near the center of 41.7% of the flecks, distorted or fragmented spores and particles not readily distinguishable from fungal structures were associated with 34.4%, and no particulate matter or spores were observed in 23.8%. On the premise that flecks are host responses to certain fungi, we suggest that the inciting spores in the last group were lost by weathering or were dislodged in the laboratory fixing process.

Fungi were isolated from a small proportion of flecks (Table 2). *B. cinerea* and *A. alternata* were recovered from 0.6 and 11.4% of the flecks, respectively. The combined number of *B. setariae, B. urochloae, B. hawaiiensis,* and *E. rostratum* isolated was only 12, or 0.6% of the total number of lesions cultured. *C. gloeosporioides* was recovered from 7.4% of the lesions, and species of *Stemphylium, Curvularia, Nigrospora, Pestalotia, Phyllostictina,* and unidentified fungi made up the miscellaneous group recovered from 7.9% of the lesions. No fungi were isolated from 72.1% of the flecks, indicating a high incidence of aborted infections.

Necrotic flecks were produced by artificial inoculations with *A. alternata, B. setariae, B. urochloae, B. sorokiniana, E. rostratum,* and *Stemphylium* sp. and were reisolated. *B. hawaiiensis, Curvularia, Nigrospora, Pestalotia,* and *Phyllostictina* failed to induce flecks on *Dendrobium* flowers.

Among six isolates of *C. gloeosporioides,* only the one from anthurium anthracnose induced flecking, primarily on the labellum. The role of *C. gloesporioides* as an incitant of flecks on *Dendrobium* flowers is not clear, but its frequent recovery from field specimens and the pathogenicity of the isolate from anthurium suggest the fungus may be a part of the mycofloral complex involved in the flecks.

Infections by *A. alternata* were discernible in 2 days, and in 3 days, when the first specimens were taken, the flecks were 0.2 mm or less in diameter. Some continued to enlarge to approximately 1 mm in diameter. *A. alternata* was recovered from 40.5% of 3-day-old flecks but from only 4.1% of 6-day-old lesions (Table 3). The fungus was still recoverable at 18 days but from only 0.9% of flecks. This rapid reduction in recoverable *A. alternata* supports the idea that a high proportion of *Dendrobium* blossom flecks represent aborted fungal infections, as suggested earlier (8).

Engelhard (4) reported isolating an *Alternaria* sp. from small (1–3 mm), reddish-brown lesions of King aster and chrysanthemum petals and a *Helminthosporium* sp. from similar lesions on chrysanthemum. The symptoms closely resembled those incited by *Botrytis* spp. He subsequently reported (5,6) a similar disease (1–2 mm, reddish-brown lesions) on chrysanthemum caused by *B. setariae,* although the relationship to the previously reported disease caused by *Helminthosporium* was not discussed.

Although numerous graminicolous species of *Bipolaris* (=*Euhelminthosporium*) have been described on other monocotyledons and dicotyledons (9), they generally occur on Gramineae and their occurrence on nongramineous hosts is considered unusual (6). For example, ink disease of iris occurring as black markings on leaves and bulb scales is caused by *B. iridis* (Oud.) Dickinson (3,10). Leaf spot of gladiolus caused by *B. bicolor* (Mitra) Shoemaker (= *Drechslera bicolor* [Mitra] Subram. & Jain)

TABLE 1. Association of fungal spores with blossom flecks on four *Dendrobium* cultivars

	Percentage of flecks with fungal spores				
	D. Jacquelyn Thomas		*D.* Neo Hawaii	*D.* Tomie	All
Spore type[a]	O-580	UH-44	Y-975		cultivars
Dictyospore	12.5	68.8	35.7	16.7	34.2
Phragmospore	6.2	0	4.1	0	3.9
Amerospore	0	12.5	0	61.1	3.6
Not distinguishable[b]	25.0	12.5	38.1	11.1	34.4
No spores	56.2	6.2	22.0	11.1	23.8
Total number of flecks examined	32	16	291	18	357

[a] Dictyospores were probably *Alternaria alternata.* Phragmospores were *Bipolaris* spp. and *Exserohilum rostratum.* Amerospores were probably *Botrytis cinerea;* none approached the size and shape of *Colletotrichum gloeosporioides.*
[b] Distorted or fragmented spores and particles.

TABLE 2. Fungal isolations from blossom flecks on four *Dendrobium* cultivars

	Percentage of flecks yielding fungal growth				
	D. Jacquelyn Thomas		*D.* Neo Hawaii	*D.* Tomie	All
Fungus	O-580	UH-44	Y-975		cultivars
Alternaria alternata	11.1	8.6	12.1	5.8	11.4
Biopolaris spp.[a]	0.8	0.8	0.5	1.0	0.6
Botrytis cinerea	3.0	0.0	0.0	1.9	0.6
Colletotrichum gloeosporioides	18.3	3.9	5.3	2.9	7.4
Miscellaneous[b]	11.3	3.9	7.6	5.8	7.9
No fungal growth	55.4	82.8	74.5	82.5	72.1
Total number of flecks cultured	361	128	1,448	103	2,040

[a] *B. setariae, B. urochloae, B. hawaiiensis,* and *Exserohilum rostratum.*
[b] *Stemphylium, Curvularia, Nigrospora, Pestalotia, Phyllostictina,* and unidentified species.

was induced by isolates obtained from kikuyugrass (2). In our study all three isolates of *B. setariae* from paragrass, kikuyugrass, and macadamia and the single isolate of *B. urochloae* from molassesgrass produced flecks on *Dendrobium* flowers. Thus, it would not be surprising to find that *Alternaria* spp., *Bipolaris* spp., and other hyphomycetes cause restricted lesions on other ornamentals. This possibility has been suggested by Engelhard (6) based on artificial induction of flecks on carnation, geranium, and snapdragon.

In a previous study (8), no *Botrytis* was recovered from field-obtained flecks, although 5 days after artificial inoculation, *B. cinerea* was isolated from 14.7% of the flecks. In our study, *B. cinerea* was isolated from 1.9 and 3% of flecks from *D.* Tomie and cultivar O-580 of *D.* Jacquelyn Thomas, respectively, during a serious outbreak of blossom blight, supporting the suggestion that flecks can be induced by *Botrytis* spp. but that infections often abort after formation of flecks.

Among the hyphomycetes in these studies, *Botrytis* is the most important pathogen on *Dendrobium* because of its potential to cause blossom blight. Although blight does not occur when night temperatures rise above 25 C, blossom flecks continue to form and are especially troublesome in prolonged wet periods. The dominant fungus involved in *Dendrobium* flecks is *A. alternata,* but other dematiaceous hyphomycetes, ie, *B. setariae, B. urochloae, B. sorokiniana, E. rostratum,* and *Stemphylium* sp., should not be ignored as factors in the mycofloral complex of fleck formation in *Dendrobium.*

TABLE 3. Effect of time after inoculation on recovery of *Alternaria alternata* from artificially induced flecks on *Dendrobium* cultivar Y-975

Days after inoculation	Test 1[a]	Test 2	Combined recovery (%)
3	23/63[b]	45/105	40.5
6	3/110	6/112	4.1
10	0/122	3/112	1.3
14	2/120	1/111	1.3
18	2/120	0/105	0.9

[a] Inocula for tests 1 and 2 were 2.5×10^4 and 5.0×10^4 conidia per milliliter.
[b] Numerator represents number of *A. alternata* colonies reisolated, and denominator is number of flecks plated out.

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