Botrytis Blossom Blight of Dendrobium

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ABSTRACT

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A previously unreported Botrytis blossom blight on *Dendrobium* spp. is prevalent in Manoa Valley, Oahu, Hawaii. The disease was characterized by elliptical, water-soaked, translucent, brown lesions on petals and sepals which eventually spread to cover large areas of the blossoms. *Botrytis* spp. isolates recovered from lesions were segregated into three groups on the basis of spore morphology. Group I isolates with ellipsoidal spores (10.0 × 7.7 μm) were identified as *B. cinerea*. Group II isolates were mostly ellipsoidal, large spored (17.3 × 12.7 μm), but also produced from 1-6% two-celled (19.9 × 12.0 μm) spores. Group III isolates also were mostly ellipsoidal and large spored (19.6 × 10.8 μm), but 3-14% were turbinate (15.4 × 14.6 μm). Spore germination and growth of all three types were optimal at 20-24 C. All isolates caused similar symptoms on *Dendrobium* by infecting buds and young (as well as mature) flowers. Of the three groups, *Botrytis cinerea* was the least virulent. A flecking response following artificial inoculation was attributed to aborted infection by *Botrytis* spp.

A blossom spotting first was noted in February 1974 on two hybrid cultivars of *Dendrobium* spp. orchid growing in Manoa Valley, Oahu, Hawaii. Cultivar 0-580 of the artificial hybrid *Dendrobium* Jacquelyn Thomas (*Dendrobium phalaenopsis* × *D. gouldii*) seemed to be more susceptible than cultivar Y-975 of *Dendrobium* Neo Hawaii (*D. phalaenopsis* × *D. grantii*). The disease was characterized by elliptical, watersoaked, brown lesions (5-15 mm in length) on petals and sepals which eventually rotted (Fig. 1).

Botrytis flower spotting has been reported on *Cymbidium* sp., *Cattleya* sp., *Cypripedium* sp., *Vanda* sp., and *Phalaenopsis* sp. (4, 5, 10, 15), but not on *Dendrobium* sp. *Botrytis cinerea* Pers. ex Fr., as well as *Alternaria* sp. and *Colletotrichum* sp. (1, 4, 6), have been implicated as causal agents of orchid blossom blight (10, 11).

In the present study, etiology of the disease with respect to involvement of several types of *Botrytis* isolates was investigated.

MATERIALS AND METHODS

On several occasions, isolations were made from blossom-blighted *Dendrobium* spp. 'O-580' and 'Y-975' specimens collected at one location in Manoa, Oahu, Hawaii. No relationship was discerned between host cultivars and distribution of *Botrytis* types. The pathogenicity and conidial characteristics of 27 isolates were determined, and more detailed studies were made on single-spored cultures of three representative isolates [isolate 347 (*B. cinerea*), 346 (two-celled spore type), and 348 (turbinate-spored type)].

Spores were harvested from cultures grown for 7 days on V-8 juice agar (VJA) (10% Campbell's V-8 juice, 0.2% $CaCO_3$, and 1.8% agar) at 20 C under continuous light (2,200 lux) from cool-white fluorescent lamps for all tests. Spore suspensions for inoculum were filtered through two layers of tissue paper to remove mycelial fragments, and the suspension in 1:2,000 Tween-20 solution was adjusted to 5×10^3 spores/ml. Detached spikes of cultivar Y-975 with open blooms and buds were sprayed with the spore suspension to runoff with an aerosol sprayer, covered with plastic bags to insure moisture saturation, and incubated for 18 hr at 20 C. Control spikes were sprayed with 1:2,000 Tween-20 solution. Bags then were removed and spikes were kept at room temperature (22-25 C). Disease incidence was assessed by counting the number of spots and flecks on the five youngest open blooms of each spike 4 days after inoculation. Each treatment group consisted of five spikes. For temperature-effect studies, inoculated spikes were incubated at 16, 20, 24, and 28 C. For determination of incubation time, inoculated spikes were incubated for 0, 2, 4, 6, 8, 12, 18, and 24 hr. To determine the effect of different levels of inoculum, spore suspensions were adjusted to concentrations of 10, 10^2, 10^3, 5×10^3, 10^4, and 10^5 spores/ml, and applied to three spikes per inoculum level.

Flecks resulting from artificial inoculation and flecks from flowers from the field were surface-sterilized in 0.6% sodium hypochlorite and cultured on water agar. Isolations were made by taking hyphal tips, which were placed on VJA until the isolates could be identified.

Single-spore transfers of one-celled or two-celled spores of isolate 346 were made for six consecutive cultural generations, and the distribution of spore types was determined. Distribution of spore type of isolate 348 after six consecutive single-spore generations of each spore type (turbinate and ellipsoidal) also was examined. All cultures were established on VJA and incubated at 20

C under continuous light until good sporulation had occurred (6-12 days). At least 300 spores were examined for each of three to 12 single-spored cultures of each generation.

RESULTS

Isolations made from two cultivars of *Dendrobium* spp. yielded mostly cultures of *Botrytis* spp.; a few were *B. cinerea*, but the majority were not. Natural sporulation was not observed on any of the blighted specimens collected from the field. Twenty-seven isolates of *Botrytis* spp. were obtained which were segregated into three groups on the basis of spore morphology. Two isolates (Group I) were *B. cinerea* (ellipsoidal spores 10.0 × 7.7 μm, Fig. 2-A, B); 13 were in Group II which had more than 94% ellipsoidal spores (17.3 × 12.7 μm), and the remaining (1-6%) were two-celled (19.9 × 12.0 μm) (Fig. 2-C, D); 12 in Group III, had spores (19.6 × 10.8 μm) similar to the single-celled ellipsoidal spores of Group II, but 3-14% of them were turbinate (15.4 μm in length and 14.6 μm at the widest point, Fig. 2-E, F). The turbinate spores had a wide array of shapes, ranging from nearly obovoid, cuneiform, turbinate, to distinctly lobate forms. Conidial measurements of representative isolates of each group are given in Table 1. Sclerotia produced by isolates of the three groups were similar on VJA, being ellipsoidal, 2-4 × 1-3 mm.

Artificial inoculations with each of the 27 isolates caused similar symptoms on flowers and buds of cultivar Y-975. Infection was first evident as pinhole-sized clear areas on petals and sepals after 18 hr of incubation. Three days after inoculation, tissues appeared water-soaked within a 2-mm radius around the point of infection. These areas enlarged and formed brown spots typical of those observed in the field. Many of the pinhole lesions, however, did not enlarge, and 6 days after inoculation were brown, restricted, or water-soaked flecks of less than 1 mm in diameter.

Botrytis cinerea was the least virulent; it caused flecking but only a few spots on blossoms inoculated at a concentration of 5×10^3 spores/ml. However, at higher inoculum levels (10^5 spores/ml), *B. cinerea* isolates incited typical spreading spots (Table 2). Virulence of all isolates was optimum at 20 and 24 C but was greatly reduced at 28 C (Table 3).

Incubation times of up to 24 hr at 100% relative humidity did not induce spot development by *B. cinerea* applied at 5×10^3 spores/ml, although the number of flecks increased with time (Table 4). Infection which progressed into brown spot development took place within 8 hr after inoculation with isolates 346 and 348 and increased with incubation time for up to 24 hr.

The respective *Botrytis* spp. types, but no other fungi, were reisolated from spreading spots after inoculation. Isolations from both restricted and water-soaked flecks also were attempted. Isolations from 143 tissue pieces (approximately 4 mm^2) with one or two flecks 5 days after inoculation with *B. cinerea* yielded 21 *Botrytis cinerea*, one *Alternaria* sp., one *Helminthosporium* sp., and three unidentified nonsporulating fungal colonies. Twenty-two single flecks (pieces approximately 1 mm^2) from flowers inoculated with isolate 346 (two-celled) were cultured and one isolate of *Botrytis* sp. resembling isolate 346 was recovered; similarly, 57 single flecks from flowers

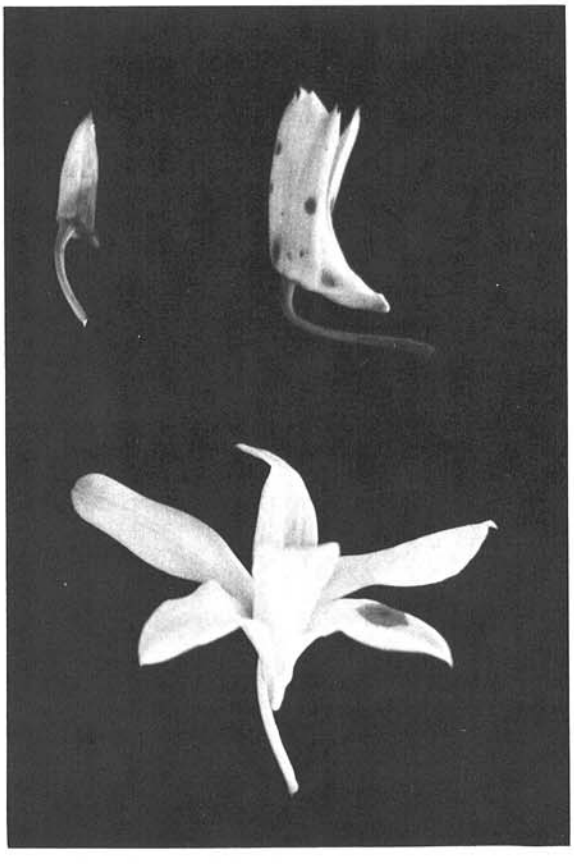

Fig. 1 Botrytis blight of *Dendrobium* sp. Spots on buds and blossom.

TABLE 1. Measurements of conidia of representative isolates of *Botrytis* spp

	Spore dimensions of isolate no.		
	347	346	348
Conidium type	(*B. cinerea*) (μm)	(two-celled) (μm)	(turbinate) (μm)
Ellipsoidal[a]			
length	10.1 ± 1.1	17.3 ± 2.8	16.6 ± 1.8
diameter	7.7 ± 0.9	12.7 ± 1.6	10.8 ± 1.1
Two-celled			
length		19.9 ± 2.1	
diameter		12.0 ± 1.1	
Turbinate			
length			15.4 ± 2.1
diameter			14.6 ± 2.4

[a]Cultures of isolates 346 and 348 which are characterized by two-celled and turbinate spores, respectively, produce mostly ellipsoidal single-celled spores.

[b]Mean and standard deviation of 100 spores of each type in μm.

TABLE 2. Inoculum level effects on incidence of spots or flecks produced on *Dendrobium* sp. by *Botrytis* spp

Spore conc.[a]	347 (*B. cinerea*)		346 (two-celled)		348 (turbinate)	
	Spots	Flecks	Spots	Flecks	Spots	Flecks
0	0[b]	1	0	1	0	1
10	0	2	0	2	0	1
10^2	0	16	6	12	1	10
10^3	0	47	24	63	14	45
5×10^3	0	120	115	217	15	88
10^4	1	343	63	317	28	367
10^5	11	533	...	...	...	...

[a]Spores per ml/ 1:2,000 Tween-20.
[b]Mean number of spots or flecks per spike. Spikes were incubated under moisture saturation at 20 C for 18 hr. The five youngest open flowers of each of three spikes were examined 4 days after inoculation.

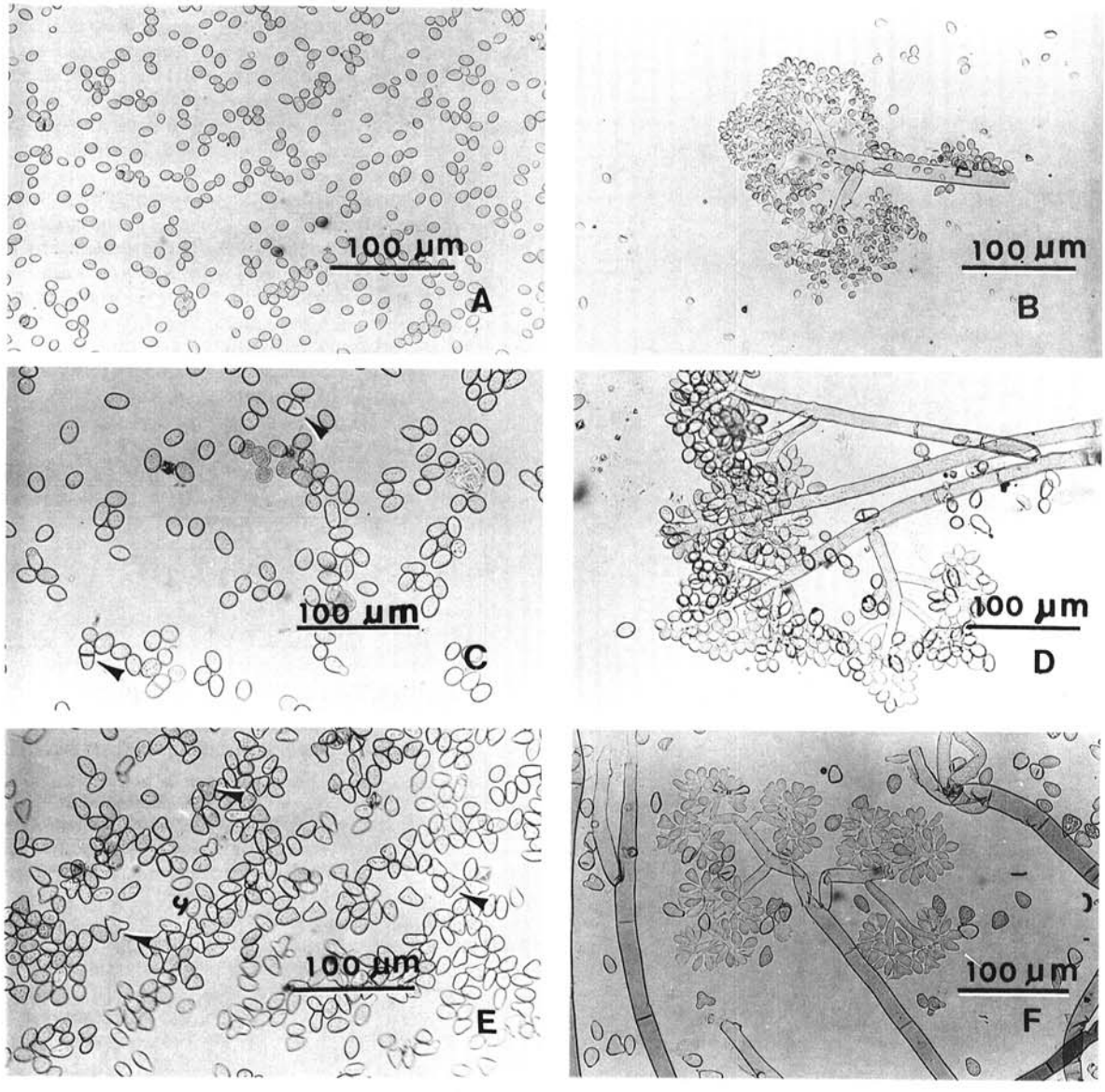

Fig. 2-(A to F). Conidia and conidiophores of *Botrytis* spp. **A, B)** Isolate 347 (*B. cinerea*); **C, D)** Isolate 346 (arrows pointing to two-celled spores) **E, F)** Isolate 348 (arrows pointing to lobate, turbinate spores).

inoculated with isolate 348 (turbinate) were cultured and one isolate of *Botrytis* sp. resembling isolate 348 was recovered. No other fungi were isolated from these flecks induced by artificial inoculations. Isolations from 108 natural flecks yielded several fungi, but none consistently. No *Botrytis* sp. was recovered from these naturally formed flecks.

The spore-type distribution of isolates 346 and 348 following serial transfers of single spores was unchanged from that of the original isolates (regression coefficient was not significantly different from 0 at $P = 0.05$) irrespective of the spore type selected.

DISCUSSION

The isolates from *Dendrobium* spp. fit the genus *Botrytis* as described by Persoon (14), Buchwald (2), Ellis (3), and Hennebert (7). Spore characteristics of *B. cinerea* isolates from *Dendrobium* spp. agree with published descriptions of the species (3, 13). The turbinate and two-celled isolates of *Botrytis*, most commonly isolated from blossom blight of *Dendrobium*, are distinct from *B. cinerea*, but these organisms were not identified. Four *Botrytis* spp. have at least some resemblance to the present isolates. *Botrytis hyacinthi* and *B. tulipae*, which may be conspecific according to Morgan (13) are characterized by ovate conidia and conidiophores with swollen basal cells which are distinct from the two-celled and turbinate-spored isolates. Conidiophores of the other two species, *B. fabae* and *B. squamosa*, are much shorter than present isolates. Further studies are needed to establish the identity of the large two-celled or turbinate-spored isolates.

Botrytis spp. conidia are described as globose to obovate to elliptical in shape. A turbinate spore of *B. tulipae* was depicted by Hopkins (9); Hickman and Ashworth (8) also have observed occasional turbinate spores of *B. squamosa*, but turbinate or septate conidia are not common in *Botrytis* spp. The turbinate and two-celled spores in certain isolates from *Dendrobium* sp. comprise small percentages of the population, but the relative numbers have been seen to be persistent in serial transfers, and therefore useful in identification of these isolates.

Botrytis cinerea usually is considered to be a weak pathogen and occurs commonly on blossoms and senescent tissues of many different hosts under favorable conditions of high humidity and low temperatures (5, 10, 11, 15). On *Dendrobium* spp. infection occurred on buds and young flowers, indicating pathogenicity of higher order, somewhat similar to that reported by McClellan and Hewitt (12) for Botrytis rot of grapes. Spot development on *Dendrobium* spp., however, occurred only at higher inoculum levels and the incidence of *B. cinerea* in naturally infected blossoms was low also (two of 27 isolates recovered); thus *B. cinerea* is of only minor importance in this disease complex.

With the exception of several unidentified fungi and single occurrences of *Helminthosporium* sp. and *Alternaria* sp., no fungi other than *Botrytis* spp. were isolated from blossom flecks on *Dendrobium* sp. The incidence of flecks was highly correlated with increased inoculum levels and incubation times, although recovery of *Botrytis* spp. from flecks induced by artificial inoculation was low. This suggests that the flecks were induced by *Botrytis* spp., but probably represent aborted

TABLE 3. Temperature effects on incidence of spots or flecks produced on *Dendrobium* sp. by *Botrytis* spp[y]

Temp	374 (*B. cinerea*)		346 (two-celled)		348 (turbinate)	
(C)	Spots	Flecks	Spots	Flecks	Spots	Flecks
16	0[z]	14ab	7ab	207b	0a	31b
20	0	112c	40c	214b	9a	62b
24	0	34b	23bc	180b	7a	43b
28	0	1a	0a	1a	0a	0a

[y]Inoculum was 5×10^3 spores/ml in 1:2,000 Tween-20; spikes were incubated for 18 hr at 100% relative humidity and 20 C.
[z]Mean number of spots or flecks per spike. The five youngest open flowers of each of five spikes were examined. Means in the same column with same subscript are not significantly different $P = 0.05$.

TABLE 4. Incubation duration effects on incidence of spots or flecks produced on *Dendrobium* sp. by *Botrytis* spp

Hours[a]	374 (*B. cinerea*)		346 (two-celled)		348 (turbinate)	
	Spots	Flecks	Spots	Flecks	Spots	Flecks
0	0[b]	0	0	0	0	0
2	0	2	0	0	0	0
4	0	3	0	0	0	1
6	0	5	0	18	0	14
8	0	10	1	50	6	122
12	0	17	12	217	7	120
18	0	133	40	310	4	70
24	2	333	58	383	52	208
Control[c]	0	1	0	7	0	1

[a]Hours of incubation at 20 C and 100% relative humidity after inoculation with 5×10^3 spores/ml suspended in 1:2,000 Tween-20.
[b]Mean number of spots or flecks per spike. The five youngest flowers of each of three spikes were examined.
[c]Sprayed with 1:2,000 Tween-20 and incubated for 24 hr.

infections. Similar flecking symptoms attributable to *B. cinerea* have been reported on *Cymbidium* spp. (10).

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