Effects of Long-Wave UV Light on *Monilinia* Growth and Identification of Species

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ABSTRACT

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The effect of long-wave UV/dark period on mycelial growth of 46 isolates of *Monilinia* sp. collected in Spain and 16 isolates collected from other parts of the world was investigated. Typical isolates of *M. laxa*, *M. fructigena*, and *M. fructicola* were grown in the dark and identified by morphological characteristics. Long-wave UV/dark conditions reduced the growth rates of *M. laxa*, *M. fructigena*, and *M. fructicola* on potato dextrose agar. All isolates of *M. fructigena* grew more slowly than those of *M. fructicola*. Typical and atypical isolates of *M. fructigena* and *M. fructicola* were placed in their respective species based on long-wave UV/dark growth rate data. *M. laxa* isolates were readily distinguished by the short distance from their conidium to the first germ tube branch. The involvement of different photoreceptors in photoresponses by *M. fructicola* and *M. fructigena* is discussed. Differences in mycelial growth under long-wave UV may be a useful tool to identify *Monilinia* spp.

Additional keywords: brown rot of fruit, classification, quarantine organism, taxonomy, wavelength

Brown rot of stone and pome fruit is caused by species of the genus *Monilinia*, *M. laxa*, *M. fructigena*, and *M. fructicola* (2). These fungi infect aerial parts of host plants inducing blight of blossoms and leaves, cankers on woody tissues, and rot of fruits.

The brown rot fungi cause economically important losses in most temperate regions of the world. *M. laxa* and *M. fructigena* were initially European species. However, the distribution area of *M. laxa* now covers most regions where stone and pome fruits are cultivated. *M. fructigena* has been found outside Europe in only a few cases. *M. fructicola* is restricted to Australia, South Africa, and North and South America, and is a European quarantine listed organism (7).

Identification of *Monilinia* spp. is based on cultural and morphological characters (2). *M. fructicola* is distinguished from *M. laxa* in having entire colony margins, by color of conidia, absence of hyphal anastomoses between germinating conidia, and longer conidial germ tube extension before branching (17,18,21). *M. fructicola* produces more abundant conidia, stromata, and spermatia, and has slightly smaller conidia and more narrow hyphal diameter than *M. fructigena* (17,19). *M. fructigena* has entire colony margins, different conidium color, and longer conidium germ tubes than *M. laxa* (18,19). These characteristics assist in the identification of typical cultures of *Monilinia* spp., but problems arise with atypical cultures. Many differences have been observed among isolates of the same species (1,2,11,25). This variability is especially important when quarantine measures apply to one species but not the other. More reliable and stable characteristics are needed to clearly distinguish one species from another.

Growth, stromatal formation, and sporulation of the three species are affected by illumination (11). The effect of alternate periods of light/dark on growth and sporulation of the three species has been previously studied, especially using daylight (6,10,11,14,26) and different wavelengths from 440 to 760 µm, but not the ultraviolet range (12,14). However, these studies were not carried out for species identification.

The aim of this work was to determine the influence of long-wave UV light on mycelial growth of *Monilinia* spp. and its usefulness in differentiating *Monilinia* species.

MATERIALS AND METHODS

Isolates. Forty-six isolates of *Monilinia* spp. were collected from several hosts in different orchards in Spain (Spanish Collection) (Table 1). Mass isolation of the fungus was made in potato dextrose agar (PDA) amended with 0.5 g of streptomycin sulfate per liter. Sixteen isolates of presumed *Monilinia* spp. from different parts of the world were also used (World Collection) (Table 2). All isolates were grown in 9-cm-diameter plastic petri dishes containing 12.5 ml of PDA (Oxoid Ltd., Basingstoke, Hampshire, Eng.) at 22°C in the dark for conidial and mycelial production. All isolates were stored on PDA slants at 4°C in the dark.

Identification of isolates. After 10 days incubation on PDA, the following characteristics were recorded for each isolate: presence or absence of conidia, concentric rings of sporulation, lobed colonies, black rings associated with lobes, and color of the sporogenous tissue. To produce conidia for germination studies, isolates were grown on PDA or PDA amended with 1% acetone for 10 days in the dark at 22°C, as recommended by Pascual et al. (20). Colonies that did not produce any sporulation were maintained at 5 to 10°C for 60 days, as recommended by Masri (16) and Tamm and Flückiger (23). Conidia were removed from the surface of the colony with a sterile scalpel, suspended in sterile distilled water, and sonicated for 1 min. A 100-µl droplet of the conidia suspension (10^3 conidia/ml) was spread on petri dishes containing distilled water agar (DWA, 2% agar, Oxoid). These dishes were incubated for 24 h at 22°C in the dark. Three replicates were made per isolate. The distance in micrometers from the conidium to the first germ tube branch was measured for 25 conidia, using Zeiss optical microscopy (×100) equipped with an ocular micrometer. A conidia was considered germinated when the germ tube was longer than the length of the conidia. This procedure was performed twice.

Effect of long-wave UV light on mycelial growth. PDA dishes were centrally inoculated with 4-mm-diameter plugs from the margin of 4-day-old PDA cultures. Six dishes were used for each isolate. Three dishes were wrapped with aluminum foil and placed in unlighted incubators at 22°C. The other three plates were incubated at 16-h long-wave UV/8-h dark photoperiod under a Phillips TLD 18W/08 light (black light 320 to 380 nm). These plates were placed approximately 15 cm from the light source. Temperatures were maintained at 18°C in the dark and 22°C in the light. Colony growth in each replicate plate was recorded 3 and 5 days after inoculation by measuring two perpendicular diameters. Growth rates (difference between diameters at days 5 and 3) were calculated for each replicate in both incubation conditions. The experiment was conducted once for the Spanish collection and twice for the world collection. The effect of long-wave UV light was assessed by calculating the percent inhibition of colony growth rate (PIGR) for each isolate using the formula

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PIGR = [1 – (GR_L/GR_D)] × 100, where GR_L is the growth rate in 16-h long-wave UV/8-h dark, and GR_D is the growth rate in the dark conditions, respectively.

Data analysis. Distance from conidia to first germ tube branch, colony growth rate, and PIGR were analyzed by protected analysis of variance, and means were compared by a least significant difference (LSD) test at $P = 0.05$. Distance from conidia to first germ tube branch and growth rate were x/Σx transformed before analysis to improve homogeneity of variances. PIGR was arcsine transformed.

RESULTS

Identification of isolates. Typical isolates of *M. laxa*, *M. fructigena*, and *M. fructicola* were identified on PDA by the cultural and morphological characteristics described previously. It was not possible to identify atypical isolates (W-1, W-9, and W-12) by the cultural and morphological characteristics. Isolate W-12 did not sporulate.

The distance from the conidium to the first germ tube branch in DWA clearly separated isolates into two groups (Tables 1 and 2). In the first group, germ tubes branched soon after conidia germination. The distance from the conidium to the first germ tube branch was less than 60 µm. Isolates within this group are considered to be *M. laxa* (2,8,13,18,27). There were 36 Spanish isolates (ES-1 to ES-40, ES-50, and ES-52) and five world isolates (W-3, W-6, W-8, W-10, and W-15) in this group. Only 26 *M. laxa* isolates of the Spanish collection and four of the world collection produced colonies of lobed margins (Tables 1 and 2), one of the characteristics defining this species. Sporogenous tissue of the Spanish *M. laxa* isolates were gray or gray/buff. Sporogenous tissue of the isolates from the world collection identified as *M. laxa* was gray or gray-buff, except for isolate W-15, which was buff.

The isolates in the second group had germ tubes that grew as single, almost straight hyphae, at least 220 µm long before branching, sometimes reaching 960 µm when this group was further characterized using the morphological characteristics described above. Differences between isolates were evident, particularly with regard to color of the sporogenous tissue and the characteristics of the margins of colonies (Tables 1 and 2). Among these isolates, ES-41 to Es-49, ES-51, W-13, W-14, W-17, and

Table 1. Characteristics of *Monilinia* isolates from Spanish collection

Isolate	Host	Year of isolation	Origin	Species	Color of sporogenous tissues[a]	Colony margin lobed[a,b]	Germ tube branch (µm)[a]	Growth rate 24-h dark (mm)[a]	Growth rate 16-h black light/8-h dark (mm)[a]
ES-1	Cherry	1988	Salamanca	*laxa*	Gray	+	29 (0.0030)	11.83 (0.0076)	8.33 (0.0140)
ES-2	Cherry	1988	Guadalajara	*laxa*	Gray	+	25 (0.0026)	17.67 ((0.0113)	11.17 (0.0188)
ES-3	Apricot	1988	Madrid	*laxa*		+	29 (0.0026)	10.67 (0.0068)	4.67 (0.0078)
ES-4	Cherry	1990	Cáceres	*laxa*		+	21 (0.0021)	6.17 (0.0039)	1.67 (0.0028)
ES-6	Apricot	1988	Toledo	*laxa*		+	46 (0.0047)	16.00 (0.0103)	6.00 (0.0101)
ES-7	Peach	1987	Zaragoza	*laxa*	Gray	+	18 (0.0019)	10.17 (0.0065)	2.67 (0.0044)
ES-8	Peach	1988	Madrid	*laxa*	Gr/bu	+	30 (0.0030)	18.83 (0.0121)	2.83 (0.0047)
ES-9	Peach	1988	Madrid	*laxa*	Gray	+	37 (0.0038)	13.67 (0.0088)	1.17 (0.0019)
ES-10	Peach	1988	Madrid	*laxa*	Gr/bu	–	23 (0.0023)	15.50 (0.0099)	7.50(0.0126)
ES-12	Apricot	1996	Zaragoza	*laxa*	Gr/bu	+	29 (0.0029)	12.83 (0.0082)	3.67 (0.0061)
ES-13	Apricot	1996	Zaragoza	*laxa*	Gr/bu	+	22 (0.0023)	10.17 (0.0065)	2.33 (0.0039)
ES-14	Apricot	1996	Murcia	*laxa*		+	40 (0.0041)	7.67 (0.0049)	9.83 (0.0165)
ES-15	Almond	1996	Murcia	*laxa*	Gr/bu	+	25 (0.0025)	4.17 (0.0026)	2.83 (0.0047)
ES-16	Apricot	1996	Murcia	*laxa*	Gray	+	36 (0.0037)	6.50 (0.0041)	0.83 (0.0014)
ES-17	Apricot	1996	Murcia	*laxa*		+	28 (0.0029)	13.17 (0.0084)	9.10 (0.0153)
ES-18	Apricot	1996	Murcia	*laxa*		+	36 (0.0037)	10.83 (0.0069)	5.75 (0.0096)
ES-19	Apricot	1996	Murcia	*laxa*	Gr/bu	+	26 (0.0026)	10.50 (0.0067)	4.33 (0.0072)
ES-21	Almond	1996	Murcia	*laxa*	Gr/bu	+	35 (0.0036)	14.33 (0.0092)	4.50 (0.0075)
ES-22	Apricot	1996	Murcia	*laxa*	Gr/bu	+	28 (0.0029)	3.83 (0.0024)	7.00 (0.0117)
ES-23	Almond	1996	Murcia	*laxa*	Gr/bu	–	24 (0.0024)	21.00 (0.0135)	8.50 (0.0143)
ES-24	Apricot	1996	Murcia	*laxa*		+	26 (0.0027)	4.00 (0.0025)	0.17 (0.0002)
ES-25	Apricot	1996	Murcia	*laxa*	Gray	+	38 (0.0039)	5.83 (0.0037)	0.00 (0.0)
ES-27	Apricot	1996	Murcia	*laxa*	Gr/bu	–	32 (0.0033)	3.00 (0.0019)	5.83 (0.0098)
ES-28	Apricot	1996	Murcia	*laxa*	Gr/bu	+	22 (0.0023)	8.00 (0.0051)	5.17 (0.0087)
ES-30	Apricot	1996	Murcia	*laxa*	Gr/bu	+	26 (0.0026)	19.67 (0.0126)	1.00 (0.0016)
ES-31	Apricot	1996	Murcia	*laxa*		–	21 (0.0022)	12.33 (0.0079)	6.33 (0.0106)
ES-34	Apricot	1996	Murcia	*laxa*	Gr/bu	–	23 (0.0023)	4.67 (0.0030)	8.17 (0.0137)
ES-35	Almond	1996	Murcia	*laxa*		+	35 (0.0036)	7.67 (0.0049)	11.33 (0.0190)
ES-36	Apricot	1996	Murcia	*laxa*	Gr/bu	+	23 (0.0024)	12.17 (0.0078)	4.33 (0.0072)
ES-37	Apricot	1996	Murcia	*laxa*		+	22 (0.0023)	9.33 (0.0060)	6.33 ((0.0106)
ES-38	Almond	1996	Murcia	*laxa*	Gr/bu	–	26 (0.0027)	17.00 (0.0109)	8.67 (0.0146)
ES-39	Apricot	1996	Pamplona	*laxa*	Gr/bu	–	30 (0.0031)	9.33 (0.0060)	5.00 (0.0084)
ES-40	Peach	1996	Pamplona	*laxa*	Gr/bu	–	24 (0.0025)	14.17 (0.0091)	5.83 (0.0098)
ES-41	Apple	1996	La Coruña	*fructigena*	Buff	–	400 (0.041)	8.17 (0.052)	1.33 (0.0022)
ES-42	Pear	1996	La Coruña	*fructigena*		–	500 (0.0513)	4.67 (0.0030)	0.00 (0.0)
ES-43	Pear	1996	La Coruña	*fructigena*		+	500 (0.0513)	4.67 (0.0030)	0.00 (0.0)
ES-44	Peach	1996	La Coruña	*fructigena*	Buff	–	312 (0.0320)	20.67 (0.0133)	0.17 (0.0002)
ES-45	Peach	1996	La Coruña	*fructigena*	Buff	–	242 (0.0249)	13.17 (0.0084)	3.17 (0.0053)
ES-46	Peach	1996	La Coruña	*fructigena*	Gr/bu	+	500 (0.0513)	5.17 (0.0033)	1.00 (0.0016)
ES-47	Peach	1996	La Coruña	*fructigena*		+	500 (0.0513)	3.50 (0.0022)	0.00 (0.0)
ES-48	Plum	1996	La Coruña	*fructigena*	Buff	–	233 (0.0239)	17.67 (0.0113)	1.17 (0.0019)
ES-49	Plum	1996	La Coruña	*fructigena*		–	283 (0.0290)	9.83 (0.0063)	0.00 (0.0)
ES-50	Almond	1996	Zaragoza	*laxa*	Buff	–	26 (0.0027)	9.17 (0.0059)	6.50 (0.0109)
ES-51	Apple	1997	Zaragoza	*fructigena*		–	231 (0.0237)	23.00 (0.0148)	0.17 (0.0002)
ES-52	Almond	1997	Zaragoza	*laxa*		+	24 (0.0025)	15.52 (0.0099)	7.17 (0.0120)
ES-231	Almond	1996	Murcia	*laxa*	Gr/bu	–	29 (0.0030)	19.17 (0.0123)	6.33 (0.0106)
LSD ($P = 0.05$)							(0.0126)	(0.0034)	(0.0064)

[a] Data of color of the sporogenous tissue, presence of lobed colonies, germ tube branch, and growth rate are the means of three replicates. gr = gray, bu = buff. Data in brackets were (x/Σx) normalized before analysis.

[b] None of the isolates exhibited concentric rings of sporulation.

Table 2. Characteristics of *Monilinia* isolates from the world collection growing in the darkness

Isolate	Host	Origin	Year of isolation	Species	Color of sporogenous tissues[a]	Concentric rings of sporulation[a]	Colony margin lobed[a]	Germ tube branch (µm)[a]
W-1	Apple	Japan	1989	*fructicola*	Gr/bu	–/+	+	226 (0.011)
W-2	Apricot	New Zealand	1994	*fructicola*	Gr/bu	–/+	–	953 (0.047)
W-3	Peach	Italy	1992	*laxa*	Gray	–	–/+	30 (0.001)
W-6	Almond	USA	…	*laxa*	Gr/bu	–	+	34 (0.002)
W-7	Plum	USA	1994	*fructicola*	Gr/wh	–/+	–	767 (0.036)
W-8	Apricot	Australia	1978	*laxa*	Gray	–	+	31 (0.002)
W-9	*Prunus* sp.	Australia	1971	*fructigena*	Gr/bu	–	+	783 (0.032)
W-10	Peach	South Africa	1996	*laxa*	Gray	–	+	36 (0.002)
W-11	Plum	USA	1996	*fructicola*	Gray	–/+	–	721 (0.034)
W-12	Apple	UK	1969	…	…	–	–	…
W-13	Quince	Portugal	1996	*fructigena*	White	–	–/+	564 (0.026)
W-14	Apple	Japan	1995	*fructigena*	Buff	–	–	734 (0.033)
W-15	Plum	Japan	1989	*laxa*	Buff	–	–	55 (0.003)
W-16	Peach	Japan	1992	*fructicola*	Gr/bu	–/+	–	801 (0.038)
W-17	Plum	Poland	1993	*fructigena*	White	–	+	389 (0.019)
W-18	Peach	Netherlands	1996	*fructigena*	Wh/bu	–	–/+	499 (0.025)
LSD (P = 0.05)								(0.008)

[a] Data of concentric rings of sporulation, color of the sporogenous tissue, presence of lobed colonies, and germ tube branch are the means of three replicates. gr = gray, bu = buff, wh = white. Data in brackets were (x/Σx) normalized before analysis.

Table 3. Growth rate (mm) of *Monilinia* isolates from the world collection grown on potato dextrose agar (PDA) in 16-h black light/8-h dark and 24-h dark in two experiments

	16-h black light/8-h dark		24-h dark	
Isolate	Experiment 1	Experiment 2	Experiment 1	Experiment 2
W-1	11.67 (0.0158)[a]	18.50 (0.0251)	13.83 (0.0087)	25.00 (0.0158)
W-2	16.30 (0.0221)	20.00 (0.0272)	22.33 (0.0141)	25.67 (0.0162)
W-3	5.00 (0.0068)	10.00 (0.0136)	17.33 (0.0109)	15.33 (0.0097)
W-6	8.50 (0.0115)	11.33 (0.0154)	12.33 (0.0078)	11.33 (0.0071)
W-7	13.17 (0.0179)	15.50 (0.0211)	25.50 (0.0161)	24.83 (0.0157)
W-8	8.50 (0.0115)	8.17 (0.0111)	11.67 (0.0073)	13.50 (0.0085)
W-9	2.83 (0.0038)	3.33 (0.0045)	8.50 (0.0053)	11.00 (0.0069)
W-10	8.17 (0.0111)	7.50 (0.0102)	6.17 (0.0039)	9.50 (0.0060)
W-11	9.33 (0.0127)	14.67 (0.0199)	23.67 (0.0149)	20.83 (0.0132)
W-13	0.17 (0.0002)	1.50 (0.0020)	3.00 (0.0019)	4.50 (0.0028)
W-14	3.33 (0.0045)	7.67 (0.0104)	11.17 (0.0070)	22.17 (0.0140)
W-15	1.67 (0.0022)	5.67 (0.0077)	10.17 (0.0064)	5.67 (0.0035)
W-16	13.00 (0.0176)	17.33 (0.0235)	27.33 (0.0173)	31.00 (0.0196)
W-17	0.0 (0.0)	1.5 (0.0020)	5.67 (0.0035)	6.17 (0.0039)
W-18	0.17 (0.0002)	0.5 (0.0006)	3.33 (0.0021)	2.83 (0.0017)
LSD (P = 0.05)	(0.0061)	(0.0062)	(0.0020)	(0.0026)

[a] Data are the means of three replicates. Colony growth was recorded 3 and 5 days after inoculation by measuring two perpendicular diameters in each replicate. Growth rate (difference between diameters at days 3 and 5) was calculated for each replicate under both sets of incubation conditions. Data in brackets were (x/Σx) normalized before analysis.

W-18 were identified as *M. fructigena*, while isolates W-2, W-7, W-11, and W-16 were identified as *M. fructicola*.

Effect of long-wave UV light on mycelial growth. Growth rates of all isolates were significantly affected by long-wave UV (Tables 1 and 3). Growth in the dark was faster (P = 0.05) than growth under long-wave UV/darkness. The mean growth rates for *M. laxa* were 11.3 ± 0.82 and 5.36 ± 0.5 (Spanish collection), and 11.53 ± 1.8 and 6.37 ± 1.34 (world collection) in the dark or long-wave UV/dark, respectively. For *M. fructigena*, growth rates were 11.05 ± 2.27 and 0.7 ± 0.32 (Spanish collection) and 6.33 ± 1.55 and 1.3 ± 0.73 (world collection) in the dark or long-wave UV/dark, respectively. For *M. fructicola*, growth rates were 22.53 ± 2.33 and 11.83 ± 1.86 (world collection) in the dark or long-wave UV/dark, respectively. Growth rates of *M. fructicola* were faster than those of *M. fructigena* or *M. laxa* under both test conditions. However, there were some Spanish isolates of *M. fructigena* (ES-44 and ES-51) and *M. laxa* (ES-23) with similar growth rates to those of *M. fructicola*, when grown in the dark. Under alternating long-wave UV/darkness conditions, all the isolates of *M. fructigena* grew more slowly (up to 8 mm) than those of *M. fructicola.*

The PIGR of *M. fructigena* by the long-wave UV/dark cycle was the highest (93 and 80% in Spanish and world isolates, respectively), followed by that of *M. laxa* (63 and 31% in Spanish and world isolates, respectively) and *M. fructicola* (38% in world isolates) (Fig. 1).

DISCUSSION

M. laxa isolates can be easily distinguished from *M. fructigena* and *M. fructicola* in DWA by the characteristic short-distance germ tube elongation from the conidium to the first germ tube branch (shorter than 60 µm). *M. fructigena* and *M. fructicola* both produced germ tubes that grew for at least 220 µm before branching. These two species were clearly differentiated from each other under long-wave UV/dark conditions. Maximum growth rate of *M. fructigena* was 8 mm, while maximum growth rate of *M. fructicola* was 20 mm. This difference may indicate that different photoreceptors may be present in the two species (15,22,24). Isolate W-1, which was similar in appearance to *M. fructigena* when grown on PDA in the dark, was identified as *M. fructicola* based on growth in long-wave UV/dark cycle. Isolate W-9, which was similar in appearance to *M. fructicola* when grown on PDA in the dark, was identified as *M. fructigena.*

Long-wave UV/dark conditions reduced the growth rates (P = 0.05) of the three *Monilinia* species on PDA. The small fluctuations in temperature associated with the light cycle did not affect growth rate. Temperature has been shown to affect growth of *Monilinia* spp., but for wider fluctuations than the ones registered in our experiment (11,23). Fungal responses to light frequently include changes in growth, reproduction, or both (5). Wavelengths producing these responses are usually in the ultraviolet and blue regions of the spectrum (4). As reported by Hall (10) and Janitor (12), *M. fructigena* showed faster growth and formation of fruiting organs in light of wavelength between 440 and 490 µm or under alternating light/dark conditions. Alternation of diurnal illumination and darkness (16-h light/8-h dark) did not affect growth of *M. fructicola* and *M. laxa* (26). Harada (11) also observed no inhibitory effect of daylight fluorescent light on mycelial growth of *M. laxa*, *M. fructigena*, and *M. fructicola.*

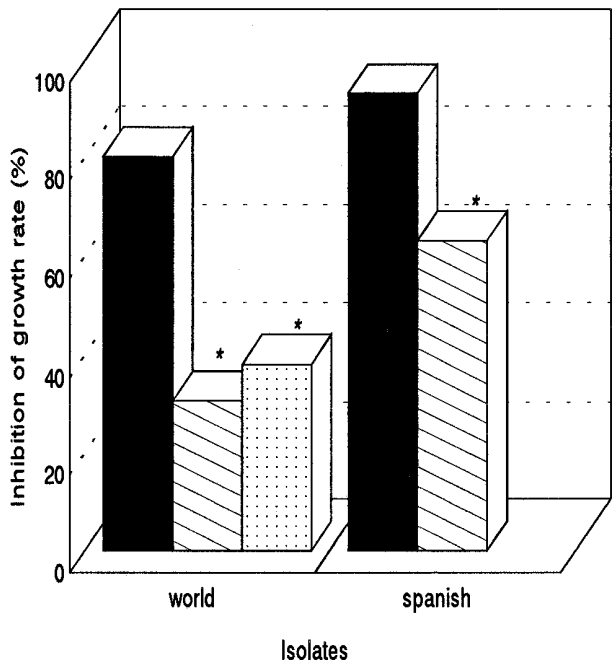

Fig. 1. Percent inhibition of growth rate (PIGR) of *Monilinia fructigena* (solid), *M. laxa* (diagonal lines), and *M. fructicola* (dots) in Spanish and world isolates by exposure to long-wave UV light. Columns with an asterisk for each collection are significantly different (P = 0.05). PIGR for each isolate was calculated by the formula PIGR = $[1 - (GR_L/GR_D)] \times 100$, where GR_L and GR_D are the growth rates in 16-h long-wave UV/8-h dark conditions, respectively. Values are the means of isolates of each species in each collection.

The type of germ tube growth has been previously used to separate the brown rot species (2). Wormald (27) observed that germ tubes of *M. fructigena* grow from 400 to 1,200 µm before branching, while germ tubes of *M. laxa* (*M. cinerea*) become geniculate and produce early branches at a short distance from its point of origin. *M fructicola* resembles *M. fructigena* in the unbranched germ tube at least 200 µm long but sometimes up to 750 µm (2). The differences in the growth patterns of germ tubes of *M. fructicola* and *M. laxa* have been confirmed by Ezekiel (8) and Jenkins (13), who found that germ tubes of *Sclerotinia laxa* commonly branched before they were 50 µm long; whereas *S. fructicola* remained unbranched up to 700 µm. Mordue (18) reported that *M. laxa* germ tubes branched before those of *M. fructigena* and *M. fructicola*. However, Calavan and Keitt (3) indicated that differences in germ tube branching can be affected by nutritional conditions. Our results were obtained using a culture medium with very few nutrients, and they clearly distinguished *M. laxa* from the other two species.

The study of mycelial growth under long-wave UV may be a useful tool to identify *Monilinia* spp. A combination of both observations (distance from the conidium to the first germ tube branch and growth rate under long-wave UV/dark cycle) produced a satisfactory method for differentiating species of *Monilinia*, although at least 9 days are needed to obtain results. Molecular and enzymatic techniques may be a more rapid method for the identification of the species. A species-specific primer for *M. fructicola* is already available (9).

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