Induction of Conidia Production by *Monilinia laxa* on Agar Media by Acetone

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ABSTRACT

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Spore production in four isolates of *Monilinia laxa* was greater when grown on potato-dextrose agar (PDA) amended with acetone (0.5 and 1% v/v) than when grown on nonamended PDA or agar amended with 50 μg/ml indolyl-3 acetic acid. The size of the fungal colony decreased as the acetone concentration increased from 0.5 to 5%, v/v, in the PDA. Sizes, viability, and aggressiveness of spores produced on PDA amended with 0.5%, v/v, acetone were not altered.

Additional keywords: conidia production.

Laboratory bioassays and other experiments may require large numbers of conidia of *Monilinia laxa* (Aderh. & Ruhl.) Honey. The inability to produce large numbers of conidia on potato-dextrose agar (PDA) in the dark has led to the addition of indolyl-3 acetic acid (IAA) to the media and to the use of other media, such as steamed potato soaked in malic acid (12), or the use of fresh or canned fruits (3,7,8).

To further improve spore production, we studied the sporulation of several isolates of *M. laxa* when grown on PDA amended with acetone.

MATERIALS AND METHODS

Four isolates of *M. laxa*, ZA-1, PA-1, MA-1, and TO-1, were collected from different stone fruit orchards in Spain (ZA-1 from apricots and the others from peaches). All of them were pathogenic on peach twigs and fruits and were typical of the species (2,6,9).

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The fungi were stored on PDA slants at 5 C and were grown on PDA at 25 ± 1 C in the dark for conidial and mycelial production.

Each isolate of *M. laxa* was grown in 90-mm-diameter plastic petri dishes containing 20 ml of PDA, PDA amended with different concentrations of acetone (ranging from 0.5 to 5%, v/v), or PDA amended with 50 μg/ml of IAA in unlighted incubators at 25 ± 1 C for 8 days. PDA was prepared as recommended by Booth (1) with slight modifications. Two hundred grams of scrubbed and diced potatoes was boiled in distilled water for 1 hr and then passed through a fine cheesecloth, squeezing through as much pulp as possible. Agar (20 g) was added, boiled until dissolved, and removed from the heat. Dextrose (20 g) then was added and stirred until dissolved. Distilled water was added to complete 1 L. The medium then was autoclaved at 15 psi for 20 min and cooled at 40–45 C afterwards. Acetone or IAA then was added and dissolved before the medium was plated into petri dishes. The following day each plate was inoculated with a 3-mm^2 mycelial plug of *M. laxa* (cut from the margin of actively growing 5-day-old colonies of the fungus on PDA) and sealed with Parafilm.

Spores of each colony grown in each plate were removed from the surface of each colony with a sterile scalpel, suspended in 25 ml of 1%, v/v, Tween 80 in sterile distilled water, and sonicated for 5 min. The number of conidia produced on one plate was counted with a hemacytometer in 20 subsamples per plate. Ten plates were made of each treatment and isolate. The complete experiment was repeated once.

To size the conidia of each isolate and treatment, suspensions of spores were prepared in sterile water, and the length and width of 50 conidia were measured with a microscope equipped with an ocular micrometer.

Germination and germ tube growth of each isolate and each treatment were estimated in sterile Czapek broth as described elsewhere (4) with slight modifications. Sterile glass slides were placed on 15-mm-diameter glass petri dishes lined with moist filter paper. On a slide, a 30-μl droplet of a spore suspension of *M. laxa* (1×10^6 spores/ml) on Czapek broth was incubated for 8 hr at 25 ± 1 C. Then the percentage of germination of 50 conidia and the length of germ tubes of 25 conidia were counted in each replicate. Four replicates were made for each treatment, and the complete experiment was repeated at least once. A spore was considered germinated when a germ tube was longer than the length of the spore.

To test conidia aggressiveness (rot-causing ability), 30-μl drops of conidia suspended in sterile distilled water (10^3–10^4 conidia/ml) of each isolate and treatment was placed onto three equidistant points of an uninjured surface of a peach or on three equidistant 2-mm-deep wounds, made by puncturing the surface, with a glass tube 2 mm in diameter (10). Fruits were sterilized before application of treatments as recommended by Sauer and Burroughs (11). The liquid in the drop of inoculum suspension was allowed to evaporate to near dryness, which required about 1 hr in a sterile chamber. The fruit then was introduced in a plastic tray with moistened filter paper at the bottom and covered with an inverted glass tray that did not touch the inoculated surface of the fruit.

The area of lesions (wounded fruits) or the number of infections in fruits (unwounded fruits) were recorded after 2 and 3 days of incubation at 25 ± 1 C in the dark. Four replicates were made per treatment and isolate.

RESULTS

Spore production and size of colonies. The production of spores by the four isolates of *M. laxa* on PDA was influenced significantly by the acetone amended to the media (Table 1). Low doses of acetone (0.5 and 1%) greatly increased this production. However, higher doses did not affect it. The addition of 50 μg/ml of IAA to the media also did not increase the production of spores.

The ability to form spores in PDA was different for each isolate. The TO-1 isolate produced the fewest number of spores, and the ZA-1 isolate produced the largest number (Table 1). However, the addition of 0.5% acetone to PDA induced a similar increase in production of spores in all four isolates (ranging from 8 to 9 times more).

The size of colonies also was influenced by acetone (Table 2). Reduction in the areas was apparent with 0.5% and increased with higher doses. Isolate TO-1 was the most sensitive to acetone, showing no growth in media amended with 3% acetone. The addition of IAA to PDA also reduced the growth of colonies (Table 2).

Size and viability of conidia. Neither acetone nor IAA affected the size of conidia with the sole exception of isolate MA-1 with 5% acetone, which increased the size significantly ($P \leq 0.05$) (data not shown). The size of conidia of the four isolates was 14 × 10 μm, 11 × 7 μm, 13 × 8 μm, and 11 × 6 μm for ZA-1, PA-1, TO-1, and MA-1, respectively.

The addition of IAA to PDA did not affect the germination of spores or the germ tube growth (data not shown). Although acetone did not influence the germination, it did increase ($P \leq 0.05$) the germ tube growth of PA-1 and TO-1 spore isolates at concentrations of 1% acetone (data not shown).

Aggressiveness of spores. Aggressiveness of spores of isolate ZA-1 was increased by addition of 5% acetone to the media (Table 3). Results obtained after 2 days of inoculation were similar to those obtained after 3 days (Table 3).

DISCUSSION

The addition of low doses of acetone to PDA media enhanced the production of spores of four isolates of *M. laxa* grown in darkness. Although the inherent ability of each isolate to sporulate is different, acetone induces in all of them a similar increase in spore production (ranging from 8 to 9 times more).

TABLE 1. Sporulation (thousands of spores produced by colony) of isolates of *Monilinia laxa* after 8 days of growth on potato-dextrose agar (PDA) and PDA amended with acetone or indolyl-3 acetic acid (IAA)[y]

	Isolates			
Compound	ZA-1	PA-1	TO-1	MA-1
Control	569 c	272 bc	37 c	177 c
0.5% acetone	4,916 a	2,783 a	305 a	1,151 a
1% acetone	3,446 b	816 b	139 b	567 b
2% acetone	993 c	112 bc	14 d	140 c
3% acetone	309 c	55 c	...[z]	53 d
4% acetone	446 c	6 c	...	45 d
5% acetone	92 c	...	...	...
50 μg/ml IAA	383 c	458 b	48 c	190 c

[y]Data are means of 10 replicates; each replicate is the average of 20 values. Means followed by the same letter in each column are not significantly different ($P = 0.05$) by Duncan's multiple range test.
[z]No growth.

TABLE 2. Area (cm^2) of colonies of four isolates of *Monilinia laxa* after 8 days of growth on potato-dextrose agar (PDA) and PDA amended with acetone or indolyl-3 acetic acid (IAA)[z]

	Isolates			
Compound	ZA-1	PA-1	TO-1	MA-1
Control	35.09 a	31.97 a	25.27 a	41.30 a
0.5% acetone	11.14 b	13.95 b	11.24 b	22.44 b
1% acetone	8.49 bc	8.73 c	5.48 c	10.88 c
2% acetone	6.99 c	3.80 cd	1.48 d	7.58 c
3% acetone	5.27 cd	2.13 de	0 d	1.79 d
4% acetone	2.43 de	0.44 e	0 d	3.39 d
5% acetone	1.09 e	0 e	0 d	0 d
50 μg/ml IAA	5.66 c	7.26 cd	4.53 c	9.45 c

[z]Data are the means of 10 replicates. Area was calculated by measuring the diameter (d) of colony in each plate and by applying the formula $\pi d^2/4$. Means followed by the same letter in each column are not significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 3. Aggressiveness of spores produced by four isolates of *Monilinia laxa* after 8 days of growth on potato-dextrose agar (PDA) and PDA amended with acetone or indolyl-3 acetic acid (IAA)[x]

	Isolates							
	ZA-1		PA-1		TO-1		MA-1	
Compound	Lesion[y] (cm^2)	Infection number[z]	Lesion (cm^2)	Infection number	Lesion (cm^2)	Infection number	Lesion (cm^2)	Infection number
Control	9 a	1.2 a	17 a	2.0 a	12 a	2.0 a	12 a	0.2 a
1% acetone	8 a	0.7 a	19 a	1.5 a	12 a	1.7 a	12 a	0.0 a
5% acetone	15 b	1.7 a	...	...	...	...	15 a	0.5 a
50 μg/ml IAA	9 a	1.2 a	19 a	0.7 a	15 a	0.7 a	15 a	1.0 a

[x]Aggressiveness of spores was estimated by recording the lesion areas produced in wounded fruits and the number of successful infections in unwounded fruits 3 days after their inoculation.

[y]Data are means of 12 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$) by Duncan's multiple range test.

[z]Data are means of four replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$) by Wilcoxon nonparametric test.

Khan (5) reported that the addition of IAA to PDA induces sporulation in darkness when very little sporulation occurs normally in cultures of *Sclerotinia fructigena* Aderh. & Ruhl. He also described that light and IAA appear to have the same effect on sporulation and that one can be replaced by the other. However, our cultures of *M. laxa* did not sporulate more when grown in darkness on PDA amended with IAA than when grown on PDA alone. We also have conducted experiments under light conditions with isolate ZA-1, showing that light stimulates sporulation more than acetone. The addition of 0.5 or 1% acetone to PDA enhances sporulation of colonies grown in lighted incubators, but it enhances sporulation less than when colonies are grown in darkness.

Acetone largely reduced the growth of colonies. Because of this reduction in the size of colonies, the number of spores per colony grown in PDA amended with high doses of acetone is low, despite the fact that acetone enhances sporulation (which was shown by the number of spores per square centimeter of colony surface). On the other hand, the induction of sporulation may be a survival mechanism of colonies that react against the negative effect of acetone.

The size, viability, and aggressiveness of spores produced by this method should not be affected by acetone. We have demonstrated that addition of 0.5% acetone to PDA did not affect these parameters.

The technique reported here quickly produces masses of conidia of *M. laxa* and has been used to produce spores for our biological studies in the laboratory and for inoculation of peach twigs and fruits.

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