Use of Interactions of Cultures to Distinguish *Monilinia laxa* from *M. fructicola*

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ABSTRACT

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Monilinia laxa and *M. fructicola* were distinguished by characteristic interactions between isolates when grown on oatmeal agar. Distinct black lines formed between *Monilinia fructicola* and *M. laxa* colonies within 10 days and profuse microconidia by 20 days of incubation. Light, double lines were visible between some of the *M. laxa* isolates after 15–20 days of incubation. The formation of distinct lines within 10 days was used as one of the criteria that a benomyl-resistant *Monilinia* isolate with scalloped margins on potato-dextrose agar was *M. fructicola*.

Additional key words: brown rot

Monilinia laxa (Aderh. & Ruhl.) Honey and *M. fructicola* (Wint.) Honey attack stone fruits in California, as well as in other areas of the world (7). In California, the two species have exhibited host specificity: *M. laxa* occurs more frequently on apricot and almond and less frequently on peach and nectarine, and *M. fructicola* occurs more often on peach and nectarine and less frequently on apricot and almond (7). Both species, however, can occur on all these hosts and in some cases are difficult to differentiate.

Isolates of *M. fructicola* and *M. laxa* are usually differentiated on the basis of cultural characteristics on potato-dextrose agar (PDA). *M. laxa* produces lobed growth on PDA with poor production of conidia, whereas *M. fructicola* generally produces abundant conidia in culture with entire or nearly entire colony margins (2). However, growth on PDA of some isolates of *M. fructicola* is very similar to growth of *M. laxa*. Other criteria used to differentiate the two species are the more frequent formation of hyphal anastomosis between germinating conidia of *M. fructicola* (4), the formation of branched germ tubes in *M. laxa* (3), and electrophoretic patterns (5).

Willets (6) reported that when different isolates of *M. laxa* are grown on the same PDA plate, the mycelia of the isolates are separated by narrow, clear zones; however, when *M. laxa* isolates are grown in the same plate with *M. laxa* f. sp. *mali*, a dark line forms between the margins of the two species. *M. laxa* isolates were not grown by Willets in the same plate with *M. fructicola*. We found that when *M. fructicola* was grown on the same oatmeal agar plate as *M. laxa*, distinct, sometimes black lines formed at the junction between mycelia of the two species. We report here the use of this phenomenon as a criterion for separating *M. laxa* from *M. fructicola*.

MATERIALS AND METHODS

Nineteen isolates of *M. laxa* and ten of *M. fructicola* from diseased stone fruit tissue from northern and central California orchards, and one isolate each of *M. laxa* from Oregon and the countries of Iraq and Argentina, were used to determine whether the formation of a line between isolates of the two species was a consistent phenomenon. The isolates were maintained on PDA (Difco). Four-millimeter disks of 4- to 7-day-old cultures of *M. laxa* and *M. fructicola* were transferred to thin layers (4–5 mm thick) of oatmeal agar in disposable plastic petri dishes measuring 150 × 15 mm. The mycelial disks were placed 2.5 cm apart in the pattern shown in Figure 1. The plates were placed in a plastic container to prevent contamination and incubated on the laboratory bench (21 ± 1 C). Characteristics of the interaction between isolates were noted at 10 and 20 days after transfer.

RESULTS

After 10 days of incubation, there were

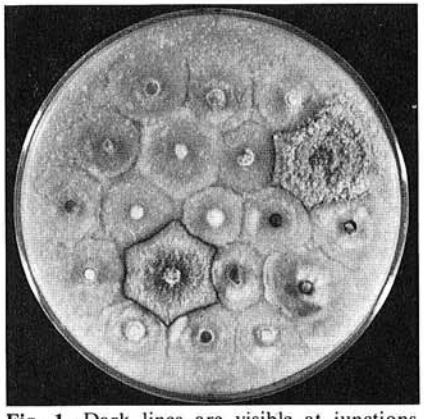

Fig. 1. Dark lines are visible at junctions between *Monilinia laxa* and two *M. fructicola* isolates but not between the *M. laxa* isolates. Mycelial disks were placed 2.5 cm apart on oatmeal agar.

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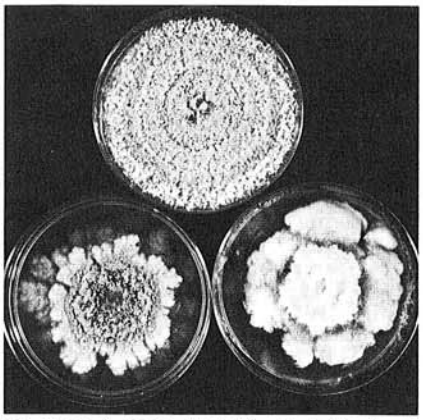

Fig. 2. Typical *Monilinia fructicola* (top) and *M. laxa* (lower right), compared with *Monilinia* isolate with lobed margins identified as *M. fructicola*.

no visible lines of interaction between *M. laxa* isolates. All *M. laxa* isolates appeared whitish on oatmeal agar except the isolate from Argentina, which had some dark-pigmented areas. After 20 days of incubation, some light, double lines were visible between some of the *M. laxa* isolates.

After 10 days of incubation, distinct, sometimes black lines were visible at junctions between an isolate of *M. laxa* and one of *M. fructicola* (Fig. 1). After 20 days of incubation, profuse microconidia formed on the lines. Lines also formed at the junction between some pairs of *M. fructicola* isolates but not others. Wherever a line occurred between pairs of *M. fructicola* isolates, profuse microconidial formation also occurred. All 10 *M. fructicola* isolates produced dark pigments in oatmeal agar, whereas *M. laxa* isolates did not.

An unknown *Monilinia* sp. isolate suspected to be *M. fructicola*, which produced lobed colony margins on PDA (Fig. 2) and was resistant to the fungicide benomyl, was inoculated on the same plate as *M. laxa* isolates. Distinct lines formed at the junction of the unknown *Monilinia* sp. and tester *M. laxa* isolates after 6 days of incubation, indicating that the unknown *Monilinia* sp. was not *M. laxa*.

DISCUSSION

M. fructicola and *M. laxa* isolates were distinguished by the characteristic interaction between the two species on oatmeal agar medium.

On the basis of the pairing test, we concluded that the benomyl-resistant *Monilinia* sp. isolate, which was similar in appearance to *M. laxa* on PDA medium, was *M. fructicola*. These results were obtained before resistance to benomyl had been detected in isolates of *M. laxa* in California. More recently, this technique has been used to confirm that there are isolates of *M. laxa* resistant to benomyl in California (Ogawa et al, *unpublished*).

Further tests with more isolates from other areas of the world are warranted to determine whether this interaction between *M. laxa* and *M. fructicola* is universal. Further studies on the interactions between *M. laxa* f. sp. *mali*, *M. fructicola*, and *M. fructigena* are needed. The distinct line formed between *M. laxa* and *M. fructicola* isolates and between some pairs of *M. fructicola* isolates and the light, double line between some *M. laxa* isolates after 20 days of incubation are similar to the phenomenon attributed to vegetative incompatibility in *Endothia parasitica* and other fungi (1). It is being further investigated in this laboratory.

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