FARM ADVISORS

Multiple-fungicide Resistant *Botrytis cinerea* Documented in Strawberry in the Central Coast Region

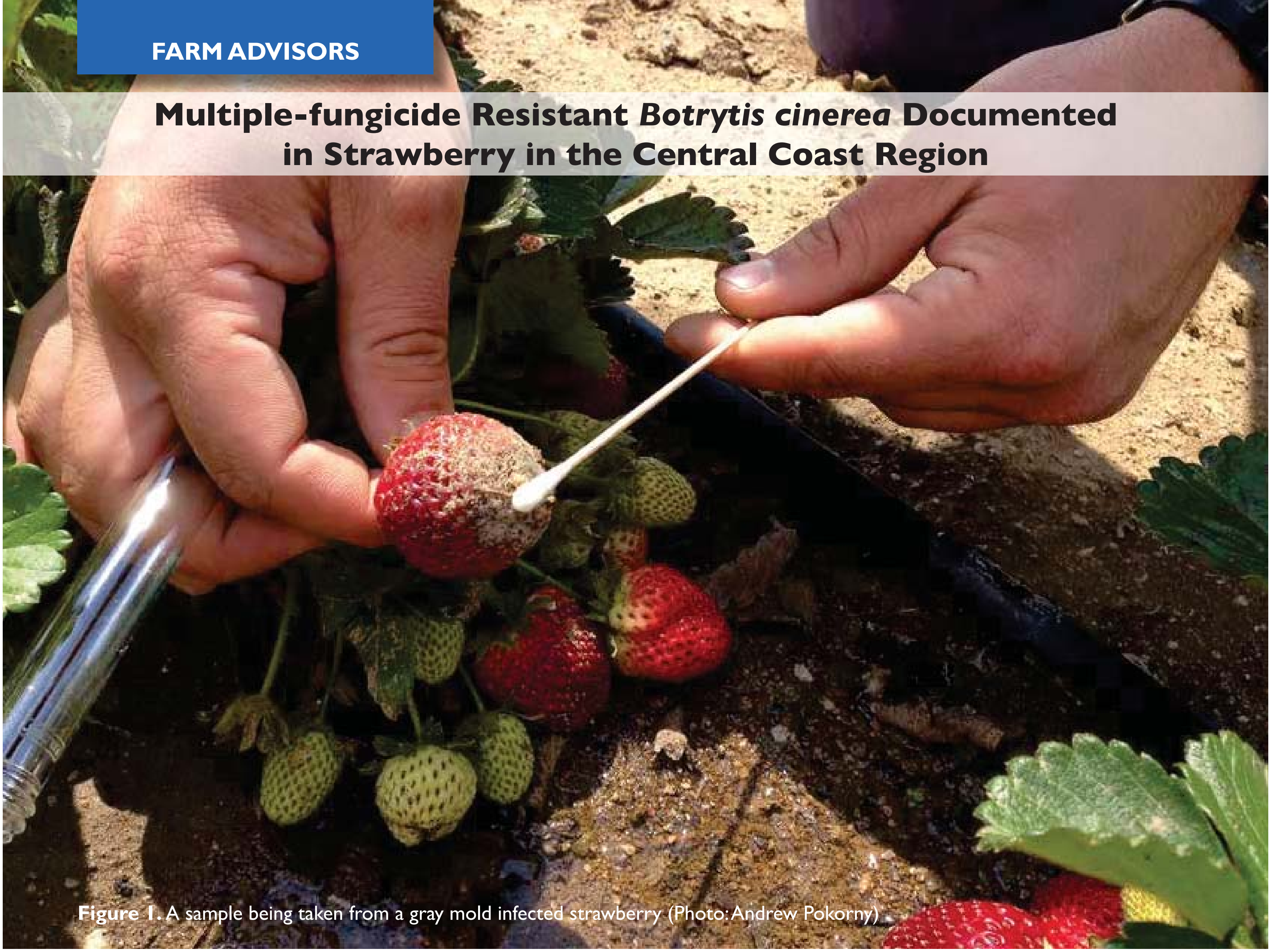

Figure 1. A sample being taken from a gray mold infected strawberry (Photo: Andrew Pokorny)

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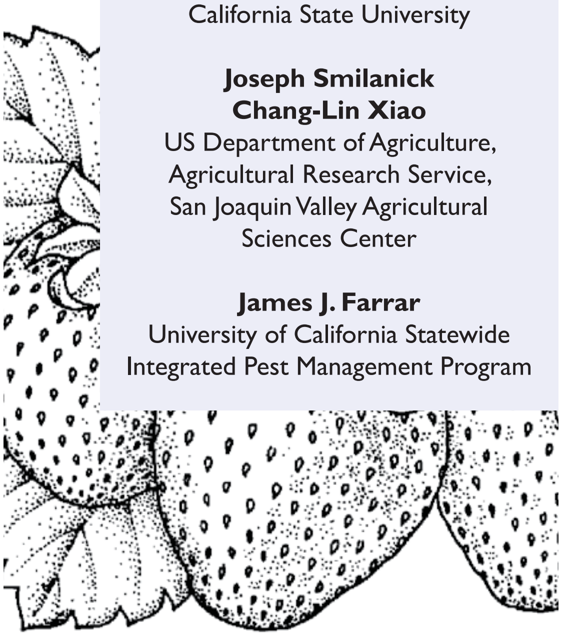

ABSTRACT

Gray mold, caused by *Botrytis cinerea*, is one of the most important diseases of strawberry in California which is typically managed by repeated application of fungicides. Resistance to fungicides in *B. cinerea* was suspected and examined in 59 samples from six different locations in the central coast strawberry production region in May 2013. Cultures were used for mycelial growth assays to compare sensitivity to boscalid, fenhexamid, iprodione, and pyraclostrobin. All of the 59 isolates were sensitive to iprodione, but 37% were resistant to pyraclostrobin, 31% were resistant to fenhexamid, and 29% were resistant to boscalid. In some instances the isolates were resistant to two or three of these fungicides. The concentration of fungicide required to reduce mycelial growth by 50% (EC_{50}) was often higher than 100 mg/L, which was the highest concentration used. Therefore, appropriate fungicide resistance management measures should be employed in strawberry growing areas of the central coast region.

Gray mold of strawberries, caused by *Botrytis cinerea*, is a particularly destructive disease that causes considerable losses of the crop (Figure 1, above). In terms of economic impact, gray mold is ranked as the most important strawberry disease.

Fungicides from a number of different mode-of-action groups are used for management of gray mold of strawberry. The University of California's Statewide Integrated Pest Management Program recommends seven commercially available fungicides with nine different modes-of-action for gray mold on strawberries in California (UC IPM 2014). Fungicides are the most used in-season pesticide by weight in strawberry production in California (CDPR 2014), resulting in heavy fungicide resistance selection pressure on the fungus. Fungicide resistance in *B. cinerea* from strawberries is an increasingly important problem (Fernández-Ortuno et al. 2014; Weber 2011). Thus, we were interested in knowing if fungicide-resistant strains of *B. cinerea* were present in the strawberry producing region of the Central Coast.

We collected samples randomly from six different strawberry fields in Monterey County, California (Figure 2). Twenty-two isolates were collected from a site north of Gonzalez, nine isolates from a site west of Gonzalez, eight isolates from a site southwest of Gonzalez, seven isolates from a site west of Soledad, ten isolates from site southwest of Soledad and three isolates from a site south of Chualar.

A total of 59 isolate samples of *B. cinerea* were collected from these locations and screened against boscalid, fenhexamid, iprodione and pyraclostrobin to determine the relative sensitivity of each isolate to each active ingredient. One of the fungicides tested included a combination of boscalid and pyraclostrobin. These active ingredients were selected to represent components of a typical fungicide spray program in strawberries in California. Each sample was collected by rubbing a sterile cotton swab against a sporulating lesion and standard procedures and protocols were used to obtain cultures that was free of contamination.

Fungicide resistance assays were conducted against the fungicides mentioned earlier using standard resistance testing protocols and culture procedures. The isolate responses were then classified as *highly resistant*, *moderately resistant*, *moderately sensitive*, and *highly sensitive*. Complete details of the methods are available in Pokorny et al. (2016).

Figure 2. The locations from where the samples were taken were enclosed within the black circle.

The sensitivity of the 59 isolates to the various fungicides are shown in Table 1. While all isolates were sensitive to iprodione, fungicide-resistant isolates were discovered from all sampling locations. Of the 59 isolates screened, 17, 18, and 22 isolates were either moderately or highly resistant to boscalid, fenhexamid, or pyraclostrobin, respectively (Table 1).

Twelve isolates were found with resistance to a single active ingredient with seven isolates resistant to only pyraclostrobin, three isolates resistant to only fenhexamid, and two isolates resistant to only boscalid. Twelve isolates were discovered with resistance to two active ingredients with four isolates resistant to boscalid and fenhexamid, four isolates resistant to boscalid and pyraclostrobin, and four isolates resistant to fenhexamid and pyraclostrobin. Seven isolates were observed with triple resistance to boscalid, fenhexamid, and pyraclostrobin.

Boscalid and pyraclostrobin are applied together in strawberries as a two active ingredient pre-mixed product. Four of the 59 isolates in this study were resistant to both boscalid and pyraclostrobin. This suggests the value of pre-mixed products in retarding the emergence and

Table 1. Classification of the 59 isolates collected from six locations and their sensitivity to the various fungicides tested.

	Sensitivity			
Fungicide	**Highly resistant**	**Moderately resistant**	**Moderately sensitive**	**Highly sensitive**
Boscalid	8	9	24	18
Fenhexamid	5	13	10	31
Iprodione	0	0	0	59
Pyraclostrobin	12	10	16	21

proliferation of fungicide resistant isolates should be investigated. Label restrictions allow the application of boscalid and pyraclostrobin for control of gray mold five times in a growing season with no more than two sequential applications without switching to a different fungicidal active ingredient.

EC_{50} of the fungicides was also determined. EC_{50} is the effective concentration of fungicide necessary to reduce mycelial growth by 50% in comparison to growth in the absence of fungicide. The EC_{50} values for isolates sensitive to boscalid were 0.13 and 0.29 mg/L, whereas EC_{50} values for isolates resistant to boscalid were greater than the highest concentration tested (>100 mg/L). The EC_{50} values for isolates sensitive to fenhexamid were 0.81 and 1.51 mg/L, whereas the estimated EC_{50} values for isolates resistant to fenhexamid were 36.3 mg/L and greater than the highest concentration tested (>100 mg/L). The EC_{50} values calculated for the isolate sensitive to pyraclostrobin were 0.32 and 1.63 mg/L, whereas the EC_{50} for the isolates resistant to pyraclostrobin were greater than the highest concentration tested (>100 mg/L). As mentioned earlier, none of the isolates showed resistance to iprodione and the EC_{50} values of the sensitive isolates were 0.36 and 1.41 mg/L.

Although this study had a relatively small sample, 37% of isolates were highly or moderately resistant to pyraclostrobin, 31% were highly or moderately resistant to fenhexamid, and 29% were highly or moderately resistant to boscalid. In addition, 37% of isolates were resistant to two fungicides and 12% were resistant to three fungicides. Although continued adherence to fungicide resistance management guidelines and fungicide label requirements is necessary, identification and development of additional effective practices is required to maintain current strawberry production levels in the central coast region of California.

Complete information on this paper is available at: Pokorny, A., J. Smilanick, C. Xiao, J. J. Farrar, and A. Shrestha. 2016. *Determination of fungicide resistance in Botrytis cinerea from strawberry in the Central Coast Region of California*. Plant Health Progress 17:30-34.

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