

ing their LD₅₀ values (the dosage of insecticide required to kill 50 percent of the test weevils; the larger the LD₅₀ value, the less toxic the material is to the black vine weevil). All materials killed adult weevils, with carbofuran and bendiocarb being the most toxic (LD₅₀ values of 0.09 and 0.18 mg/ml, respectively). Chlorpyrifos and acephate achieved greater mortality than did isofenphos (LD₅₀ values of 0.88, 1.01, and 1.93 mg/ml, respectively). The pattern of toxicity was the same when the data were extrapolated to compare the recommended field rates of all toxicants. Because adult weevils feed on foliage, all insecticides might have been more toxic if ingestion toxicity had also been considered.

This study provides comparative toxicity data for selected insecticides to adults. It cannot be presumed that toxicity will be the same to larvae. Larvae are often deep among the roots of plants and, despite a thorough drenching, it may be difficult to obtain coverage with the toxicant. In addition, different soil types used in various nurseries could significantly affect the activity of an insecticide applied to soil. In general, newly hatched larvae are more susceptible to insecticides than are older larvae. Therefore, insecticides applied soon after egg hatch will probably provide the greatest larval control. Researchers at UC Davis and at research laboratories in other countries are evaluating the control potential of parasitic nematodes and fungi against black vine weevil larvae. Parasitic nematodes have been used successfully in Australia, and results appear promising in California.

At present, trials suggest that consistently good results can be obtained through foliar applications of insecticides against adults. A foliar spray with long residual properties, applied at night, will kill weevils both through direct contact and ingestion. In addition, applications when adult activity is first observed may provide appreciable adult mortality before egg laying occurs because of the relatively long, 20- to 30-day, prereproductive period. Finally, once the primary infestation area is identified, treating only that location may be sufficient to reduce the problem; treating the entire nursery probably is not necessary, unless the weevils have been established for a long time and are widespread.

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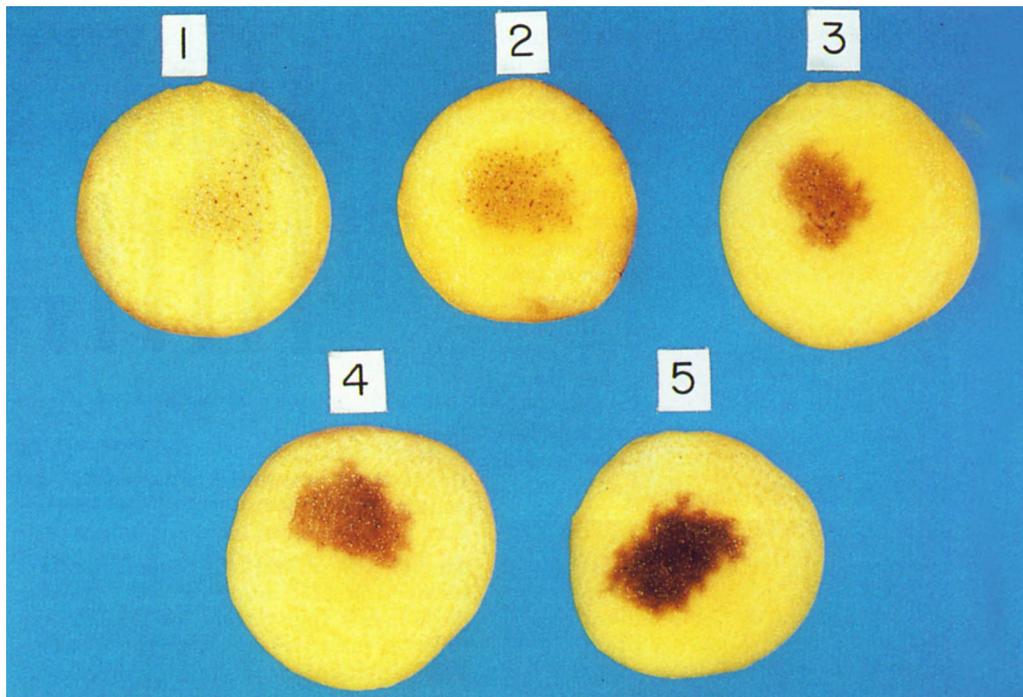


Fig. 1: Phenolic compounds and activity of polyphenol oxidase (PPO) enzyme cause undesirable browning in peaches. Catechol added to peach slices (above) tests level of

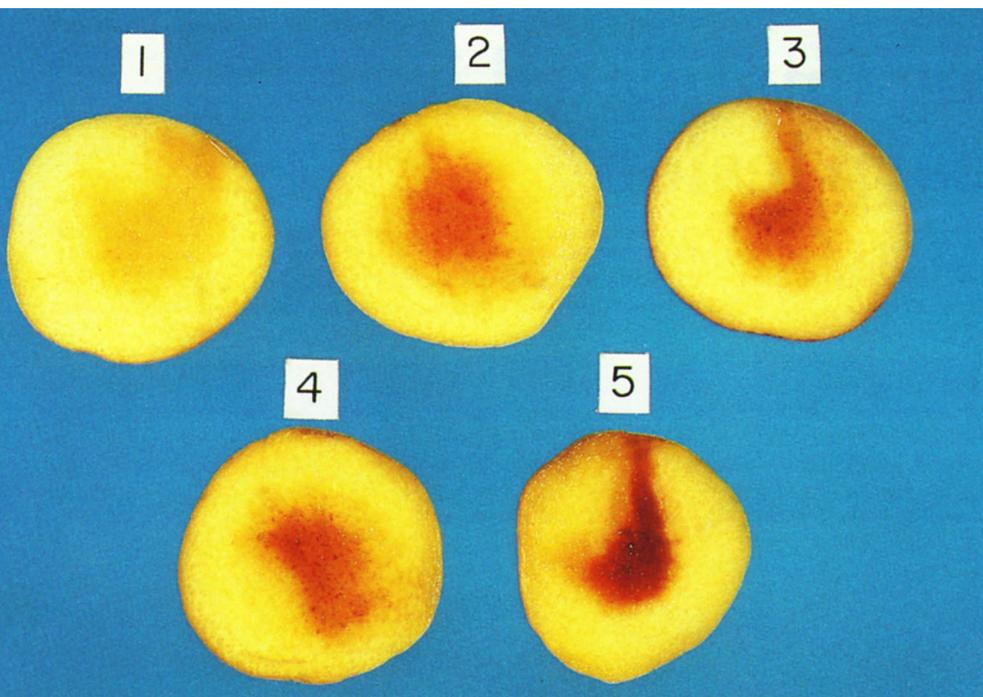
Evaluating the browning potential of peaches

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Fresh fruit tissues turn brown when bruised, cut, or otherwise damaged during harvesting and postharvest handling. This browning detracts from the appearance of fruits marketed fresh or used for processing. The extent of the discoloration depends not only on the severity of bruising, but also on the inherent browning potential of the fruits. Browning potential depends upon the total amount of phenolic compounds and level of activity of the polyphenol oxidase (PPO) enzyme, which catalyzes enzymic browning in fresh fruits. Normally, phenolic compounds are separated from PPO enzyme in the

intact tissue. Once the fruit tissue is damaged, PPO gets access to the phenolic compounds, and reactions leading to browning occur. These reactions involve the oxidation of phenolic compounds to form quinones, which are tightly unstable and polymerize quickly to form brown-colored products.

In a study of peach bruising and browning initiated in 1978, we found large differences in browning potential, total phenolics content, and PPO activity among cultivars and within a given cultivar in relation to environmental conditions and cultural practices. These results indicate that it is possible to



PPO activity. Fig. 2 (above): A dark cherry-red color develops in a nitroso test, indicating phenolics content in fruit. Combined scores measure browning potential.

select new cultivars with lower browning potential and to manipulate production, harvesting, and postharvest handling practices to reduce the fruit's browning potential.

Since chemical analyses for total phenolic compounds and PPO activity involve rather complicated laboratory procedures, they are not practical for plant breeders and field researchers to use for screening large numbers of breeding lines or pre- and post-harvest treatments. Thus, we evaluated several histochemical tests and adapted two of them for use as quick assays for PPO activity and phenolic compounds content. This procedure involves two quick tests (described in more detail later in this report) that can be carried out on fruit slices as follows:

1. To test for PPO activity, catechol is added to the slice, then the degree of brown discoloration is rated on a 1-to-5 scale.
2. The Nitroso test is used for estimating total phenolics content. A dark cherry-red color develops and is rated on a 1-to-5 scale.
3. The two scores are added. Browning potential is considered low if the combined score is less than 2, moderate if the score is 2 to 4, high if the score is 5 to 7, and very high if the score exceeds 7.

During the past three years, we have evaluated these two quick tests and

have found that they correlate very well with actual bruising tests (correlation coefficient values of 0.8 to 0.9) of peaches.

Actual bruising tests involve applying a consistent bruising treatment to the fruits, holding them for 24 hours at 20° to 25°C (68° to 77°F), then evaluating the bruised area size and degree of browning (subjectively or by a light reflectance measuring instrument). Compared with this procedure, the quick tests are faster, do not require laboratory equipment, and can be carried out in the field. A more detailed description of these two quick tests follows:

Polyphenol oxidase activity

This test involves providing a substrate for PPO action and determining the extent of browning resulting from its oxidation and formation of brown products. Catechol was selected from among several phenolic compounds (caffeic acid, chlorogenic acid, gallic acid, catechin) because of its greater susceptibility to PPO activity and its solubility in cold water. The optimum pH for this reaction is between 5.9 and 6.3 and is provided by the citric acid-phosphate buffer used for preparation of the catechol solution.

Reagents needed:

1. Citric acid-phosphate buffer solution: Prepare 0.1 M citric acid solution

(21.0 g $C_6H_8O_7 \cdot H_2O$ per liter), and 0.2 M disodium phosphate solution (28.3 g Na_2HPO_4 anhydrous per 1 liter). Mix 339 ml of 0.1 M citric acid solution with 661 ml of 0.2 M disodium phosphate solution; pH of this buffer solution should be 6.2. It should be refrigerated when not in use.

2. Catechol solution: Prepare 0.1 M catechol solution (0.55 g catechol in 50 ml of citric acid-phosphate buffer). Catechol solution should be freshly prepared on the day it is used.

Test procedures:

Slice fruit to expose an area about 3 to 4 cm in diameter. Apply one drop of 0.1 M catechol solution. After 6 minutes, score intensity of browning relative to the standard chart shown in fig. 1. The extent of fruit tissue darkening due to their endogenous phenolic content in six minutes is very limited and does not interfere with this test.

Phenolic compounds

In this test, sodium nitrite in presence of acetic acid releases nitrous acid, which subsequently reacts with phenolic compounds, forming nitroso derivatives. Urea is used as a stabilizer. Nitroso derivatives, after addition of base (sodium hydroxide), are converted into sodium salts characterized by deep cherry-red color. The intensity of this color depends on the amount of phenolic compounds in the fruit tissues.

Reagents needed:

1. Sodium nitrite ($NaNO_2$), 10 percent solution: Weigh 10 g $NaNO_2$ and dissolve in distilled water in a 100 ml volumetric flask.
2. Urea ($CO [NH_2]_2$), 20 percent solution: Weigh 20 g urea and dissolve in 100 ml distilled water in a volumetric flask.
3. Acetic acid, 10 percent solution: Transfer 10 ml of glacial acetic acid into a 100 ml volumetric flask and bring up to volume with distilled water.
4. Sodium hydroxide, 8 percent solution: Weigh 8 g NaOH. Transfer to a 100 ml volumetric flask, and bring up to volume with distilled water.

Test procedure:

To each slice of peach flesh (3 to 4 cm in diameter) apply one drop of each of the following reagents in succession: 10 percent sodium nitrite, 20 percent urea, and 10 percent acetic acid.

After four minutes, apply two drops of 8 percent sodium hydroxide solution.

Evaluate the intensity of cherry-red color according to the standard chart shown in fig. 2.

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