# Preliminary testing shows strobilurin fungicides are no longer effective against anthracnose

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Azoxystrobin was first registered for use in avocado by Syngenta in 2003 as Amistar®WG (wettable granule), with later registration as the soluble concentrate (SC) formulation in 2005. It was registered for control of a range of fungal diseases in many crops, including anthracnose, caused by *Colletotrichum* spp. and stem end rot in avocado and mango.

Group 11 (strobilurin or QoI) fungicides, including azoxystrobin and many others, kill fungi by targeting a specific gene involved in fungal respiration. This single-site mode of action classifies them as being "high risk", for the fungus developing resistance to its action, through genetic mutation. The longer that these single-site chemicals are used, the higher the chance that target fungi could develop resistance. For this reason, the labels for Amistar and other strobilurin fungicides, have very strict instructions for their use, as part of an anti-resistance strategy, to reduce the risk of resistance and prolong the life of the fungicide. While these fungicides are best used preventatively, they are considered translaminar, and remain active within the leaf and cuticle of the peel for several weeks, and thus have a useful post-infection activity.

Our AV16007 project work during 2020 - 2022 showed that pre-harvest applications of Amistar 250SC, at label rates, did not result in reduced severity of anthracnose, compared with untreated or limited copper-only controls. This supported anecdotal evidence from several growers who felt that azoxystrobin sprays were not as effective as they once were. This suggested that *Colletotrichum* sp., the fungus causing anthracnose, had possibly mutated to become resistant to the QoI strobilurin group of fungicides.

Resistance to strobilurins is well known in *Colletotrichum* spp. causing diseases in other crops. From these previous studies we optimised a molecular test to detect the mutation, and thus screen for resistance in individual isolates of *Colletotrichum* from avocado. So far, we have screened only a small number of isolates, but confirm that a high proportion of isolates sourced from orchards which have a history of using azoxystrobin fungicides, were resistant. Three of these are from south east Queensland, and 2 from south west WA. This was confirmed in agar plate tests, where the resistant isolates grew very well on plates amended with high concentrations of azoxystrobin (Fig. 2). Only 1 isolate from an orchard in NSW with history of azoxystrobin did not have the gene mutation for resistance. Isolates from pre-Amistar days (available from the QLD Dept. Agriculture and Fisheries fungal collection), and those from organic and minimally-sprayed orchards, are still sensitive to azoxystrobin, and do not have the mutation.

## What are our next steps?

We have a very large collection of *Colletotrichum* isolates from most of the growing regions, and will be screening more isolates in coming months to determine the range and extent of the resistance. Resistance will be confirmed using the molecular detection assay as well as the *in vitro* (agar plate) screening method. It is likely that resistance is widespread.

It is likely that there is resistance to the whole group of strobilurin fungicides, including trifloxystrobin, one of the actives in Luna Sensation. We will be checking growth on agar plates amended with Luna Sensation.

## What does this mean for growers?

- If the orchard has a history of using strobilurin fungicides for several years, there is a high chance the *Colletotrichum*, which is always present in orchards, has developed resistance
  - Your field sprays will no longer be effective
  - Graduate A+ in the packingshed will no longer be effective (except possibly for reducing stem end rot)
  - Do not be tempted to use ultra-high concentrations, or multiple applications, as this will only make the problem worse
- Canopy management is even more important to
  - a) reduce the source of *Colletotrichum* spores (dead twigs, branches and mummified fruit) and
  - b) to improve airflow in the canopy to hasten drying out after rain events, and improve spray coverage of fruit
- Field sprays with protectant fungicides throughout fruit development are important to prevent infection from occurring in the first place
- There is no evidence to suggest that the other fungicide groups registered in avocado are no longer effective

## How do you know if you have resistant isolates?

Our suggestion would be to select and tag a few trees or a row or two which have fruit at commercial maturity (i.e. over 23% dry matter for Hass). Spray the tagged trees with fungicide, (azoxystrobin formulations or Luna Sensation), wait a week. Harvest a few trays of fruit from both sprayed *and* unsprayed trees (label the trays), and keep them in a warm place to ripen. When fruit are fully ripe, or even a day or so over-ripe, cut them and peel to check for anthracnose lesions. It is difficult to determine the extent of the rot in Hass, which is why the recommendation is to peel the fruit to assess the damage (see Fig. 1). If there is a similar level of disease in strobilurin-sprayed compared with the unsprayed fruit, then it is likely the orchard has fungicide-resistant *Colletotrichum*, and there would be little point continuing with those sprays.

In most situations, if the crop has been well managed throughout fruit development, and given optimal picking, packing, storage and transport practices, there will be minimal problem with anthracnose disease, particularly in early season fruit. Breakdown problems may occur in late season fruit.

## What is industry doing about it?

Some alternative fungicides from other chemical groups, not currently registered in avocado, were identified in AV16007, which could be effective as pre-harvest sprays. Avocados Australia is working with Hort Innovation and agrichemical companies to progress potential label registrations or minor use permits for these other options.

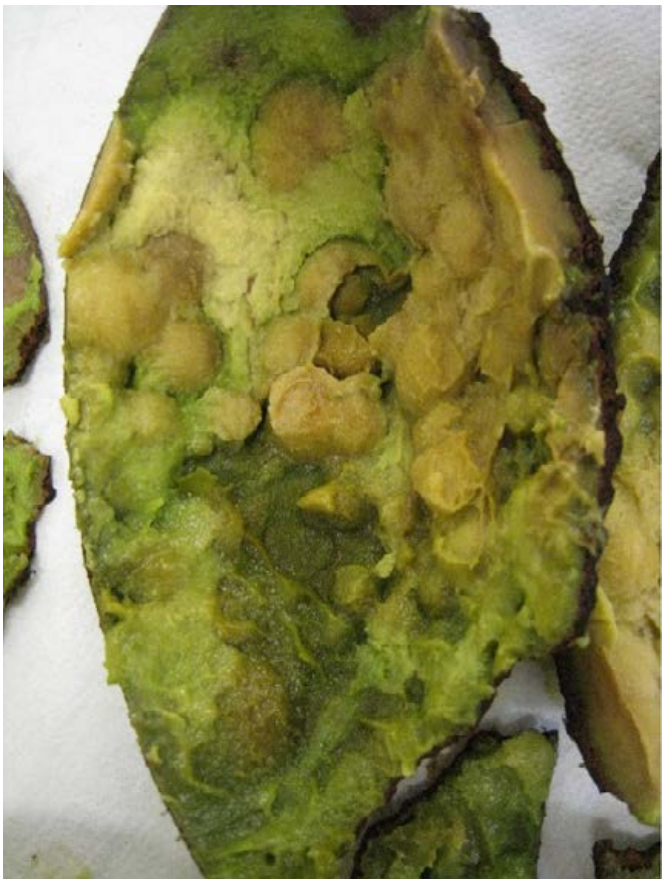

**Figure 1.** Anthracnose lesions observed on the inner peel of over-ripe Hass fruit. Each hemispherical lesion represents a single infection by a *Colletotrichum* sp. spore.

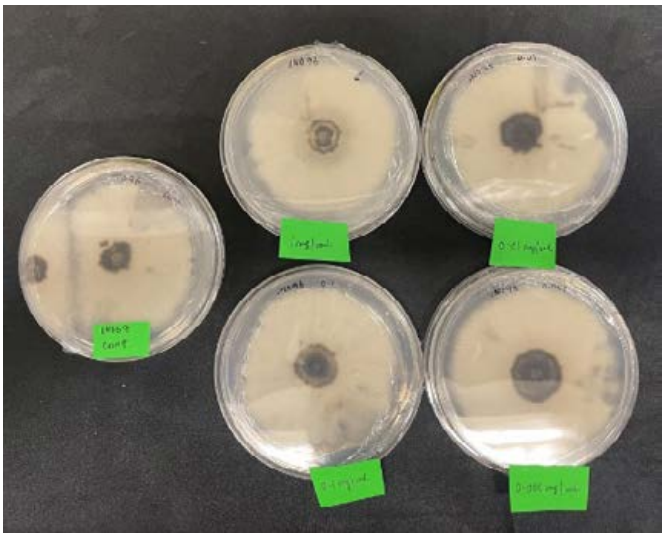

**Figure 2.** Growth of a QoI resistant *Colletotrichum* sp. isolate on agar plates amended with different concentrations of Amistar, (labelled as mg (azoxystrobin)/mL), compared with water control (left). For comparison, the label rate for field spray application is 80mL/100L, equivalent to 0.0002 mg azoxystrobin/mL.

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