

Avocado Black Streak Disease Revisited: An Unsolved Mystery

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Avoocado black streak (ABS) has long been a disease of concern for California avocado growers – first reported as early as 1934 and later becoming a topic of research for George Zentmyer, Howard Ohr, and Ramon Jordan at the University of California, Riverside (UCR) during the 1970s and 1980s. They discovered that the disease mainly occurs on Guatemalan cultivars, including the market-dominant and mass-cultivated ‘Hass’ variety. There also have been reports from Florida in the early 1990s of the disease on Mexican and West Indian cultivars, indicating the disease affects all races. Interestingly, the budwood from symptomatic trees in Florida was reportedly brought in from California. Outside the United States, the disease was reported in Israel on ‘Hass’ in the 1980s.

The research performed by the aforementioned researchers at UCR found viral entities from diseased and healthy tissue, but they were unable to reproduce the symptoms when inoculating healthy avocados with the viral entities in greenhouse conditions. They ruled out bacteria as the cause since antibiotic treated trees still produced symptoms of the disease and the fungi recovered from diseased tissue also

were being recovered from healthy tissue. This enigmatic disease is still an unsolved mystery, likely due to the lack of robust research on the topic since the 1980s, and the apparent minor economic importance of the disease in the current day. The work presented here was done to revisit the potential involvement of fungi in cankers formed in trees exhibiting ABS symptoms and their pathogenicity in healthy mature avocados.

Symptoms

The most obvious symptoms of ABS are trunk and branch cankers that usually appear on the underside of large branches. The canker is made visible by the accumulation of dry sugar exudate present in small cracks in the bark along the canker (Figure 1 A-B). This symptom has recently been confused with similar white sugar exudate that appears after attacks from invasive shot hole borers. The cankers may range in



Figure 1: Symptoms of ABS showing sugar exudation from small cracks in the bark. Typical symptoms will appear on the underside of the branch in older branches (A) and occasionally younger branches (B).

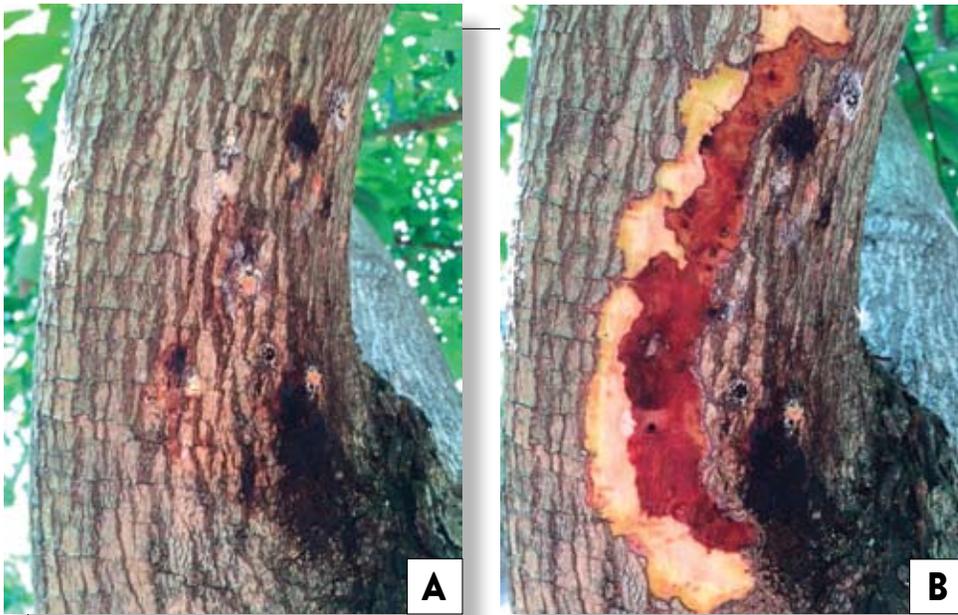


Figure 2: An older branch exhibiting ABS Symptoms (A) and the resulting canker (B) developing underneath the bark.

size from a few centimeters to the entirety of a branch or trunk. When the canker first appears, the resulting lesion under the outer bark is reddish brown and is usually limited to the phloem but can extend past the vascular cambium into the xylem tissue (Figure 2 A-B). Besides the obvious cankers present, symptoms including chlorosis, early bloom, branch dieback, leaf blotching, zinc deficiency, wilting of foliage and rapid death of new growth may occur. There are currently no known treatments to effectively treat this disease after symptoms are observed, although there are management strategies, such as good fertilization and irrigation practices, aimed at preventing stress in the host and reducing occurrence of this disease.

UCR Survey and Pathogenicity Tests

In 2015-2016, we conducted surveys in five avocado groves within San Diego County and Orange County where black streak symptoms were reported. Symptomatic and asymptomatic tissue samples were obtained from three to five trees within each

grove and isolated on culture media to recover fungi and bacteria. The most consistently recovered organisms from the survey included *Lasiodiplodia* spp., *Neofusicoccum luteum*, *Diplodia mutila*, and *Phaeoacremonium* spp. (Figure 3) with the former three hailing from the Botryosphaeriaceae, a family of fungi

with species known to cause Botryosphaeria canker and stem end rot in avocado. *Phaeoacremonium* spp. have not been reported on avocado but are widely studied in phytopathology and are known to cause grapevine decline.

To determine the pathogenicity of the isolated fungi and attempt to reproduce symptoms of ABS, two isolates from each fungal species were used to inoculate mature avocado branches, 20 branches per fungal species, at Pine Tree Ranch, Ventura County. Twenty trees in total were inoculated with the suspect fungi in January 2017 on the underside of the branches with a cork borer to make a 5mm diameter circular wound past the bark to the cambium where fungal tissue of the same size was deposited and allowed to colonize for three months. In March 2017, lesion lengths were recorded and wood samples were taken at the tip of the lesions to recover the fungi to fulfill Koch's postulates.

Lesion lengths for all fungi inoculated were significantly greater than

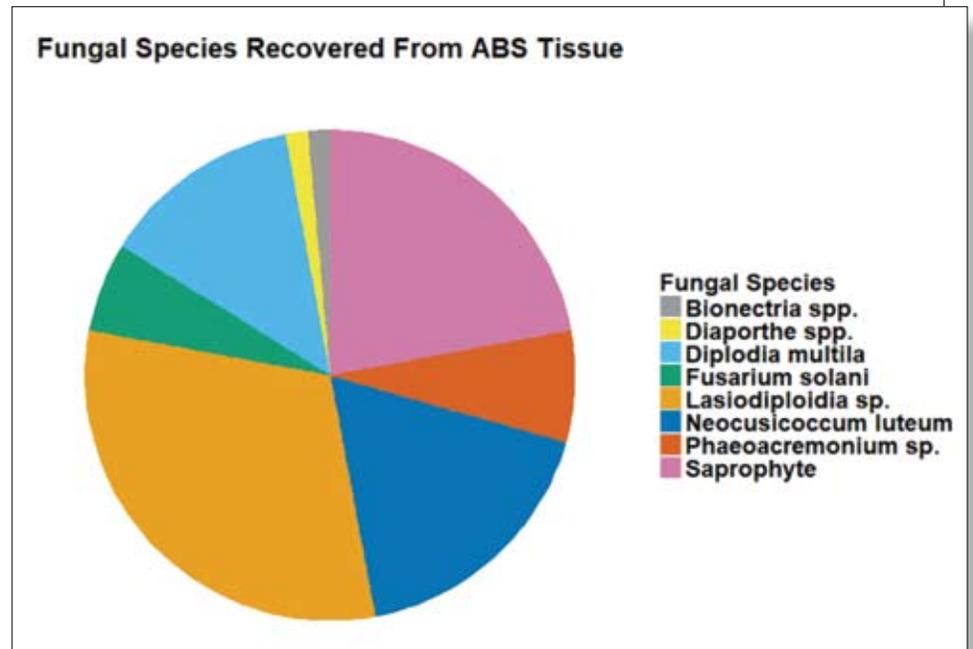


Figure 3: Proportion of species identified from woody tissue showing ABS symptoms on initial surveys done in 2015-2016.

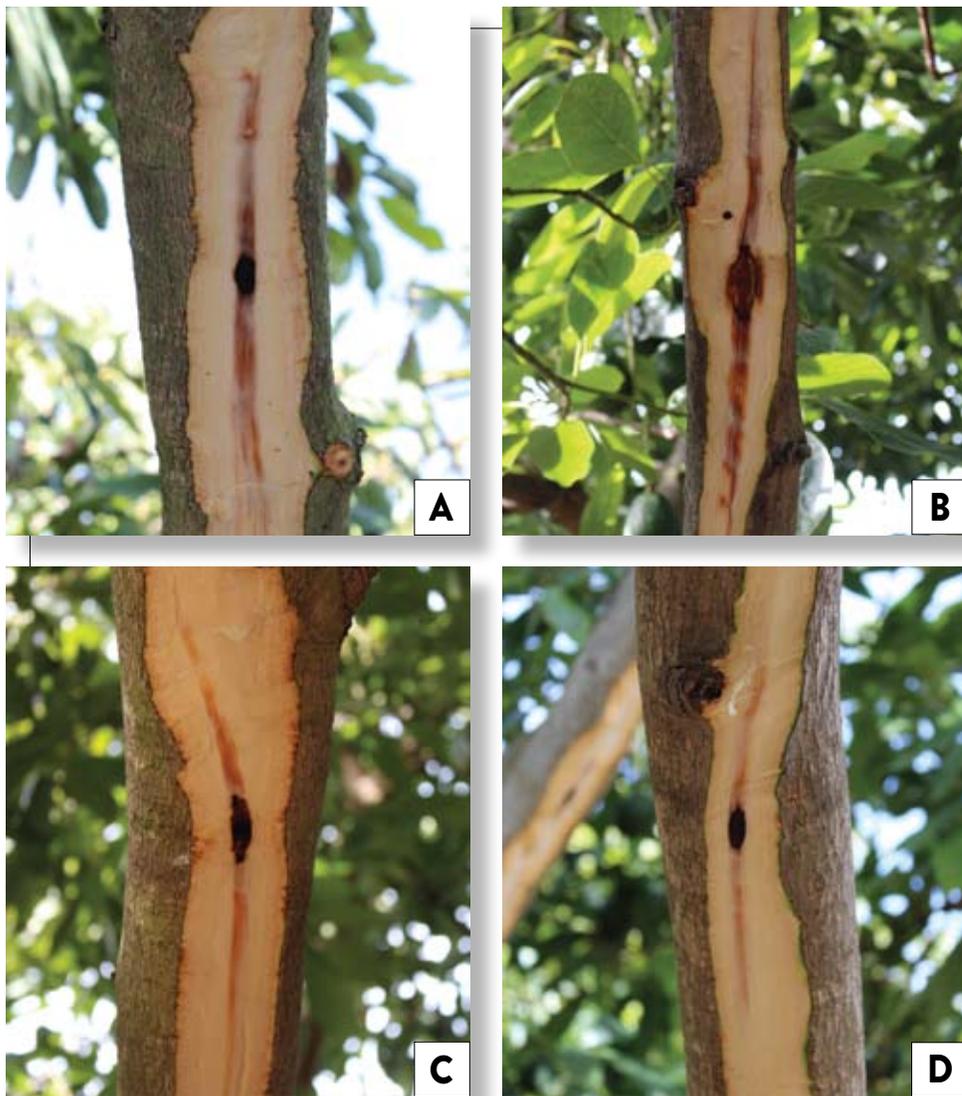


Figure 4: Lesions developed from treatments of fungi recovered from symptomatic black streak tissue. A. *Phaeoacremonium* sp. B. *Lasiodiplodia* sp. C. *Neofusicoccum luteum* D. *Diplodia mutila*.

the control. The damage resulting from inoculation of the suspect fungi (Figure 4) recovered from ABS tissue produce significant lesions and cause necrosis in the phloem and xylem tissue based on our method of inoculation. However, the developed lesions and necrosis are not consistent with symptoms of ABS, as there was widespread exudation along the underside in *some* but not *all* of the treated branches. Nevertheless, this experiment does show that *Lasiodiplodia* sp. and *Phaeoacremonium* sp. can colonize and progress through host tissue through open wounds. It would have been interesting to see the progression of the pathogens over a longer

period of time and the resulting damage and canker formation, but planned removal of the block for replanting prevented this.

Conclusions

Botryosphaeriaceae members are known to be latent pathogens in a wide variety of hosts – present, but not causing any observable symptoms when the host is in good health. This may explain why previous researchers working on ABS overlooked these fungi, since they also were recovering them from asymptomatic tissue as well as symptomatic. The unobservable symptoms in hosts already exposed to these fungi, how-

ever, can progress to form cankers and cause branch dieback upon physiological stress from environmental conditions. It is thought that environmental stress triggers symptom development of ABS, leading to formation of small open wounds and visible exudation of sugar. These openings can serve as entry points for secondary pathogens and opportunists, such as the fungi we have been recovering from this tissue.

Botryosphaeriaceae members in particular have been previously reported as latent pathogens on a diverse group of woody hosts, present on virtually all major plant organs. Physiological stress during ABS development could create opportunities for latent pathogens that already exist in the host, allowing them to become pathogenic in times of stress. Although the cause of ABS is *still* unknown, we can conclude from our surveys and pathogenicity experiments that *Lasiodiplodia* sp. and *Phaeoacremonium* sp. are examples of secondary fungi that can cause damage upon introduction into the host from open wounds formed during ABS disease development.

The causal agent of ABS was attributed to have potential viral etiology by Howard Ohr in 1981, but the agents driving canker formation are likely a complex community of fungi based on our survey results. It is a topic worth revisiting in order to investigate what pathogens are initiating ABS symptoms and what pathogens are contributing to the progression of the disease and resulting damage. Conventional methods of culturing from wood tissue are not able to represent *every* organism in the sample, which is why we plan to take a molecular approach to this issue looking for nucleic acid traces of fungi, bacteria and virus in the samples to investigate if there is any correlation in ABS and healthy samples to attempt to resolve the causal agent(s) of this disease. 🍌