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Blackstreak disease of avocado in California

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SYNOPSIS
Blackstreak of avocado is a disease of unknown etiology. Attempts to define a causal agent have been unsuccessful. The discovery of several viruses in avocado have not led to the correlation of any dsRNA pattern(s) and the incidence of Avocado Blackstreak. Current work is being focused on several fungi isolated from diseased trees.

INTRODUCTION

The disease known as Avocado Blackstreak (ABS) may have been observed as early as 1934, when Horne (1) described symptoms similar to those currently seen. Later observations by Pehrson (4) and Zentmyer (5) also describe symptoms similar to those of ABS, while Zentmyer (5) first used the name Avocado Blackstreak. These early observations indicated that the disease may have been present in California over 50 years ago, but was not a serious problem at that time. The most probable reason for the increase of ABS as an important disease of avocado, is the increase in acreage of the susceptible Hass variety, which accounts for 70 per cent of California's 75 000 acres of avocados. ABS appears to be a problem unique to California as reports of the disease from other areas have not been brought to the authors' attention.

In 1975, the senior author became interested in the disease when it was brought to his attention in Ventura County. It soon became apparent that ABS was no longer a minor problem to the avocado industry but one which had become extensive, occurring in all the avocado growing areas of the state where Guatemalan varieties are grown.

GEOGRAPHICAL DISTRIBUTION

To date, the disease has only been observed on Guatemalan varieties such as Hass, Reed and Nabal. It may affect the Mexican or West Indian varieties but, while many of the associate symptoms have been observed on these varieties, the canker never has.

The disease occurs in all areas of the state where Guatemalan varieties are grown, from San Luis Obispo County south to San Diego County and inland to Riverside County. All ages of trees are affected and symptoms have been observed on trees as young as one year in the field, to trees older than 35 years. Not all groves in an area will have the disease, but those groves that do, may have from a few to a high percentage of their trees affected.

SYMPTOMS

Appearance of the disease in a grove often follows a period of environmental or cultural stress, such as insufficient water. In the normal progression of the disease, an affected tree usually gradually declines and may eventually die. Rapid tree collapse has been observed. Even though some trees may linger or 'recover', observations indicate that fruit production is usually poor.

The symptoms associated with ABS are varied and can be attributed to other causes. Due to this, the canker that appears on the trunk and branches. was chosen as the indicator symptom of ABS. Choosing this symptom as the disease indicator, may explain why the disease has not been observed on Mexican or West Indian varieties, as these do not show the canker.

The canker usually first appears on the lower trunk or on the underside of a lower branch, although it has been observed to occasionally appear on the upper trunk first. Lower trunk canker can be confused with that caused by the fungus *Phytophthora citricola*, but there are enough differences to readily distinguish between them. The canker is made visible by the accumulation of a dry, powdery sugar (3) that appears to exude through minute cracks in the bark. This is in contrast to the powder that accumulates around a wound, which can be readily seen when the sugary substance is removed. The powder is water soluble and easily removed by rain. In the absence of the powder, the canker is difficult to find. Cankers may range in size from a few millimetres to encompassing most of the trunk. The cankers do not seem to favour any specific side of the tree. When the canker first appears, the exudate is often a cinnamon colour which changes to white, as more powder accumulates. As time passes, the area darkens somewhat. The name blackstreak is a misnomer that will likely be changed when the ultimate cause of the disease is determined.

Scraping of the bark surface over the lesion reveals polymorphic, reddish-brown necrotic areas which are usually shallow, but sometimes extend into the cambium. These areas can often be easily removed by inserting a knife blade under them and lifting them. It is likely that the lesions are the result of the disease and not the cause of death, as trees often die with few lesions, or the lesions present mostly do not extend deep or far enough around the tree to girdle it.

Other symptoms of the disease include chlorosis, early bloom, branch dieback, leaf blotching, zinc deficiency, bunchy growth due to shortened internodes, wilting of foliage and rapid death of new growth. Chlorosis may occur on the whole tree or on a single branch. Often a branch may turn chlorotic, decline and die while the rest of the tree continues to look healthy and bear fruit. Eventually though, the rest of the tree usually declines and dies. Affected trees often bloom early, producing chlorotic flowers five to 10 days before the rest of the grove. In one ABS survey affected trees were easily spotted by the early bloom. Leaf blotching is characterised by necrotic areas that first appear on the bottom and then the upper leaf surface. They are not confined by the veins and resemble damage caused by sodium toxicity, although tests for excessive

sodium have been inconsistent. Zinc deficiency occurs on the diseased trees, but is most likely a result and not the cause of ABS. Bunchy growth appears on some of the trees, giving terminals a dense growth habit. Internodes appear shortened in these areas. Some affected trees have been observed to have 'flagging' foliage over the complete tree or on some branches, while neighbouring trees were unaffected. Regrowth on trees defoliated due to the disease and on diseased trees severely cut back to reduce picking height, commonly enters a shock syndrome and dies. When a diseased tree that has been reduced in height does regrow vigorously, the disease usually reappears within several years and the tree declines again.

RESULTS

Surveys

While early surveys described the geographical distribution of the disease within the state, other surveys were undertaken to determine the extent of the disease within selected groves and its pattern of increase, if any. Two groves were selected, one in Ventura County containing 1 731 10-year-old Hass trees on mixed rootstocks and the other in San Diego County, containing 374 22-year-old trees on Topa-Topa rootstocks (Ohr, unpublished). Three surveys were made at yearly intervals and were terminated only when environmental conditions made it impossible to observe lesions on the trunks. In Ventura County (1 731 trees), the surveys began with 6,7 per cent (116 trees), increasing to 15,1 per cent (261 trees) the second year and to 19,9 per cent (344 trees) the third survey. It must be emphasised that the survey was based on the appearance of the trunk lesion only. Many diseased trees may have been overlooked due to the lack of a lesion (Ohr, unpublished).

Analysis of the data by Jordan using Converse's modification of Vanderplank's doublet method, found that in the second and third surveys 74 and 87 per cent respectively of the newly detected trees, were adjacent to previously diseased trees. This fact rejects random distribution and indicates the disease may be due to a causal agent (Jordan, unpublished). There were a number of different rootstocks in the Ventura County grove, but there were no correlations between rootstock variety and disease incidence (Ohr, unpublished).

In the San Diego County surveys (374 trees), the first survey revealed that 17,1 per cent (64 trees) had the disease, increasing to 30,2 per cent (113 trees) during the second survey and 37,2 per cent (139 trees) at the third survey. In the second and third surveys in this grove, 83 and 96 per cent respectively of new trees with symptoms were adjacent to previously affected trees. Analysis again concluded that distribution was nonrandom (Jordan, unpublished). The surveys have not been continued, due to adverse conditions for symptom expression and the removal of trees from the groves. Conclusions from the surveys were that the disease appears to be spread in the grove consistent with the spread expected of a soilborne pathogen.

Transmission

Early attempts at transmission were made in a greenhouse and later in the field. In greenhouse studies, budwood was collected from trees showing the bark symptom of

the disease and from apparently healthy trees, Recipient trees were Hass on Topa-Topa rootstock and Topa-Topa seedlings. Trees were grown in the greenhouse for 22 months, while being periodically observed for symptoms. None of the trees developed the typical symptoms of ABS disease. Termination of the experiment occurred upon the discovery, in the field, of pitting in the trunk below the bud union in a number of trees affected with ABS. Upon termination the stems were peeled, revealing pitting in a number of the trees inoculated from ABS source trees. Sixteen of 44 trees inoculated from ABS trees had pitting, while none of the 28 control trees exhibited any pitting. Pitting in these experiments was not confined to the rootstock, but occasionally occurred on the scion around the point of graft inoculation. An interesting note is that in the ABS source grove, it was difficult to find an ABS tree with pitting below the bud union (Ohr, unpublished).

Field transmission attempts were made by reciprocal bark and scion cross inoculations. Cross inoculations were made scion to scion; scion to rootstock; rootstock to scion and rootstock to rootstock. The grafts were made in 1979 and to date there has been no evidence of ABS in the recipient grove.

Another transmission attempt was made following a field observation where young Reed trees, planted into sites after removal of young ABS infected Hass trees, also developed the disease. In this attempt 20 Reed trees were planted into sites where diseased Hass had been removed. Half of the planting sites were fumigated with four pounds of methyl bromide per 100 square feet before planting. There has been no evidence of disease transmission, but the trees planted in the fumigated sites have grown at a much better rate than those planted in nonfumigated sites (Munnecke, personal communication).

Attempts to transmit an ABS causal agent to herbaceous plants, using plant extracts and nematodes, have not been successful and although Tobacco Mosaic Virus (TMV) was found in some plants, it is not considered to be a cause of ABS (Jordan, unpublished).

Fungi-bacteria-mycoplasms

Early isolations for organisms were done using common media and techniques. Isolations were made from roots, canker tissues, twigs and leaves. The isolations were inconsistent for fungi and bacteria. Mycoplasm isolations were negative. Attempts to detect a pathogen in the above tissues by light and electron microscopy, were all negative (Jordan, unpublished).

Injections of tetracycline and oxytetracycline using gravity flow and pressure injection, were done to determine if bacteria or mycoplasms were involved. Twenty seven ABS affected trees were injected with the antibiotics at rates ranging from 300-1 200 µg/m/. Controls were six trees injected with water and 30 untreated trees. There were no detectable differences in growth of the injected trees and no evidence of symptom remission (Ohr, unpublished).

Nematodes
Nematode surveys were made in several groves around both ABS and apparently healthy trees. Samples were collected from around 106 trees in 10 different groves. *Xiphinema americanum* nematodes were found at all 10 locations and while the numbers detected from tree to tree ranged from zero to more than 300 per 400 cc of soil, there was no correlation to ABS. Several other nematode species were detected but their numbers were low and there was no correlation with ABS (Ohr, unpublished).

Pitting
As mentioned previously, pitting below the bud union occurs on many trees affected with ABS. The pits range from a few necrotic areas that appear to be covered by subsequent growth to very severe, deep pits that cover the wood below the bud union. The pits do not occur in every grove that has ABS, but pitting has been transmitted to young trees from budwood taken from a grove with little detectable pitting. When pitting occurs within a grove, it may occur on as many as 90 per cent of the trees with ABS. Pitting has not yet been detected on trees appearing to be healthy. It has not been determined whether pitting is part of the ABS syndrome, or whether the more plausible explanation is that trees with pitting are stressed and therefore are more likely to develop ABS (Ohr, unpublished).

Viruses-viroids
As noted previously, viruses and viroids were not detected in electron micro-scope studies, even though the pitting suggested that viruses might be present. With the advent of techniques to detect double stranded ribonucleic acid (dsRNA) particles on polyacrylimide gels, efforts were again made to detect viruses and viroids. There were no viroids detected, but three separate dsRNA patterns were detected indicating that as many as three viruses might be present in avocados. Subsequent surveys demonstrated that most avocado varieties have at least one of the patterns and that some have all three (2).

Graft transmission of dsRNA patterns
Jordan (2) reported on the rootstock to scion transmission of two of the three dsRNA patterns found in avocado, and that the transmission of pitting suggested that some agent could be transmitted from scion to rootstock. However, Jordan did not design studies to test this, due to the difficulties associated with analysing rootstock material.

Pollen and seed transmission
Seed transmission of dsRNA patterns in avocado has been previously reported by Jordan (2). Studies on pollen transmission of the dsRNA patterns were carried out using seed in the avocado registration block at Riverside, which has been completely indexed for the dsRNA pattern of each tree. Six varieties were chosen depending on the dsRNA pattern contained in the parent tree. One hundred seeds from each variety were planted and from 43 to 76 seedlings were analysed for their dsRNA content.

It can be seen from Table 1 that all three dsRNA patterns are apparently transmitted via pollen to seeds of adjacent trees. There is no evidence that pollen transmission can carry the dsRNA to the seed parent.

TABLE 1 Pollen transmission of the dsRNA patterns.

Seedling dsRNA Pattern	Parent and parent dsRNA patterns					
	Duke 7 0	G-6 1	Zutano 2	Rincon 3	Ganter 1-2-3	Teague 1-2-3
1	0	36	1	1	0	0
2	0	0	17	0	24	0
3	0	0	0	22	3	29
1&2	0	2	1	1	1	2
1&3	0	36	0	2	0	3
2&3	0	0	28	15	14	20
1,2&3	0	2	4	10	1	3
Total seedlings	50	76	51	51	43	57
% transmission parent dsRNA	100	100	98	96	2	5
% apparent pollen transmission	0	53	67	57	NA	NA

dsRNA-ABS correlation

In another effort, scion material from 343 trees in a grove with a known ABS distribution were sampled and analysed for dsRNA. The authors were unable to find any correlation between dsRNA content and ABS. They were unable to repeat this on a large scale for rootstocks, due to the difficulty of getting green tissue to analyse. The very few rootstocks that they were able to analyse, did not support a correlation between ABS and dsRNA (Ohr, unpublished).

Current efforts

With the apparent lack of evidence pointing to a viral etiology for ABS, the authors returned to some earlier work to determine whether they had passed anything by. This included isolating for fungi from canker tissues, roots and twigs. These isolations were made periodically from two selected groves and have resulted in fairly consistent isolations of Verticillium lateritiurn, Cladosporum sp and Fusarium sp. These fungi are currently being tested in a field trial, both singly and in all combinations. Four trees were inoculated by trunk injection for each fungus and combination. The trees will be water-stressed to determine whether ABS can be linked to any of the organisms.

DISCUSSION

Avocado blackstreak disease appears to be a disease unique to California that has, to date, eluded attempts at a solution. The discovery of three dsRNA patterns, presumably due to viruses, in avocado has been investigated and at present there is no apparent correlation with ABS, although they may be causing other problems such as pitting.

With the apparent disfavour of viruses as the causal agent of ABS, the authors have returned to some of the earlier work for further investigation. Because many of the symptoms of the disease are similar to those caused by vascular pathogens, this premise is being investigated. The finding of three fungi in correlation with ABS, supports the need for further investigation in this area.

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