AGROBACTERIUM WILT AND BRONZING: THE NEW CHALLENGE

A. A. Bell, Y. Cui, C. Magill,
H. Orta and M. Hawkins
USDA, Southern Crops Research Laboratory
and Texas A&M University
College Station, TX

Abstract

Plants showing symptoms of sudden wilt and bronzing during fruit development show extensive root deterioration caused by *Agrobacterium* biovar 1 isolates. Methods for evaluating cultivar resistance to this disease are described. The bacterium appears to interfere with uptake of nutrient anions (phosphate and sulfate). Consequently, the disease can be largely controlled by fertilizers containing phosphate and sulfate, providing that excess nitrogen is avoided. Biological control of this disease also may be possible.

In 1995 and 1996, many short-season varieties grown in short-season production systems were severely affected by a disease referred to variously as "sudden wilt", "copper top", "red top", "bronzing", "early fade out", and "Pseudomonas wilt" depending on locality. Fields in Texas, Louisiana, Arkansas, Mississippi, Tennessee, and North Carolina suffered losses of as much as 50% of the potential crop. In 1997 the same disease appeared extensively in California and Georgia in addition to the previous locations. Plants affected with the disease show extensive deterioration of the secondary and tertiary root systems, often leaving necrotic scars on the larger root where the smaller root was girdled and rotted at its point of origin. The root scars invariably contain high concentrations of a bacterium identified as an *Agrobacterium* belonging to the Biovar 1 group. *Agrobacterium* isolates obtained from seed of 43 cotton cultivars grown across the Cotton Belt showed considerable diversity in chromosome structure, fatty acid profiles, and plasmid content. Most isolates contained 200 kb plasmids and many showed amplification of the *vir C* gene which occurs in Ti and Ri plasmids. However, the bacterial isolates failed to induce tumors or typical hairy roots on 10 different plant species. Thus, it is uncertain whether they cause transformation of root tissue. More than 100 isolates of *Agrobacterium* from cotton produced abundant polygalacturonase (pH 4.5), grew at 43°C, and caused rapid swelling of cotton hypocotyls indicating the production of plant hormones. We conclude that the disease is caused by a unique group of *Agrobacterium* but considerable genetic diversity occurs in this group.

The *Agrobacterium* parasite is seed borne and occurs in all cultivars, often in 100% of the seed. The bacteria move systemically in the xylem and can be obtained from xylem fluid of about 5% of the plants on any given day. The bacteria in leaves often caused small lesions that vary in appearance depending on the nutritional status of the plant.

The disease is best created in the greenhouse by wetting germination towels with bacterial suspensions and then transplanting 24-hour-old seedlings started at 30°C into pasteurized natural soils that contain 10% or more clay. The most severe disease develops when phosphorous is omitted from the fertilizer. The disease can be attenuated and controlled by progressive increases of phosphorus in the fertilizer mix. Symptoms also depend strongly on the sulfate content of fertilizers. Most commercial "complete fertilizers" do not contain adequate sulfur to prevent chlorosis, copper top, and bronzing symptoms in the presence of *Agrobacterium*. Gypsum, epsom salts or sodium sulfate can be included in fertilizers to prevent these symptoms. The bacteria appear to interfere with phosphate and sulfate uptake. Consequently, greater amounts of these nutrients than normally recommended are needed if the disease is prevalent.

When the 64 major cultivars grown in the USA were inoculated and grown on pasteurized soil with 25% clay, none were able to set and carry fruit during a 3-month period when nitrogen alone was used as fertilizer (the soils laboratory recommendation for this soil). Adding sodium phosphate as a single pulse or at low concentrations in fertilizer applied twice monthly provided considerable segregation of cultivars. Excess superphosphate applied as granules to the surface of the pot allowed all cultivars to fruit readily. Thus, careful control of phosphorus nutrition showed cultivar differences in susceptibility to sudden wilt and development of leaf necrosis.

Additional studies of nutrition confirmed that bronzing and copper top are favored by low availability of sulfur, while sudden wilt and interveinal leaf necrosis are favored by low availability of phosphorus. The nitrogen level relative to the phosphorus level also is very critical. Any increase in the N/P ratio above 2 in the fertilizer caused progressive increases in *Agrobacterium* concentrations in roots and in disease severity. These changes were most severe when phosphate was already low in soil, but they also occurred in soils that tested high for phosphate. The results indicate that greater amounts of phosphorous and sulfur may be needed in both starter and late season fertilizers when sudden wilt and bronzing are a problem.

Under field conditions *Agrobacterium* bronzing and wilt are usually suppressed by microbial antagonists of the bacterium, which keep its population in balance. In controlled tests, many *Fusarium* species, *Phoma* species, *Talaromyces flavus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and some isolates of *Bacillus subtilis* isolated from cotton roots suppressed development of the bacteria in roots

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and decreased disease severity. In contrast, *Rhizoctonia solani* and root-knot nematodes increased bacterial multiplication and disease severity. Evaluation of antagonists as seed treatments for Agrobacterium bronzing and wilt is underway.