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A New Color Variant of the Dry Bean Bacterial Wilt Pathogen (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) Found in Western Nebraska

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Bacterial wilt of dry beans, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*), has been a sporadic but often serious production problem in dry beans throughout the irrigated High Plains since first being reported from South Dakota in 1922. It was first observed in western Nebraska dry bean production fields in the early-mid 1950s, and continued to be economically important throughout the 1960s and early 1970s. The disease then only periodically appeared in seed, but had little detectable effect on yields after the implementation of crop rotation and seed sanitation practices.

Because of the systemic nature of this disease, the pathogen will often color or stain seeds, but not in all situations. This is particularly conspicuous on white seeded cultivars when it does occur. Colony growth and staining of seeds reported for original isolates of *Cff* were always yellow until orange (3) and purple colored (4) variants were found in western Nebraska. The purple variant maintains a yellow colored colony in culture, but produces an extracellular, bright purple water-soluble pigment that diffuses into growth medium within 2 to 3 days and also discolors seed. The purple variant is very rare, and has only been reported once outside of the western Nebraska Panhandle - from cull bean seeds in Alberta, Canada in 2006 (2). The pigment produced by the purple variant is often unstable and inconsistently expressed, which may explain this variant's lower reported incidence in nature.

Isolation and Identification

During the last 4 years, the wilt pathogen's presence has increased (> 300 fields) throughout the dry bean-producing areas of western Nebraska and other areas of the central High Plains, including Colorado and Wyoming (1). Since 2005, all three pathogen color variants have been isolated from infected dry bean plants in western Nebraska fields during the season, with more than 90% of collected isolates during this time consisting of the yellow and orange variants. Following the 2007 growing season, a pink bacterial isolate closely resembling the wilt pathogen was recovered on isolation media from orange-stained seeds (market class Great Northern) that originated from research plots affiliated with the University of Nebraska's Panhandle Research and Extension Center (Scottsbluff Ag Lab) near Mitchell, NE.

Recovery of the pink isolate was accomplished by soaking discolored seeds overnight in sterile deionized water. The leachate was streaked on NBY (nutrient broth yeast extract) medium. Pink, mucoid colonies were observed after 36 to 48 h at 25°C (Fig. 1). The bacterium was identified as *Cff* based on several morphological and physiological characteristics. It reacted positively for both the Gram stain and KOH test, and exhibited short, pleomorphic rods with the characteristic coryneform-shape. Identification of the isolate was based on carbon source utilization using Biolog GP2 microplates comparing outputs to

the Microlog system 2 database, release 4.01B (Biolog Inc., Hayward, CA). This system matched the pink isolate and *Cff* with a probability of 100 and 98% after 24 and 48 h, respectively.

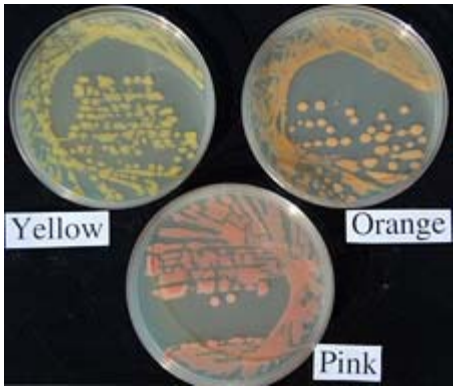

Fig. 1. The pink wilt variant growing in culture compared to the typical yellow and orange variants on nutrient broth yeast extract medium (NBY). The orange variant is the same Great Northern isolate used in these studies as a comparison.

Testing Pathogenicity

Sterile needles were dipped into bacterial colonies from 48-hour-old cultures and inserted into stems just below the first fully expanded trifoliolate of Great Northern "Orion" dry bean plants. A known highly virulent *Cff* orange variant isolate (hereafter referred to as GN) originally obtained from an infected Great Northern plant was used as a comparison. Plants punctured with needles dipped in sterile water served as controls. Plants were incubated in lighted growth chambers with a 12-h light/dark cycle utilizing two different temperature regimes, including: (i) a constant 30°C, and (ii) 12-h fluctuations between 23°C (night) and 30°C (day). Plants were watered daily to maintain turgor. Five replications per isolate/temperature regime were used, and all tests were repeated twice.

Symptoms for both isolates at the constant 30°C treatment first appeared within 4 to 5 days after inoculation. These early symptoms consisted of limp leaves with slight curling on the edges (Fig. 2). True wilting was observed after 7 days (Fig. 3, right), followed 5 to 7 days later by yellowing and interveinal necrosis (firing) symptoms (Fig. 3, left), characteristic of bacterial wilt. Death of inoculated plants occurred within 18 to 21 days after inoculation. Those plants incubated at the fluctuating temperatures were slower to develop wilting, yellowing and firing symptoms than those at the constant 30°C (approximately 2 to 3 days later for each successive symptom). Inoculation with both the pink and GN isolates resulted in the same symptoms (Fig. 4) and similar disease development time intervals for various symptoms, indicating both were pathogenic and equally virulent. Identical orange- (Great Northern) and pink-colored bacterial colonies were re-isolated from symptomatic plants, but not from uninoculated controls, thus completing Koch's postulates.

Fig. 2. Early symptoms of bacterial wilt 4 to 5 days after inoculation, consisting of slight wilting and curling of leaf edges of the young trifoliolate.

Fig. 3. Advanced symptoms consisting of severe wilting (right) and interveinal necrosis (firing) on left 15 days post-inoculation. Newest leaves tend to wilt first with older leaves later producing the firing symptoms as disease progresses.

Fig. 4. Comparison of symptoms on inoculated plants with pink (left) and GN (right) isolates 15 days post-inoculation.

Conclusions

The pink isolate variant of bacterial wilt is very stable and appears to retain its color from the original isolation through multiple transfers and re-isolations from inoculated plants. Interestingly, this isolate was obtained from the same research farm that has yielded all three pathogen variants over the last three years, and the same general area of western Nebraska from where the original orange and purple variants were first found. As noted, however, seed color discoloration can be independent of the colony color expressed in culture, but in general, yellow-, purple-, and orange-colored seeds will yield yellow-, purple-, and orange-colored isolates in culture. This paper represents the first report of another color variant for *Cff*, and combined with the consistent occurrence of the other three pathogen variants for more than 50 years, further illustrates the high degree of microbial variation within this pathogen and Nebraska dry bean production fields.

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