BACTERIAL WILT

[Summarized by Howard F. Schwartz and Robert M. Harveson – December 2011]

Fig. 1. Bacterial wilt symptoms on leaves of common bean (Photograph provided by H. F. Schwartz – Colorado State University)

Fig. 2. Bacterial wilt symptoms on leaves of common bean (Photograph provided by R. M. Harveson – University of Nebraska)

INTRODUCTION: Although few in number, bacterial diseases have had major impacts on common bean production, and significant efforts have been made to manage them. Each disease is favored by high moisture conditions. Additionally factors such as storms, planting non-certified seed, proximity to infected volunteers, and equipment or irrigation water that cause wounding and move pathogens and infected residue between and within fields will all contribute to enhancing disease problems (Harveson and Schwartz, 2007; Schuster and Coyne, 1974; Schwartz, 1980).

Daily temperatures favoring development of each disease vary: halo blight - less than 27^{o} C (80^{o}F); bacterial brown spot – less than 30^{o}C (85^{o}F); common bacterial blight and bacterial wilt – greater than 27^{o}C (80^{o}F) (Ishimaru et al., 2005; Schwartz et al., 2005). This environmental information may be used in combination with developing symptoms and diagnostic methods to help to identify and distinguish between the various bacterial diseases that may be encountered in dry bean production. This research paper will focus on bacterial wilt.

DISEASE: Wilt is caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*), and was first reported from a South Dakota navy bean field in 1922 (Hedges, 1926; Zaumeyer and Thomas, 1957). It then became one of the most problematic bacterial diseases in the USA, particularly throughout the irrigated high plains and Midwest. Bacterial wilt was commonly found in dry bean production in western Nebraska during the 1960's and early 1970's but until recently has not been observed in the Central High Plains since that time (Harveson et al., 2005).

In 2003, the disease was found in two Nebraska (Scotts Bluff Co.) Great Northern fields and was widely observed throughout Colorado, Wyoming, and Nebraska from multiple (>200) fields during 2004-2005 (Harveson et al., 2005 and 2006). Affected fields were planted with beans from many different dry bean market classes and seed sources, including yellow, Great Northern, pinto, kidney, black, navy, small red, and Anasazi (Harveson et al., 2006). Wilt is most destructive after periods of plant stress and temperatures exceeding 32^{o}C (Ishimaru et al., 2005; Schwartz, 1980; Schwartz et al., 2005). The disease is also a problem in regions of Canada and other countries, and poses additional threats as a quarantine disease listed by EPPO (Conner et al., 2008; Ishimaru et al., 2005; Schwartz, 2009).

PATHOGEN: *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

PRIMARY HOSTS: Common bean (*Phaseolus vulgaris* L.)

SYMPTOMS AND SIGNS: Field symptoms consist of leaf wilting during periods of warm, dry weather or periods of moisture stress (Hagedorn and Inglis, 1986; Ishimaru et al., 2005; Schwartz et al., 2005) **(Figures 1** and **2)**. Plants often recover during evening hours when temperatures are lower, but wilt again during the heat of the day. Infected plants in the Central High Plains have additionally exhibited symptoms consisting of interveinal, necrotic lesions surrounded by bright yellow borders. These symptoms may be confused with those caused by the common bacterial blight pathogen, but bacterial

wilt lesions tend to be more irregular, and severely infected plants will die (Harveson et al., 2005; Schwartz et al., 2005).

If plants survive to produce mature seed, they are often discolored as a result of bacterial infection and colonization, particularly in the white-seeded market classes such as navy and Great Northern **(Figure 3)**. Infection can occur on pod sutures, but seldom causes circular spots (Schwartz, 1980; Schwartz et al., 2005). Seeds also may become infected even while pods appear to remain healthy, due to pathogen movement into developing seeds through the vascular system (Harveson et al., 2005).

Fig. 3. Bacterial wilt symptoms on Great Northern seed (Photograph courtesy of R. M. Harveson – University of Nebraska)

HOST RANGE: Known host range for *Cff* include scarlet runner bean (*Phaseolus coccineus*), lima bean (*P. lunatus*), pea (*Pisum sativa*), soybean (*Glycines max*), Azuki bean (*Vigna angularis*), *V. mungo*, mung bean (*V. radiata*), hyacinth bean (*Lablab purpureus*), and cowpea (*V. unguiculata*) (EPPO/CABI, 1997; Harveson and Vidaver, 2007; Ishimaru et al., 2005).

GEOGRAPHIC DISTRIBUTION: Bacterial wilt has been infrequently, but repeatedly observed as field infections in the Central High Plains of the U.S., although not until recently (Harveson et al., 2005 and 2006) after more than 30 years. It has also been reported from numerous countries representing widespread distribution across the world, including Canada (Alberta and Ontario), Tunisia, Turkey, Bulgaria, Greece, Hungary, Romania, Russia, former Yugoslavia, Belgium, Australia, Mexico, and Columbia (Hsieh et al., 2002; Ishimaru et al., 2005).

PATHOGEN ISOLATION: Isolation of the pathogen can be accomplished successfully in several ways. Lesion margins of infected leaves may be abraded, or punctured with a dissecting needle, and streaked onto medium plates (Harveson et al., 2005; Rickard and Walker, 1965). Another method is to squeeze the sap out of petioles attached to wilted or necrotic leaves and blot onto medium, followed by streaking with a sterilized inoculation loop (Harveson et al., 2006). Alternatively, the bacterium can easily be isolated from infected, discolored seeds. Discolored seeds are soaked overnight in water or buffer. Plates are then streaked with the leachate and/or imbibed seeds are plated after splitting in half (R. M. Harveson, unpublished). Fluidal colony growth will be seen emerging from margins of seed and on media surface after streaking**.**

PATHOGEN IDENTIFICATION: The pathogen is aerobic, gram-positive, with short, Coryneform-shaped, rods that characteristically bend or snap. Colony growth on nutrient broth yeast extract medium is slow and fluidal (Ishimaru et al., 2005; Lelliott and Stead, 1987; Vidaver and Davis, 1988). Pathogen color variants have been reported that stain seeds, and are particularly conspicuous on white seeded cultivars**,** including the original type strain yellow (Hedges, 1926), orange (Schuster and Christiansen, 1957), and purple (Huang et al., 2006; Schuster et al., 1968) (**Figure 4**). The purple pathogen variant produces an extracellular, pigment that diffuses into growth media after 7-10 days. Over time, the pigments still remain in media, but the initial orange or yellow colonies are not as obscured, and can be visualized much easier than with younger cultures.

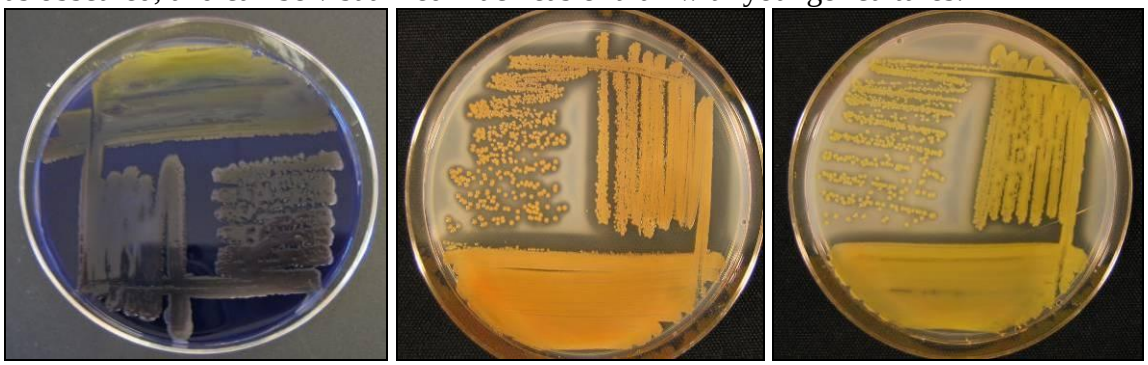

Fig 4. Bacterial wilt colonies and color variants on agar (Photograph courtesy of H. F. Schwartz, M. S. McMillan & K. Otto – Colorado State University)

PATHOGEN STORAGE: Long term preservation can be achieved by suspending bacterial cells in sterile glycerol (30-50%) or 10% dry milk and storing at -70 to -80^{o}C (Ishimaru et al., 2005; Schaad and Stall, 1988). Another readily available method is through commercially available kits. Microbank^{TM} (PRO-LAB Diagnostics, Austin TX) sterile vials contain porous beads serving as carriers in a liquid growth medium. Vials are inoculated with young cultures (18-24 hrs old), closed and inverted 4-5 times; so that bacterial cells will adhere to beads. Store at -70^{o}C until needed. Inoculated beads may be removed and used to streak directly to solid medium or dropped into an appropriate liquid medium. The pathogen may also be successfully stored long term on seeds. Due to a strong resistance to drying, the pathogen has been demonstrated to remain viable up to 24 years in seed stored under cool conditions in the laboratory (EPPO/CABI, 1997).

PATHOGENICITY TESTS: Due to the systemic nature of bacterial wilt, inoculation tests need to be conducted differently from the other bacterial pathogens of bean. Inoculations may be made with either: 1) a 26 gauge syringe using 2-3 day old liquid culture, or 2) inserting dissecting needles into plants dipped with bacterial growth from cultures (48 hours preferred) into plants. Syringes are inserted and contents injected into plant tissues (**Figure 5**), while needles are pushed through stems and petioles, and withdrawn back through the newly created holes. Incubation at 30^{o}C is optimal for disease development, and virulent isolates generally induce wilting and necrotic leaf symptoms with 14-21 days (R. M. Harveson, unpublished). Other inoculation methods of seed, cotyledons, and stems have also been used (Conner et al., 2008; Hsieh et al., 2003; Huang et al., 2007).

Schwartz et al. (2010) screened germplasm using the cotyledonary node inoculation method. Seven to 8 seeds were sown 2.5 cm deep into potting mix in a 15-cm wide plastic pot and thinned to 5 emerged seedlings prior to inoculation. The point of a sterile dissecting needle or syringe bearing inoculum was inserted into the stem at the cotyledonary node of 7 to 10 day old seedlings. The inoculated plants were incubated in a growth chamber (with high humidity) or greenhouse with a daily temperature of 28^{o}C / 22^{o}C – 16 hr day / 8 hr night photoperiod with watering as needed. Screening consisted of planting to emergence to inoculation to final evaluation 4 weeks later.

Fig 5. Inoculum preparation for inoculation of stem and cotylendary tissue (Photograph courtesy of CIAT – Bean Pathology Program)

EVALUATING REACTION OF GERMPLASM: Various researchers (Conner et al., 2008; Hsieh et al., 2005; Huang et al., 2007; Schwartz et al., 2010) have evaluated bean germplasm for resistance to bacterial wilt under controlled conditions, and the following materials (presumed to be *P. vulgaris*) have looked promising for resistance to one or more local isolates (color variants) of bacterial wilt:

	Resistant to Local Variants of Bacterial Wilt			
Entry	**Orange**	**Yellow**	**Purple**	**Citation**
AC Litekid (LRK)	R	R	R	Conner et al., 2008
Chinook 2000 (LRK)	R	R	R	
Redkanner (LRK)	R	R	R	
Cabernet (DRK)	R	R	R	
Red Hawk (DRK)	R	R	R	
L02E317 (GN)	R	R	-	Hsieh et al., 2003
Resolute (GN)	R	R	R	Huang et al., 2007
L02B662 (pinto)	R	R	-	
999S-2A (pinto)	R	R	-	
AC Agrinto (pinto)	R	R	R	
L02F132 (black)	R	R	R	
AC Early Rose (pink)	R	R	R	
PI 165078 (white)	R	R	R	Schwartz et al., 2010
PI 201010 (tan)	R	R	R	
PI 201329 (brown)	R	R	R	
PI 203958 (black)	R	R	R	
PI 207182 (brown)	R	R	R	
PI 207322 (black)	R	R	R	
PI 207336 (yellow)	R	R	R	
PI 309701 (red tan)	R	R	R	
PI 310611 (black)	R	R	R	
PI 310778 (gold)	R	R	R	
PI 311843 (pink mot)	R	R	R	
PI 311982 (red tan)	R	R	R	
PI 312018 (black)	R	R	R	
PI 313429 (red tan)	R	R	R	
PI 313501 (brown)	R	R	R	
PI 313512 (yellow)	R	R	R	
PI 313531 (red tan)	R	R	R	
PI 317350 (grey mot)	R	R	R	
PI 318695 (grey mot)	R	R	R	
PI 325614 (black)	R	R	R	
PI 325687 (brown)	R	R	R	
PI 451889 (grey mot)	R	R	R	
Emerson (white)	R	R	R	
Hungerford (white)	R	R	R	

Germplasm and cultivars were evaluated using a 1 – 4 rating scale: 1 (highly resistant) = no wilt or discoloration, 2 (moderately resistant) = wilt or discoloration at one of the unifoliolate leaves, 3 (susceptible) = wilt or discoloration on both unifoliolate leaves with no symptoms on the 1st trifoliolate leaf, and 4 (highly susceptible) = wilt or discoloration on the 1st trifoliolate leaf. Data were reported as an average severity for the replicated plants per isolate; and entries considered resistant had less than 1.51 rating to one or more bacterial wilt isolates (Schwartz et al., 2010).

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