**FUSARIUM WILT (YELLOWS)**

[ Summarized by Howard F. Schwartz and Kris Otto – December 2011 ]

**Fig. 1**. Fusarium wilt affected plants in common bean field during pod fill (Photographs provided by H. F. Schwartz – Colorado State University)

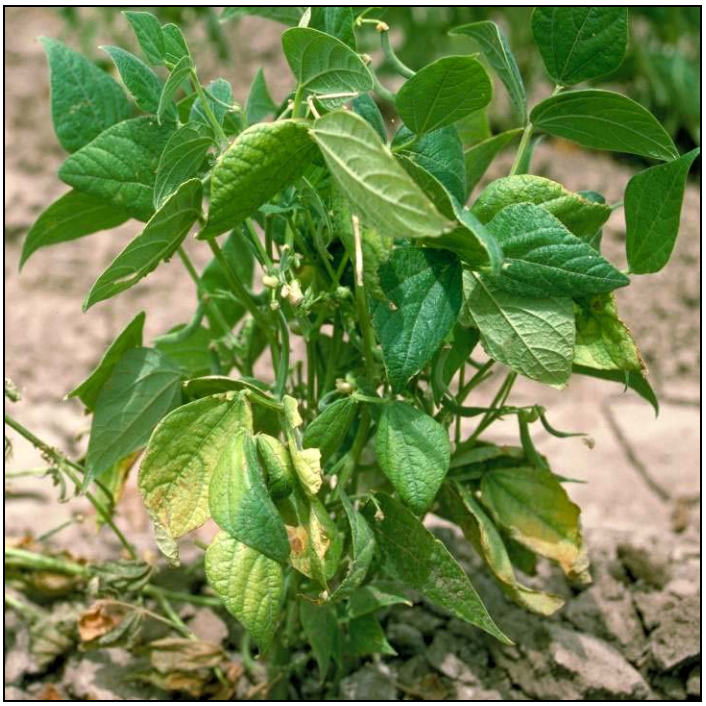

**Fig. 2**. Symptoms of Fusarium wilting and yellowing on leaves of common bean

**Fig. 3**. Fusarium wilt symptoms of vascular discoloration and streaks in hypocotyl and stem of common bean

**INTRODUCTION:** Fusarium yellows was originally discovered in dry beans in California in 1928. It has since been found elsewhere in the United States, South and Central America, Spain, and Africa. The disease is becoming more important in the midwestern United States, and is considered to be important in Brazil. A similar disease of scarlet runner bean has been reported in England and the Netherlands.
**DISEASE:** The pathogen inhabits soil in the form of chlamydospores and may also be transmitted as spores adhering to seed. Although symptom development occurs primarily on *Phaseolus* spp., the pathogen can colonize the roots of other plants, particularly legumes, and produce chlamydospores without causing symptoms on "reservoir" hosts. Infection of *Phaseolus* beans occurs through roots and hypocotyls, most commonly through wounds. Thereafter, the fungus grows through (and plugs) the vascular tissue, causing the plant to become chlorotic and drop its leaves (Figures 1 and 2).

The optimum temperature for disease development is 20°C. Extremes of soil moisture do not appear to be needed for the disease to occur but can influence disease severity. Soil compaction and poor drainage also appear to aggravate plant symptoms, with evidence of discoloration apparent in vascular tissues in the hypocotyl and stem (Figure 3).

**PATHOGEN:** *Fusarium oxysporum* f. sp. *phaseoli*

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

**SYMPTOMS AND SIGNS:** Initial symptoms are slight yellowing and premature senescence of the lower leaves. The chlorotic symptoms then appear on the next higher leaves on the stem and continue progressively up the plant. As the disease becomes more severe, leaves become increasingly yellow and finally bright yellow. If young plants are infected they remain stunted. The vascular tissues of the entire plant usually become reddish brown, often beyond the second node. Water-soaked lesions differing in size and shape may occur on pods.

**HOST RANGE:** Known hosts for Fusarium wilt include common bean (*Phaseolus vulgaris*), scarlet runner bean (*P. coccineus*), and tepary bean (*P. acutifolius*).

**GEOGRAPHIC DISTRIBUTION:** Fusarium yellows was originally discovered in dry beans in California in 1928. It has since been found elsewhere in the United States, South and Central America, Spain, and Africa. The disease is becoming more important in the midwestern United States, and is considered to be important in Brazil. A similar disease of scarlet runner bean has been reported in England and the Netherlands.

**PATHOGEN ISOLATION:** Samples for disease diagnosis should be taken when the problem is active or increasing. Areas should be selected for sampling where the damage or symptoms are representative of the entire affected area. Samples should be collected at the edge of an infected area, and they should include both healthy and infected plants exhibiting varying stages of infection. As soon as possible after collection, samples should be wrapped in dry paper toweling, placed in a container such as a self-sealing plastic bag to prevent dessication, and delivered to the clinic. In cases where the whole plant is collected, it is helpful to wrap the root system in plastic to prevent contamination of foliage with soil particles during handling and shipment (McMillan, 2005)..

Excise a portion (1 -2 mm) of diseased tissue from the hyptocotyl, root or vascular system, and place in 0.5% sodium hypochlorite solution (1 part bleach to 9 parts sterile distilled water or SDW) for 20 – 30 seconds. Rinse twice in SDW and shake or blot dry before transferring to agar. Plate on 2% water agar (WA) or potato dextrose agar (PDA), and incubate in the dark at 24-28° C for 5 – 7 days until sporulation is visible. Then transfer spores (single macroconidium if possible) to a new plate of PDA or other suitable medium for growth of the fungal colony and production of fresh macroconidia in 10 – 14 days. If bacterial contamination occurs during the Fusarium isolation from plant tissue, a 25% solution of lactic acid may be added to agar before pouring plates.

**PATHOGEN IDENTIFICATION:** Fusarium yellows is caused by the fungus *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *phaseoli* J. B. Kendrick & W. C. Snyder. At least seven pathogenic races are now known to occur in one or more bean growing regions of the world. Maximum mycelial growth occurs in culture at 28° C. The fungus typically has hyaline, nonseptate chlamydospores (2-4 X 6-15 u*m)* and elongate, two- or three-septate macroconidia that are slightly curved and 3-6 X 25-35 u*m.*

**PATHOGENICITY TESTS:** Start, grow and store cultures on agar plates or slants at 25-27$^{o}$C under variable light (Percival Incubator). They can be stored long term on artificial media at -80$^{o}$C. Fungal spore suspensions for inoculation are prepared in sterile distilled water by gently scraping plates to remove mycelium and conidia. The mycelium and macroconidia are passed through 2 layers of cheesecloth to remove mycelium, and then counted in a hemacytometer. Concentrations for inoculation are adjusted to 5 X $10^{5-7}$ conidia per ml, in a total suspension of 500 ml. Ten sporulating plates generally provide sufficient inoculum for suspension in 500 ml of sterile distilled water to inoculate 250 plants; additional 500 ml suspensions of fresh inoculum should be prepared for larger experiments.

Bean seedlings are grown to 10 – 14 days of age either in a greenhouse or a controlled growth chamber. Seedlings are removed from the potting medium, roots clipped (25% removed), and root systems placed in a container with water for 5 – 10 minutes prior to inoculation. Plants are then inoculated by placing clipped root systems in the spinning spore suspension for 2 – 5 minutes (**Figure 4**). Inoculated (and water-control) plants are then transplanted into sterile potting mix or soil in pots or flats; and placed in a growth chamber or greenhouse bench. Plants are then maintained with a normal photoperiod, and day / night temperature regime that favors normal plant development (e.g., 25 - 30°C / 18 – 22°C). After incubation for 14 – 28 days (**Figure 5**), non-wilted plants are pulled from the soil, and root/hypocotyl tissues split to observe for symptom incidence and severity; wilted and dead plants are noted earlier or at this time as well. Reisolation from infected or dead plants will confirm identity and pathogenicity of suspected *Fusarium oxysporum* cultures.

**Fig. 4.** Inoculation of bean root systems by dipping in a suspension of Fusarium macrocondia, followed by transplanting in autoclaved soil mixture

**Fig. 5.** Response of bean seedlings to inoculation of bean root systems 14 days earlier by dipping in a suspension of Fusarium macrocondia

**HOST PLANT RESISTANCE:**

Isolates of Fusarium wilt can be classified into a race structure based on isolate reaction on different bean genotypes (Woo et al., 1996). Two pathogenic races of wilt were confirmed based on differential reaction of bean germplasm to isolates from Brazil, the Netherlands and the U.S. (Ribeiro and Hagedorn, 1979). Since the publication of this work in 1979, other races of Fusarium wilt have been identified in the U.S. (Schwartz et al., 1989). Pathogenic variability of 32 isolates was characterized into five pathogenic races based on differential reaction on 10 bean genotypes (Woo et al., 1996). Races 1 and 2, previously described by Ribeiro and Hagedorn (1979) from the U.S. and Brazil, respectively, were confirmed to be distinct from race 3 from Colombia, race 4 from Colorado and race 5 from Greece.

Resistance to Fusarium wilt in common bean is conferred by major resistance genes. The *Fop-1* gene confers resistance to the Brazilian race 2 of Fop, whereas *Fop-2* confers resistance to the U.S. race 1 from S. Carolina (Ribeiro and Hagedorn, 1979). In addition to the single dominant resistance gene model, Salgado et al. (1995) reported on recessive and polygene resistance to race 4 from Colorado. The Colorado race was virulent on most bean cultivars grown in the U.S., but new sources of resistance were detected in Durango race beans from Mexico including Lef2RB and Sierra pinto (Velasquez-Valle and Schwartz, 1997). In Rwanda, the cultivars Vuninkingi (G685 originally from Guatemala) and Flor de Mayo (Mexico) were resistant to Fop (Buruchara and Camacho, 2000) which suggests that different races are present as Flor de Mayo is very susceptible to race 4 from Colorado and Spain (Velasquez-Valle et al., 1997). The *Phaseolus* core collection was screened for resistance to races 1, 4, and 5 and only five PI accessions were resistant to all three races (Brick et al., 2006). Cross et al. (2000) showed that resistance to race 4 is controlled by single dominant gene in race Durango beans whereas resistance in Mesoamerican (MA) race beans is under quantitative inheritance, similar to the findings of Salgado et al. (1995). Given the quantitative nature of resistance in MA germplasm, QTL analysis was conducted using race 4 wilt and a major QTL that accounted for over 63% of the genotypic variability was detected on chromosome B10 suggesting that resistance acts largely in a qualitative manner (Fall et al., 2001). Current work is focused on mapping the other major QTL for resistance from Lef2RB and Sierra pinto germplasm sources (Roman-Avilés et al., 2012).

**EVALUATING REACTION OF GERMPLASM:** Various researchers have evaluated bean germplasm for resistance under controlled conditions. The following materials have looked promising for resistance to one or more local isolates (races) of Fusarium wilt:

| **Entry (Species)** | **Reaction to 1 or more Races** | **Citation** |
|---|---|---|
| PI 200749 (Pa) | R | Salgado et al., 1994 |
| PI 209480 (Pa) | R | |
| PI 310606 (Pa) | R | |
| PI 310800 (Pa) | R | |
| PI 310801 (Pa) | R | |
| PI 310802 (Pa) | R | |
| PI 312122 (Pa) | R | |
| PI 319438 (Pa) | R | |
| PI 319442 (Pa) | R | |
| PI 331181 (Pa) | R | |
| PI 440787 (Pa) | R | |
| PI 440791 (Pa) | R | |
| PI 440794 (Pa) | R | |
| PI 440803 (Pa) | R | |
| PI 440804 (Pa) | R | |
| PI 462025 (Pa) | R | |
| PI 477033 (Pa) | R | |
| PI 477039 (Pa)4 | R | |
| PI 477035 (Pa) | R | |
| PI 477039 (Pa) | R | |

| | | |
|---|---|---|
| PI 527334 (Pa) | R | |
| PI 535214 (Pa) | R | |
| PI 321637-s (Pa) | I | Miklas et al., 1998 |
| PI 321638-a (Pa) | I | |
| PI 440788-s (Pa) | I | |
| PI 440806-s (Pa) | I | |
| PI 502217-s (Pa) | I | |
| Mex 114 (Pa) | I | |
| Neb-T-5-s (Pa) | I | |
| BAT 336, 477, 1385, 1400 (Pv) | R | Pastor-Corrales & Abawi, 1987 |
| G 4000 = NEP Bayo 22 (Pv) | R | |
| A 55, 107, 170, 195, 295, 300, 301 (Pv) | R | |
| AND 286, 323, 357 (Pv) | | |
| LM 21525 (Pv) | R | |
| V 8025 (Pv) | R | |
| WAF 4 (Pv) | R | |
| EMP 81 (Pv) | R | |
| XAN 112, 195 (Pv) | R | |
| HF 465-63-1 (Pv) | R | |
| MCD-025 (Pv) | R | |

| | | |
|---|---|---|
| Bayo Rio Grande (Pv) | R | |
| Cacahuete 72 (Pv) | R | |
| Calima (Pv) | R | |
| Ecuador 605, 1056 (Pv) | R | |
| Mortino (Pv) | R | |
| Nima (Pv) | R | |
| San Cristobal 83 (Pv) | R | |
| Sanilac (Pv) | R | |
| Top Crop (Pv) | R | |
| Tundama (Pv) | R | |
| CSU CO33142 (Pv) | R | Salgado et al., 1995 |
| CSU CO59196 (Pv) | R | |
| Jamapa (Pv) | R | |
| Porrillo Sintetico (Pv) | R | |
| Rio Tibagi (Pv) | I | |
| LEF2RB (Pv) | R | Velasquez-Valle et al., 2002 |

Species: Pv = *Phaseolus vulgaris*, Pa = *Phaseolus acutifolius*

Reaction: Germplasm and cultivars were evaluated using the CIAT 1 – 9 rating scale (Pastor Corrales and Abawi, 1987): 1 (no external symptoms) to 9 (plant > 75% diseased). R = resistant (1 – 3), I = intermediate (3.1 – 6), S = susceptible (6.1 – 9).

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