BEAN ROOT ROT EVALUATION PROTOCOLS

Root rot diseases are widespread and often considered a major constraint to bean production, reducing both yield and profitability worldwide. Depending on the pathogen(s) involved, general root rot symptoms might include any combination of the following: poor seedling establishment, damping-off, uneven growth, chlorosis, premature defoliation, death of severely infected plants, and lower yield (see above). The roots of infected plants are reduced in size, discolored, and exhibit various stages of decay. Several soilborne pathogens are known to cause root rot diseases on beans, but their prevalence and damage varies from one production region to another. In New York State, the major soilborne pathogens found causing damage to beans are *Fusarium solani* f. sp. *phaseoli*, *Pythium ultimum*, *Rhizoctonia solani*, *Thielaviopsis basicola* and *Pratylenchus penetrans* (lesion nematode).

The involvement of multiple soilborne pathogens with different mechanisms of pathogenicity has made it difficult to develop a simple and effective disease management program. Currently, the management of root rot diseases is possible only through the use of a combination of control options (cultural, chemical and biological) which utilize the concept of Integrated Pest Management (SOIL-IPM). Therefore, a thorough knowledge of the fields' cropping and management history, including the identity of causal pathogen(s), is critical for the formulation of practical and effective IPM program.

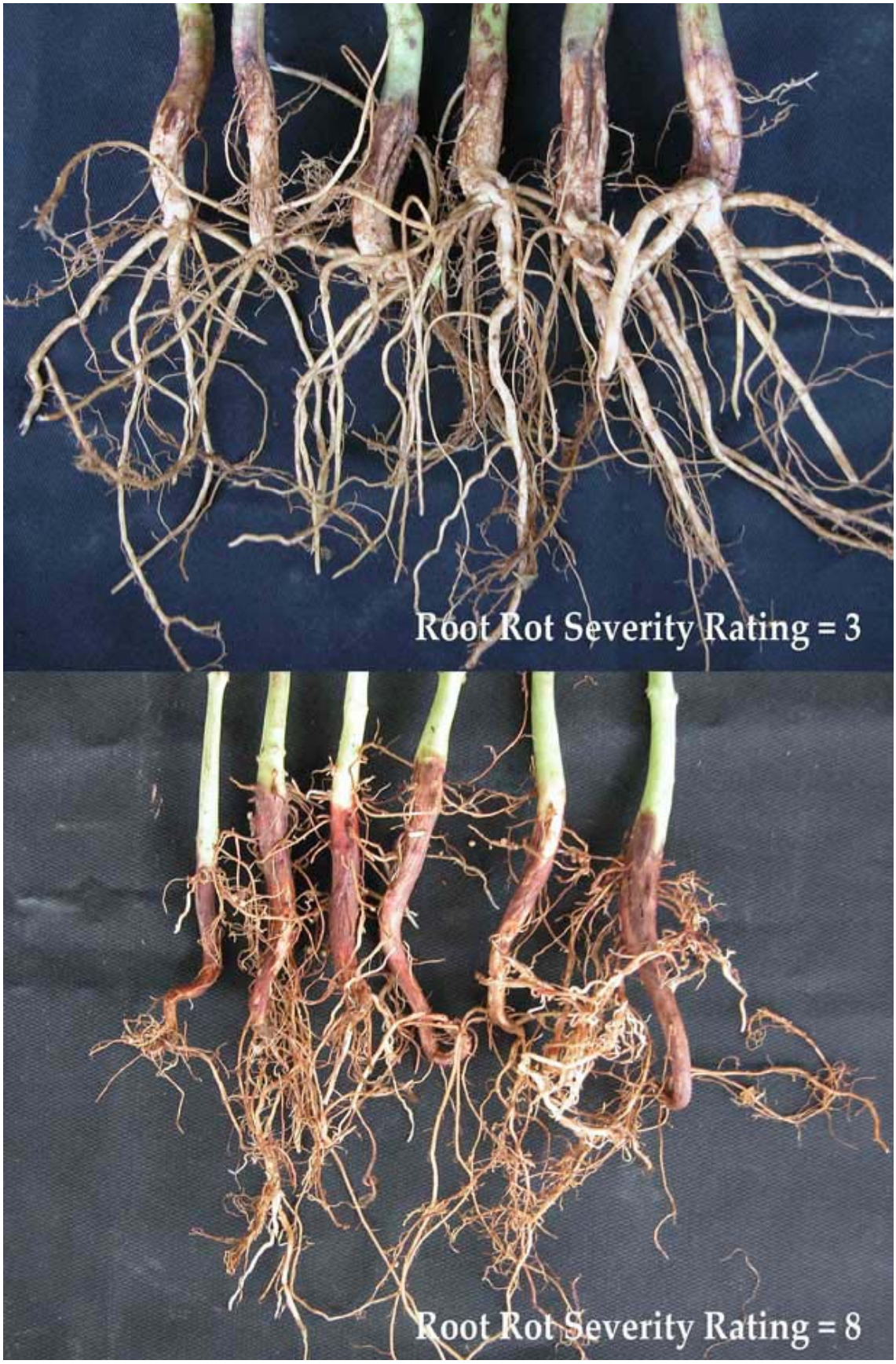
However, the single most effective and practical management strategy is the use of bean cultivars that are resistant to the most common soilborne pathogen(s) in a production region. Bean germplasm lines with resistance to a single or multiple pathogens have been reported and in few cases, fully characterized. Unfortunately, commercial bean varieties currently grown in New York State still do not exhibit a high level of tolerance to the prevailing root pathogens and similar needs exist in the majority of the bean producing regions of the world. The following is a field and greenhouse protocols which can be used to screen germplasm for resistance to soilborne pathogens. The techniques can be adapted based on the predominant pathogens in the region of interest.

Field Evaluation Protocol

To effectively screen germplasm for resistance to the predominate soilborne bean pathogens in New York, a root rot nursery (~ 1 ha) was established twelve years ago at the Vegetable Research Farm, NYSAES, Geneva, NY. To build-up pathogen inoculum, the field was double cropped to a susceptible bean variety during each of the first two years of establishment. In the first year, emerging seedlings in each planting were inoculated with *F. solani*. f. sp. *phaseoli* (Fsp), *T. basicola* (Tb), *P. ultimum* (Pu) and *R. solani* (Rs). Inocula of Fsp and Tb consisted of conidial suspensions that were applied to the base of the seedling stems. Inoculum of Pu consisted of a suspension of sporangia or colonized rye or wheat seeds, whereas the inoculum of Rs was a suspension of hyphal fragments or colonized rye or wheat seeds. All seedlings were hilled immediately after inoculation and irrigated. After two years (4 bean crops), root incidence and severity became uniform and severe enough that additional infestations have not been required during the subsequent 10 years of continuous bean germplasm plantings.

Seeds of all the bean germplasm to be evaluated are first treated with two fungicides (Apron + Captan) and an insecticide (Lorsban) at recommended commercial rates. The latter seed treatments make it possible to evaluate bean germplasm for the root rot phase and not the seed-decay and damping-off stages which are effectively controlled by chemical or biological products. Seeds of each germplasm are then planted in two 4-m-long-rows, 76-cm apart and replicated a minimum of 4 times and arranged in a completely randomized block design.

The plots are irrigated as needed and all additional maintenance practices are performed according to recommended commercial guidelines. Seedling emergence and stand establishment are recorded at 3 and 6 weeks after planting, respectively. The number of productive plants and seed or pod weight are recorded at harvest. Root rot severity is assessed at the full-bloom stage (usually 6 weeks after planting) on 20 or more plants dug from one of the two rows. The washed roots are rated on a scale of 1 (normal root/healthy) to 9 (75% of root and stem tissues affected and decaying). Generally, germplasm lines with an average root rot severity ratings of 1-3, >3-6, and >6-9 are described as resistant, intermediate, and susceptible, respectively. Known susceptible and known or reported resistant germplasm (if available) are included for comparison and for determining fluctuations in root rot severity between growing seasons and monitoring which pathogen(s) are present.


Root Rot Severity Rating = 3
Root Rot Severity Rating = 8

Greenhouse Evaluation Protocol

Bean germplasm lines that exhibit field resistance to root rot pathogen(s), are adapted to local conditions, and/or exhibit other desirable traits are candidates for greenhouse root rot screening. Their reaction to a single pathogen, specific race/strain of a pathogen or combination of pathogens can be tested under greenhouse conditions. Seeds or seedlings of bean germplasm are planted in clay pots ≥(10 cm in diameter) containing pasteurized soil (30 min at 60ºC). Depending on the target pathogen, the soil can be infested with the inoculum prior to planting, at planting time or at a specific seedling stage. However, several suggested methods for inocula preparation and inoculation techniques for the various root rot pathogens are available (Abawi and Pastor-Corrales, 1990). For example, soil-potato inoculum is effective for screening for resistance to Rs. Bean seeds are planted in pasteurized soil mixed thoroughly with this inoculum preparation at a rate of 1-5% (vol. to vol.) or the infested soil is placed around the stem of emerging seedlings in a plastic or paper collar placed on top of the pots. Seed of grain crops or beet seed colonized by Rs can also be used as an inoculum source and mixed with pasteurized soil or placed directly next to the seedling stems near the soil surface. For screening for resistance to the Fusarium Yellows pathogen, it is best to first plant seeds in sterile sand or light soil. After one week, seedlings are removed; 1-cm segments are cut from the root tips, seedlings are dipped in a spore suspension of Fop (10^6 conidia/ml) and then transplanted into pots filled with pasteurized soil. Greenhouse evaluations have resulted in uniform, high infection rates and are generally simple, cost-effective and rapid. Greenhouse evaluations are ideal for characterization of resistance gene(s) to specific pathogens and assist in the development of molecular markers for such factors. However, greenhouse results are dependable in the development of resistant cultivars only if they correlate closely to the reaction of bean germplasm under field conditions. In contrast, field screening under naturally fluctuating conditions accurately measure the reaction of bean germplasm to root rot pathogens and to assess the impact on the quantity and quality of marketable yield (seeds or pods). Field screening also permits the selection for local adaptation, reaction to other disease pathogens and pests, and to tolerance abiotic stresses.

Selected References

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[Information and photos provided by George S. Abawi, John w. Ludwig, and Beth K. Gigino]

Rhizoctonia Root Rot Screening Protocol for Dry Beans

1. Grow *Rhizoctonia solani* isolate* (stored in sugar beet seeds) in water agar for 3 to 5 days at room temperature (22±1°C).
2. Transfer one plug (6 mm) into a Petri dish containing Potato Dextrose Agar and incubate for 3 to 5 days at room temperature (22±1°C).
3. Autoclave soil** and Potato Dextrose Broth for 15 to 20 min.
4. Place 100cc of soil**, 30 ml of PD broth and 20 plugs (6 mm each) of *R. solani* from the margins of the PDA plates in a deep Petri dish.
5. Incubate 15 days at 22°C.
6. Mix each Petri dish with steamed soil in a ratio 1:10 (one Petri dish mixture per 900cc of soil**)
7. Plant 5 bean seeds per 4 in pot and cover them with 100cc of inoculum.
8. Water daily or as needed without flooding the pots.
9. Grow inoculated plants 15 days in greenhouse (22-26°C).
10. Remove plants from the pots and wash the roots gently.
11. Use 1 to 9 scale to score disease (See Picture)
    - 1 to 3 = resistant
    - 3 to 6 = moderately resistant
    - 7 to 9 = susceptible
12. Use at least 4 replications of each pot experiment
13. Perform an ANOVA, comparisons among treatments, isolates, varieties, replications using SAS

* We use the isolates WN-11 (AG-2-2 IV), WN-116 (AG-2-2 IIIB/LP, WN-293 (AG-4 HGIII).
** If preferred, mix 3 parts soil and 1 part sand to simulate sandy loam field conditions

[Information and photos provided by Pamela Pena, J.R. Steadman, Carlos Urrea, University of Nebraska, Lincoln]

Root Rot Rating Scale

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