**Arizona Iceberg Lettuce Research Council**
**Annual Research Report**
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**Project title:** Biocontrol strategies for sustained management of Fusarium wilt of lettuce in Arizona

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**Introduction:** In 2001, Fusarium wilt of lettuce caused by the soilborne fungus *F. oxysporum* f. sp. *lactucae* (FOL) was detected in 6 fields near Wellton, AZ. This was the first report of Fusarium wilt of lettuce in Arizona. Since 2001, the fungus has continued to spread in the Yuma area where lettuce is produced. During this time, much has been learned about the epidemiology of the disease, particularly that it is a warm season pathogen, which has allowed for a certain level of disease management through avoidance of infested fields during optimal periods for disease development. But FOL is an opportunistic soil inhabitant. Once introduced in an area, it can persist in the soil almost indefinitely, even in the absence of a lettuce crop, and this requires a broader approach to long-term management of this disease including strategies based on host genetics, cultural practices, and biological control.

Experience with biocontrol of Fusarium wilt diseases of other crop reveals promise in non-chemical management of these destructive diseases. What is now necessary for Arizona lettuce production is to select the most novel/promising means to control Fusarium diseases in other crops and to examine their efficacy on wilt of lettuce, and specifically Fusarium wilt of lettuce in Arizona. The continued development of improved and flexible strategies for management of this disease will insure the Arizona's prominence as the premier winter lettuce production region.

Previous studies have shown a number of biocontrol agents effective in reducing the incidence of Fusarium wilt on other crops including cotton, tomato, melon, lentil, radish, etc. Biocontrol organisms include both *Fusarium*-antagonistic bacteria and fungi. Further, rhizosphere-competent biocontrol agents are flexible in their utility because they can protect not only the seed and seedling but also the mature plant. Thus, control could be through application in seed coatings or as seed bed applications at planting, and through continued application through the growing season. Biological control with introduced antagonists is economically viable, due to the limited amount of product needed because many of these agents reproduce effectively in the field following application.

Other advantages of biological control agents include efficacy against fungicide-resistant pathogens, reduced probability of resistance development, and use in organic farming situations where the use of chemicals is restricted. Thus, biocontrol strategies are considered a critical component in any integrated disease management program for Fusarium wilt. However, the challenge with biocontrol is matching the right organism with the right pathogen in the environment under the proper application techniques. An improper match will result in non-efficacy and failure to control the disease under specific conditions. This is not to say that the biocontrol strategy is generally ineffective, just that the strategy is ineffective under the conditions of the improper match. The distinction between these conditions and the potential

application for disease management is the kind of information that only comes about through testing under local conditions. However, once established, locally effective biocontrol strategies are enduring.

Our proposed study evaluated a number of commercially available biocontrol products against different *Fusarium* sp. that have shown efficacy under varied cropping systems and under varied environmental conditions. In addition to products that have shown efficacy under field conditions, we examined several products that have shown efficacy under greenhouse condition, but have not been evaluated under field conditions. Thus, we examined these products against Fusarium wilt of lettuce both in field cropping system and in controlled greenhouse studies.

**Materials and Methods.**

**Field trials.** Two field trials were conducted near Yuma, AZ, each in a field with a history of lettuce production and of Fusarium wilt of lettuce disease. The soils in both fields were a silty clay loam. Lettuce cv Raider was seeded in double rows 12 in. apart on beds with 42 in. centers, then sprinkler-irrigated to germinate seed in on 10 Sep and 16 Sep for trial 1 and trial 2, respectively. Treatment plots were replicated three times in a randomized complete block design with each replicate treatment plot consisting of 25 ft of bed and a 5 ft of bed buffer between plots. Plants were thinned 28 Sep and 07 Oct for trial 1 and trial 2, respectively, at the 3-4 leaf stage to an approximate spacing of 12 in. All plots were managed from production similarly regarding irrigation, fertilization, and insect pest management. All products were applied twice. Products tested and rates appear in the table below. The first application was at planting (9/10/15 and 9/16/15 for Trial 1 and 2, respectively) with sprinkle irrigation following within 4 hours. The second application was post-thinning. In Trial 1, thinning was on 9/28/15 and the application was on 10/5/15. In Trial 2, thinning was on 10/7/15 and the application was on 10/15/15. Furrow irrigation followed the second application within 4 hours.

**Greenhouse trials.** The lettuce cultivar Raider was used as host for all greenhouse studies and seeds were planted in a sterile soil mix (1 part peat: 1 part vermiculite: 2 parts sand) in 12”x18” metal trays. At 8 days post emergence, seedlings were thinned to 100 seedlings per tray and trays were arranged in the greenhouse in a RCBD with 4 blocks. At 10 days post emergence, plants were inoculated with the same biocontrol products as used in the field trials at the same rate. For inoculation, soil in each tray was soaked with each respective treatment. At 28 days post inoculation, 10-day-old cultures of the *F. o.* f. sp. *lactuca isolate* (isolate HL2) were used to make a spore suspension ($10^4$ spores/ml $H_2O$) and inoculate each tray using the same soil drench method as for each treatment. The trays were incubated in the greenhouse at day and night temperatures of 25 ± 3$^o$C and 19.0 ± 3 $^o$C, respectively. All trays were managed similarly using standard production regarding irrigation, fertilization, and insect pest management appropriate for greenhouse lettuce production. The lettuce was grown for another 28 days, at which time the incidence of disease (dead plants) was recorded.

**Results.**

**Field trials.** Symptoms of Fusarium wilt, including stunting and chlorotic leaves, were first observed on 1 and 8 Oct in trial 1 and 2, respectively. Maximum and minimum (EF) soil temperatures at the 4 in depth recorded at a nearby University of Arizona AZMET (Arizona Meteorological Network) weather station were as follows: 97-89 during Sep; 92-76 during Oct; and 77-57 during Nov. Monthly rainfall in inches was as follows: Sep, 0.30; Oct, 0.21; Nov,

0.24. Disease severity was recorded at crop maturity (from 16 to 20 Nov) for each trial by counting both the remaining lettuce plants in each plot (total) and the number of plants that were marketable at the time of disease assessment. Disease incident data were subjected to analysis of variance (ANOVA), then compared for significance using Fisher's Protected LSD test.

Overall compared to disease levels in Trial 1, the incidence of Fusarium wilt in Trial 2 was lower (48% and 13%, respectively, among products tested). Thus, only the results of Trial 1 are presented belose. A review of all treatments found that only Rootshield Plus/low rate had a significant increase in number of total heads per plot over that found in the control plots. Regarding the number of marketable heads, only Serenade/low rate had a significant increase in number of marketable heads per plot over that found in the control plots. No symptoms of phytotoxicity were observed on lettuce treated with any of the products. Table 1.

**Greenhouse trials.** Symptoms of Fusarium wilt, including stunting and chlorotic leaves, were first observed on seedlings 14 days post inoculution. Disease incidence (dead plants) was recorded at 42 days post inoculation for each treatment. Disease incident data of treatments were compared to incidence data from control (no i) and were subjected to analysis of variance (ANOVA), then compared for significance using Fisher's Protected LSD test.

Overall compared to disease levels in the field trials, the incidence of Fusarium wilt in greenhouse trails was higher that field trials. A review of all treatments found that only Rootshield Plus/high rate had a significant decrease in the incidence of disease (# diseased plants/ # plants in control) over no treatment. Similar to field trials, Serenade/low rate also had a significant decrease in the incidence of disease No symptoms of phytotoxicity were observed on lettuce treated with any of the products. Table 2.

**Discussion.** Results from both the field trials and greenhouse studies were not conclusive and an additional year of trials would be necessary to confirm these findings. However, these additional trials will be conducted in the same manner as in 2015 with the exception of the location of the field trials and the quantity of disease inoculum applied in the greenhouse trials. In 2015, the fields selected for our trials did not have sufficient natural inoculum to generate the level of disease necessary for a comprehensive evaluation of products. While it was known that these fields had a history of Fusarium wilt, the level of disease expressed in 2015, even with highly susceptible cultivars did not permit a definitive separation of product performance. While it is difficult to predict in advance the impact of soilborne inoculum based upon the previous year's incidence of disease, we now know that a history of moderate levels of disease is not enough and we need to select fields with the very highest disease incidence history. Now there is a risk in conducting trials in such fields in that the trial may conclude with all plants being dead and no harvest data is generated. However, if disease is scored at intervals over the course of the season, this will allow the selection of a time period where a separation in product performance is most optimal. And although harvest data might not be possible under these conditions, a comparison of products to suppress disease for the longest period of time will be possible.

Similarly, trials conducted in the greenhouse in 2015 did not result in a significant separation in product performance. While we were able to generate data that revealed a difference between any treatment (doing something) and the control (doing nothing), the data did not reveal significant difference between most products. The fact that variation in results among many of the treatments was rather low may be evidence of this. In future studies, we hope to

conduct trials with higher levels of disease inoculum. In this way, we will be able to evaluate product performance under varied conditions and better determine if the effects and benefits of each product are disease pressure dependent. This information will be most valuable to growers for projecting product performance under conditions more common to typical Yuma lettuce production.

**Table 1. Field trials**

| Treatment | Number of healthy heads per plot at crop maturity[x] | |
|---|---|---|
| | Total | Marketable |
| Rootshield Plus /low (2 tsp/gal) | 58 a | 41 ab |
| Great White /high (5.2 tsp/gal) | 56 ab | 42 ab |
| Serenade/low (60 ml/gal) | 55 ab | 48 a |
| Rootshield Plus /high (8 tsp/gal) | 55 ab | 42 ab |
| Hydroguard/low (2 ml/gal) | 54 ab | 39 ab |
| Root Pack/high (16 tsp/gal) | 53 b | 42 ab |
| Actinovate/low (2 tsp/gal) | 53 b | 38 ab |
| Great White/low (1.3 tsp/gal) | 53 b | 39 ab |
| MycoStop/low (0.66 tsp/gal) | 52 b | 38 ab |
| Hydroguard /high (8 ml/gal) | 52 b | 36 ab |
| **Control** | 48 b | 34 b |
| MycoStop/high (2.4 tsp/gal) | 47 b | 37ab |
| Serenade/high (240 ml/gal) | 46 b | 29 b |
| Root Pack/low (4 tsp/gal) | 45 b | 30 b |
| Acinovate/high (8 tsp/gal) | 44 b | 28 b |

Values alues followed by a separate letter are statistically different according to Tukey's test ($P <$ ı.05%).

**Table 2. Greenhouse Trial**

| Treatment | | |
|---|---|---|
| | Total # of plants | Incidence of disease[x] |
| Rootshield Plus /high (8 tsp/gal) | 76 | 24 a |
| Serenade/low (60 ml/gal) | 68 | 32 a |
| Great White /high (5.2 tsp/gal) | 67 | 33 ab |
| Root Pack/high (16 tsp/gal) | 67 | 33 ab |
| Hydroguard/low (2 ml/gal) | 58 | 42 ab |
| Acinovate/high (8 tsp/gal) | 57 | 43 ab |
| Rootshield Plus /low (2 tsp/gal) | 53 | 47 ab |
| MycoStop/high (2.4 tsp/gal) | 53 | 47 ab |
| Great White/low (1.3 tsp/gal) | 53 | 47 ab |
| MycoStop/low (0.66 tsp/gal) | 52 | 48 ab |
| Hydroguard /high (8 ml/gal) | 52 | 48 ab |
| Serenade/high (60 ml/gal) | 46 | 54 b |
| Root Pack/low (4 tsp/gal) | 45 | 55 b |
| MycoStop/low (0.66 tsp/gal) | 44 | 56 b |
| **Control (no treatment)** | 41 | 59 b |
| **Control (no inoculum)** | 100 | 0 a |