

# Improved Screening of the Arizona Population of *Fusarium oxysporum* f.sp. *lactucae* Final Report

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## Executive Summary

To examine genetic variation in *Fusarium oxysporum* f. sp. *lactucae* (FOL), the causal agent of Fusarium wilt of lettuce, a collection of isolates was recovered from symptomatic lettuce plants in Arizona. Additionally, isolates from California were added to the collection to determine if the Arizona isolates differed from the CA isolates. Over 150 isolates were assembled from 42 locations and production companies in AZ and CA isolates in 2021 and early 2022 (Table 1). The collection included reference isolates from California and also the original isolate of FOL first recovered from lettuce in the U.S. (KP02). Several isolates from Italy and the Netherlands, were examined including several FOL race 4 isolates. More recently, reference isolates of FOL race 2 and race 3 (designated *F. odoratissimum* and *F. cugenangense* respectively) were obtained for examination.

Isolates were tested for pathogenicity and grouped into vegetative compatibility groups (VCGs). Pathogenicity tests indicated that the isolates could be clearly identified as *Fusarium oxysporum* f.sp. *lactucae* (Table 2), and all AZ and CA isolates examined collected, including the original isolate of FOL from 2002 (KP02), belong to VCG 0300 (Table 1). Vegetative compatibility tests are a non-molecular test to assess relatedness of fungal isolates by examining the ability of two isolates to fuse. Isolates that share a VCG have a higher genetic similarity than isolates of another VCG. The four FOL races have been found to be genetically distinct, with each as a separate VCG subgroup (Fujinaga et al., 2005; Mbofung et al., 2007; Pintore et al., 2017).

A subset of isolates was examined for molecular diversity by examining sequence variation in the elongation factor target sequence (Figure 1). The majority of the isolates examined show little to no sequence variation. Interestingly, two isolates of FOL race 4 from Italy (GG41 and GG42) could be distinguished from the rest of the isolates examined based on vegetative compatibility (Table 3).

Based on the vegetative compatibility and molecular assays, the isolates representing the Arizona *Fusarium oxysporum* f.sp. *lactucae* population are highly similar. This suggests that changes in disease severity observed in fields in Arizona are due to an increase of the pathogen levels in the soil. However, changes in the native population may still occur, or material contaminated with a pathogen variant may enter the growing region.

Isolates have been collected in California that do not conform to the race 1 phenotype. Care should be taken to not introduce this pathogen variant into Arizona on plant material including seed, and in soil on equipment. Growers should continue to report unexpected disease incidence or severity, especially on cultivars with a history of tolerance or resistance to FOL. A robust pipeline for evaluating FOL populations is in development, and the pathogenicity of FOL isolates from Arizona and California continues to be examined outside of the scope of this project.

## References

- Fujinaga, M., Ogiso, H., Tsuchiya, N., and Hideki, S. 2001. Physiological specialization of *Fusarium oxysporum* f. sp. *lactucae*, a causal organism of fusarium root rot of crisp head lettuce in Japan. J. Gen. Plant Pathol. 67:205-206.
- Fujinaga, M., Ogiso, H., Shinohara, H., Tsushima, S., Nishimura, N., Togawa, M., Saito, H., and Nozue, M. 2005. Phylogenetic relationships between the lettuce root rot pathogen *Fusarium oxysporum* f. sp. *lactucae* races 1, 2, and 3 based on the sequence of the intergenic spacer region of its ribosomal DNA. Journal of General Plant Pathology 71:402-407.
- Mbofung, G. Y., Hong, S. G., and Pryor, B. M. 2007. Phylogeny of *Fusarium oxysporum* f. sp. *lactucae* inferred from mitochondrial small subunit, elongation factor 1- $\alpha$ , and nuclear ribosomal intergenic spacer sequence data. Phytopathology 97:87-98.
- Pintore, I., Gilardi, G., Gullino, M., and Garibaldi, A. 2017. Analysis of vegetative compatibility groups of Italian and Dutch isolates of *Fusarium oxysporum* f. sp. *lactucae*. Journal of Plant Pathology (Rivista di patologia vegetale) 99:517-521.

**Table 1. Master list of isolates of *Fusarium oxysporum* f. sp. *lactucae* VCGs Updated: 06/27/2022**

| Isolate <sup>a</sup> | VCG ID | Race | Collected <sup>b</sup> | Location | Company/<br>Contact |
|----------------------|--------|------|------------------------|----------|---------------------|
| SS01                 | 0300   |      | 10/26/20               | AZ       | 1                   |
| SS05                 | 0300   |      | 10/26/20               | AZ       | 2                   |
| SS06                 |        |      | 10/26/20               | AZ       | 2                   |
| SS09                 | 0300   |      | 10/26/20               | AZ       | 3                   |
| SS10                 |        |      | 10/26/20               | AZ       | 4                   |
| SS12                 | 0300   |      | 10/26/20               | AZ       | 5                   |
| SS13                 | 0300   |      | 10/26/20               | AZ       | 5                   |
| SS14                 | 0300   |      | 10/26/20               | AZ       | 5                   |
| SS15                 | 0300   |      | 10/26/20               | AZ       | 6                   |
| SS16                 | 0300   |      | 10/26/20               | AZ       | 6                   |
| SS17                 | 0300   |      | 10/26/20               | AZ       | 6                   |
| SS18                 |        |      |                        | CA       | 7                   |
| SS19                 | 0300   |      |                        | CA       | 7                   |
| SS20                 | 0300   |      |                        | CA       | 7                   |
| SS21                 | 0300   |      | 11/04/20               | AZ       | 8                   |
| SS22                 |        |      | 11/04/20               | AZ       | 8                   |
| SS23                 |        |      | 11/04/20               | AZ       | 8                   |
| SS24                 | 0300   |      | 11/04/20               | AZ       | 9                   |
| SS25                 | 0300   |      | 11/04/20               | AZ       | 9                   |
| SS26                 |        |      | 11/04/20               | AZ       | 9                   |
| SS27                 |        |      | 11/04/20               | AZ       | 10                  |
| SS28                 | 0300   |      | 11/04/20               | AZ       | 10                  |
| SS29                 |        |      | 11/04/20               | AZ       | 10                  |
| SS30                 |        |      | 11/04/20               | AZ       | 11                  |
| SS31                 | 0300   |      | 11/04/20               | AZ       | 11                  |
| SS32                 | 0300   |      | 11/04/20               | AZ       | 12                  |
| SS33                 | 0300   |      | 11/04/20               | AZ       | 12                  |
| SS34                 | 0300   |      | 11/04/20               | AZ       | 12                  |
| SS36                 | 0300   |      | 11/04/20               | AZ       | 13                  |
| SS37                 | 0300   |      | 11/04/20               | AZ       | 13                  |
| SS38                 |        |      | 11/04/20               | AZ       | 13                  |
| SS39                 | 0300   |      | 11/04/20               | AZ       | 14                  |
| SS40                 |        |      | 11/04/20               | AZ       | 14                  |
| SS41                 |        |      | 11/04/20               | AZ       | 14                  |
| SS42                 |        |      | 11/04/20               | AZ       | 15                  |
| SS43                 | 0300   |      | 11/04/20               | AZ       | 15                  |
| SS44                 | 0300   |      | 11/04/20               | AZ       | 15                  |
| SS45                 | 0300   |      | 11/13/20               | AZ       | 16                  |
| SS46                 | 0300   |      | 11/13/20               | AZ       | 16                  |
| SS47                 | 0300   |      | 12/31/20               | AZ       | 17                  |
| SS48                 | 0300   |      | 12/31/20               | AZ       | 17                  |
| SS49                 | 0300   |      | 12/31/20               | AZ       | 18                  |
| SS50                 | 0300   |      | 12/31/20               | AZ       | 18                  |
| SS51                 |        |      | 12/31/20               | AZ       | 19                  |
| SS52                 |        |      | 12/31/20               | AZ       | 19                  |
| SS53                 |        |      | 12/31/20               | AZ       | 20                  |
| SS54                 | 0300   |      | 12/31/20               | AZ       | 20                  |
| SS55                 |        |      | 12/31/20               | AZ       | 21                  |
| SS56                 | 0300   |      | 12/31/20               | AZ       | 21                  |
| SS57                 |        |      | 12/31/20               | AZ       | 22                  |
| SS58                 | 3000   |      | 12/31/20               | AZ       | 22                  |
| SS59                 |        |      | 12/31/20               | AZ       | 23                  |
| SS60                 |        |      | 12/31/20               | AZ       | 23                  |
| SS61                 | 0300   |      | 03/15/21               | AZ       | 24                  |
| SS62                 |        |      | 03/15/21               | AZ       | 24                  |
| SS63                 |        |      | 03/15/21               | AZ       | 24                  |
| SS64                 |        |      | 03/15/21               | AZ       | 24                  |
| SS65                 |        |      | 03/15/21               |          | 25                  |
| SS66                 |        |      | 03/15/21               |          | 25                  |
| SS67                 |        |      | 03/15/21               |          | 25                  |
| SS68                 |        |      | 03/15/21               |          | 25                  |
| SS69                 | 0300   |      | 03/22/21               |          | 26                  |

|        |       |   |            |                 |                             |
|--------|-------|---|------------|-----------------|-----------------------------|
| SS70   | 0300  |   | 03/22/21   |                 | 26                          |
| SS71   |       |   | 03/22/21   |                 | 26                          |
| SS72   | 0300  |   | 03/22/21   |                 | 26                          |
| SS73   |       |   | 11/23/21   |                 | 27                          |
| SS74   |       |   | 11/23/21   |                 | 27                          |
| SS75   | 0300  |   | 11/23/21   |                 | 27                          |
| SS76   | 0300  |   | 11/23/21   |                 | 28                          |
| SS77   | 0300  |   | 11/23/21   |                 | 28                          |
| SS78   | 0300  |   | 11/23/21   |                 | 28                          |
| SS80   |       |   |            |                 | 29                          |
| SS81   | 0300  |   |            |                 | 29                          |
| SS82   | 0300  |   | 11/23/21   |                 | 30                          |
| SS83   | 0300  |   | 11/23/21   |                 | 30                          |
| SS84   | 0300  |   | 11/23/21   |                 | 30                          |
| SS85   | 0300  |   | 11/23/21   | AZ              | 31                          |
| SS86   |       |   | 11/23/21   | AZ              | 31                          |
| SS87   |       |   | 11/23/21   | AZ              | 31                          |
| JV 192 | 0300  |   | 01/08/20   | AZ              | 32                          |
| JV 198 | 0300  |   | 01/08/20   | AZ              | 33                          |
| JV 199 | 0300  |   | 01/08/20   | AZ              | 33                          |
| JV 200 | 0300  |   | 01/08/20   | AZ              | 34                          |
| JV 201 | 0300  |   | 01/08/20   | AZ              | 35                          |
| JV 202 | 0300  |   | 01/08/20   | AZ              | 36                          |
| Q1A1   |       |   | 02/26/20   | AZ              | 36                          |
| Q1A2   | 0300  |   | 02/26/20   | AZ              | 36                          |
| Q1B1   | 0300  |   | 02/26/20   | AZ              | 37                          |
| Q3D1   |       |   | 02/26/20   | AZ              | 37                          |
| Q3D2   |       |   | 02/26/20   | AZ              | 37                          |
| Q4A1   | 0300  |   | 02/26/20   |                 | 38                          |
| Q4D2   |       |   | 02/26/20   |                 | 38                          |
| Q4E2   |       |   | 02/26/20   |                 | 38                          |
| JQ11   | 0300  |   | 02/23/21   |                 | 39                          |
| JQ12   | 0300  |   | 02/23/21   |                 | 39                          |
| JQ21   |       |   | 02/23/21   |                 | 39                          |
| JQ22   | 0300  |   | 02/23/21   |                 | 40                          |
| JQ31   | 0300  |   | 02/23/21   |                 | 40                          |
| JQ32   |       |   | 02/23/21   |                 | 40                          |
| JQ41   |       |   | 02/23/21   |                 | 41                          |
| JQ42   | 0300  |   | 02/23/21   |                 | 41                          |
| JQ51   | 0300  |   | 02/23/21   |                 | 41                          |
| JQ52   |       |   | 02/23/21   |                 | 42                          |
| KP01   |       |   | 2004       | Wellton, AZ     | Kelly Paugh/UC Davis        |
| KP02   | 0300  |   | 2002       | Huron, CA       | Kelly Paugh/UC Davis        |
| KP03   |       |   | 2002       | Yuma, AZ        | Kelly Paugh/UC Davis        |
| KP04   | 0300  |   | 2002       | Huron, CA       | Kelly Paugh/UC Davis        |
| KP05   |       |   | 2002       | Huron, CA       | Kelly Paugh/UC Davis        |
| KP06   |       |   | 2015       | Davis, CA       | Kelly Paugh/UC Davis        |
| KP07   | 0300  |   | 2015       | Watsonville, CA | Kelly Paugh/UC Davis        |
| KP08   |       |   | 2016       | Chualar, CA     | Kelly Paugh/UC Davis        |
| KP09   |       |   | 02/18/2022 | Davis, CA       | Kelley Paugh/UC Davis       |
| GG11   |       | 1 |            | Italy           | Giovanna Gilardi-Agroinnova |
| GG12   |       | 1 |            | France          | Giovanna Gilardi-Agroinnova |
| GG13   |       | 1 |            | Italy           | Giovanna Gilardi-Agroinnova |
| GG41   | 0304? | 4 |            | Italy           | Giovanna Gilardi-Agroinnova |
| GG42   | 0304? | 4 |            | Italy           | Giovanna Gilardi-Agroinnova |
| GG43   |       | 4 |            | Netherlands     | Giovanna Gilardi-Agroinnova |
| GG44   |       | 4 |            | Netherlands     | Giovanna Gilardi-Agroinnova |
| OC01   |       |   | 11/10/2020 | AZ              | 43                          |
| OC02   | 0300  |   | 12/03/2020 | AZ              | 43                          |
| OC03   | 0300  |   | 12/03/2020 | AZ              | 43                          |

<sup>a</sup> Isolates with the nomenclature "SS" are from Dr. Stephanie Slinski, "KP" isolates are from Kelly Paugh, "GG" are isolates sent from Giovanna Gilardi-Agroinnova.

<sup>b</sup> Date collected correlates with the day the lettuce sample was taken from the field.

**Table 2. Mean disease severity in greenhouse inoculations tests with *Fusarium oxysporum* f. sp. *lactucae* on the cultivar Grizzly.**

| Isolate           | Mean Disease Severity (Scale – 0.0 - 4.0) <sup>1</sup> |                    |                    |                    |                    |                    |
|-------------------|--|--------------------|--------------------|--------------------|--------------------|--------------------|
|                   | Test 1<br>06/09/20                                     | Test 2<br>06/19/20 | Test 3<br>09/01/20 | Test 4<br>09/25/20 | Test 5<br>11/16/20 | Test 6<br>12/20/20 |
| H <sub>2</sub> O* | 1.6  | 1.3                | 0.0                | 1.0                | 0.0                | 0.0                |
| BR35B**           | 0.7  | 1.0                | 0.1                | 0.6                | 0.1                | 0.7                |
| JV 202            | 3.6  | 2.7                | 0.9                | 2.1                | 2.8                | 3.1                |
| Q3D1              | 2.0  | 1.9                | 1.7                | 2.9                | 2.2                | 3.1                |
| Q3D2              | 3.3  | 1.9                | 1.6                | 1.6                | 2.1                | 3.1                |
| Q4A1              | 3.3  | 3.1                | 2.3                | 2.4                | 2.8                | 3.1                |
| JV 192            | 3.5  | 2.8                | 1.8                | 1.9                | 3.8                | 3.2                |
| JV 199            | 2.5  | 2.5                | 0.2                | 2.6                | 3.4                | 3.4                |
| Q4D2              | 0.8  | 2.1                | 1.8                | 2.4                | 1.2                | 3.4                |
| JV 198            | 3.3  | 1.9                | 1.7                | 2.8                | 3.3                | 3.7                |
| Q1A2              | 3.2  | 1.5                | 1.7                | 2.5                | 3.3                | 3.7                |
| Q1B1              | 3.8  | 2.9                | 1.1                | 1.2                | 2.1                | 3.7                |
| Q4E2              | 3.4  | 2.7                | 1.0                | 3.0                | 1.5                | 3.8                |
| JV 200            | 2.8  | 2.6                | 0.6                | 1.2                | 3.4                | 4.0                |
| JV 201            | 3.3  | 2.1                | 3.0                | 2.6                | 2.8                | 4.0                |
| Q1A1              | 3.0  | 3.1                | 2.1                | 2.2                | 2.7                | 4.0                |

<sup>1</sup>The mean disease severity was 4-5 weeks post inoculation. Disease was rated on a scale of 0 – 4 with zero indicating no symptoms; 4 = Severe Fusarium wilt symptoms.

\* H<sub>2</sub>O water negative control.

\*\* BR35B is an isolate of *F. oxysporum* f. sp. *coriandrii* used as a negative control.

**Table 3. Reference isolates and passport information for isolates of FOL collected and characterized by the Correll Lab.**

| Isolate<br>(Correll Lab Code) | Received<br>As Code | Collected | Location    | VCG    | Pathogenicity |
|-------------------------------|---------------------|-----------|-------------|--------|---------------|
| JV198                         |                     | 01/08/20  | AZ          | 0300   | +             |
| JV199                         |                     | 01/08/20  | AZ          | 0300   | +             |
| JV201                         |                     | 01/08/20  | AZ          | 0300   | +             |
| JV202                         |                     | 01/08/20  | AZ          | 0300   | +             |
| 244141*                       | F9501               |           | Japan       | Race 2 |               |
| 744085*                       | N3                  |           | Japan       | Race 3 |               |
| 744086*                       | M7                  |           | Japan       | Race 3 |               |
| Q1A2                          |                     | 02/26/20  | AZ          | 0300   | +             |
| GG41                          | FL3/19              |           | Italy       | 0304?  |               |
| GG42                          | FL6/19              |           | Italy       | 0304?  |               |
| GG43                          | PD015/0470896       |           | Netherlands | 0304?  |               |
| GG44                          | PD015/04750888      |           | Netherlands | 0304?  |               |
| HS                            |                     |           |             |        |               |
| HS                            |                     |           |             |        |               |
| HS                            |                     |           |             |        |               |

\* Isolate designation is the MAFF number listed by Naro Genebank and can be found at [https://www.gene.affrc.go.jp/databases-micro\\_search\\_en.php](https://www.gene.affrc.go.jp/databases-micro_search_en.php)

References for Japanese isolates:

Race 2: Mbofung et al, 2007

Race 3: Fujinaga et al, 2001

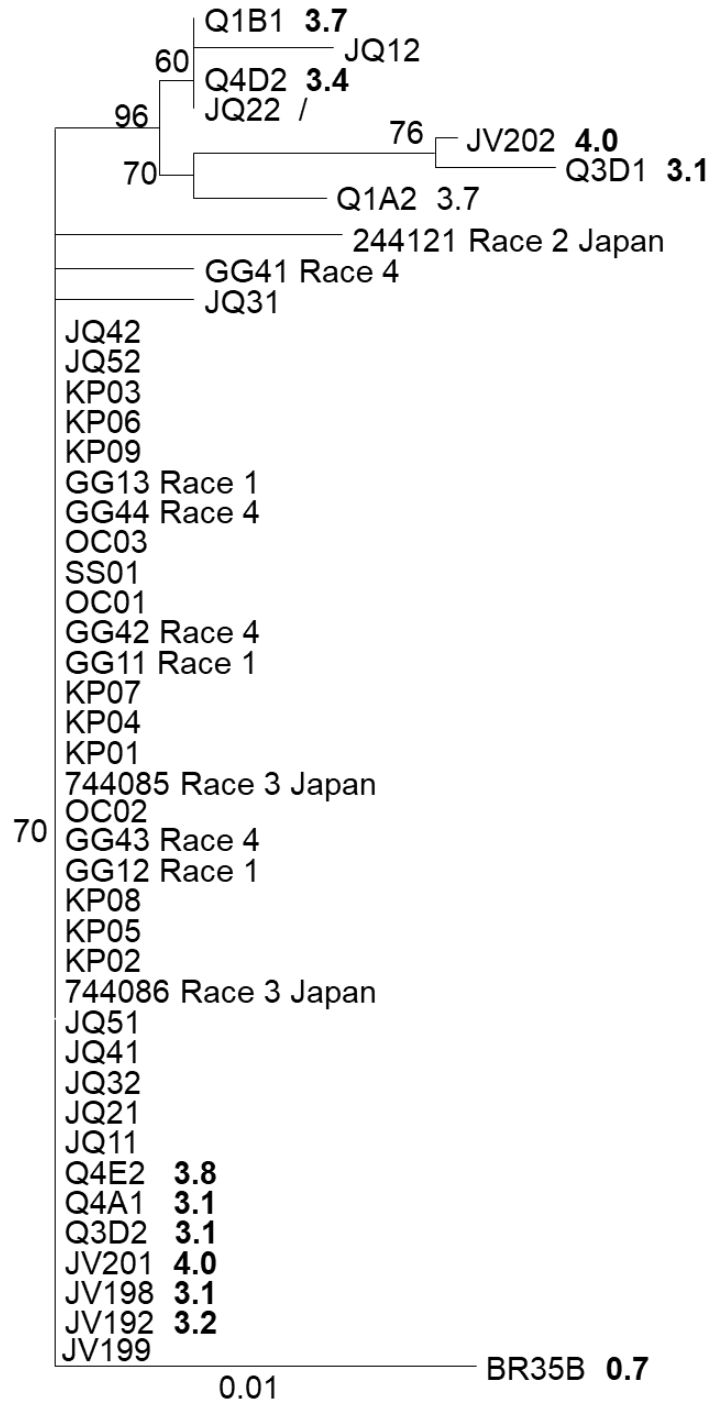


Fig. 1. Phylogenetic tree based on Neighbor Joining (NJ) analysis of the elongation factor sequences with the isolates of *Fusarium oxysporum* f. sp. *lactucae* (FOL); the out-group BR35B is *F. oxysporum* f. sp. *coriandrii* (FOC) from cilantro. The isolate of 244121 is race 2, and the isolates of 744085 and 744086 are race 3 from Japan. Bootstrap values were labeled on the branch of the tree. Scale bars represents the average number of substitutions per site over time, and the numbers in bold on the figure are the disease severity ratings from pathogenicity tests on lettuce on a scale of 0.0 (no disease) to 4.0 (severe disease).