2014 Bacterial Ring Rot (BRR) Variety Inoculations

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Introduction

Clavibacter michiganensis subsp. *sepedonicus* (Cms) causes bacterial ring rot (BRR) in potatoes. The bacteria are spread by seed cutting equipment, conveyors, planters, cultivators, harvesters, trucks, and on storage equipment. The bacteria can also survive as extremely durable biofilms on equipment, trucks, boxes, pallets, etc. for several years, and only require rehydration to become active again. Cms is often managed as a zero-tolerance disease when found in seed potatoes. However, asymptomatic infections allow the bacteria to evade detection, and spread to new areas. Both the disease and the measures to manage it cause substantial economic losses.

Foliar symptoms include wilting of leaves after midseason, usually the lower leaves, which can recover when soil moisture levels are improved. Leaves may become pale green and are slightly rolled at the margins and, as the disease progresses, pronounced wilting of the plant may become apparent. The bacteria may cause symptoms in only one or two of the infected stems. Milky white bacterial ooze may be observed when the lower portion of a cut stem is squeezed. Symptoms vary by variety of potato, and also depend on environmental conditions. Symptoms of BRR often do not express until the latter part of the growing season, and generally are increased under hot, dry conditions. Asymptomatic infections also occur. The above ground portions of infected plants can serve as inoculum sources if they are damaged during field cultivation.

Tuber symptoms include discoloration of the vascular tissue, often an orange or red arc developing to the classic symptom of complete degradation of the vascular ring and oozing of bacteria. Symptomatic tubers can show external cracking and rot. Symptoms are somewhat suppressed during cool storage, and may progress rapidly upon warming. These symptoms may be masked by invasion of the tuber with opportunistic organisms. Many tuber infections are asymptomatic. Cms can overwinter in stored tubers and volunteer plants, providing initial inoculum for the following season.

The goal of this study is to use common potato varieties to demonstrate the symptoms likely to be seen under Alaskan growing conditions, as well as any differences in the rate of infection.

Materials and Methods

In 2014, tuber core samples were taken from growers' lots around the state, 6 growers submitted 30 lots for testing. Samples were washed and soaked overnight in distilled water on a shaker table at room temperature to release bacteria. Soak fluid was tested for the presence of Cms by real-time PCR, using the CelA primers and probe (Gudmestad, et al., 2009), which detects the Cellulase A gene, required for pathogenicity (Nissinen, et al., 2001). Cms was found in varieties 'Pike', 'Russet Norkotah', and 'Cherry

Red', one lot of each. The identity of the bacteria was confirmed by real-time PCR using the modified Mills primers, Cms50 and Cms72a (Gudmestad, et al., 2009; Mills, et al., 1997). Additional confirmation was provided by Immunofluorescent Antibody Stain (IFAS), and Enzyme Linked Immunosorbent Assay (ELISA) (Agdia, Elkhart, IN).

Bacteria from the 'Pike' tubers was isolated and maintained on NCP-88 media (de la Cruz, et al., 1992). Plates of nutrient broth-yeast extract agar (Vidaver, 1967) were streaked with pure bacteria on May 30, 2014. The bacteria were harvested on June 4, 2014, (5dpi), by agitation with a bent glass rod and rinsing plates with distilled water. Clumps were disrupted with a Dounce-style tissue homogenizer, and diluted with additional distilled water to form a turbid solution. Inoculations consisted of 10µl of a 4.35×10^8 cfu/ml suspension in distilled water. This is approximately 4.35×10^6 (=4,350,000) viable bacteria per inoculation. The inoculum concentration was calculated from plated serial dilutions. Inoculations were performed as a modification of the method described by Bishop and Slack in 1987. Inoculum was stabbed near the rose-end eye of the tubers, 0.5-1.0cm deep, with a dissecting needle. The needle was reinserted twice more at right angles to the first, through the inoculum under the eye.

The varieties used in this trial are among the most commonly grown in Alaska: 'Bake-King', 'CalWhite', 'Cherry Red', 'Russet Norkotah', 'Shepody', and 'Yukon Gold'. Ten tubers of each variety were planted on June 4th at the UAF Palmer Center for Sustainable Living, in Palmer, AK. All varieties were planted as single drop tubers except for 'Cherry Red' where seed was cut due to lack of sufficient tubers. All seed pieces were planted within 1 hour of inoculation. Plantings were spaced on 12 inch drop in rows, six feet apart to facilitate observations. The planting was not irrigated and was under drought stress much of the growing season. Hot, dry conditions should cause greater symptom expression. These plots were located near the Seed Lot Source Trial, in a field separated from the rest of the farm.

Results

Please note that the temperature and precipitation measurements for the 2014 field were taken at the Palmer Center for Sustainable Living. The 30-year average measurements were taken from the Palmer Municipal Airport, roughly 8 miles away (NOAA dataset).

June 2014 was cooler than normal, with average high temperature of 61.6°F, and average low of 43.8°F (as compared with the 30-year averages of 65.1°F and 46.2°F). Soil temperature averaged 53.3°F. Infection of seed pieces is favored by wet soil and soil temperatures of 62-72°F. Precipitation was much higher than normal, with 2.40" compared to 0.42" average.

All plants had emerged on July 3rd, 10 hills each. July 2014 temperatures were near normal, with average high temperature of 67.8°F, and average low of 50.2°F (as compared with the 30-year averages of 66.2°F and 50.3°F). BRR symptoms are optimally expressed at air temperatures of 75-90°F. Soil temperature averaged 60.2°F. Precipitation was higher than normal, with 2.31" compared to 1.66" average.

On August 8th, one plant of 'Bake-King' showed mild chlorosis and wilt. All other varieties were symptomless. On August 18th, four varieties were symptomatic. 'Russet Norkotah' had one plant wilted on the 18th but recovered by the following week. 'Yukon Gold' had one plant with a mild wilt and mild chlorosis. 'CalWhite' had one plant wilted with interveinal chlorosis. 'Bake-King' had two plants with green wilt. August 2014 was slightly warmer than normal, with average high temperature of 65.7°F, and average low of 49.5°F (as compared with the 30-year averages of 64.5°F and 47.8°F). Soil temperature averaged 59.6°F. Precipitation was about ½" less than normal, with 1.82" compared to 2.38" average.

On September 2nd, all 'Russet Norkotah' plants were dead; 'CalWhite' and 'Bake-King' showed increased wilt and chlorosis. 'Cherry Red' had one plant showing a mild chlorosis. 'Shepody' never showed any symptoms and the mildly chlorotic 'Yukon Gold' plant had recovered and showed no symptoms.

On September 15th, 2014, the field plots were harvested. All stems from each hill were harvested by cutting at the soil line. Tubers were dug from each hill, and the largest 5 tubers were kept for testing. It was not possible to keep tubers from each stem separate. It was thought that the largest tubers had developed for the longest time, and therefore were likely to have higher bacterial concentrations and greater symptom expression. The only tubers to show symptoms of BRR at harvest were from the varieties 'Bake-King' and 'CalWhite'. Through harvest day, the month was warmer than normal, with average high temperature of 59.8°F, and average low of 45.3°F (as compared with the 30-year averages of 55.7°F and 40.0°F). Soil temperature averaged 55°F. Precipitation was slightly lower than normal, with 1.27" compared to 1.67" average.

Stems were recut in the laboratory, individually washed and soaked overnight in distilled water. The soak fluid of individual stems was tested by real-time PCR, using the CelA primers developed by Gudmestad, et al. (2009). This was intended to give indication of how readily the bacteria colonize the above-ground portions of the plant; it is well known that Cms can be present in some stems and absent in others from the same seed piece. This is of importance in detection, as well as spread of the bacteria on field equipment during the current season.

Tubers were cored in the laboratory, with 13mm diameter melon ballers, at the stem-end attachment. Tubers from the first hill of each variety were processed individually, while the remaining tubers were bulked for each hill. They were washed, soaked, and tested by real-time PCR the same as the stem cuttings.

'Bake-King' had at least one infected stem, and at least one infected tuber in 100% of the hills (Fig.1). 38 of 39 stems were infected. Of the tubers collected, 10 of 50 were visibly rotten.

'CalWhite' had at least one infected stem in 100% of the hills, and at least one infected tuber in 80% of the hills. 60 of 75 stems were infected. Of the tubers collected, 5 of 50 were visibly rotten.

'Cherry Red' had at least one infected stem in 60% of the hills, and at least one infected tuber in 40% of the hills. 13 of 28 stems were infected.

'Russet Norkotah' had at least one infected stem in 90% of the hills, and at least one infected tuber in 50% of the hills. 25 of 52 stems were infected.

'Shepody' had at least one infected stem in 100% of the hills, and at least one infected tuber in 70% of the hills. 52 of 76 stems were infected.

'Yukon Gold' had at least one infected stem in 100% of the hills, and at least one infected tuber in 70% of the hills. 37 of 50 stems were infected.

Discussion

Seed potato certification requires a minimum of two field inspections. These inspections in Alaska are typically completed by mid-August, as killing frosts have been recorded by that time. The majority of plants and tubers were infected, but did not show foliar or tuber symptoms. This increases the risk of harboring and spreading the bacteria to other lots and other farms.

The six potato varieties in this study showed varying susceptibilities to stem and tuber infection. However, the resistance is not enough to be useful in disease management. All infected tubers came from hills with infected stems. However, a few hills with infected stems produced tubers which did not test positive for bacteria. Since we only harvested the largest tubers from the hill, we may have missed additional smaller tubers.

Core sampling and real-time PCR testing of random tubers from seed lots is extremely sensitive, and able to discover bacteria in asymptomatic plant material. The identification of BRR infected tubers prior to cutting and planting enabled growers to adjust their planting schedules and procedures to minimize the risk of infecting other lots. These infections can then be managed by destruction of materials, sanitation procedures, lot tracing, and flush-out of other lots on the farm.

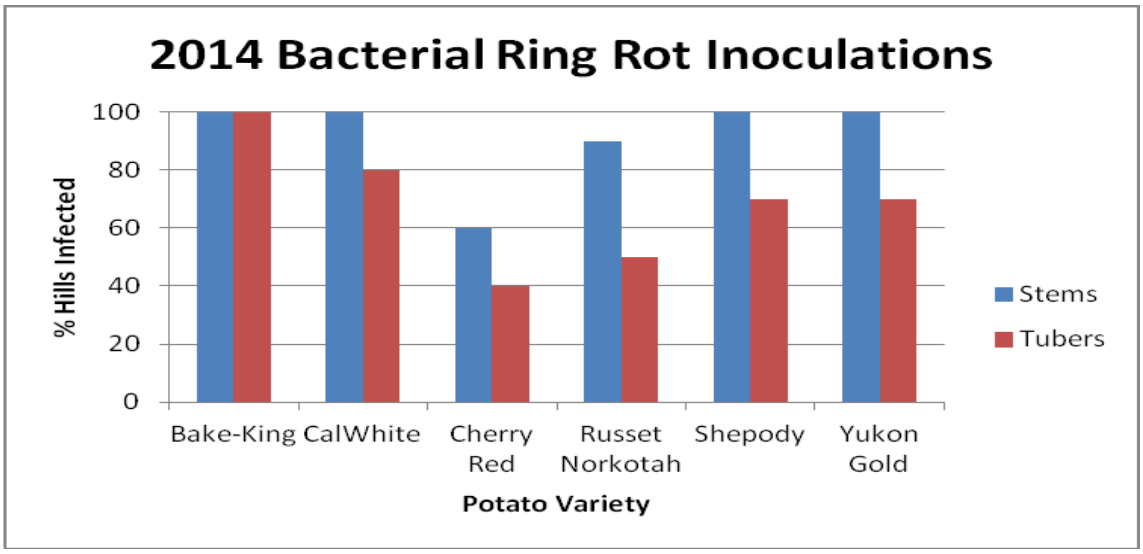

Fig. 1. 2014 Bacterial ring rot inoculations. Ten seed pieces of each variety were inoculated, one seed piece planted per hill. One infected stem (or tuber) from a hill categorizes that hill as infected.

Literature cited

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