Yeast Soft Rot of Onion in the Walla Walla Valley of Washington and Oregon

DENNIS A. JOHNSON, Extension and Associate Plant Pathologist, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350; K. M. REGNER, Research Associate, Department of Plant Pathology, Washington State University, Pullman 99164; and J. D. LUNDEN, Research Technician, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350

ABSTRACT

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Soft rot of onion disks inoculated with the yeast *Kluyveromyces marxianus* var. *marxianus* increased significantly ($P = 0.01$) as temperature increased from 10 to 30 C. Rot was not evident in onion disks inoculated with the yeast and incubated for 12 days at 2 C. A temperature of 2 C or less during transit and storage of bulbs will reduce damage caused by the yeast. The amount of soft rot caused by seven isolates of *K. m.* var. *marxianus* from the Walla Walla Valley of southeastern Washington and northeastern Oregon did not vary significantly ($P = 0.05$) when tested on onion disks. A strain of the bacterium *Erwinia carotovora* subsp. *carotovora* from onion produced significantly ($P = 0.01$) more soft rot in onion than *K. m.* var. *marxianus* at 25 and 15 C, whereas *K. m.* var. *marxianus* produced significantly more soft rot in onion than a strain of *E. c.* subsp. *carotovora* from potato. Resistance to *K. m.* var. *marxianus* was not evident in 25 onion cultivars grown in the Pacific Northwest.

A soft rot of onion bulbs (*Allium cepa* L.) caused by the yeast *Kluyveromyces marxianus* (Hansen) van der Walt var. *marxianus* was identified in Walla Walla sweet onion bulbs (Yellow Globe variety) from southeastern Washington and northeastern Oregon during and after harvest in 1986 and 1987 (2). Little is known of the distribution, epidemiology, and control of this postharvest yeast-pathogen of onion bulbs. Severity of rot increased as the inoculum concentration increased from 0.9×10^2 to 1.2×10^5/ml of yeast cells (2). There was less soft rot in onions inoculated with *K. m.* var. *marxianus* and kept at 5 and 10 C as compared with 20 or 27 C (1). Additional information on this disease would help in developing disease management strategies. The purposes of this work were to 1) define the effect of low temperatures on soft rot development to determine temperatures at which active rot ceases in infected onions, 2) compare virulence among various isolates of *K. m.* var. *marxianus*, 3) compare the virulence of *K. m.* var. *marxianus* with that of *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al, and 4) test bulbs of 25 onion cultivars and breeder's lines from the Pacific Northwest for resistance to soft rot caused by *K. m.* var. *marxianus*.

MATERIALS AND METHODS

Seven isolates of *K. m.* var. *marxianus* collected in 1986, 1987, and 1988 from northeastern Oregon and southeastern Washington (near Walla Walla, WA) were used in this study. They were isolated from the margin of water-soaked tissue of onions with soft rot symptoms on either nutrient broth-yeast extract agar (NBY) (2) or on Difco malt agar (MA) as previously described (2). Single, well-isolated colonies were then selected and recultured on MA. Two cycles of plating were completed for each isolate (6). Isolate Yo A originated from an onion collected in a field in 1986, isolates Yo 20 and Yo 29 from fields in 1987, and isolates Yo 82 and Yo 83 from fields in 1988. Isolate Yo 19 was isolated from an onion in a 10-mo-old cull pile in 1987. Yo 31 originated from a packinghouse in 1987.

All yeast cultures were maintained in the dark on Difco potato-dextrose agar slants in glass tubes at 5 C, and inoculum was increased on MA at room temperature at 23 ± 2 C. Onion disks were cut about 2 cm thick perpendicular to the main axis of the bulb with a sterile knife after outer scales of bulbs were peeled off and bulbs were washed and dried. Onion disks were placed in sterile glass petri dishes and inoculated by applying 0.1 ml of inoculum to the center of the disk. Inoculum concentrations of the yeast were determined using a hemacytometer. Sterile water (0.1 ml) was applied to disks as a control. After each incubation period, the volume of rot of the disk was determined from the diameter and depth of the macerated tissue.

Whole onion bulbs were inoculated after their outer scales had been peeled and after the bulbs had been washed and swabbed with ethanol. Bulbs were injected with 0.5 ml of either a water suspension of yeast cells or sterile water with a disposable hypodermic syringe and needle. The injection was 15 mm deep at 3–5 cm from the neck and at approximately 45° to the main axis of the bulb. After an incubation period, inoculated and noninoculated bulbs were cut in half at the point of injection from the neck to the base. The rotted area of the cut surface was estimated visually (6% rot = one of 16 scales rotted).

To determine the effect of temperature on soft rot development, two yeast isolates, Yo 20 and Yo 29 at 4×10^6 cells per ml, were applied to onion disks of the cultivar Armada. Petri dishes with inoculated disks were placed in temperature chambers for 3 days at 2, 5, 10, 15, 20, and 30 C. Treatments were arranged in a randomized complete block design with five replicates. Additional inoculated disks were placed at 2, 5, and 10 C for 12 days in a randomized complete block design with five replicates. The disks at 2 C were then placed at 30 C for 3 days.

Virulence of isolates Yo A, Yo 19, Yo 20, Yo 29, Yo 31, Yo 82, and Yo 83 of *K. m.* var. *marxianus* was compared by inoculating the cut surfaces of disks of Walla Walla sweet onions with a concentration of 5×10^6 cells/ml. The inoculated disks were arranged in a randomized complete block design with six replicates and were incubated at 29 C for 3 days.

Two strains of *E. c.* subsp. *carotovora* (Wo 57, Wo 105) and an isolate of *K. m.* var. *marxianus* (Yo A) were compared for ability to cause soft rot by inoculating onto disks of the cultivars Armada and Walla Walla sweet onion at a concentration of 4×10^6 cfu/ml. One strain of *E. c.* subsp. *carotovora* (Wo 57) was isolated from the margin of water-soaked tissue from a soft rotted onion collected near Walla Walla, WA. A small tissue segment was placed in 5 ml of sterile distilled water and macerated with the blunt end of a flame-sterilized glass rod. The macerate (0.1 ml) was spread on NBY in a petri dish, and the bacteria were identified (1,4). A second strain (Wo 105) was originally isolated from a seed potato collected in North Dakota (Dr. D. C. Gross, Dept. of Plant Pathology, Washington State Univ.). Bacterial

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strains were grown overnight at 25 C on plates containing NBY. Single colonies were transferred with an inoculation loop to 10 ml of sterile phosphate buffer. Cell suspensions were adjusted to an optical density of 0.3 at 420 nm on a spectrophotometer (Spectronic 20). A 100-fold dilution was completed to give a concentration of approximately 10^6 cfu/ml. Standard serial dilutions were completed to determine inoculum concentrations. The amount of inoculum applied to disks was 0.1 ml. Sterile phosphate buffer and sterile water were used as noninoculated controls. The experiment was arranged in a factorial design with four replications at 15 and 25 C.

To obtain bulbs for pathogenicity tests, seed of 24 onion cultivars was obtained from seed companies: Granada, Maya, XPH 3373, XPH 3326, and XPH 3374 from Asgrow, P.O. Box 278, Warden, WA 98857; Red Baron, Sweet Amber, XPH 85N39, XPH 86N61, and XPH 87N11 from Crookham, P.O. Box 520, Caldwell, ID 83606; Bronze Reserve, Bullseye, Oro Grande, Redman, and Rip Van Winkle from Ferry-Morse, 3015 E. Comstock, Nampa, ID 83651; Brahma, Bravado, Cima, Golden Cascade, Valiant, Tango, and Carmen from Sunseeds, P.O. Box 1666, Nyssa, OR 97913; and Banner 80 and Capra from Alf Christianson, P.O. Box 98, Mt. Vernon, WA 98273. Seeds were planted in a sandy loam soil near Quincy, WA, in April 1988 and were harvested in August. Walla Walla sweet onions from a commercial field near Walla Walla, WA, were also used. Six onion disks (each from a separate bulb) of each cultivar were inoculated with 5×10^6 cells/ml of *K. m.* var. *marxianus* isolate Yo A. Disks were arranged in a completely random design at 29 C for 3 days. Six bulbs of each cultivar were also injected with 0.5 ml of 5×10^6 cells/ml of isolate Yo A, arranged in a completely random design, and incubated at 25 C for 18 days.

All experiments were completed twice. Analysis of variance and regression analysis of the temperature studies were used to analyze data.

RESULTS AND DISCUSSION

K. m. var. *marxianus* was reisolated from disks and bulbs that were inoculated with the yeast after each experiment. *E. c.* subsp. *carotovora* was reisolated from disks inoculated with the bacterium. None of the noninoculated disks and bulbs developed soft rot symptoms. Results from the repeated experiments were similar to those reported here.

The amount of rot of onion disks 3 days following inoculation with Yo 20 or Yo 29 increased significantly ($P = 0.01$) as temperature increased from 2 to 30 C (Fig. 1), as determined by both linear and curvilinear regressions. Rot was not evident at 2 and 5 C, and mean amount of rot was less than 1% at 10 C. The two yeast isolates did not differ significantly ($P = 0.05$) in the amount of rot produced. The quadratic equation $\hat{Y} = 4.1 - 1.5X + 0.1X^2$ best described the relationship when data for isolates were combined. Coefficient of determination was 0.80.

The percentage of rot for disks increased significantly ($P = 0.01$) as temperature increased from 2 to 10 C (Fig. 2), with both linear and curvilinear regressions, when disks were inoculated with Yo 29 or Yo 20 and incubated for 12 days. Amount of rot produced by the two yeast isolates was not significantly different ($P = 0.05$). Rot was not evident at 2 C. The quadratic equation was $\hat{Y} = -1.1 + 0.45X + 0.04X^2$ when data for isolates were combined. The coefficient of determination was 0.70. When disks that were inoculated and kept at 2 C for 12 days were later placed at 30 C for 3 days, the amount of soft rot increased from 0 to 20%.

Our data suggest that low temperatures (2 C) during transit and storage can reduce damage caused by *K. m.* var. *marxianus*. Even though storage at 2 C stopped soft rot development, rot resumed when infected onions were placed in warmer temperatures. Therefore, additional control practices are needed to integrate with refrigeration during transit and storage. Storage temperatures from 0.6 to 2 C are recommended for onions in the Pacific Northwest (3).

The percentage of rot produced in disks did not vary significantly ($P = 0.05$) among the seven isolates of *K. m.* var. *marxianus*. The mean amount of rot ranged from 32 to 57%. Pathogenic strains of *K. m.* var. *marxianus* have been isolated from the Walla Walla Valley of southeastern Washington and northeastern Oregon over a 3-yr period. Attempts to isolate the yeast from other onion-growing areas in Washington have failed. More information on the disease cycle, methods of overwintering, and presence of the yeast during additional seasons needs to be established to determine if *K. m.* var. *marxianus* is endemic in the Walla Walla Valley.

In 1987, onions with soft rot symptoms and external bruises and those that were flaccid when hand-pressed where collected from the Walla Walla Valley to determine the prevalence of pathogenic yeast and bacteria (2). Of 114 samples, *K. m.* var. *marxianus* was isolated from four bulbs and soft-rotting *Erwinia* was isolated from another four bulbs (2). Both organisms were isolated only from bulbs with soft rot symptoms.

E. c. subsp. *carotovora*, originally isolated from onion (Wo 57), caused significantly ($P = 0.01$) more soft rot in two onion cultivars than did *K. m.* var. *marxianus* Isolate Yo A at 15 and 25 C. In contrast, *K. m.* var. *marxianus* caused significantly ($P = 0.01$) more soft rot than the *E. c.* subsp. *carotovora* isolate Wo 105 from potato (Fig. 3). The amount of soft rot of the two onion cultivars was not significantly different ($P = 0.05$). Therefore, data for cultivars were combined.

Soft rot in onion caused by *K. m.* var. *marxianus* has probably occurred in the Walla Walla Valley for some time but has been confused with bacterial soft rot caused by *E. c.* subsp. *carotovora*. Symptoms caused by *K. m.* var. *marxianus* and *E. c.* subsp. *carotovora* are similar (2,7,8). Isolation from infected tissue, microscopic exami-

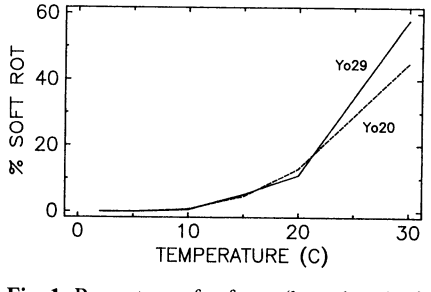

Fig. 1. Percentage of soft rot (by volume) of onion disks inoculated with isolates Yo 29 and Yo 20 of *Kluyveromyces marxianus* var. *marxianus* and incubated at temperatures from 2 to 30 C for 3 days.

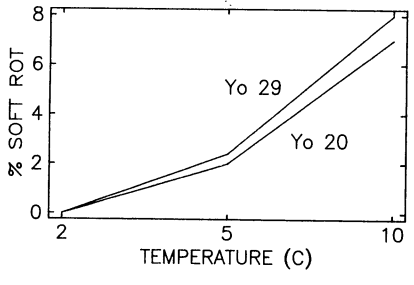

Fig. 2. Percentage of soft rot (by volume) of onion disks inoculated with isolates Yo 29 and Yo 20 of *Kluyveromyces marxianus* var. *marxianus* and incubated at 2, 5, or 10 C for 12 days.

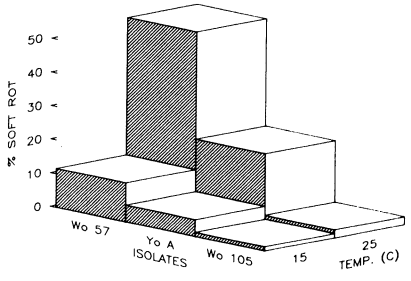

Fig. 3. Percentage of soft rot (by volume) of onion disks inoculated with isolate Yo A of *Kluyveromyces marxianus* var. *marxianus* or isolates Wo 57 and Wo 105 of *Erwinia carotovora* subsp. *carotovora* and incubated at 15 or 25 C for 3 days.

nations, and pathogenicity tests are needed to differentiate the yeast from soft rot bacteria (2). More strains of *E. c.* subsp. *carotovora* need to be tested before concluding the relative virulences in onion of *E. c.* subsp. *carotovora* from potato and onion.

All disks and bulbs of the onion cultivars inoculated with *K. m.* var. *marxianus* developed soft rot symptoms. Cultivars did not differ significantly ($P = 0.05$) in susceptibility to soft rot when either inoculated disks or bulbs were evaluated. Mean soft rot varied from 30 to 56% for the disks and 15 to 33% for the bulbs. Walla Walla sweet onions were no more susceptible to rot caused by *K. m.* var. *marxianus* than any of the onion cultivars tested. The fact that genetic resistance to the yeast was not found in a limited number of cultivars is not surprising given the wide diversity of substrates, including soil, sorghum, beer, lungs, feces, sputum, tonsils, and maize dough (5), that *K. m.* var. *marxianus* has occupied.

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