A Soft Rot of Onion Caused by the Yeast *Kluyveromyces marxianus* var. *marxianus*

D. A. JOHNSON, Extension and Associate Plant Pathologist, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350, and J. D. ROGERS, Professor and Chairman, and K. M. REGNER, Research Assistant, Department of Plant Pathology, Washington State University, Pullman 99164

ABSTRACT

Johnson, D. A., Rogers, J. D., and Regner, K. M. 1988. A soft rot of onion caused by the yeast *Kluyveromyces marxianus* var. *marxianus*. Plant Disease 72:359-361.

The yeast *Kluyveromyces marxianus* var. *marxianus* isolated from onion bulbs showing soft rot symptoms from commercial fields, a cull pile, and packinghouse was confirmed as a soft rot pathogen of onion bulbs by completing Koch's postulates. Resultant soft rot symptoms were significantly ($P = 0.05$) more severe at 27 and 20 C than at 10 or 5 C. Decay was significantly ($P = 0.05$) less in bulbs injected with 1 ml of 90 and 1,000 yeast cells per milliliter than bulbs inoculated with 11,000 or more yeast cells per milliliter. This is the first report known to us of a true yeast being pathogenic to onion bulbs.

Onions (*Allium cepa* L.) are continuously grown in southeastern Washington and northeastern Oregon. Walla Walla sweet onions (Yellow Globe variety) are planted in the fall (beginning in September) and harvested from the end of June throughout August. Several onion hybrid cultivars are planted in the spring (April) and are harvested from the end of July throughout August. Bulbs to produce seed of Walla Walla sweet onions are planted in August, and seed is harvested the next fall.

Scientific Paper No. 7782, College of Agriculture and Home Economics, Washington State University, Pullman 99164.

Accepted for publication 16 November 1987 (submitted for electronic processing).

In July 1986, a soft rot was observed in Walla Walla sweet onions after harvest. Affected bulbs had a soft, watery rot in the outer and/or inner scales. Fluid frequently exuded from the neck when bulbs were squeezed. Water-soaking of tissue, followed by soft rot, usually progressed internally in one or a few scales. Sometimes a large proportion of a bulb was affected. Rot also occurred around bruises on the outer scales. *Kluyveromyces marxianus* (Hansen) van der Walt var. *marxianus* was isolated. The isolated yeast rotted onion bulb disks in preliminary pathogenicity tests. The purpose of this work was to further establish the pathogenicity of the isolated yeast and to determine its prevalence in onion in southeastern Washington and northeastern Oregon.

MATERIALS AND METHODS

The yeast was originally isolated from a Walla Walla sweet onion bulb grown in a commercial field near Umapine, Umatilla County, Oregon, that was seeded in the fall of 1985 and harvested June 1986. Bulbs with soft rot symptoms were observed soon after harvest, and an attempt to isolate the causal agent was made.

Isolations were made from the margin of water-soaked tissue of an affected onion. 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 nutrient broth-yeast extract agar (NBY) (3) in a petri dish. Dishes were placed in an incubator at 25 C for 24 hr. Yeast colonies were observed after 12 hr of incubation.

K. m. var. *marxianus* has the following characteristics: Colonies on solid media at 20–25 C in 12-hr fluorescent light white or grayish white, at first entirely mucoid, but after several weeks the margin fringed with immersed hyphae. Vegetative cells reniform or somewhat allantoid, 3–5 × 1.5–2 μm, globose to ellipsoid, 4–6 × 4–6 μm, and rectangular to hyphal. Asexual reproduction by multipolar budding and disarticulation of hyphae. Asci formed by copulation of vegetative cells or, apparently, by conversion of short lengths of hyphae immersed in agar. Ascospores ellipsoid to globoid to, most commonly, reniform, smooth, two to four (to eight) per ascus. Physiological characteristics of the yeast are listed elsewhere (1). Identification was made with the aid of D. Yarrow (Centraalbureau voor Schimmelcultures, Delft, Netherlands). A culture has been deposited in the American Type Culture Collection.

Inoculation procedures. The yeast was increased in pure culture on Difco malt agar (MA) at room temperature on a laboratory bench (23 ± 2 C). Onion bulbs were inoculated with the yeast as follows:

Cut bulb. The outer scales of two hybrid yellow onions of cv. Titan (8–11 cm in diameter) were peeled. The bulbs were dipped in 0.5% sodium hypochlorite for 2 min, then cut in half (perpendicular to main axis of the bulb) with a sterilized knife. Yeast cells from a MA plate were spread across one-half of the four cut surfaces. Each onion was placed cut surface up in a sealed plastic bag at 23 C for 6 days.

Injection. Yeast cells were washed from a MA plate, and a 1.0-ml suspension of 1.6×10^8 cells per milliliter was injected with a disposable hypodermic syringe and needle 15 mm deep into Titan bulbs at 3–5 cm from the neck and angled toward the main axis of the bulb.

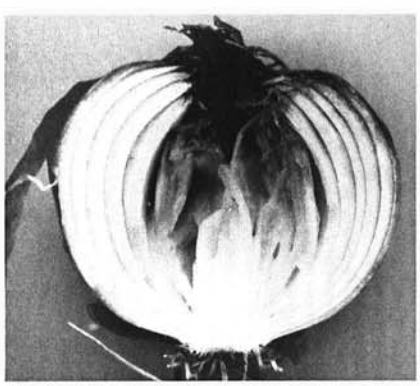

Fig. 1. Onion bulb with water-soaking and soft rot caused by *Kluyveromyces marxianus* var. *marxianus*.

Four inoculated bulbs and three bulbs injected with sterile water were each placed in chambers at 27 and 20 C for 6 days and at 10 and 5 C for 15 days.

Sets of three Titan bulbs were injected with a 1.0-ml suspension of 1.5×10^7, 1.3×10^6, 1.2×10^5, 1.1×10^4, 1.0×10^3, or 0.9×10^2 yeast cells per milliliter and placed in a chamber at 20 C. A 1.0-ml injection of sterile water served as a control. Onions inoculated with the three higher yeast cell concentrations became soft and were removed after 8 days. Onions inoculated with the three lower concentrations were firm at 8 days and were not removed until after 13 days.

Nail puncture. A sterilized nail was used to make holes 10 mm deep and 3 mm in diameter into the side of eight Titan bulbs. Four holes were filled with 0.1 ml of 1.6×10^8 yeast cells per milliliter and four were filled with sterile water. The test bulbs were placed in a chamber at 20 C for 13 days.

No wound. The outer scales of seven Titan bulbs were peeled to expose succulent scales. A 1-cm^2 section of MA culture was placed on the side of each of five bulbs so that yeast cells were between the onion and MA. A 1-cm^2 section of MA with no yeast was placed on the side of two bulbs as a check. Onions were placed in separate plastic bags, and the bags were sealed and placed at 23 C for 13 days.

Postincubation procedures. After an incubation period, all inoculated and noninoculated bulbs were cut in half from the neck to the base with a sterile knife. The rotted area was estimated visually. Another cut with a sterile scalpel was made near the margin of decay separating healthy and decayed tissue and a 2- to 3-mm^2 section of tissue from the margin was cut out, disinfected in 0.5% sodium hypochlorite for 20 sec, rinsed in sterile water, macerated in 5 ml of sterile water, and plated on malt agar with a sterilized inoculation loop.

The yeast was reisolated from all inoculated bulbs. Bacteria were also sometimes isolated from disintegrated tissue. A 1-ml cell suspension of $4–5 \times 10^8$ cells per milliliter of five bacterial isolates was injected with a hypodermic syringe into two Titan onions per isolate. Three onions were injected with 1 ml of 2.8×10^8 yeast cells per milliliter. Onions were placed in a chamber at 23 C for 8 days.

Disease prevalence. Onion bulbs with soft rot symptoms were collected during the spring and summer of 1987 from southeastern Washington and northeastern Oregon to determine the occurrence of pathogenic yeast and bacteria. Collections were made in March and April from three seed onion fields, in June from a 10-mo-old cull pile, in July through September from six onion fields near the time of harvest, and in July and August at 7- to 10-day intervals from three packinghouses. Isolations were made as previously stated, except that MA was used as the growth medium.

Yeast and bacteria isolated from affected bulbs were increased on MA and NBY, respectively, and placed with an inoculation loop on five spots (at center and at four corners to make a square) of cut onion bulb slices (1 cm thick) of Walla Walla sweet onion, Titan, or Golden Cascade in petri dishes, replicated twice, and incubated for 2 days at 27 C to test for pathogenicity.

RESULTS

Pathogenicity tests. Water-soaking followed by a soft, watery tissue disintegration of the parenchyma of the scales developed in the four halves of the two onions cut crosswise and inoculated with the yeast. The rot progressed downward from the inoculated half of a cut surface and also around to the surface of the noninoculated half. Only 5–15 mm of the center of the outermost scales remained healthy. Pure cultures of the yeast were reisolated from the margin of water-soaked tissue.

Soft rot (Fig. 1) developed in all bulbs injected with 1.6×10^8 yeast cells per milliliter and placed at various temperatures. The mean area of rot estimated on the surface of the internal bulb tissue after a longitudinal cut through the center of the bulbs was 70, 50, 7, and 4% (4% rot = one of 16 scales was two-thirds rotted) for bulbs kept at 27, 20, 10, and 5 C, respectively. There was no significant difference in severity of rot of bulbs in the 27 and 20 C temperatures and between the 10 and 5 C temperatures, but there was between the 20 and 10 C temperatures using Student's t test ($P = 0.05$). Rot did not develop in bulbs injected with sterile water. The yeast was reisolated from all inoculated bulbs.

Soft rot developed in all bulbs injected with 1.5×10^7, 1.3×10^6, and 1.2×10^5 yeast cells per milliliter. The mean areas of rot were 50, 43, and 42%, respectively. A significant difference was not observed among these treatments using linear regression ($P = 0.05$). Soft rot developed in all three bulbs injected with 1.1×10^4 yeast cells per milliliter, in two of the three injected with 1.0×10^3 cells, in two of the three injected with 0.9×10^2 cells, and in none of the bulbs injected with sterile water. The mean areas of rot in descending order of concentration of yeast cells were 58, 2, 2, and 0%, respectively. A significant reduction in rot was seen with a reduction in concentration of yeast cell suspension using linear regression ($P = 0.05$). The yeast was reisolated from all inoculated bulbs with soft rot symptoms.

The four bulbs punctured with a nail and inoculated became infected. The scale below the nail puncture was water-soaked, and rot progressed 3–5 cm from the puncture within scales that were wounded. The yeast was reisolated from

the diseased margin of scales wounded and inoculated and from the water-soaked tissue below the wound. Non-inoculated check bulbs did not become infected.

Soft rot developed in the outer scale of two of the five bulbs inoculated by sandwiching yeast cells between a nonwounded onion surface and a 1-cm^2 piece of malt agar. Area of rot was 3×2 cm and 2×1 cm in the two onions. The yeast was reisolated from the parenchyma of the outer scale. Rot did not develop in the two check bulbs.

Soft rot did not develop in the onions injected with bacteria that were isolated from some inoculated test bulbs. A light brown discoloration occurred in 1- to 8-cm sections of the scale where the bacterial cell suspension was injected. There was no tissue disintegration, however, indicating that bacteria isolated from inoculated test bulbs were secondary. Soft rot developed in the three onions inoculated with the yeast, affecting 50, 75, and 95%, respectively, of the bulb area.

Prevalence of *K. m.* var. *marxianus* and other organisms. *K. m.* var. *marxianus* was isolated from samples from the cull pile, two fields (including the original field), and one packinghouse. Isolates from these samples completely disintegrated tissue of onion slices but not of potato slices. Three other yeast species that also produced creamy white, convex colonies on MA were isolated from samples from several locations, but isolates of these yeasts produced only superficial rots that extended just a few millimeters into the onion slices. One of these was a bipolar budding yeast reminiscent of *Saccharomycodes*. Another was a multipolar budding yeast that produced abundant asci and probably represented a species of *Saccharomyces*. The third was a multipolar budding yeast of uncertain affinity. A filamentous yeast, possibly a *Saccharomycopsis*, was isolated from several samples; it also produced a superficial rot in inoculated onions. A soft-rotting *Erwinia* was isolated from samples from one field, from a packinghouse in June, and twice from another packinghouse in August. The bacterial isolates completely disintegrated tissue of onion slices and potato slices, produced pits on crystal violet pectate medium (3), and were facultatively anaerobic.

DISCUSSION

Many diseases of onion are known (4,5), but this is the first report known to us of a pathogenic yeast on onion bulbs. A yeast ally, *Geotrichum* sp., was recently reported to cause a soft rot in onion (2).

Symptoms in onion bulbs caused by *K. m.* var. *marxianus* are very similar to bacterial soft rots (4,5). Isolation of the causal organism from affected tissue, microscopic examinations, and pathogenicity tests were needed to differentiate the yeast from bacterial pathogens.

Decay caused by the yeast did not readily spread from scale to scale. Soft rot severity caused by the yeast was reduced in onions kept at 5 and 10 C and proceeded rapidly at 20 and 27 C. Severity of soft rot increased in inoculated bulbs as 1 ml of inoculum increased from 1.0×10^3 to 1.2×10^5 yeast cells, then leveled off as inoculum concentration increased.

K. m. var. *marxianus* was found throughout the onion-growing area near Walla Walla, WA. Pathogenic isolates were obtained from bulbs collected from a 10-mo-old cull pile, onion fields, and a packinghouse. Because onions are grown throughout the year in southeastern Washington and northeastern Oregon, the yeast is able to survive from one growing season to the next in infected bulbs in the current crop as well as in cull piles. Method of dissemination is unknown. In fact, much remains to be learned on the distribution, importance, epidemiology, and control of this yeast-caused soft rot of onion bulb.

K. marxianus is a complex species with seven described varieties. The typical variety, *marxianus*, of interest here, has been isolated from a number of substrates, including soil, sorghum beer, lungs, feces, sputum, tonsils, and maize dough from various parts of the world (1). Its capacity to decay onions thus is not entirely surprising. Given its wide substrate and geographic distribution, it could become a more serious problem on onions.

LITERATURE CITED

1. Kreger-van Rig, N. J. W., ed. 1984. The Yeasts: A Taxonomic Study. 3rd ed. Elsevier, Amsterdam. 1,082 pp.
2. Miller, M. E., Camp, T. H., Taber, R. A., and Pike, L. M. 1987. A rot of onion bulbs caused by *Geotrichum* sp. (Abstr.) Phytopathology 77:642.
3. Schaad, N. W., ed. 1980. Laboratory Guide for Identification of Plant Pathogenic Bacteria. American Phytopathological Society, St. Paul, MN. 72 pp.
4. Walker, J. C. 1952. Diseases of Vegetable Crops. McGraw-Hill, New York. 529 pp.
5. Walker, J. C., and Larson, R. H. 1961. Onion diseases and their control. U.S. Dep. Agric. Handb. 208. 27 pp.