Involvement of *Aureobasidium pullulans* and *Rhodotorula glutinis* in Russet of d'Anjou Pear Fruit

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ABSTRACT

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d'Anjou pear, the main cultivar grown in the Mid-Columbia Region of Oregon, is subject to russetting of the fruit surface, resulting in reduced quality and value. The role of *Aureobasidium pullulans* and *Rhodotorula glutinis* in russet of pear fruit was studied. Inoculations were done at full bloom in 1998 and 1999 and petal fall in 1999 with a log range of concentrations up to 10^8 CFU/ml. Populations of *A. pullulans* on floral and fruit tissue were monitored during spring 1999 and 2000 in six orchards with a history of russet. Russet of fruit in both studies was evaluated at harvest. In 1998 neither fungus increased russet. In 1999, inoculation with two strains of each fungus at 10^8 CFU/ml increased russet. Inoculation with 10^4 or 10^6 CFU/ml did not increase russet in either year. In commercial orchards, there was no correlation between fruit russet and the populations of *A. pullulans* on floral and fruit tissue. Populations were less than 10^3 CFU/g of tissue. We conclude that *A. pullulans* and *R. glutinis* are not major contributors to russet of d'Anjou pear fruit in the Mid-Columbia Region.

Additional keywords: *Pyrus communis*, yeast

Russet of d'Anjou pear is a disorder of the fruit surface that lowers the market value. Several causes of pear fruit russet have been identified and include frost (3), various chemicals (4,6), indole acetic acid-producing strains of several bacteria (7), and powdery mildew (12). Other possible causes include extended periods of surface wetness and improper nutrition (3). Recently, in New York, the yeast *Rhodotorula glutinis* (Fresen.) F.C. Harrison and the hyphomycete *Aureobasidium pullulans* (deBary) G. Arnaud were implicated as causal agents of russet of apple fruit (10). *A. pullulans* caused the most russet and was the most commonly detected microorganism on russeted apple fruit. In a study of epiphytic yeasts and yeast-like fungi on the surface of mature pear fruit in the Pacific Northwest, *A. pullulans* and *R. glutinis* accounted for 59 and 22%, respectively, of the total population on the surface of d'Anjou pear fruit from the Hood River area of Oregon (2).

The objectives of this study were to (i) examine the relationships between various

concentrations of strains of *A. pullulans* and *R. glutinis* and fruit russet in orchard inoculation experiments, and (ii) determine the populations of *A. pullulans* and *R. glutinis* and the severity of russet in six commercial orchards with histories of fruit russet. An abstract presenting preliminary results has been published (13).

MATERIALS AND METHODS

Inoculations with *A. pullulans* and *R. glutinis*. Two strains of *R. glutinis* (HRA4 and YMB4) and two strains of *A. pullulans* (HRA1 and YY1) were obtained from the surface of mature pear fruit in September 1993 (2). The fungi were removed from -70°C storage, transferred to yeast malt dextrose agar (YMDA), and grown for 48 h. Cells were removed from the dishes with a sterile rubber spatula, transferred to sterile distilled water (SDW), and centrifuged at 8,000 rpm for 15 min. Supernatant was decanted and cells resuspended in SDW. Concentrations were adjusted based on spectrophotometer readings to obtain a range from approximately 2×10^4 to 2×10^8 CFU/ml (Tables 1 and 2). Cell concentration was verified by dilution plating on YMDA.

On mature d'Anjou pear trees, limb sections with 25 to 50 blossom clusters were flagged. Five replicate limbs, each on separate trees, were marked per concentration. Yeast suspensions were applied to the clusters with a hand atomizer. Approximately 2 ml of suspension was applied per cluster. Inoculations were done on 17 April (bloom) 1998 and 19 April (bloom) and 3 May (petal fall) 1999.

Populations of viable yeasts on blossom and fruit surfaces were determined 0, 10, and 20 days after inoculation. Three blossom or fruit clusters were removed from each limb, combined, and weighed. Each bulked sample was immersed in 70 ml of sterile phosphate buffer with Tween 80, shaken on a rotary shaker at 150 rpm for 10 min, then placed in an ultrasonic bath (L & H Manufacturing Company, Kearny, NJ) for 10 min. Yeasts were recovered by spreading aliquots of 0.1 ml from serial dilutions of the cell suspensions on duplicate dishes of a semi-selective yeast medium. The medium consisted of 6.7 g of Bacto yeast nitrogen base, 10.0 g dextrose, and 18.0 g Bacto agar per liter. After autoclaving, the following were added per liter: 0.01 g iprodione, 0.00267 g dichloran, and 0.10 g chloramphenicol dissolved in 2.0 ml ethanol. Dishes were incubated 7 to 10 days at 22°C and colonies counted. Colony forming units per gram fresh weight of tissue were calculated.

The distribution of yeast cells on the flower receptacle surface was determined microscopically in 1999. One flower was removed from each replicate limb inoculated with the highest rate of *R. glutinis* (HRA4) and *A. pullulans* (HRA1) and from the unsprayed control limbs. Samples were taken four (bloom) and one (petal fall) days after inoculation, then weekly for five (bloom inoculation) and three (petal fall inoculation) weeks. Two thin sections of epidermal tissue were cut by hand with a razor blade from opposite sides of the receptacle. Tissue pieces were mounted in 0.1% cotton blue on a microscope slide, and stained yeast cells were counted at $\times 250$. Cell density was determined with the aid of an ocular micrometer.

Russet on the side of the fruit was evaluated on 20 August 1998 and 10 September 1999. Russet near the fruit calyx caused by rust mite and markings from limb rub and other mechanical causes were not included in the russet assessment. The percentage of the surface affected with russet was estimated for each fruit after training with DiseasePro 5.1 disease assessment software (F. W. Nutter, Jr., A. Worawitlikit, and D. Litwiller, Iowa State University, Ames) and viewing a series of photos of fruit with a range of russet severity, where the actual percentage of area russeted was determined with an area meter (Li-Cor, Inc., Lincoln, NE). Data were transformed to the square root of the percent russet and analyzed with analysis of

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variance. Means were separated with the protected least significant difference test. The relationships between fruit russet and concentrations of *A. pullulans* and *R. glutinis* on blossom and fruitlet surfaces immediately after inoculation were determined with correlation analysis.

Populations of *A. pullulans* and russet on fruit in commercial orchards. Six commercial orchards with a history of fruit russet were selected from the lower and upper Hood River Valley. In each orchard, three blossom or fruit clusters were removed from five d'Anjou pear trees starting at full bloom and at 10 to 15 day intervals. Samples were processed as in the inoculation experiments above for determination of CFU/g. Nondiluted tissue washes were plated in triplicate.

Russet on fruit was evaluated in late August to mid-September, approximately 1

week before commercial harvest. Fifty fruit were removed from each of the five replicate trees in each orchard, and russet severity was determined as in the inoculation experiment above.

RESULTS

Inoculations with *A. pullulans*. d'Anjou pear blossoms inoculated with a range of concentrations of *A. pullulans* from approximately 2.1×10^4 to 1.9×10^8 CFU/ml in increments of 10^2 resulted in initial populations on the blossoms and developing fruitlets of 1.2×10^4 to 2.5×10^7 CFU/g fresh weight. Initial populations on noninoculated control blossoms averaged 1.3×10^4 CFU/g. Inoculum concentrations and populations on blossoms are presented in detail for both strains of *A. pullulans* in 1998 and 1999 (Table 1). The population of *A. pullulans*

on the blossom and fruitlet surfaces generally remained stable for the control and lower inoculum concentrations, but declined at the highest inoculum concentration during the 20 days after inoculation (Table 1).

In 1999, an increase in the proportion of the fruit surface russeted at harvest (approximately 5 months after inoculation) occurred with the highest concentration (10^8 CFU/ml) of *A. pullulans* strain YY1 applied at bloom, and strains YY1 and HRA1 inoculated on trees at petal fall (Table 1). The 1998 bloom inoculations did not cause an increase in russet. Correlations between percent area of fruit surface with russet and the log of CFU of *A. pullulans* HRA1/g fresh weight were not significant ($r = 0.274$, $P = 0.725$) in the 1998 bloom inoculation but were significant in both inoculations in 1999 ($r = 0.983$, $P =$

Table 1. Populations of *Aureobasidium pullulans* on pear blossom and fruitlet tissue after inoculation in 1998 and 1999

Time of inoculation	<i>A. pullulans</i> strain	Inoculum concentration log(CFU/ml)	log CFU <i>A. pullulans</i> /g blossom or fruitlet ^x			Fruit area rus- setted (%) ^y
			Sample time (days) after inoculation			
			0	10	20	
Bloom, 1998	Control ^z	...	4.47 ± 0.15	4.39 ± 0.13	3.57 ± 0.08	3.0 ab
	HRA1	3.84	3.58 ± 0.17	4.42 ± 0.12	4.08 ± 0.19	2.9 ab
	HRA1	5.84	4.63 ± 0.15	5.05 ± 0.31	4.34 ± 0.19	4.4 ab
	HRA1	7.84	7.15 ± 0.07	5.63 ± 0.15	5.00 ± 0.13	2.7 a
	YY1	8.04	7.04 ± 0.05	5.55 ± 0.21	4.71 ± 0.28	5.3 b
Bloom, 1999	Control ^z	...	3.26 ± 0.35	3.30 ± 0.27	4.10 ± 0.10	0.9 a
	HRA1	4.39	3.77 ± 0.20	4.34 ± 0.35	4.00 ± 0.13	0.9 a
	HRA1	6.39	6.57 ± 0.28	5.43 ± 0.43	5.22 ± 0.34	1.2 ab
	HRA1	8.39	7.51 ± 0.05	5.13 ± 0.48	6.11 ± 0.07	1.4 ab
	YY1	8.30	7.71 ± 0.06	6.95 ± 0.07	6.40 ± 0.31	1.9 b
Petal fall, 1999	Control ^z	...	3.86 ± 0.14	4.14 ± 0.10	4.46 ± 0.15	0.6 a
	HRA1	4.51	4.41 ± 0.14	4.32 ± 0.13	4.28 ± 0.06	0.7 a
	HRA1	6.51	4.65 ± 0.31	5.17 ± 0.35	4.59 ± 0.20	0.9 ab
	HRA1	8.51	6.43 ± 0.43	5.39 ± 0.55	5.31 ± 0.12	1.5 bc
	YY1	8.32	7.59 ± 0.08	6.69 ± 0.06	6.27 ± 0.11	1.9 c

^x Means ± standard errors of the means.

^y Russet determined prior to harvest on 20 August 1998 and 10 September 1999. Means followed by the same letter at each inoculation time are not significantly different at $P = 0.05$ according to protected LSD.

^z Sterile distilled water applied as the control.

Table 2. Populations of *Rhodotorula glutinis* on pear blossom and fruitlet tissue after inoculation in 1998 and 1999

Time of inoculation	<i>A. pullulans</i> strain	Inoculum concentration log(CFU/ml)	log CFU <i>R. glutinis</i> /g blossom or fruitlet ^x			Fruit area rus- seted (%) ^y
			Sample time (days) after inoculation			
			0	10	20	
Bloom, 1998	Control ^z	...	3.43 ± 0.22	3.58 ± 0.16	1.90 ± 0.16	3.0 a
	HRA4	4.49	3.67 ± 0.16	3.46 ± 0.21	2.23 ± 0.16	4.1 a
	HRA4	6.49	4.75 ± .04	4.40 ± 0.10	3.45 ± 0.16	2.7 a
	HRA4	8.49	7.19 ± 0.06	5.99 ± 0.14	4.90 ± 0.11	4.0 a
	YMB4	7.78	7.05 ± 0.09	5.76 ± 0.08	4.42 ± 0.25	4.7 a
Bloom, 1999	Control ^z	...	4.38 ± 0.33	4.27 ± 0.14	4.08 ± 0.26	0.9 ab
	HRA4	3.65	4.14 ± 0.36	4.54 ± 0.07	4.45 ± 0.07	0.6 a
	HRA4	5.65	5.04 ± 0.05	5.22 ± 0.07	5.00 ± 0.19	0.8 a
	HRA4	7.65	7.11 ± 0.04	6.99 ± 0.07	6.04 ± 0.12	1.6 b
	YMB4	8.77	7.43 ± 0.07	7.36 ± 0.09	6.98 ± 0.15	2.7 c
Petal fall, 1999	Control ^z	...	4.21 ± 0.31	3.52 ± 0.52	3.39 ± 0.54	0.6 a
	HRA4	4.29	4.35 ± 0.33	3.75 ± 0.27	3.67 ± 0.49	0.7 a
	HRA4	6.29	5.23 ± 0.05	4.03 ± 0.17	3.61 ± 0.11	1.0 a
	HRA4	8.29	7.27 ± 0.13	6.30 ± 0.15	6.17 ± 0.19	2.6 b
	YMB4	7.78	7.38 ± 0.11	6.79 ± 0.11	6.20 ± 0.11	5.6 c

^x Means ± standard errors of the means.

^y Russet determined prior to harvest on 20 August 1998 and 10 September 1999. Means followed by the same letter at each inoculation time are not significantly different at $P = 0.05$ according to protected LSD.

^z Sterile distilled water applied as the control.

0.017 and $r = 0.992$, $P = 0.008$ at bloom and petal fall, respectively).

Inoculations with *R. glutinis*. Inoculation with a range of concentrations from 1.8×10^4 to 2.1×10^8 CFU/ml resulted in initial populations on blossoms and fruitlets of 1.3×10^4 to 1.9×10^7 CFU/g. Control populations initially were approximately 0.9×10^3 CFU/g. Inoculum concentrations and populations on blossoms are presented in detail for both strains of *R. glutinis* in 1998 and 1999 (Table 2). Most populations declined during the first 20 days after inoculation (Table 2).

Increased russet of fruit surface area compared with the control occurred at the highest inoculum level of 6.0×10^7 CFU/ml or higher in 1999 for *R. glutinis* YMB4 at bloom and YMB4 and HRA4 at petal fall (Table 2). The 1998 bloom inoculation did not cause an increase in fruit russet. Correlations between percent russet of fruit surface area and the log of CFU of *R. glutinis* HRA4/g fresh weight were not significant ($r = 0.340$, $P = 0.660$) in the 1998 bloom inoculation but were signifi-

cant for both inoculations in 1999 ($r = 0.954$, $P = 0.046$ and $r = 0.989$, $P = 0.010$ at bloom and petal fall, respectively).

Density of yeasts on receptacle surfaces. The densities of *A. pullulans* on the receptacles of blossoms inoculated with the highest concentration of strain HRA1 at bloom and petal fall were 8.4 and 6.8 cells per $1,000 \mu\text{m}^2$, respectively (Table 3). Cell density decreased with time, and population changes corresponded to changes in CFU/g (Table 1). Similarly, cell densities on receptacle surfaces inoculated with the highest concentration of *R. glutinis* strain HRA4 declined from over five cells per $1,000 \mu\text{m}^2$ shortly after inoculation to 1.34 to 1.64 per $1,000 \mu\text{m}^2$ after 3 to 5 weeks (Table 3).

Populations of *A. pullulans* and russet on fruit in commercial orchards. Populations of *A. pullulans* varied from 101 to 2.6×10^4 CFU/g in 1999 and 0 to 4.2×10^3 CFU/g in 2000 (Table 4). The highest single replicate sample contained 1.1×10^5 and 8.7×10^3 CFU/g in 1999 and 2000, respectively. Although some of the highest

populations occurred 22 to 29 days after full bloom, changes in *A. pullulans* populations varied considerably among orchards with no clear trend from full bloom to the final sampling. During 1999 and 2000, *A. pullulans* accounted for over 98% of the total microorganism population recovered from blossom tissue onto a semi-selective medium. The highest population of *R. glutinis* was 67 CFU/g, but *R. glutinis* usually was not detectable.

The percentage of fruit area with russet varied from 0.8 to 3.1 in 1999 and from 0.7 to 10.1 in 2000 (Table 4). Significant differences ($P = 0.05$) occurred among orchards in both years. No correlations between fruit russet and *A. pullulans* populations were significant ($P = 0.05$) for any of the sample times in either year.

DISCUSSION

A. pullulans is commonly found on the surfaces of many plant species, including leaves of oak (14), *Acer platanoides* (1), and *Hippophae rhamnoides* (8), bud scales and leaves of plane tree (9), twigs, buds, and leaves of sycamore (11), and twigs of horse chestnut and lime (11). It often is the predominant epiphytic microorganism, especially early in the growing season (1,5,8,9). Previously, we found that *A. pullulans* was present in all pear orchards surveyed, and its relative abundance was highest among the yeast and yeast-like fungi on the fruit surface (2). In that study, *R. glutinis* was present in 80% of the orchards. In the research described herein, *A. pullulans* accounted for over 98% of the yeast and yeast-like microorganisms recovered from blossom and fruitlet surfaces in six commercial orchards during spring.

A. pullulans and *R. glutinis* previously have been implicated in russet of apple fruit (10). In that study, inoculations were done up to seven times during the spring, and populations were determined at harvest. In contrast, we inoculated limbs only once, either at full bloom or petal fall and monitored populations from shortly after

Table 3. Density of yeast cells on the surface of pear flower receptacles after inoculation in 1999

Time of inoculation ^x	Time of sampling after inoculation (weeks)	No. cells per 1,000 μm^2	
		<i>Aureobasidium pullulans</i> ^y	<i>Rhodotorula glutinis</i> ^y
Bloom	0 ^z	8.41 $\pm$ 1.17	5.03 $\pm$ 0.80
	1	2.72 $\pm$ 0.64	3.81 $\pm$ 0.62
	2	3.25 $\pm$ 0.68	3.03 $\pm$ 0.66
	3	3.53 $\pm$ 1.05	2.66 $\pm$ 1.05
	4	2.00 $\pm$ 0.79	0.94 $\pm$ 0.42
	5	1.31 $\pm$ 0.64	1.34 $\pm$ 0.67
Petal fall	0 ^z	6.84 $\pm$ 0.85	5.75 $\pm$ 0.95
	1	4.13 $\pm$ 0.99	5.16 $\pm$ 1.09
	2	2.94 $\pm$ 1.06	2.91 $\pm$ 0.86
	3	1.79 $\pm$ 0.68	1.64 $\pm$ 0.75

^x Bloom inoculation on 19 April, petal fall on 3 May with *A. pullulans* HRA1 and *R. glutinis* HRA4. *A. pullulans* concentrations were 2.45×10^8 (bloom) and 3.23×10^8 (petal fall) CFU/ml. *R. glutinis* concentrations were 4.47×10^7 (bloom) and 1.69×10^8 (petal fall) CFU/ml.

^y Linear regression equations for bloom inoculation are: cell density (*A. pullulans*) = $6.027 - 1.068x$ ($R^2 = 0.627$, $P = 0.060$), cell density (*R. glutinis*) = $4.761 - 0.784x$ ($R^2 = 0.922$, $P = 0.002$). Equations for petal fall inoculation are: cell density (*A. pullulans*) = $6.376 - 1.634x$ ($R^2 = 0.949$, $P = 0.026$), cell density (*R. glutinis*) = $6.052 - 1.458x$ ($R^2 = 0.958$, $P = 0.021$). Time in weeks = x .

^z Bloom time 0 was sampled 4 days after inoculation, petal fall 0 was sampled 1 day after inoculation.

Table 4. Populations of *Aureobasidium pullulans* and percentage of d'Anjou fruit surface with russet in six commercial orchards in the Hood River valley in 1999 and 2000

Orchard	Year	<i>A. pullulans</i> CFU/g blossom or fruitlet ^x					Fruit area with russet (%) ^z
		FB ^y	FB + 10 days	FB + 15 days	FB + 22 days	FB + 29 days	
A	1999	211 $\pm$ 71	891 $\pm$ 228	...	4988 $\pm$ 732	...	2.0 bc
B		215 $\pm$ 84	1714 $\pm$ 940	...	3973 $\pm$ 1484	...	2.1 c
C		3904 $\pm$ 2931	2101 $\pm$ 711	...	19621 $\pm$ 4241	...	3.1 d
D		2725 $\pm$ 647	4986 $\pm$ 2269	...	25950 $\pm$ 21099	...	1.6 b
E		139 $\pm$ 49	1373 $\pm$ 722	...	2091 $\pm$ 768	...	1.8 bc
F		101 $\pm$ 23	4148 $\pm$ 1091	...	327 $\pm$ 101	...	0.8 a
A	2000	2800 $\pm$ 908	...	3662 $\pm$ 322	...	4203 $\pm$ 305	1.2 b
B		15 $\pm$ 15	...	0 $\pm$ 0	...	108 $\pm$ 62	3.0 d
C		1912 $\pm$ 1704	...	0 $\pm$ 0	...	16 $\pm$ 9	10.1 e
D		3224 $\pm$ 805	...	1387 $\pm$ 216	...	4269 $\pm$ 199	0.7 a
E		928 $\pm$ 443	...	0 $\pm$ 0	...	114 $\pm$ 59	3.5 d
F		725 $\pm$ 463	...	54 $\pm$ 31	...	66 $\pm$ 52	2.1 c

^x Values are the mean $\pm$ standard error or the mean.

^y FB = full bloom.

^z Means followed by the same letter for each year are not different at $P = 0.05$ according to protected LSD.

inoculation for up to 4 weeks. Russet is considered to occur during and shortly after bloom (3). Russet of d'Anjou pear fruit caused by the powdery mildew fungus *Podosphaera leucomyces* occurs during the 6-week period beginning 3 weeks before bloom (12). By June, all traces of mycelium are gone, leaving only the russeted area on the fruit surface. Thus, we focused our inoculation and recovery studies with *A. pullulans* and *R. glutinis* from bloom to petal fall when fruit are considered most susceptible to russet.

In addition to the presence of potential russet-causing microorganisms at the time when fruit are susceptible, the concentration or population density of the microorganisms is important. Most previous studies do not include quantitative data (CFU per unit weight or area). In the apple fruit russet study, population data were presented at harvest but not during the inoculations in spring (10). Although we found that *A. pullulans* is present on pear blossoms and fruitlet tissue in the Hood River Valley, the populations in commercial orchards were well below the levels needed to cause russet as determined in the inoculation experiments. Some aggregation or clustering was observed on inoculated surfaces (data not shown), but the number of clusters and the cell density within clusters decreased over time, indicating that the clusters were not areas of growth but artifacts of the inoculation process. Thus, while high concentrations of *A. pullulans*

and *R. glutinis* have the potential to induce russet of pear fruit, populations in the six commercial orchards in both years of the study were too low to attribute russet to these fungi. Russet has been related to rain, dew, and high relative humidity (3), but since these factors also promote the growth of microorganisms, it is difficult to determine the relative importance of each factor (7). In the Hood River Valley in April and May, the 1999 growing season was somewhat drier than in 2000 (24 versus 30 days of rain and 36.8 versus 46.2 mm of precipitation), and russet was less in commercial orchards in 1999 than 2000 (1.9 versus 3.4%). However, *A. pullulans* populations were higher at both postbloom sample times in 1999, which also may indicate that the russet was related more closely to moisture than to *A. pullulans*.

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