Influence of Inoculation with *Pseudomonas andropogonis* or *P. gladioli* on the Occurrence of Post-harvest Diseases of Tulip Bulbs: Bulb Rot, Bacterial Black Rot and Bacterial Brown Rot

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Bulb rot caused by *Fusarium oxysporum* f. sp. *tulipae*, bacterial black rot caused by *Pseudomonas andropogonis*⁶⁾ and bacterial brown rot caused by *P. gladioli*⁴⁾ are important post-harvest diseases of tulip in Toyama Prefecture. During maintenance of seed bulbs, we frequently observed secondary infection or succession on lesions of bacterial black rot by other pathogens such as *F. oxysporum*, *P. gladioli*, *Penicillium* spp. and *Aspergillus niger*. Because, we rarely observed secondary infection by other pathogens on bacterial brown rot lesions, we hypothesized that *P. gladioli* had an antagonistic effect against other post-harvest pathogens. Therefore, we examined the effects of post-harvest inoculation of *P. andropogonis* or *P. gladioli* on the disease development of bacterial black rot, bacterial brown rot and bulb rot of tulip bulbs.

Strains and inocula. *P. andropogonis* strain Qn21, *P. gladioli* strain BRA4 and *F. oxysporum* f. sp. *tulipae* strain Tu: 4-2 were used in this study. The bacterial strains were grown on PPGA⁵⁾ medium (decoction of 200 g potato 1 liter, peptone 5 g, glucose 5 g, Na₂HPO₄·12 H₂O 3 g, KH₂PO₄ 0.5 g, NaCl 3 g, agar 15 g) for one to two days at 25°C in the dark. *F. oxysporum* was grown on PDA medium (decoction of 200 g potato 1 liter, glucose 20 g, agar 18 g) for three weeks at 25°C in the dark.

Influence of inoculation with two bacterial pathogens on natural development of three post-harvest diseases. Tulip bulbs of 17 cultivars (Fig. 1) were lifted from the field in June 1991, the root systems and seed-bulb scales (debris) removed and main-bulbs collected and washed with running water. Fifty bulbs per cultivar were inoculated by submerging in a suspension of *P. andropogonis* or *P. gladioli* (2×10^8 cfu/ml) for 30 min. Subsequently the inoculated bulbs were kept in a plastic bag for moist condition for 24 hr at 25°C, dried, and then stored at room temperature. Control bulbs were treated with water only. The experiment was done once.

In mid-October, after two months in storage, disease

development of bacterial black rot, bacterial brown rot and bulb rot was recorded as the percentage of diseased bulbs. Bacterial black rot occurred in most cultivars after inoculation with *P. andropogonis* (Fig. 1-B). Bacterial brown rot, however, occurred in only five cultivars after inoculation with *P. gladioli*; the other 12 cultivars might be resistant to the disease (Fig. 1-B, C). Tulip bulb rot apparently increased after inoculation with *P. andropogonis* in six cultivars. However, the effect of *P. gladioli* was inconclusive (Fig. 1-A). The incidence of bacterial black rot greatly decreased following inoculation with *P. gladioli* in 14 cultivars (Fig. 1-B). However, the effect of *P. andropogonis* on the development of bacterial brown rot was inconclusive, because of the generally low occurrence of bacterial brown rot in this experiment.

To further examine the effects of *P. andropogonis* or *P. gladioli* on the natural incidence of bulb rot, 30 bulbs were artificially inoculated. Controls were either untreated or submerged in fungicide (benomyl WP, 1/250 solution) for 15 min. The experiment was repeated six times over a five-year period, and each treatment replicated three times. The natural incidence of bulb rot was recorded as the percentage of diseased bulbs in mid-October. Bulb rot incidence increased after inoculation of *P. andropogonis* in all experiments, whereas it decreased after inoculation with *P. gladioli*, except in 1994 (Fig. 2).

Influence of mixed inoculations on the interactions between pathogens. Tulip bulbs (cv. 'Merry Widow') were inoculated with mixed suspensions of various concentrations of *P. andropogonis*, *P. gladioli* and *F. oxysporum* using two methods. Wound inoculation was made by puncturing the outer bulb scale with a bundle of 20 needles dipped into inoculum. Dip inoculation was made by submerging the bulb into a suspension of pathogens for 15 min. Application rates and pathogen combinations are shown in Figs. 3, 4. Ten bulbs were used for wound inoculations, 30 bulbs were used for the dip treatments, and each treatment was replicated three

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Fig. 1. Effect of dip inoculation with at 2×10^8 cfu/ml of *P. andropogonis*, *P. gladioli* or water on the development of three post-harvest diseases on 17 tulip cultivars. Percentage of diseased bulbs after two months in storage at room temperature: A, bulb rot; B, bacterial black rot; C, bacterial brown rot.

Fig. 2. Effect of *P. andropogonis*, *P. gladioli* or fungicide dip treatments on the natural development of bulb rot after two months in storage at room temperature. MW, cultivar 'Merry Widow'; RB, cultivar 'Rose Beauty'. Within each experiment, bars with the same letters are not significantly different at $p=0.05$, according to Duncan's multiple-range test.

Fig. 3. Effect of mixed suspensions of *P. andropogonis* or *P. gladioli* with 10^6 conidia/ml of *F. oxysporum* f.sp. *tulipae* on the occurrence of bulb rot after wound inoculation.

times. Inoculated bulbs were maintained as in the previous experiments. Disease incidence was recorded as percentage of diseased bulbs or number of lesions per bulbs in mid-October.

Bulb rot increased as the concentration of *P. andropogonis* increased in the mixed suspensions of *P. andropogonis* and *F. oxysporum* after wound inoculation. Bulb rot decreased, however, with increasing concentrations of *P. gladioli* in mixed suspensions of *P. gladioli* and *F. oxysporum* (Fig. 3). Similarly, bacterial brown rot increased as the concentration of *P. andropogonis* increased in the mixed suspensions of *P. andropogonis* and *P. gladioli* after wound inoculation. Bacterial black rot decreased, however, with increasing concentrations of *P. gladioli* in mixed suspensions of *P. andropogonis* and *P. gladioli* (data not shown).

Results from dip inoculations were similar to those from wound inoculation. Bacterial black rot development was suppressed by relatively higher proportions of *P. gladioli* to *P. andropogonis*, and bacterial brown rot was induced by *P. andropogonis* at the highest concentration 10^8 cfu/ml (Fig. 4).

In vitro assay for antibiotic activity. The antibiotic activity of the three pathogens was assessed *in vitro* by measuring growth inhibition in dual culture on PPGA medium for *F. oxysporum*, and using the plate-chloroform method⁷⁾ on YPGA medium for *P. andropogonis* and *P. gladioli*. *P. gladioli* strongly inhibited the growth of both *P. andropogonis* and *F. oxysporum*, but *P. andropogonis* did not inhibit *P. gladioli* or *F. oxysporum*.

Some of *P. gladioli* strains are well known to produce antibiotic substances such as pyrrolnitrin³⁾, and have been used as biocontrol agents of some soil-borne diseases in Japan¹⁻³⁾. Perhaps *P. gladioli* suppressed bulb rot and bacterial black rot in this study by producing an antibiotic, although the mechanism of biocontrol is uncertain.

Our results show that bulb rot and bacterial brown rot

Fig. 4. Effect of mixed suspensions of *P. andropogonis* and *P. gladioli* on the occurrence of **A**, bacterial black rot and **B**, bacterial brown rot after dip inoculation. Bars with the same letters are not significantly different at $p=0.05$, according to Duncan's multiple-range test.

were increased by inoculation with *P. andropogonis*, whereas bulb rot and bacterial black rot were reduced by inoculation with *P. gladioli*. The effects of inoculation with *P. gladioli* on the development of bulb rot were variable, depending on the cultivar and year, for an unknown reason. The time of infection and relative inoculum density of the microorganisms may be important, since bulb rot occurred both in the field and in storage, whereas bacterial brown rot occurred only after lifting. Further study is needed to ascertain the population dynamics of these three pathogens in the infection courts and the induction of resistance or susceptibility in tulip bulbs.

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和 文 摘 要

守川俊幸・築尾嘉章・野村良邦：チューリップ黒腐病細菌と褐色腐敗病細菌の接種が両病害と球根腐敗病の発生に及ぼす影響

収穫後のチューリップ球根への褐色腐敗病細菌 *Pseudomonas gladioli* や黒腐病細菌 *P. andropogonis* の接種が、貯蔵中の両病害と *Fusarium oxysporum* f. sp. *tulipae* による球根腐敗病の発生に及ぼす影響を調査した。チューリップ 17 品種を用いた浸漬接種試験では、黒腐病細菌を接種すると球根腐敗病の自然発病が助長され、褐色腐敗病細菌を接種すると黒腐病の自然発病が抑制された。さらに、5 カ年の両病原細菌の接種試験の結果から、褐色腐敗病細菌の接種によって球根腐敗病の自然発病が抑制される傾向を認めた。また、これら 3 病害の病原の混合接種試験の結果、黒腐病細菌の共存下で、褐色腐敗病と球根腐敗病の発病率が高まる傾向が認められ、褐色腐敗病細菌の共存下では黒腐病と球根腐敗病の発病は抑制された。培地上で、褐色腐敗病細菌は球根腐敗病細菌と黒腐病細菌の生育を著しく阻害したが、黒腐病細菌にはこのような抗菌活性は認められなかった。

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