Influence of Inoculum Concentration, Temperature and Humidity on the Disease of Annual Bluegrass Caused by *Xanthomonas campestris* pv. *poae* JT-P482

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**Key words:** *Xanthomonas campestris* pv. *poae*, annual bluegrass, biological control.

Annual bluegrass (*Poa annua* L.), a common winter annual, is one of the most difficult weeds to control in intensively managed golf turf. Chemical herbicides are rarely used to control this weed in cool-season turf, because of their phytotoxicity. Bacteria which are highly pathogenic to annual bluegrass have been isolated in the United States and in Japan. Identified as *Xanthomonas campestris* pv. *poae*, these bacteria are localized in the xylem and cause severe wilt on annual bluegrass. The efficacy of these bacteria in the biological control of annual bluegrass has been investigated in the United States and Japan.

To elucidate the potential of the Japanese isolate JT-P482 as a biological control agent of annual bluegrass, we studied the effect of inoculum concentration, air temperature and relative air humidity (RH) on disease development in growth chambers.

Seeds of annual bluegrass were purchased from Herbiseed (Wokingham, England, UK) and stored at 4°C. After sowing the seeds in organic soil in plastic pots, pots were placed in a greenhouse at 25°C/20°C (day/night). The plants were inoculated 3-4 weeks after sowing.

For inoculum preparation, *X. c. pv. poae* JT-P482 was cultured in YNB liquid medium (yeast extract, 5 g; nutrient broth (Difco), 8 g; 1 liter of distilled water) at 28°C for 2 days on a rotary shaker. The bacterial suspensions were diluted to a final concentration of 10⁹ cells/ml by comparison with an absorption curve at 575 nm.

**Effect of inoculum concentration and air temperature on disease development and bacterial multiplication in annual bluegrass.** Plants were inoculated with bacterial suspensions containing 10⁴, 10⁶ or 10⁸ cells/ml by means of leaf clipping-inoculation. Inoculated plants were placed in growth chambers (MLR-350; Sanyo, Tokyo, Japan) which were kept at 30°C/25°C, 20°C/15°C or 10°C/5°C (day/night temperature regime) under 16 hr photoluminescence conditions. Disease development was estimated by the disease severity rating method (disease index). Bacterial multiplication in inoculated plants was measured as follows: Inoculated plants were harvested and surface-sterilized by wipping with cotton dipped in 70% ethanol. Tissue samples, 5 mm of the lowest part of the culm, were collected from harvested plants and cut into five pieces using sterile scissors. The samples were placed in 200 µl of sterilized and distilled water and shaken gently for ca. 30 min to isolate the bacteria in the xylem. The suspensions were diluted serially and spread onto YNA plates containing 200 µg/ml of cycloheximide to count the number of viable JT-P482 cells.

Severe wilt (disease index of 75%) was observed two weeks after plant inoculation with bacterial suspensions of 1.0×10⁶ cfu/ml or 1.0×10⁸ cfu/ml and subsequent incubation at 30°C/25°C (Fig. 1). While under the 20°C/15°C temperature condition, plants had severe wilt 4-5 weeks after inoculation with concentrations over 1.0×10⁶ cfu/ml (Fig. 1). Under the 10°C/5°C condition, wilt developed more slowly when the inoculum concentration was over 10⁶ cfu/ml; severe wilt was observed 7 weeks after inoculation (Fig. 1). When the inoculum concentration was decreased to 10⁴ cfu/ml, the wilt of annual bluegrass hardly developed at any temperature (Fig. 1).

Bacterial multiplication in tissue at the lowest part of the culm correlated to the disease severity. High air temperature (30°C/25°C) accelerated bacterial multiplication in the tissue but little multiplication occurred regardless of temperature in the case of 10⁴ cfu/ml of inocula (Fig. 2). At all air temperatures tested, 10⁶ cfu/ml of inocula accelerated disease development and bacterial multiplication more than 10⁸ cfu/ml of inocula. However, differences in the acceleration of disease development and bacterial multiplication influenced by inoculum concentrations were not as great as the acceleration influenced by air temperature. From these results, inoculum concentrations less than 10⁴ cfu/ml of JT-P482 do not seem to infect the annual bluegrass effectively but high temperature increases the disease development in annual bluegrass. This acceleration of disease development influenced by air temperature is generally known for bacterial wilt of forage grasses caused by *X. c. pv. graminis*.

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Fig. 1. The effect of inoculum concentration and temperature on the development of wilt caused by *Xanthomonas campestris* pv. *poae* JT-P482. Annual bluegrass was inoculated with $1.0 \times 10^4$ cfu/ml (∇), $1.0 \times 10^6$ cfu/ml (○) or $1.0 \times 10^8$ cfu/ml (●) of a bacterial suspension of isolate JT-P482. Plants were then grown in growth chambers at $10^\circ$/5°C, 20°C/15°C or 30°C/25°C (day/night). Bars indicate standard errors (n=34).

Fig. 2. The effect of inoculum concentration and temperature on the multiplication of isolate JT-P482 cells in 5 mm of tissue from the lowest part of the culm. Annual bluegrass was inoculated with $1.0 \times 10^4$ cfu/ml (∇), $1.0 \times 10^6$ cfu/ml (○) or $1.0 \times 10^8$ cfu/ml (●) of a bacterial suspension of isolate JT-P482. Plants were then grown in growth chambers at $10^\circ$/5°C, 20°C/15°C or 30°C/25°C (day/night). Bars indicate standard errors (n=6).

**Effect of humidity on disease development and multiplication of JT-P482 cells in annual bluegrass.** Plants were inoculated with bacterial suspensions containing $10^8$ cfu/ml by the method mentioned above. Inoculated plants were placed in growth chambers which were kept at a relative humidity (RH) of either 30-40% or 90% at 20°C/15°C (day/night temperature regime) under 16 hr of photoluminescence.

Wilt of annual bluegrass caused by isolate JT-P482 at 90% RH developed more slowly than at 30-40% RH until four weeks after inoculation. The means of the disease index were significantly different using Duncan’s multiple range test ($p=0.05$). Inoculated plants under both dry and humid conditions died five weeks after inoculation (Table 1). The symptoms of inoculated plants under both dry and humid conditions were same. The humidity effect on disease severity seemed to be caused by the predisposition of host plant rather than by the pathogenicity of isolate JT-P482. This is because the multiplication of isolate JT-P482 cell in the tissue at the lowest part of the culm was similar under both dry and humid conditions of air (Fig. 3). This humidity effect on disease severity is also known in the case of other

### Table 1. The effect of relative humidity on the development of wilt caused by *Xanthomonas campestris* pv. *poae* JT-P482

<table>
<thead>
<tr>
<th>Humidity</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40% RH</td>
<td>8.8±2.1</td>
<td>22.8±2.7</td>
<td>56.6±3.0</td>
<td>87.5±3.0</td>
<td>100.0±0.0</td>
</tr>
<tr>
<td>90% RH</td>
<td>2.2±1.2</td>
<td>13.2±2.2</td>
<td>39.7±2.8</td>
<td>71.3±3.7</td>
<td>100.0±0.0</td>
</tr>
</tbody>
</table>

a) Each value is the mean disease index±standard error of 34 inoculated plants.
b) Weeks after inoculation.
Fig. 3. The effect of relative humidity on the multiplication of isolate JT-P482 cells in 5 mm of tissue excised from the lowest part of the culm. Annual bluegrass was inoculated with 1.0 x 10^8 cfu/ml of a bacterial suspension of isolate JT-P482. Plants were then grown at 20°C/15°C (day/night) in growth chambers with 30-40% RH (•) or 90% RH (○). Bars indicate standard errors (n=12).

Our results indicate isolate JT-P482 has sufficient potential as a biological control agent of annual bluegrass when inoculum concentration is over 10^6 cfu/ml. Further, both high air temperature and low humidity are favorable environment for biological control of annual bluegrass with isolate JT-P482.

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Literature cited