CONTROL OF CROWN GALL ON CHRYSANTHEMUM WITH A NONPATHOGENIC BACTERIUM AND WITH SELECTED ANTIBIOTICS1

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Additional index words. Agrobacterium tumefaciens, Agrobacterium radiobacter.

Abstract. Crown gall on stems, roots and leaves of chrysanthemum was controlled by drenching Agrobacterium tumefaciens infested soil with an avirulent strain of Agrobacterium (Agrobacterium radiobacter). Inoculating plants by hypodermic needle injection with the virulent strain 24 hrs after inoculation with the avirulent strain controlled or greatly inhibited crown gall development. Control of crown gall was also obtained by soil drenches and plant sprays with oxytetracycline. Drenching infested soil with Vancomycin HCI 500 ppm significantly reduced crown gall development.

Crown Gall on chrysanthemum (Chrysanthemum morifolium) caused by Agrobacterium tumefaciens (Smith & Townsend) Conn. has occurred sporadically for many years. Galls on the basal stems of chrysanthemum were described by Smith and Townsend in 1907 (6). The disease is primarily confined to the crown and roots of older plants; however, leaf and stem galls have been observed in Florida plantings. The etiology and symptomatology of crown gall on stems, crowns and roots of chrysanthemum have been described by Kohn (2) and by the senior author (3). The relative susceptibility of commonly grown chrysanthemum cultivars to Agrobacterium tumefaciens has been determined by inoculation studies by Miller et al. (4). Although the disease is not now of major significance to growers in Florida it is a potential threat to the industry.

Kerr recently reported on the effectiveness of a nonpathogenic strain of Agrobacterium which protected seeds and seedlings of peach against the gall forming strain (1). Moller and Schroth (5) described biological control of crown gall on deciduous fruit trees by the use of this non-gall forming bacterium. The purpose of this study was to determine possible control of crown gall on chrysanthemum with the nonpathogenic strain of Agrobacterium and by selected antibiotics.

Materials and Methods
Indexed, rooted cuttings of the crown gall susceptible chrysanthemum cultivar 'Mountain Sun' were obtained from Yoder Bro. of Ft. Myers for these tests. All cuttings were planted in a 50-50 peat-vermiculite mixture in 10.2 cm pots and maintained in a greenhouse. Fifteen plants (1 plant/pot) arranged on the greenhouse bench as three replications of 5 plants each were used for each treatment. The plants were fertilized and maintained according to recommended procedures. Thirteen treatments (Table 1) were applied and gall readings were made six weeks after inoculation and treatment.

Table 1. Effects of an avirulent strain of Agrobacterium and selected antibiotics on crown gall development on chrysanthemums inoculated with the pathogenic strain.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease index, average of 3 replications*</th>
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<tbody>
<tr>
<td>1. Plant roots injured before potting, soil drenched with avirulent strain, stems and leaves injected with virulent strain.</td>
<td>9.15*</td>
</tr>
<tr>
<td>2. Same as treatment 1 except plants injected 24 hrs later.</td>
<td>6.02*</td>
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<tr>
<td>3. Plants injected with avirulent strain at time of planting, followed by injection with virulent strain.</td>
<td>4.61*</td>
</tr>
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<td>4. Same as treatment 3 except injected with virulent strain 24 hrs later.</td>
<td>1.08*2</td>
</tr>
<tr>
<td>5. Plants injected with a combination of avirulent and virulent strains at a 3:1 ratio.</td>
<td>5.89*</td>
</tr>
<tr>
<td>6. Plant roots injured, soaked in water soln. of avirulent strain for 30 min. planted, injected with the virulent strain.</td>
<td>8.66*</td>
</tr>
<tr>
<td>7. Same as treatment 6 except injected with virulent strain 24 hrs later.</td>
<td>4.66*</td>
</tr>
<tr>
<td>8. Plants sprayed with Oxytetracycline (400 ppm), injected with virulent strain.</td>
<td>0.01*2</td>
</tr>
<tr>
<td>9. Same as treatment 8 except injected with virulent strain 24 hrs later.</td>
<td>0.16*2</td>
</tr>
<tr>
<td>10. Soil infested with ground agar cultures of virulent strain, plant roots injured, planted, soil drenched with avirulent strain.</td>
<td>0.66*2</td>
</tr>
<tr>
<td>11. Same as treatment 10 except soil drenched with Oxytetracycline (400 ppm).</td>
<td>1.16*2</td>
</tr>
<tr>
<td>12. Same as treatment 10 except soil drenched with Vancomycin HCI (500 ppm).</td>
<td>2.65*2</td>
</tr>
<tr>
<td>13. Control plants injected with virulent strain only.</td>
<td>12.69</td>
</tr>
</tbody>
</table>

*Disease index: average no. galls per plant (5 plants/replication) X average size of galls in mm. 
*All treatments followed by the letter * are significantly better than the control according to Dunnett's test. 
*All treatments followed by the letter 2 are significantly better than the other treatments according to Duncan's multiple range test.

1Florida Agricultural Experiment Station Journal Series No. 169.
The pathogenic strain of *Agrobacterium tumefaciens* (Smith & Townsend) Conn. used in these tests was isolated by the authors from naturally infected chrysanthemum growing in Florida. The antagonist was a non-pathogenic Agrobacterium, *A. radiobacter* (Beijerinck & Van Delden) Conn. var radiobacter biotype 2 supplied by Dr. A. Kerr, Waite Agricultural Research Institute, University of Adelaide, Glenn Osmund, Australia.

In one series of tests the soil was infested with a macerated agar culture of the chrysanthemum strain of *A. tumefaciens*. One lima bean agar slant of the organism was used per pot. The roots on the plant were clipped and the crowns were injured by needle punctures just before planting in the infested soil. After planting, the soil and plant roots were drenched with the avirulent strain of the organism or by the antibiotics oxytetracycline HCl (Uri-Tet, Key Pharmaceuticals Inc., Miami, Florida) and Vancomycin (Vancocin HCl, Eli Lilly & Co., Indianapolis, Ind.). Drenching was immediately after planting or 24 hrs later as indicated in Table 1. The concn of the avirulent strain used for drenching was 10⁶ cells per ml. Oxytetractline was used at the rate of 400 ppm and Vancomycin at the rate of 500 ppm (Table 1).

All above ground inoculations in the following tests using either the virulent or avirulent strain were made by hypodermic needle injections into the crowns, stems and leaves of the test plants. The pathogenic organism was used at approximately 10⁶ cells per ml. The antibiotic oxytetracycline was sprayed on the plants at the rate of 400 ppm either immediately before or 24 hrs before needle injections with the virulent strain. The avirulent and virulent strains in a 3:1 ratio were injected into host plants. Also, the avirulent strain was used either as a soil drench on plants following root injury, or a preplant root soak following root injury, or needle injected into chrysanthemum plants. The treatments were followed by needle injection with the virulent strain either immediately or 24 hrs later.

The tests were read 39 days after treatment. A disease index was obtained by multiplying the average number of galls/plant times the average gall size in mm.

### Results and Discussion

All treatments caused a highly significant (p=0.01) reduction of crown gall when compared to the control, according to Dunnett's test (Table 1). Six treatments were highly significantly better (p=0.01) than the other treatments according to Duncan's multiple range test. These were; oxytetracycline used as a spray or drench when the plants were inoculated either immediately or 24 hrs later with the virulent strain of the pathogen, Vancomycin when used as a soil drench on plants in infested soil, and the avirulent strain when drenched into infested soil planted with injured chrysanthemum plants or when injected into plants inoculated with the virulent strain 24 hrs later.

It is interesting to note that treatment with the biological agent was more effective when the plants were subjected to the pathogen 24 hrs later rather than immediately. This appears to indicate that some time lag is necessary to allow the inhibiting agent to become trans-located into the plant to protect it from subsequent infection. Results obtained from other tests not reported here indicated that the crown gall inhibiting agent was present in the plant roots and stems before becoming concentrated in sufficient amounts in the leaves to protect them from infection.

Chemical control of crown gall with oxytetracycline appears to be very effective and economically feasible.

Drenching the soil with the avirulent strain at the time of planting also was effective and appears to be a potential method to control crown gall of chrysanthemum.

### Literature Cited


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**SPHAEROPSIS WITCHES' BROOM OF NERIUM OLEANDER**

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*Abstract*. Sphaeropsis tumefaciens was isolated from witches' broom and galls of oleander, Nerium oleander. Inoculations of branch terminals reproduced the symptoms of this disease within 4 months. The fungus was isolated consistently from inoculated, diseased plants.

Oleander, *Nerium oleander* L., is a popular ornamental shrub grown in subtropical areas. One of the most serious diseases of oleander in southern Florida is witches' broom which kills entire branches. The disease usually occurs on older, flowering plants. In 1937, West (2) reported the causal agent as *Sphaeropsis* sp. but provided no experimental evidence concerning pathogenicity. The fungus *Sphaeropsis tumefaciens* Hedges, reported to cause galling of *Callistemon viminalis* G. Don (1), has been isolated consistently from witches' broom of oleander in several locations of Florida. This paper reports the pathogenicity of this fungus to oleander.

*Materials and Methods*

The isolate of *Sphaeropsis tumefaciens* was cultured on...