Temporary Symptom Remission of Strawberry June Yellows and Witches'-Broom by use of Oxytetracycline

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ABSTRACT

Oxytetracycline applied as a root spray was effective in some cases in causing June yellows-infected strawberry plants to produce significantly greener leaves than untreated plants. When applied to strawberry plants with witches'-broom disease, oxytetracycline caused leaf areas to increase significantly, as compared to untreated, infected plants in some cases. These observations support the hypothesis that mycoplasmalike agents are involved in the etiology of these disorders. Phytopathology 61:1137-1139.

Additional key word: mycoplasma.

Application of tetracyclines has moderated the symptoms of several plant diseases which are thought to have mycoplasma involved in their etiology. The rapidly growing literature on the effects of tetracyclines on such diseases has been recently reviewed (10). We report the effects of oxytetracycline on the symptomatology of strawberry plants chronically infected with June yellows (JY) (7) and witches'-broom (WB) (12). JY has not been experimentally transmitted by sap, insect vectors, or grafting (7), but can be predicted in seedlings obtained by controlled pollinations (11). In his review of June yellows, Plakidas (8) states: "It is now accepted beyond any doubt that June yellows is a non-infectious disease of genetic origin . . . due to a recessive mutating gene or genes which cause somatic mutations". Wills (11) recognizes this possibility, but concludes that ". . . more probably it is an exogenous, virus-like particle". WB has been transmitted by grafting (6), but not by aphids (5).

MATERIALS AND METHODS.—Propagant lines of the following strawberries were used: Nisqually showing JY symptoms; Nisqually doubly infected with JY and WB; *Fragaria vesca* var. *semperflorens* 'Alpine' (Duchesne) Ser. infected with WB; and healthy Alpine strawberry.

To determine the effects of oxytetracycline treatments on Nisqually plants, leaf color was recorded. We rated the degree of leaf variegation in JY-diseased Nisqually plants on a 1 to 10 scale (1 = completely yellow; 10 = completely green), taking 3 recently matured leaves/plant and using 18 to 36 plants/treatment. Because the severity of symptoms of JY in Nisqually plants tends to vary from plant to plant, from leaf to leaf, and with time, we determined ratings before and at intervals after oxytetracycline treatment. The differences in pre- and post-treatment ratings were then compared statistically with the differences found in Nisqually that had received no oxytetracycline over the same time period. If the treatment caused a net increased chlorosis with respect to controls, a negative difference would result; if it made the plants relatively greener, a positive difference would occur.

One of the most characteristic symptoms of WB is the proliferation of crowns with the development of numerous small leaves. Therefore, the effects of oxytetracycline on the severity of WB in Alpine and Nisqually were determined by making leaf-area measurements. We selected the 10 largest recently matured leaves from the plants used in each experiment. The number of plants per treatment varied from 8 to 27, but in each case the number of control plants was equal to the number of plants treated.

Quantitative differences between treatments for both JY and WB were then compared by the unpaired t-test. As a check on our methods, strawberry mottle virus (SMV) (5) was used as an example of a typical strawberry virus which is presumably not influenced in its transmissibility by oxytetracycline. This virus was transmitted to and from Alpine by the strawberry aphid *Chaetosiphon fragaefolii* (Cock.). Acquisition feeding periods of 3 days and transmission feeding periods of 1 day were employed.

Aster plants (*Callistephus chinensis* [L.] Nees), healthy or infected with aster yellows disease, were included in the treatments as a measure of the effectiveness of oxytetracycline, which has been shown to cause remission of symptoms of this disease (1).

Treatments consisted of applications of 0, 5, 10, or 50 μg/ml of calcium oxytetracycline (OT) (Agricultural Terramycin, Pfizer Incorporated, New York, N.Y.) in half-strength Hoagland's solution (3) pH 6.6, as an intermittent, recycled, fine spray to plant roots in plastic containers in a greenhouse. Two storage tanks and pumps were used, one for the Hoagland's solution and the other for Hoagland's solution plus OT. The concentrations of OT in the Hoagland's solution storage tank were determined by standard bioassay (9) using *Bacillus cereus* Frankland & Frankland (ATCC 11778). The determinations were made by Pfizer Incorporated. The half life of oxytetracycline in this

TABLE 1. Influence of various levels of oxytetracycline (OT) on the leaf color rating of Nisqually strawberry plants showing June yellows symptoms

Experiment no.	Oxytetracycline, μg/ml	Duration of treatment, days	Comparisons of leaf color rating changes between OT treatments and controls[a]: Just after OT treatment	49 days after OT treatment
1	50	35	−1.8**	+1.4**
2	50	35	−2.5**	−0.5 N.S.
3	10	28	+1.0**	X
4	10	28	+1.6**	X
5	5	18	−1.2*	X

[a] + = The change in color before and after treatment in leaves from the OT treatment was greater in the direction of increasing green color than the comparable change in control plants in Hoagland's solution without antibiotic; − = the reverse. Statistical significance of the differences is indicated by ** (1% level); * (5% level); N.S. = not significant; X = not tested.

system was about 2 days. Initial concentrations of 50 μg/ml tended to stabilize at ca. 15 μg/ml from 4 to 14 days. The OT solutions were changed every 3 days during the tests.

RESULTS.—Results of tests with OT on JY are presented in Table 1. In infected strawberry plants treated with 50 μg/ml OT (Experiment No. 1), the difference in leaf color was significantly greater (1% level) in the direction of chlorosis after 35 days of treatment than was the comparable difference in control (diseased, untreated) plants. However, 49 days after the final application of 50 μg/ml OT, the leaf color change of OT-treated plants in Experiment No. 1 was significantly greater (1% level) than the change in control plants in the direction of greener leaves. In a repeat test (Experiment No. 2), leaves of plants treated with 50 μg/ml OT had a change in color rating after 49 days that did not differ significantly from the comparable change in color rating in the controls. Treatment with 10 μg/ml OT resulted in a change in color rating toward greener leaves that was significantly greater (1% level) than the change in color rating in control plants after 28 days of treatment in repeated tests (Experiments No. 3, 4). Infected plants treated with 5 μg/ml OT for 18 days had a change in color rating that was significant (5% level) compared to the change in control plants (Experiment No. 5) in the direction of increased chlorosis.

Results of tests with OT on WB are presented in Table 2. Recently matured leaves of WB-infected Nisqually plants treated with 50 μg/ml OT for 35 days were significantly (1% level) larger in leaf area than those of control (diseased, untreated) plants (Experiment No. 6) when measured 49 days after OT treatment (Fig. 1). However, leaves formed later and measured 154 days after treatment ended (Experiment No. 7) were as small as comparable leaves on control plants. Leaf areas of Nisqually WB plants treated with 10 μg/ml OT were not significantly different from leaf areas of control plants (Experiment No. 8) when measured 112 days after the end of OT treatment. Alpine strawberry plants infected with WB and treated with 10 μg/ml OT had significantly smaller leaves (1% level) when they were compared to untreated plants 112 days after OT treatment had ceased (Experiment No. 9). Infected plants treated with 5 μg/ml OT had significantly larger (1% level) leaves than untreated leaves when measured 98 days after OT treatment had ceased (Experiment No. 10).

At all concentrations tested, OT caused a striking temporary remission of symptoms in aster infected with aster yellows. Alpine strawberry plants infected with SMV showed no change in symptoms when treated with 10 μg/ml OT, and this treatment did not interfere with aphid transmission of SMV.

The influence of various levels of OT on the areas of healthy Alpine strawberry leaves is shown in Table 3. Measurements were made at intervals comparable to those used following OT treatment of WB-infected plants. OT at 50 and 10 μg/ml significantly reduced leaf area compared to untreated plants (Experiments No. 11, 12), but reduction following 5 μg/ml OT treatment was only significant at $P = .075$ (Experiment No. 13).

DISCUSSION.—Phytotoxicity of OT was a major problem in this study. All strawberry plants were weakened by OT. The higher concentrations induced necrosis in root systems of healthy seedling strawberry plants, and leaves became very chlorotic. The action of OT was to cause JY leaves to become relatively greener, and to cause WB leaves to become larger,

TABLE 2. Influence of various levels of oxytetracycline on the leaf area of strawberry plants infected with witches'-broom disease

Experiment no.	Test cultivar	Avg leaf area (cm²) from indicated treatment with oxytetracycline (μg/ml): μg/ml	cm²	μg/ml	cm²	Duration of treatment, days	Holding period, days[a]
6	Nisqually	50	20.2**[b]	0	9.7	35	49
7	Nisqually	50	7.9 N.S.	0	8.2	35	154
8	Nisqually	10	4.3 N.S.	0	6.3	28	112
9	Alpine	10	6.3**	0	8.2	28	112
10	Alpine	5	11.5**	0	3.4	18	98

[a] Indicates the length of time the plants were held in the greenhouse bench in potting soil after oxytetracycline mist treatment before leaf area measurements were made.

[b] Statistical significance of the differences between oxytetracycline-treated plants and their comparable Hoagland's solution checks is indicated by ** (1% level); * (5% level); N.S. = not significant.

TABLE 3. Influence of various levels of oxytetracycline on the leaf area of healthy Alpine strawberry plants[a]

Experiment no.	Avg leaf area (cm^2) from indicated treatment with oxytetracycline (μg/ml)				Holding period, days[b]
	μg/ml	cm^2	μg/ml	cm^2	
11	50	17.7	0	27.1**[c]	154
12	10	19.4	0	25.9*	112
13	5	18.6	0	24.9 N.S.	98

[a] Three to four plants were used/treatment.
[b] Indicates the length of time the plants were held in the greenhouse bench in potting soil after oxytetracycline mist treatment before leaf area measurements were made. Exp. No. 11 was grown in oxytetracycline mist for 35 days, Exp. No. 12 for 28 days, and Exp. No. 13 for 18 days.
[c] Statistical significance of the differences between oxytetracycline-treated plants and their comparable Hoagland's solution checks is indicated by ** (1% level); * (5% level); N.S. = not significant.

than those in comparable untreated control plants. These effects occurred over and above the phytotoxic effects of the chemical, which tended to produce chlorosis and smaller leaves in healthy plants. The effects of OT in causing some remission of symptoms with both of these diseases appeared to be temporary, and the phytotoxic effects of OT seemed to last longer (Table 3) than its symptom-remission effects. Concentration of OT, duration of the treatment, duration of the observation period, and microenvironmental factors all interact with the phytotoxic and the symptom-remission effects of OT in establishing the symptomatology. Thus, we find reversal of effects of OT on JY in the same experiment, depending on how long after treatment the data are taken (Experiment No. 1). In the case of WB, remission occurred at 50 μg/ml OT at 84 but not at 189 days after treatment began (Experiments No. 6, 7). Remission of WB also occurred at 5 μg/ml when measured 116 days after treatment began (Experiment No. 10), but not at 10 μg/ml when measured 140 days after treatment began (Experiment No. 11).

Experimental alteration of JY symptoms has been previously accomplished only by clonal selection (8) and by heat treatment (2), although the effect was not permanent (11). We feel that expanded studies of the effects of antibiotics on the June yellows disease will be justified, regardless of the etiology of this disorder. The symptoms of June yellows sometimes do not appear in a strawberry cultivar for many years after it has been developed. There is an interesting parallel in *Drosophila paulistorum,* in which crosses of the Santa Marta and Mesitas strains produce sterile males in the F_1 and in several backcross generations (4). A tetracycline-sensitive mycoplasmalike symbiont is present in degenerating spermatids of sterile males. This mycoplasmalike symbiont is favored by the Santa Marta genome and is repressed by the Mesitas genome, so that sufficient backcrosses to the Mesitas genome eventually suppress the symbiont.

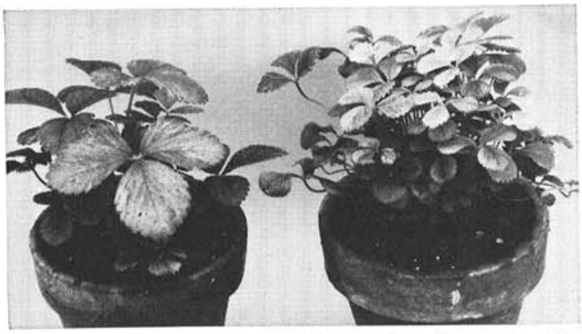

Fig. 1. Strawberry plants of the Nisqually cultivar infected with witches'-broom photographed 49 days after treatment with 50 μg/ml oxytetracycline; (left) treated plant; (right) untreated infected plant.

While we have shown that OT can cause partial and temporary remission of symptoms of JY and WB in strawberries in some instances, the explanation of these findings must await further studies. Symptom remission of JY and WB, but not of SMV, by a tetracycline antibiotic suggests that mycoplasmalike agents may be involved in the etiology of JY and WB. However, we recognize that additional lines of evidence, certainly including electron microscope studies, will be required to clarify this possibility.

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