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Biological Control of Fusarium Wilt of Carnations: Progress and Prospects

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The control of fusarium wilt of carnation is of primary concern to commercial growers in California and worldwide. While it is the major disease affecting carnation production, disease resistant cultivars and fungicides are not available yet for its effective control. In this light, biological control is being given increasing attention as a possible solution. Research is being conducted through the University of California at Berkeley in conjunction with several offices of Cooperative Extension to explore the potential of biological control.

Fusarium wilt of carnation is caused by a fungus, *Fusarium oxysporum* f. sp. *dianthi*. The disease can be prevented by eradicating the fungus from carnation beds, maintaining strict sanitation, and planting disease-free cuttings. The usual cause of disease in steamed raised beds is poor sanitation practices, as the fungus can be readily eradicated from raised beds by thorough steaming. Steaming of ground beds for control of fusarium wilt, however, is ineffective. Complete eradication of the fungus in raised or ground beds by treatment with chemical fumigants is seldom achieved because the fungus can persist in areas of the soil not reached by the fumigants.

Treatment of soil with steam or chemical fumigants destroys many beneficial bacteria and fungi that normally help to protect plants from infection by pathogens. In the absence of these beneficial microorganisms, the wilt fungus left in soil after ineffective soil treatment or reintroduced into treated soil through poor sanitation can spread unchecked and readily infect susceptible plants.

The biological control strategy being tested in our research is the reintroduction of beneficial organisms into treated soil or onto carnation root systems. We have been investigating fusarium wilt-suppressive soils—soils in which fusarium wilt cannot establish due to high population of “antagonists” or disease-inhibitory microorganisms. These soils are being tested both as a medium by which antagonists can be reintroduced into treated soil and as a source from which antagonistic organisms can be isolated and utilized separately.

Suppressive Soils

Earlier researchers at the University of California found that fusarium wilts develop at reduced levels when susceptible plants are planted into particular soils from the Salinas Valley.

These “wilt-suppressive” soils, in contrast to “wilt-conductive” soils, inhibited different pathogenic forms of *Fusarium oxysporum*, including those which cause wilt in sweet potato, tomato, and cotton. In 1980, separate studies at the University of California and at Colorado State University demonstrated that fusarium wilt of carnations can be controlled by the addition of small amounts of suppressive soil to sterilized carnation beds.

Subsequent studies at the University of California have led to a refinement in the method of applying suppressive soils. In the new method, suppressive soil is applied directly to the root system by dipping the root ball of rooted cuttings into a slurry of suppressive soil and water mixed at a ratio of two parts soil to one part water by volume. A greater number of plants can be protected with a given volume of soil, while eliminating the need for adding suppressive soil directly to treated soil. In a number of field trials the application of a suppressive soil slurry was as effective in reducing fusarium wilt in carnations as an amendment of suppressive soil to the entire bed.

A survey was conducted of soils from the Salinas Valley to locate some of the more suppressive ones. Seven

soils were collected from sites in the central portion of the valley near the towns of Chualar and Soledad, and tested along with three soils taken from the coastal area near Moss Landing. Each soil was applied as a slurry to rooted cuttings which were then planted into a conducive soil infested with the wilt fungus.

The 10 soils displayed different levels of fusarium wilt protection. Five months after planting, disease severity in plants treated with three soils from the Chualar-Soledad area and one soil from Moss Landing was over 60 percent below that recorded in plants given no soil slurry treatment (table 1). The remaining six had less effect on fusarium wilt.

Before this survey, it was believed that fusarium wilt suppressiveness was an exclusive trait of soils from the central portion of the valley and was not found in the coastal soils. However, the findings from the survey indicate that the level of wilt suppressiveness varies markedly among soils, and that no generalization can be made as to the suppressiveness of soils from any one region.

There are drawbacks to using suppressive soils. The populations of antagonistic bacteria within suppressive soil are highly unstable, fluctuating markedly with changes in soil conditions while in the field and in storage. Because suppressive soils cannot be sterilized before use without destroying the suppressiveness, there is the possibility that pathogens and insect pests of carnations may be carried in a suppressive soil and be introduced into treated beds. A more effective and safer alternative is to isolate the antagonistic organisms from the soil and to use them alone or in combination. Surveys, such as the one conducted in this study, can be useful in locating the most effective suppressive soil sites to serve as sources of biological control organisms.

Antagonistic Bacteria

Bacteria were isolated from the root surface of carnations grown in wilt-suppressive soils with the intent of selecting antagonists to *F. oxysporum* which can also colonize the root systems. Some of these bacteria, when grown on nutrient media, produce substances which inhibit the germination of spores and the growth of mycelia of the wilt pathogen.

These strains were evaluated in pot tests on carnation plants. Rooted cuttings of carnation were treated by

TABLE 1. Effect of various Salinas Valley soils* on fusarium wilt of carnations

Soil	Origin†	Disease rating‡
Elkhorn fine sandy loam	ML	0.8 a
Pico fine sandy loam	S	1.0 a
Mocho silty clay loam	S	1.3 ab
Salinas loam	S	1.3 ab
Arroyo Seco gravelly sandy loam	S	1.7 b
Chualar sandy loam	C	1.7 b
Elder loam	S	1.8 b
Metz fine sandy loam	S	1.8 b
Oceano loamy sand	ML	2.3 bc
Santa Ynez fine sandy loam	ML	2.7 c
Untreated control	—	3.5 d

*Soils were applied as slurries to roots of "White Improved Sim" cuttings before planting.

†ML = Moss Landing; S = Soledad; C = Chualar.

‡Average of 8 plants rated on a scale of 0 (symptomless) to 4 (dead), 5 months after planting in soil infested with fusarium wilt pathogen. Values followed by the same letters are not significantly different at the 95% confidence level.

TABLE 2. Effect of Mocho suppressive soil and bacteria on fusarium wilt of carnations

Treatment	Disease rating*	
	3 mo.	5 mo.
Mocho soil slurry	1.8 a	2.4 a
Bacterial suspensions:		
C88	1.7 a	3.6 b
MFA1	1.8 a	3.8 b
B10	2.6 b	4.0 b
Untreated control	3.2 c	4.0 b

*Average of 10 plants rated on a scale of 0 (symptomless) to 4 (dead). Values followed by the same letter are not significantly different at the 95% confidence level.

dipping the roots in suspensions of bacterial cells and then planting into fusarium-infested soil. Treatment with some strains of bacteria proved to be effective in the pot tests in reducing fusarium wilt. However, the protection imparted by these bacteria proved to be short-lived. In one experiment, cuttings were treated with one of three bacteria or with a suppressive soil slurry, and compared with cuttings left untreated. Three months after planting, disease levels in plants treated with bacteria or with the suppressive soil were significantly lower than levels observed in untreated plants. When rated again for disease 2 months later, disease levels in bacteria-treated plants were as high as those of untreated plants. The suppressive soil slurry treatment, in contrast, continued to reduce the level of disease (table 2).

The shortened duration of wilt protection provided by the bacterial treatments has been related to the time span of survival of the bacteria in the carnation root system. The survival of the bacteria may have been limited by adverse physical and chemical factors in the soil. The longer effective period of the suppressive soil treatment suggests that antagonistic microorganisms were applied to the root systems which

were more capable of establishing under adverse and changing soil conditions.

Soil texture and soil pH can be major factors determining the length of survival of antagonistic bacteria applied to carnation root systems. In one experiment in which bacterium strain MFA1 was applied to cuttings planted into different soils, greater numbers of the bacterium were reisolated from the root system of plants grown in soils with a fine texture and neutral pH than from plants grown in soils of coarse texture and low pH. The populations of MFA1 4 months after planting ranged from 20 bacteria per cm of root to as high as 95,000 per cm, depending upon the soil type.

Conceivably, the protective effect of the antagonistic bacteria can be extended to last through the productive life of carnations by selecting or altering soils such that conditions are favorable to the survival and activity of the bacteria. Another strategy would be to screen for antagonistic organisms more adapted to diverse soil conditions.

Commercial Greenhouse Trials

Experiments are underway in commercial carnation greenhouses in the San Jose, Salinas, and San Diego areas testing one fusarium wilt-suppressive

soil and several strains of bacteria. The suppressive soil, Mocho silty clay loam from the Salinas Valley, was applied as an amendment to carnation beds at a rate of 5 liters per square meter, or as a slurry to rooted cuttings of susceptible 'Sim' cultivars. The bacteria were applied as cell suspensions of single strains or of several strains in combination.

In four out of five trials, suppressive soil treatment reduced the amount of loss due to fusarium wilt. Treatment of cuttings with suspensions of bacteria proved far less effective in controlling the disease. Some treatments reduced disease in some trials, but not in others. Treatment involving combinations of bacteria were not better than treatments with single strains.

Preliminary data from one trial are presented to illustrate the effects of some treatments (table 3). In this trial, treatments include Mocho soil applied both as an amendment and as a slurry, and two bacterial treatments—A13 as a single strain, and MFA1 and B10 in combination. As the beds were fumigated with methylbromide before planting, disease inoculum came from natural infestation. A reduced level of mortality was recorded in both bacteria treatments after 7 months, with A13 providing protection through 12 months. The effects of the suppressive soil treatments became apparent after 12 months. At this time, while the checks suffered 60 percent loss, losses of approximately

TABLE 3. Effect of various treatments on loss of carnations due to fusarium wilt in a commercial greenhouse

Treatment	Percent mortality	
	7 mo.	12 mo.
Mocho suppressive soil:		
applied to cuttings as a slurry	16	48*
added to greenhouse soil at 5 liter/m ² rate	16	51*
Bacteria suspension applied to cuttings:		
A13	11***	43***
MFA1 and B10 in combination	11**	55
Untreated control	19	60

*, **, *** Difference from check is statistically significant at $p = .10$, $.05$, and $.01$.

50 percent were measured in the two suppressive soil treatments and 43 percent in the A13 treatment.

Potentials for Biological Control

Fusarium wilt of carnations can be reduced, under carefully controlled conditions, by applying antagonistic bacteria isolated from wilt-suppressive soils. However, the bacteria have shown erratic performance under commercial greenhouse conditions, indicating that, when taken outside of their native soils, these organisms are subject to many conditions that may reduce their ability to survive and inhibit the wilt fungus. A better understanding of the effects of different environmental parameters on these activities will be required before the use of bacteria for the control of fusarium wilt in carnation can become practical.

Antagonistic bacteria cannot be expected to provide adequate protection against fusarium wilt if they are used as the sole means of control. To be useful, they will have to be applied in conjunction with proven methods of disease control, including soil pasteurization and sanitation. The use of bacteria may also have potential if combined with other biological control methods currently being investigated, such as disease tolerant carnation cultivars and antagonistic soil fungi.

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