Final Project Report to the NYS IPM Program, Agricultural IPM 2000-2001

Title: Prevention of Diseases in Zinnia Plug Production

Project Leaders: Jana Lamboy, NYS IPM Program, Geneva
Nellie Call, Flower Fields, Elba

Cooperators: Margery Daughtrey and Maria Tobiasz, Long Island Horticultural Research and Education Center, Riverhead
Karen Hall, Erie County CCE, East Aurora
Art Harrington, Harrington's Greenhouses, Elba
Roxanne McCoy, Lilies of the Field, West Falls
Deborah Kwasniewski, Taylor Greenhouse, Portland

Type of Grant: Demonstration and Implementation, Ornamentals
Use of microbials and active composts
Cultural methods, sanitation

Project Location: Ontario, Genesee, Erie, Chautauqua, and Suffolk
Results will be useful across NY, the Northeast, and nationally

Abstract:
Nearly all cut flower and bedding plant growers plant zinnias. Many growers observe losses in crop quality due to leaf spot diseases. This project entailed seed treatment trials to study source of inoculum and efficacy trials of disease suppressive microbials and compost in greenhouses and cut flower fields. Clorox at 20% for 20 minutes reduced viable bacteria on seeds to zero; hot water treatments 52°C for 20 minutes reduced the presence of fungi. Both treatments affected seed germination rate, but at an acceptable level. The cut flower growers observed an increase in plant quality when seeds were grown in BioComp compost instead of standard peat/lite mixes. Disease suppression was observed with the variety Ruffles treated with the microbial inoculants Mycostop, RootShield, and SoilGard. The results in identifying effective seed treatments and disease suppressive inoculants are very promising, but need to be repeated before making firm conclusions.

Background and Justification:
Zinnias are a very important crop for field cut flower growers. Unfortunately the plants often devlop seed-borne disease problems during transplant production. The diseases are a problem not only for the bedding plant producer but also for the cut flower grower. An effective seed treatment would be very beneficial.

Laboratory testing of zinnia seeds for the presence of bacterial and fungal pathogens with and without treatments to disinfest the seeds will let us know whether growers should consider these practices. Two important pathogens of zinnia, *Xanthomonas campestris* pv *zinniae* and *Alternaria zinniae* can be disseminated by seed.

In addition to better understanding of the sources of inoculum, the development of an IPM strategy for zinnia includes testing preventive treatments that might replace fungicides. Recent experiments with cotton seeds in Eric Nelson's laboratory showed that planting seeds in active composts can reduce disease incidence later in the field. If this method works with zinnia, it is a ready tool to reduce pesticide use and the associated labor costs. This study will include microbial treatments to suppress diseases, and comparison of standard soil mix and active compost on disease incidence. The appearance of leaf spot disease symptoms in the greenhouse and field were monitored, and several samples were sent to the Plant Disease Clinic for identification.

Objectives:

1) To compare disease incidence of zinnias from
    a. different seed sources and varieties
    b. seed treatments on zinnia seeds:
        hot water, chlorox, ZeroTol
    c. in the greenhouse and in the field
    d. plugs grown in regular potting mix and suppressive compost

2) To note the earliest appearance of disease symptoms; apply biorational or least toxic products as necessary: PlantShield, Insecticidal soap plus Latron B 1956

3) To observe greenhouse cultural practices to see if some changes are necessary- nitrogen too high? ventilation poor? sanitation adequate?

4) To evaluate the results

Procedures:

1) a and b. Zinnia seed treatments were conducted two times: at the IPM House kitchen with the seeds air dried and then sent to the Plant Diagnostic Clinic in Ithaca for testing**;** and in Margery Daughtrey's Riverhead, Long Island laboratory.

In Geneva, seeds from four different tall zinnias were treated, 50 seeds per treatment, with one replication. On Long Island, one bedding plant seed variety "Peter Pan Gold" was used. In both experiments, the treatments were soapy water, soapy water followed by Zero Tol, 52° C water for 20 minutes, and 20% Clorox for two minutes. In Long Island, there was an additional Clorox treatment for 2 hours.
At the Ithaca clinic, seeds were examined using acidified potato dextrose agar (medium for fungi) and on Tryptic soy agar (medium for bacteria). Five seeds were positioned on each agar plate; the plates were scored as percent of the total number of seeds exhibiting yellow bacterial colonies or fungi with the morphology characteristic of *Alternaria*.

In the Riverhead trial an uninoculated control, an inoculated control and 5 treatments were compared for their suppression of bacteria and fungi. Twenty-four hours prior to treatment, all but the uninoculated control seeds were sprayed with a suspension of *Xanthomonas zinniae* in mineral salt solution (50% transmittance @590nm in the spectrophotometer). The seeds were tossed to distribute inoculum, and air-dried overnight. The inoculated seeds were then moistened with water to which 1 t/gal Dawn Ultra Blue had been added before the treatments. The treatments were ZeroTol at 2% for one minute, Clorox at 20% for 2 minutes, Clorox at 20% for 2 hours, Hot water (52C) for 20 minutes and Dawn Ultra Blue for 2 minutes. 100 seeds of 'Peter Pan Gold' were given each treatment. Fifty seeds/treatment were placed on moist filter paper in plastic boxes and incubated at room temperature in the dark to check germination. 50 seeds/treatment were divided among 10 potato dextrose agar plates to check suppression of bacteria (10 replications of 5 seeds/plate). The number of seeds from which bacteria or fungi grew was recorded 5 days after plating, and the results analyzed by Fisher's Protected LSD, P=0.05.

1) c and d. BioComp compost was purchased from Penn State Seed Co. for use at the two greenhouses in comparison with their standard peat/lite mixes. Microbial inoculants (RootShield, 1 oz/gal water/2 cu ft; Mycostop, 1 g/2.5 gal water; and SoilGard, 1 oz dry/2 cu ft soil) were mixed into the media prior to seed sowing or drenched after planting, as suggested on the labels. At Harrington's, Nellie Call's 22 flats of 72 transplants were grown: Benary Giants (10), Ruffles (6), Oklahoma (2), Cut and Come Again (2), and Envy (2). At the Taylor Greenhouse, Roxanne McCoy's 10 flats of 288 plants of Benary Giants were produced.

2) Karen Hall scouted the Taylor Greenhouse, and then observed the results at Roxanne McCoy's cut flower operation. Nellie Call scouted at Harrington's greenhouse, and also in her field of cut flowers. Observations were made during transplant production, at the time of field planting, and later at time to harvest flowers. Fungicides were not applied in either trial.

3) Cultural practices in the greenhouses were observed.

4) Photographs were taken in the greenhouse and field. Preliminary results will be used to justify a second IPM grant proposal that will include use of the seed treatments prior to seeding in the greenhouses. The next project will include cost effectiveness of the seed treatments and preventive microbials.

Results and Discussion

Results from seed treatment at the IPM house kitchen and the Plant Disease Clinic in Ithaca suggested that there was some contamination between seed batches, perhaps during treatment. In spite of this error factor, some conclusions can be drawn. The fungi counted as *Alternaria* were reduced only slightly by the hot water treatment, but the yellow bacterial colonies indicated as *Xanthomonas* were reduced considerably by both the Clorox and hot water treatments. The soapy water and ZeroTol treatments did not result in consistent decontamination.

Zinnia Seed Treatment Effects, IPM House, Geneva and Plant Disease Clinic, Ithaca

Zinnia seed variety	control - no treatment		Clorox 20%		52° C		soapy water		ZeroTol	
	% A	% X	% A	% X	% A	% X	% A	% X	% A	% X
Benary Giant	4	0	12	0	4	2	28	6	20	10
Envy	6	40	0	0	4	0	16	60	10	42
Cut and Come Again	6	46	22	0	2	8	0	0	21	23
Oklahoma	0	20	12	2	4	0	16*	4*	10	2

*average percent of seeds developing colonies; only one batch Oklahoma seeds in soapy water

The results from the Riverhead Lab with "Peter Pan Gold" included comparison with inoculated seeds. The 2-min Clorox treatment gave a statistical reduction in the number of seeds yielding bacteria, while the 2-hr Clorox treatment completely eliminated bacterial growth from the treated seeds. The bacteria were not identified, but morphology of many of the colonies was typical of *X. zinniae.* Most of the plated seeds showed growth of a light pink *Fusarium* sp. that did not appear to cause damping-off symptoms. The number of seeds yielding this fungus was reduced by the 2-hr Clorox treatment, and reduced to zero by the 20-min hot water treatment. ZeroTol, hot water and soapy water treatments had no effect on bacterial recovery from treated seed, while ZeroTol, 2-min Clorox and Dawn had no effect on fungal recovery in comparison to inoculated or uninoculated controls.

Zinnia Seed Treatment Effects, Riverhead Lab

	Percentage of Seeds Yielding [1]			
Treatments	Bacteria [2]		Fungi [3]	
Not Inoc	52	b [4]	96	c
Inoc	38	b	100	c
ZeroTol 2%	46	b	96	c
Clorox 20%, 2 min	16	a	98	c
Clorox 20%, 2hrs	0	a	32	b
Hot water, 20 min @52°C	94	c	0	a
Soapy water	42	b	98	c

[1] Values represent means of 10 replications of 5 seeds
[2] All bacteria (not identified to species)
[3] All fungi (predominantly Fusarium sp.)
[4] Values followed by the same letter are not significantly different (Fisher's Protected LSD, P=0.05).

Germination effects were not sufficiently replicated to allow statistical analysis, but 96% of the seeds germinated in the uninoculated controls, 88% in the inoculated controls, and 80% in the 2-hr Clorox treatment. Germination percentages for the other treatments were ZeroTol—96%, 2-min Clorox—90%, Hot water—83% and Dawn—90%. Based on these results, only the extended period of Clorox treatment holds promise for the elimination of bacteria from surface-contaminated zinnia seeds. Further testing will be needed to establish whether naturally-contaminated seeds could be reliably and safely disinfested with 2-hr treatments with sodium hypochlorite (the active ingredient in Clorox). To our knowledge, no sodium hypochlorite materials are currently registered for zinnia seed treatment.

In both seed treatment experiments Clorox was the best disinfectant for bacteria, and hot water was the best treatment for fungi. Of the tall zinnia varieties, seeds of Benary Giant were least infected with yellow bacteria (*Xanthomonas*?), and Oklahoma carried the least *Alternaria*-like fungi. Different approaches were taken in the assay for seed-borne microorganisms in Riverhead so the results are not directly comparable. About half of the Peter Pan Gold seeds (not inoculated) carried bacteria and almost all carried fungi. The germination of seeds was reduced by the effective seed treatments. Additional testing will be necessary to see whether the seed treatments would be cost-effective for growers, i. e., whether seed treatment increases quality of seedlings and field cut flowers substantially, and if that value is worth the time and trouble and loss in germination.

Leaf Spot Diseases in the Greenhouse and Field

At Harrington's Greenhouse, microbial treatments and seed planting were done 4/23-24. For the next four weeks, the quality of the seedlings were compared. Plants in compost were generally healthier, greener, and taller than plants in the standard peat/lite mix. By 5/21, diseases (*Botrytis* and leaf spots) were apparent in almost all treatments. Percentage of plants with leaf spots:

with leaf spots:	standard mix	67.3%
	compost	52.2%
	Ruffles	42.6%
	Benary Giants	61.1%

The only flats that didn't have sporulating *Botrytis* infections were Ruffles in the standard mix with Rootshield and SoilGard, and compost with Mycostop. These same flats did not have any tan centered round lesions, but they did have angular lesions. The Ruffles seedlings in standard mix with Mycostop did have *Botrytis*, but no angular lesions or tan-centered round lesions. It is too early to say that these effects are predictable, since actual placement on the bench and falling flowers from baskets overhead contribute substantially to disease incidence.

At transplanting 6/1-4, Nellie Call noted that the best plants were in compost with Mycostop and Root Shield. Benary Giants were taller and showed more Botrytis; the most leggy and moldy plants were discarded. In the field, the seedlings from high concentrations of leaf spots acted as focal points for disease spread. Powdery mildew appeared in August and September, and spread to the flowers. No fungicides were applied. Zinnia samples sent to the Plant Diagnostic clinic were infected with Alternaria (three samples), Botrytis, Fusarium, and Rhizoctonia. Xanthomonas was not isolated.

At Taylor Greenhouses, Karen Hall rated the seedlings the first and second weeks of May, finding that the seedlings in compost with Mycostop and the standard mix with RootShield were the best quality. She wrote that plants in compost tended to be better, healthier, and taller. Percentage of seedlings with leaf spots (Jana Lamboy rating 5/16 prior to transplanting):

standard mix	13.9%	compost	4.2%
standard mix + Mycostop	9.0%	compost + Mycostop	2.8%
standard mix + RootShield	13.9%	compost + RootShield	0.7%
standard mix + SoilGard	na	compost + SoilGard	4.2%

Karen monitored Roxanne's cut flower field in June and July. She reported that damage due to slugs, katydids, and tarnished plant bug reduced production, but that leaf spot diseases were not a major problem. During the cutting season, Roxanne McCoy observed that those plants in compost and RootShield did better than the others. Plants tended to grow away from the leaf spots on the lower leaves. She saw less damping off in zinnias in 2001 than in prior years.

Cultural Practices
The main comment from Art Harrington was that the compost dried out more frequently than their standard mix, causing them inconvenience and overwatering of the plants in the standard mix. Potential conditions that might lead to greater disease include high humidity, crowding as seedlings grow larger, baskets suspended over the benches, watering late in the day, over or under fertilization, and presence of other crops in the greenhouse with leaf spot diseases.

Evaluation
This project was a collaborative effort that began as a request from a cut flower grower, Nellie Call. Two laboratories and associated diagnosticians, two greenhouse operations, two cut flower growers, one Cooperative Extension Educator, and one IPM Coordinator worked together closely to accomplish the work described at five New York sites.

Zinnia seed analysis and seed treatments at two sites confirmed the presence of bacteria and fungi, and the efficacy of 20% Clorox and hot water. The first year's results suggested that all zinnia seeds are likely to carry fungi and bacteria, but that Ruffles and Benary Giants seeds were cleaner than Cut and Come Again or Envy.
Reduction in germination rate should be expected when using 20% Clorox or hot water seed treatments. The 2002 trial will be designed to test efficacy of seed treatments and germination rates in the seedling trays instead of on moist paper.

The plants that spent more time in the greenhouse (Harrington's) suffered more from disease (and legginess). This may suggest that the seeds should be planted later, so they would be younger at time of hardening off and transplanting. The plants in compost thrived; the biological control organisms in Mycostop and RootShield treatments appear to suppress diseases. We will simplify the soil mix/microbial inoculants trial by using only compost and eliminating SoilGard. Subsequently we will continue to monitor the seed treatments and microbials in the cutting fields.

The specificity of pathogen identification should be increased in future studies both in seed testing and in leaf lesions. For example, isolated yellow bacterial colonies should be tested by antibody methods or DNA analysis to see if they are *Xanthomonas*, and/or they should be tested according to Koch's postulates to ascertain that they are pathogenic on zinnia. More testing of field samples and close-up photography will be necessary to develop a simple field key, if possible, for discrimination between *Xanthomonas, Alternaria, Cercospora, Botrytis*, and other common Zinnia pathogens in the field. We didn't collect any identified *Xanthomonas* saqmples from the field, but yellow bacterial colonies were isolated from seeds.

These results of this project provided preliminary data for an IPM strategy for zinnia leaf spot diseases that could benefit almost all of the cut flower and bedding plant growers in New York and the Northeast. We need to repeat the demonstration to validate the results, and determine cost-effectiveness of seed treatments and microbials. Eventually, our IPM strategy for zinnia will improve profitability for growers by increasing crop quality while reducing the number of pesticide applications.

References

R. K. Horst, 1979. Westcott's Plant Disease Handbook, 4th Edition. Van Nostrand Reinhold.

H. Dillard, 1999. New York State Vegetable Project Reports Relating to IPM, 1999
Also see: Managing bacterial diseases of pepper and tomato.
http://www.nysipm.cornell.edu/publications/bacterial_dse.html

D. L. Stider. 1979. Methods to eradicate *Xanthomonas* on zinnia seed. Plant Disease Reporter 63:873-876.