Multicomponent Preplanting Dips for Easter Lily Bulbs

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ABSTRACT

Bald, J. G., Lenz, J. V., Paulus, A. O., and Bricker, J. L. 1982. Multicomponent preplanting dips for Easter lily bulbs. Plant Disease 66:399-401.

Yellow discoloration of Easter lily bulbs is caused by surface invasion of the outer bulb scales by mild strains of *Fusarium oxysporum*, and basal rot is a destructive disease caused by severe strains of the same organism. These and other *Fusarium* symptoms were checked by a preplanting dip containing PCNB and ferbam. In field trials with Easter lily cultivar Ace, the two materials were found to be more effective in combination than either one was alone. Adding thymol to the dip as a bactericide helped control stem lesions, which were attributed to the combined action of *F. oxysporum* and *Pseudomonas gladioli*, and improved root condition. Benomyl was tested as an additional or alternative fungicide; as a substitute for ferbam, benomyl needs further testing.

The standard preplanting bulb dip for field-grown Easter lilies is a mixture of PCNB and ferbam (6). The disease complex that the dip is designed to control is a bulb and root rot combined with stem lesions. The disease complex is caused primarily by clones of *Fusarium oxysporum*, varying in pathogenicity from mild to severe, combined with a bacterium, *Pseudomonas gladioli* (syn. *P. marginata*). The bacterium is indistinguishable from those causing scab disease of gladiolus and scorch disease of rhizomatous iris (4). Fungi in addition to *Fusarium* that have been found occasionally as part of the disease complex are *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani*.

Easter lily bulbs are white when completely healthy. Mild strains of the lily *Fusarium* cause surface yellowing of the outer scales. Severe strains cause lesions, but in the presence of milder strains, they seem able to invade the underground tissues of lily plants without producing visible damage. Yellowing of Easter lily bulbs nearly always indicates the presence of *Fusarium* strains that are capable of damaging roots, bulbs, and stems of the lily plants. Mild strains may act in association with *Pseudomonas* (8), and severe strains may act with or without the bacterium. The degree of yellowing on the outer scales of bulbs is a convenient indicator of possible disease in subsequent crops.

Two questions have been asked about PCNB-ferbam as a dip for lily bulbs: Are both components necessary for its effective action?, and Should any other fungicide or a bactericide be added or substituted to make the dip more effective? Of several fungicides tested, none was found that could usefully modify the standard mixture, except possibly benomyl. Benomyl has been used alone, but several growers now add it to PCNB-ferbam, making the dip a three-component mixture. We tested the possibility of improving the standard dip in field trials in 1978–1980 and in laboratory and greenhouse experiments.

Accepted for publication 14 August 1981.

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0191-2917/82/05039903/$03.00/0

MATERIALS AND METHODS

Chemicals and concentrations tested as preplanting dips on Ace Easter lilies were PCNB, ferbam, benomyl, and thymol at 5.4, 2.7, 1.8, and 0.9 lb per 100 gal, respectively. The field trials were set out in balanced incomplete blocks, but because the complex design added little to the accuracy of the results, most data were analyzed as ordinary randomized blocks. Each trial had five treatments and was replicated five times. Thirty bulbs of yearling size were planted in each plot. The trials were situated in commercial fields and were cultivated as part of those fields until harvest.

A greenhouse dipping trial consisted of 36 bulbs, six for each treatment, planted in 6-in. containers of pasteurized UC mix (3). The containers were held at 10 C in a soil temperature tank and were arranged in a Latin square. Three plants from each treatment were examined during the experiment and three at the end of the experiment. Sixty plants in a parallel bench experiment were exposed to fluctuating greenhouse temperatures (mean about 23 C). They were harvested at irregular intervals to check observations made on the plants in the temperature tank.

The yellow discoloration of bulbs was estimated on a 5-point scale. The lowest number, 1, indicated that the outer surfaces of the bulbs were completely yellow; 5 indicated that they were completely white; 2, 3, and 4 indicated decreasing levels of yellow discoloration.

Height of individual plants was measured at intervals of days or weeks in greenhouse trials. In the field, height measurements were generally taken once at maturity. Four height measurements were taken at random positions in each plot and averaged.

Both preharvest and postharvest data were used to construct an index of growth and yield. Postharvest weights of bulbs plus bulblets and of foliage were measured in kilograms. The mean heights of plants at maturity in each plot were used as the preharvest component. To bring the two types of measurements into physical and statistical conformity, the height measurements were cubed and multiplied by a constant factor that brought the absolute values to about the same numerical level as the bulb yields in kilograms. Values for these indicators were added to form a composite index of growth and yield for each plot.

RESULTS

Preliminary tests with thymol. To test the toxicity of thymol to *Pseudomonas*, we added 1% of a 10% solution of thymol in ethyl alcohol, 1:1,000 thymol, to liquid and solid culture media, which were then inoculated with high concentrations of bacteria. Thymol prevented growth of *Pseudomonas*. It was bactericidal at this concentration. Tested against the lily *Fusarium*, it failed to stop growth on potato-dextrose agar (PDA).

Toxicity to Ace lily bulb tissue was tested with 1:1,000 thymol in water plus 1% alcohol, made as above. This gave a saturated solution with some thymol in suspension. Apparently healthy bulb scales were soaked for periods from 2 or 3 min to 24 hr and transferred to petri dishes in a moist atmosphere. A few brown lesions appeared, mainly on older scales, but scales soaked for the longer periods had fewer lesions. A number of cultured tissue pieces from lesions were sterile. The lesions may have been associated with small crystals of thymol adsorbed by the surfaces of bulb scales. Dipped scales produced more adventitious bulblets faster than untreated checks. Similar tests of thymol incorporated in PCNB-ferbam dipping suspension, in which the thymol seemed to be fully dispersed, gave insufficient evidence of toxicity to delay testing on a larger scale.

Bulb discoloration. Table 1 shows the level of discoloration of Ace lily bulbs after harvesting the two field trials in 1978–1979 and 1979–1980. Data for the two trials were listed together because the results were consistent; and in two greenhouse trials, similar responses were noted. Singly, PCNB and thymol failed to reduce yellowing (Table 1). Ferbam caused a significant reduction to about half yellow, half white. Any combination that included both PCNB and ferbam drastically reduced yellowing, leaving the surfaces of bulbs white or nearly white.

Benomyl, by itself or combined with thymol, reduced yellowing in the 1978–1979 trial to about the same degree as ferbam in the 1979–1980 trial. Unfortunately, the combination PCNB-benomyl was not included in either trial. In general, action of the dips on discoloration was associated with the use of fungicides, not the bactericide thymol, in agreement with previous findings (9).

Fusarium basal rot. The Ace bulbs for the greenhouse trial at 10 C soil temperature were discards from a commercial stock, and Fusarium basal rot (10) was expected among them. Bulbs were distributed at random among treatments. The treatments and numbers of plants affected by severe basal rot were as follows: control (C), 1; thymol (T), 2; benomyl (B), 1; benomyl-thymol (BT), 1; PCNB-ferbam (PF), 0; and PCNB-ferbam-thymol (PFT), 2. The C and T treatment bulbs neither sprouted nor produced roots; they rotted in the soil. The B and BT treatment bulbs with basal rot produced spindly shoots with short leaves and a few weak roots. Their weak and spindly growth distinguished them from normal plants soon after emergence.

The two PFT-treated plants with basal rot appeared healthy; the rot became evident only from direct examination of the bulbs. In one of the two bulbs, the rot involved two-thirds of the basal plate; from the other third, a white and vigorous root system with only a few small lesions developed. The second bulb had a large basal rot lesion that had been similarly checked. Although the numbers of plants were small, the extreme and consistent differences among treatments left no doubt that they were significant. Action of the dips on yellowing and basal rot was representative of their action on lesions of intermediate severity.

Stem lesions. In northern California during 1978–1979, lesions were present on Easter lily stems but were not very damaging. In the field trial of that season, the proportion of stems with lesions ranged from 60% (no preplanting dip) to 25% (PFT) (Table 2). Treatments fell into three pairs with significantly different percentages of stems with lesions: C (no treatment) and T averaged 58%; B and PF averaged 43%; and BT and PFT averaged 29%. Thymol alone had no effect, but coupled with fungicides, it contributed to the reduction of stem lesions (7).

There were indications, also, that thymol affected root growth. Production of roots generally precedes emergence of shoots above ground, and early root growth seems to be linked with rapid emergence. In the 1979–1980 field trial, emergence was most rapid after treatments including thymol.

Early in the season of the 1979–1980 field trial, the weather was exceptionally warm and dry, and few stem lesions appeared on Easter lilies except in the field where the dipping trial was planted. Unlike other fields of lilies on the same farm, this field was previously in pasture and had never grown lilies before. Yet two-thirds of the lily stems from the trial had lesions, mostly shallow depressions less than 1 cm long walled off by periderm. The only significant differences in incidence were between different areas of soil. Culturing from samples of stems yielded *Fusarium* and *Pseudomonas* but no other likely pathogen. The chances were that infection causing most of the lesions came from the soil. Carry-over of soilborne diseases (as distinct from animal parasites) from pasture to lilies has not previously been seriously considered but may be worth examining.

Effects of thymol on roots and senescing bulb scales. In the greenhouse trials, root systems of the Ace lilies were washed out carefully so that little damage occurred. Dips containing thymol reduced the number of root lesions more than corresponding dips without thymol and gave whiter, larger root systems with more copious lateral branching. Because of thymol's bactericidal action and low toxicity to *Fusarium*, these results suggested the presence of *Pseudomonas* as well as *Fusarium* in root lesions.

A root symptom that was increasingly likely to appear as a lily plant aged was premature collapse of the softer tissues inside the intact epidermis. A similar premature rot of senescing outer bulb scales often happened when these scales were losing their contents to developing shoots and buds. Preplanting dips, particularly those containing thymol, helped prevent the premature rot on both roots and outer bulb scales, allowing the outer scales to empty without rotting.

Culturing for pathogens. In the greenhouse trial at 10 C soil temperature, culturing was confined to tissue from control plants and from plants treated with combination dips containing thymol, particularly PFT. Cultures were made from tissue of outer bulb scales and from root lesions. Surface-sterilized pieces of tissue were plated on Nash and Snyder's agar (11), which is selective for *Fusarium*, and on PDA.

Results were first recorded 3 days after plating and were checked at intervals thereafter. All 13 tissue pieces from controls plated on Nash-Snyder agar and 75% of pieces on PDA yielded *Fusarium*. Percentages for dipped scales were 27 on Nash-Snyder agar and zero on PDA. *Pseudomonas* was present among the bacteria isolated, but quantitative values were not obtained.

Cylindrocarpon radicicola was isolated from treated plants of this trial and appeared on the same plates as bacteria with the colony characters of *Pseudomonas*. The two organisms showed evidence of mutual antagonism in vitro, comparable with the antagonism in Croft lily bulb tissue inoculated with both organisms (8).

Growth in height. In the greenhouse dipping trial at 10 C soil temperature, height measurements of the shoots were taken periodically as indicators of growth. Within any one treatment, except benomyl applied singly, growth rates of individual plants varied little. Benomyl-treated plants germinated either very early or late, and growth was less regular. This irregularity has not been noticed in field trials or commercial plantings where the bulbs were treated with benomyl.

Table 1. Color ratings of Easter lily bulbs (cultivar Ace) harvested from two field trials of preplanting bulb dips

Treatment[a]	Trial[b]	Rating[c]
C	1	2.4
T	1	2.5
P	2	2.6
F	2	3.3
B	1	3.0
BT	1	3.2
PF	1	4.95
	2	4.4
PFT	1	4.9
	2	4.95
PFB	2	4.9
PFBT	2	5.0

[a] C = control (dipped in water), P = PCNB, F = ferbam, B = benomyl, and T = thymol.

[b] Trial 1 was conducted in 1978–1979, trial 2 in 1979–1980.

[c] Rating 5 indicated that outer scales of bulbs were completely white, rating 1 completely yellow. The least significant difference in trial 1 was 0.42 ($P < 0.05$) and in 2 trial was 0.37 ($P < 0.05$).

Table 2. Percentages of Easter lily stems (cultivar Ace) with lesions in the 1978–1979 field trial of preplanting dips

	Stems with lesions	
Treatment[a]	Percent	$\sqrt{\text{Percent}}$[b]
C	59.5	7.62
T	56.2	7.40
B	39.6	6.20
BT	32.6	5.66
PF	45.6	6.70
PFT	25.0	4.78

[a] C = control, T = thymol, B = benomyl, P = PCNB, and F = ferbam.

[b] Percentages were transformed to square roots for statistical analysis. The least significant difference was 1.32 ($P < 0.05$).

The date of emergence of each plant from the soil was taken as zero time, and height was plotted against time in days after emergence. For the first 25 days, plants from all treatments except benomyl grew similarly (Table 3). During the 25- to 75-day period, plants from treatments C (no dip), T, and BT grew at the same rate and reached roughly the same height at maturity. The growth rate for benomyl was not significantly different. Plants from treatments PF and PFT grew faster during this period than those from other treatments and reached a greater maximum height.

Yield and growth index. In the 1979–1980 field trial, total yields of bulbs plus bulblets for the combination dips were higher than those for PCNB and ferbam applied singly (Table 4). All combination dips contained both PCNB and ferbam. There was no clear statistical evidence that total yield was further increased by adding benomyl or thymol or both to the PCNB-ferbam; however, almost significant interactions suggested that adding thymol to the PF or PFB treatment encouraged production of more bulblets rather than more bulbs. PF and PFB treatments seemed more likely to increase the yield of bulbs than of bulblets.

If any one treatment stood above the others throughout the trial it was PFBT, yet the difference from other combination dips in total yield was not significant. To estimate overall productivity, we combined measures of foliar growth for each plot with yield of bulbs plus bulblets (total yield) in a composite value that served as an index of growth and yield. The index mean for PFBT, 6.02, was significantly higher than the PF mean, 5.55. Adding benomyl or thymol to the PF treatment gave only an insignificant increase in the index. The sum of these two increases, 0.13, was well below the least significant difference, 0.29. The combination PFBT, like the combination PF, may well be an example of potency enhanced by combined action (synergism).

As might be expected, the combination dips PFB, PFT, and possibly PF gave higher index values, as well as higher yields, than PCNB or ferbam used singly. The one disappointing feature of these current-season gains was that they were not channeled, as the grower would wish, directly into the production of more or larger bulbs. However, experience suggests that increases in yield and quality of bulbs in subsequent seasons might be substantial.

Table 3. Height of Easter lily plants (cultivar Ace) in a greenhouse trial of preplanting dips at a soil temperature of 10 C in 1978

Treatments[a]	Mean height[b] (cm) Day 25	Day 65[c]	Increase in height (cm)
C, T	21.0	55.1	34.1
BT	22.0	55.3	33.3
B	12.3	42.6	30.3
PF, PFT	19.5	63.5	44.0

[a] C = control, T = thymol, B = benomyl, P = PCNB, and F = ferbam.
[b] Calculated from linear regressions of height in centimeters on time in days after germination.
[c] The 65-day estimate of height was given because growth in height systematically fell slightly below the straight-line course between days 65 and 75.

Table 4. Mean plot yields[a] and growth index of treated Easter lily bulbs in a field trial, 1979–1980

Treatment[b]	Bulbs (kg)	Bulblets (kg)	Total yield (kg)	Growth index
P	3.62	0.78	4.40	5.27
F	3.61	0.98	4.59	5.27
PF	3.79	1.07	4.86	5.55
PFB	3.90	0.96	4.86	5.78
PFT	3.66	1.13	4.79	5.73
PFBT	3.72	1.29	5.01	6.02
LSD[c]	0.225	0.265	0.246	0.291

[a] Thirty bulbs per plot were dipped in one of six preplanting dips, planted, and grown to maturity.
[b] P = PCNB, F = ferbam, B = benomyl, and T = thymol.
[c] LSD = least significant difference.

DISCUSSION

Both PCNB and ferbam contributed to the effectiveness of the current preplanting dip for Easter lily bulbs. Their action appeared to be synergistic, although a more rigorous examination would be necessary before synergism could be claimed. In the presence of PCNB and ferbam, benomyl and thymol also seemed to act in a synergistic fashion. The complexity of these reactions matched the complexities of the disease, the host anatomy, and the pattern of growth (9). The presence of vesicular-arbuscular mycorrhizae on Easter lilies may add to these complexities (1).

The PCNB-ferbam mixture might be made more effective by the addition of benomyl, or benomyl and thymol, but the evidence for this is insufficient. The combination of PCNB and benomyl still needs to be evaluated. The usefulness of thymol as a bactericide added to fungicides in the preplanting dip cannot be properly determined until it is tested under field conditions favoring *Pseudomonas* and its interaction with *Fusarium*. Meanwhile, the available evidence suggests that thymol may be a useful bactericide for *Pseudomonas*.

The protection against Fusarium basal rot given by PCNB-ferbam (plus thymol) at a soil temperature of 10 C is interesting because this temperature is below the range at which an effective barrier of periderm normally forms in storage tissue at the edge of a lesion (2). Only PCNB-ferbam controlled these lesions. In the field, slow growth of *F. oxysporum* at low temperatures and competition between mild and severe clones of the pathogen (5) may be reasons why Fusarium basal rot of Easter lilies is not more frequent and damaging.

Our data raise the general question of host reaction to invasion and when protection is most needed, not only against *Fusarium* but also against *Pseudomonas*. Damaging stem lesions and probably some root lesions, caused by the combined action of the two pathogens, are likely to be encouraged by cold, wet weather during December, January, and February, when Easter lilies are rooting, sending up shoots, and emerging above ground. Therefore, persistence of fungicidal materials on the bulb and between the bulb scales over a period of 4 or 5 mo after planting is a consideration. Also, protection of the bulb scales next to the external scales is needed when the latter are being depleted of stored nutrients for the development of the flower head. PCNB-ferbam seems to satisfy these requirements. Deposits from the preplanting dip are visible between the bulb scales after harvest. The low solubility of thymol in water may help its persistence in a similar way, particularly if the thymol is thoroughly mixed with the insoluble deposits of PCNB and ferbam on the bulb.

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