VG226
Control of lettuce downey mildew

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South Australian Primary Industries

Know-how for Horticulture™

VG226

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FINAL REPORT VG 226
Control of Lettuce Downy Mildew

1. SUMMARY

a) Industry

Downy mildew is a major problem of lettuce, particularly where there has been a breakdown of cultivar resistance or where fungicides fail due to the development of fungal strains insensitive to fungicides. In South Australia, surveys showed resistance to Ridomil and related fungicides was widespread in lettuce crops and alternative fungicides needed to be developed.

Ridomil has been formulated with mancozeb and applied to lettuce crops since 1979, and although the mixture did not prevent Ridomil resistant strains from developing, it still provided effective disease control.

Ridomil resistant strains of the downy mildew fungus were also controlled with two new fungicides dimethomorph and phosphonic acid.

Downy mildew was found wide spread in nurseries and it is likely that the fungicide resistant strains of the fungus were spread with the distribution of transplants. Because of this drenches or foliar applications of phosphonic acid are recommended in nursery production.

b) Technical

Strains of *Bremia lactucae* insensitive to 0.01g a.i. L metalaxyl have been detected in all lettuce growing districts of South Australia. Despite this, applications of a formulated mixture of 0.25g a.i. L^{-1} metalaxyl and 2g a.i. L^{-1} mancozeb every 7 to 10 days controlled downy mildew in the field where metalaxyl or mancozeb alone were ineffective. Protectant schedule of 0.3g a.i. L^{-1} dimethomorph or 2 to 2.4g a.i. L^{-1} phosphonic acid also controlled downy mildew.

Fungicide applications increased marketable yield by 60% compared to that of unsprayed plants.

Drenching seedlings with 4g a.i. L^{-1} phosphonic acid controlled downy mildew for at least 14 days and should prevent the spread of disease from plant production areas.

2. RECOMMENDATIONS

a) Apply 2.4g a.i. L^{-1} phosphonic acid as a foliar spray or drench to seedling transplants to prevent the spread of Ridomil resistant strains of downy mildew.

Apply 2.4g a.i. L^{-1} phosphonic acid, 0.3g a.i. L^{-1} dimethomorph or a mixture of 0.25g a.i. L^{-1} metalaxyl at 2g a.i. L^{-1} mancozeb every 7 to 10 days to control downy mildew.

b) **Directions of future research**

1. Epidemiological studies are required to define conditions required for infection and disease development. Knowing this fungicide sprays may be applied strategically thus reducing the number if applications needed for control.

2. Dose responses to newly developed fungicides need to be determined to monitor shifts in fungicide sensitivity.

3. Spray application - ground vs aerial and critical spray volumes per ha need to be evaluated.

4. Pesticide residues on lettuce crops needs to be determined more accurately and decay curves established on different cultivars.

5. Resistant cultivars need to be evaluated for resistance/tolerance to other diseases (eg Anthracnose, corky root etc).

6. Effect of soil fumigation on the over wintering stages of the fungus needs to be determined.

c) **Financial/Commercial Benefits**

Improved diseases control - improve marketable yields by at least 60% in some cases.

3. <u>TECHNICAL REPORT</u>

- Paper accepted by Crop Protection is attached with other associated published reports.

Fungicidal control of metalaxyl insensitive strains of *Bremia lactucae* on lettuce.

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KEYWORDS: Lettuce, downy mildew, dimethomorph, phosphonic acid and metalaxyl insensitivity.

RUNNING TITLE: Lettuce downy mildew control

Abstract Strains of *B. lactucae* insensitive to 0.01 g a.i. L^{-1} metalaxyl have been detected in all the lettuce growing districts of South Australia. Despite this, applications of a formulated mixture of 0.25 g a.i. L^{-1} metalaxyl and 2 g a.i. L^{-1} mancozeb every 7 to 10 days controlled downy mildew in field experiments, where similar applications of either metalaxyl or mancozeb alone were ineffective. Protectant schedules of either 0.3 g a.i. L^{-1} dimethomorph or 2 to 2.4 g a.i. L^{-1} phosphonic acid also controlled downy mildew, but were less effective than the metalaxyl/mancozeb formulation. Mixtures of either dimethomorph or phosphonic acid with mancozeb enabled the time between the sprays to be increased to 14 days without significantly reducing control.

Fungicide applications increased marketable yield by 60% compared to that of the unsprayed plants.

Drenching seedlings with 4 g a.i. L^{-1} phosphonic acid controlled downy mildew for at least 14 days and should prevent the spread of the disease from plant production areas.

Introduction

Downy mildew caused by *Bremia lactucae* Regel is a major disease of glasshouse and field grown lettuce crops throughout the world (Crute and Dixon, 1981). The disease is controlled by the use of cultivars resistant to downy mildew and the frequent application of fungicides. However breakdown of cultivar resistance has occurred and in some areas fungicides fail to control the disease due to the development of fungal strains insensitive to fungicides (Crute, 1987; Schettini *et al*, 1991).

Studies on this disease were undertaken in Australia as lettuce cultivars resistant to the prevalent races of *B. lactucae* are not widely grown and insensitivity to the acylalanine fungicides has recently been detected (Wicks *et al.*, 1993).

The present studies were aimed at determining the extent of insensitivity in *B. lactucae* present in South Australian lettuce crops, to establish base line levels of sensitivity of *B. lactucae* isolates to alternative fungicides, and to extend the field evaluation of fungicides and application schedules for the control of downy mildew.

Materials and methods

Laboratory Studies

An excised cotyledon technique was used to determine the dose response of *B. lactucae* to fungicides and to evaluate the fungicide sensitivity of field isolates.

Lettuce seeds were planted 3 per cell in seedling trays containing a mix of peat and sand (2:1). Preliminary tests showed that the Yates cv Cos Verdi was the most suitable of several lettuce cultivars evaluated as this cultivar produced large cotyledons susceptible to infection by downy mildew 14 - 18 days after planting . The cotyledons of Cos Verdi from another company were less susceptible to infection.

Preliminary tests showed phosphonic acid was best applied by dipping the plants in the fungicide solution and allowing these to dry without the solution wetting the soil. Cotyledons were removed approximately 24 hours after the application of the fungicide. Other fungicides were applied with a small hand held misting sprayer using approximately 0.1 ml $plant^{-1}$. After drying for 3-4 hours, at least 25 cotyledons were removed from each treatment and inoculated immediately. Cotyledons placed abaxial surface up on germination paper overlying towelling moistened with a nutrient solution were held in glass petri dishes and inoculated with a 0.04 ml drop of spore suspension. Spore suspensions were prepared by shaking freshly sporulating leaves in chilled water for 1 minute to obtain a solution containing 1×10^4 spores ml^{-1}. Cotyledons were then incubated at 15-17°C for 7-10 days on a 12 hour light/dark cycle with the first period dark.

After incubation, sporulation was assessed using a 0-4 scale, where 0 = no sporulation, 1 = 1 - 24%, 2 = 25 to 49% 3 = 50 to 74%, and 4 = 75 to 100% of leaf area sporulating. A severity rating was then calculated using the formula:

$$\frac{1*(c\ in\ rating\ 1) + 2*(c\ in\ rating\ 2) + 3*(c\ in\ rating\ 3) + 4*(c\ in\ rating\ 4)}{total\ no.\ of\ cotyledons\ assessed} \quad \frac{*100}{n}$$

where c = number of cotyledons and n = total number of ratings. The severity rating of any fungicide treated cotyledons was expressed as percent inhibition compared to the value for the untreated control. Thus 100% inhibition indicated no sporulation and 0 % inhibition indicated sporulation equal to or greater than that of the control.

Fungicide sensitivity. The sensitivity of 3 isolates of *B. lactucae* (one sensitive to metalaxyl and the other two of unknown sensitivity) was assessed over a range of concentrations of metalaxyl (methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL - alaninate), dimethomorph ((E,Z). 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl] morpholine) and phosphonic acid (H_3PO_3, a solution of 20% phosphonic acid neutralised with an equal amount of KOH). All tests were repeated at least 3 times using excised cotyledons. Sensitivity was tested using a minimum of 10 concentrations of the fungicides, metalaxyl or dimethomorph between 1×10^{-5} and 0.5 g a.i L^{-1} and phosphonic acid between 1×10^{-4} and 5 g ai L^{-1}.

Insensitivity survey. Isolates of *B.lactucae* were collected from the main lettuce growing areas of South Australia which are all situated within 100 km of Adelaide. Between 10 to 20 infected leaves were collected from each of twelve properties where poor fungicide control had been reported (Table 1).

To induce sporulation, infected leaves were first washed, then dried by blotting on a paper towel, sealed in a plastic bag with a wet paper towel to create high humidity and incubated overnight at 15-17°C in the dark. A wet paint brush was used to remove spores from the leaves and place to them into chilled water (4-10°C). The suspension was then sonicated for 30 seconds to separate the spore mass. Because sporulation on these leaves collected from the field was often sparse, inoculum was multiplied by inoculating untreated excised cotyledons as previously described.

The fresh spores were collected by shaking the sporulating cotyledons in chilled water for one minute. For each test the concentration of the spore suspensions from the different isolates were adjusted to 1×10^4 spores ml^{-1}, either by adding water or shaking more sporulating cotyledons in the suspension. The spore suspensions were then used to inoculate excised cotyledons sprayed with either 0.1, 1 or 10 mg a.i. L^{-1} metalaxyl. A strain of *B. lactucae* sensitive to metalaxyl was included in each test for comparison.

Glasshouse studies

Fungicide evaluation. Lettuce plants cv Cos Verdi (Yates) were grown to the 2 leaf stage in a mix of peat and sand (1:1). Fungicides were either applied as foliar sprays using a hand held misting sprayer, or as a drench by pouring 8 ml of the fungicide evenly onto the soil surface around the plant. Plants were inoculated either before or after the application of fungicides using a spore suspension of *B. lactucae* insensitive to metalaxyl. Leaves were sprayed with an aerosol chromatography sprayer (supplied by Sigma Chemical Co. USA) using approximately 0.2 ml plant^{-1} of spore suspension containing 10^4 spores ml^{-1}. The plants were sealed in plastic bags moistened with water to provide 100% humidity and incubated overnight in the dark at 15-17°C. The plants were removed from the bags and kept at 15-25°C in a glasshouse for 10 to 14 days before they were placed overnight in a misting unit providing 100% humidity to induce sporulation. A 0-4 rating system was used to assess sporulation where 0 = no sporulation, 1 = <50% of 1 leaf sporulating, 2 = 1 leaf fully sporulating, 3 = both leaves partially sporulating, 4 = both leaves fully sporulating.

Experiment 1. Phosphonic acid was evaluated as a soil drench using either 1.2, 2, 5, 10 or 20 g a.i. L^{-1} applied to each plant. The plants were inoculated 3 days after drenching and assessed 10 days later.

Experiment 2. A soil drench of 4 g a.i. L^{-1} phosphonic acid was compared to foliar sprays of 2.4 g a.i. L^{-1} phosphonic acid, 0.25 g a.i. L^{-1} metalaxyl and 0.375 g a.i. L^{-1} dimethomorph. Plants were treated 1, 4, 8 and 14 days before inoculation and assessed 14 days later.

Experiment 3, 4, 5. These experiments evaluated the eradicant properties of three fungicides. In experiment 3, plants were treated 3 days after inoculation with either 0.25 g a.i. L^{-1} metalaxyl, 0.375 g a.i. L^{-1} dimethomorph or 1.2 and 2.4 g a.i. L^{-1} phosphonic acid. Experiment 4 assessed the effect of 0.25 g a.i. L^{-1} metalaxyl, 0.75 g a.i. L^{-1} dimethomorph, and 2.4 g a.i. L^{-1} phosphonic acid applied as foliar sprays 1, 3 and 6 days after infection and a soil drench of 4 g a.i. L^{-1} phosphonic acid applied 1 and 3 days after infection. In experiment 5, plants were treated 1, 2, 3 and 5 days after inoculation with foliar sprays of either 0.25 g a.i. L^{-1} metalaxyl, 0.375 g a.i. L^{-1} dimethomorph or 2.4 g a.i. L^{-1} phosphonic acid.

Field evaluation

General methods. Three field experiments were conducted during 1992, two on commercial properties in the lettuce growing district of Virginia 30 km north of Adelaide and one at the Lenswood Horticultural Centre 30 km east of Adelaide. Lettuce cv Supergreen was used in experiment 1 and cv El Toro in all other plantings. Seedlings were planted mechanically on commercial properties or by hand on the Horticultural Centre in beds of 4 rows, each row 45 cm apart and plants 30 cm apart in the row. Fungicide programs were started within 8 days of planting and applied with a knapsack sprayer using 800 L ha^{-1}.

Debris infected with downy mildew was collected from naturally infected plants and scattered over the trial area to ensure an even development of disease. Operations such as weed control, irrigation and fertilizer applications were either carried out by the grower or were similar to commercial practices.

At all sites plots of at least 50 plants were replicated 5 times and arranged in a randomised block design. Twenty plants from the centre of each plot were assessed for incidence and severity of disease on 3 or more occasions after planting and at harvest. Downy mildew severity was assessed using a 0-5 scale similar to that described by Dixon *et al* (1973) where 1 = 1 to 5, 2 = 6 to 10, 3 = 11 to 25, 4 = 26 to 50 and 5 = >51% of the basal area infected with *B. lactucae*.

Fungicides and formulations used in these experiments were metalaxyl (Ridomil 25% WP); metalaxyl (8% WP) and mancozeb (manganese ethylenebis (dithiocarbamate), 64% WP) formulated as Ridomil MZ; dimethomorph (15% emulsified concentrate EC); phosphonic acid (Foli-R-Fos, 20%); a mixture of metalaxyl (30% WP) and dimethomorph (40% EC); mancozeb (Dithane M45 80% WP); and a formulation of dimethomorph (9%) and mancozeb (60%) (Acrobat). The rates chosen were based on manufacturers recommendations or were similar to those reported to control diseases in other crops.

Prochloraz (N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide) was applied at 0.23 g a.i. L^{-1} on up to 6 occasions to control *Microdochium panattonianum* (Berl.) Sutton Galea in the Virginia experiments, and on 3 occasions at the Lenswood site.

All data was analysed using analysis of variance of a randomised block design in the statistical package "Statistix" (NH Analytical Software - 1958 Eldridge Ave, Roseville MN-USA).

In experiments 1, 2 and 3 the efficacy of various fungicides, fungicide rates and formulations were compared whereas in experiment 4, fungicides were compared on either 7 or 14 days or eradicant schedules.

Experiment 1. Virginia. Dimethomorph at either 0.3375 g, 0.2475 g or 0.195 g a.i. L^{-1} was compared with 0.25 g a.i. L^{-1} metalaxyl, 0.25 g a.i. L^{-1} metalaxyl + 2 g a.i. L^{-1} mancozeb (Ridomil MZ), and 2 rates of the dimethomorph + metalaxyl combination, 0.3375 g + 0.25 g a.i. L^{-1} and 0.2475 g + 0.1875 g a.i. L^{-1}. A total of 12 sprays were applied on a 7 to 10 day schedule from four days after planting (23 July).

Experiment 2. Virginia. Lettuce seedlings were planted 30 July and sprayed a total of 9 times from 6 days after planting. Fungicide treatments shown in Table 3 were applied on a 7 to 10 day schedule. Dimethomorph at 0.3375 g a.i. L^{-1} was used as it was the most effective of the three rates of dimethomorph in experiment 1. As the two rates of dimethomorph + metalaxyl in experiment 1 were equally effective, a lower rate (0.195 g a.i. $^{-1}$L metalaxyl + 0.15 g a.i. L^{-1} dimethomorph) was compared with the high rate (0.3375 g + 0.25 g a.i. L^{-1}). The trial was harvested on 12 October when the total yield was determined by weighing 10 plants from the centre of each plot. Marketable yield was obtained by reweighing the heads after removing the diseased leaves.

Experiment 3. Lenswood. Seedlings were planted 13 October and spraying commenced 7 days later. The fungicide and application rates are outlined in Table 4. A 7-10 day schedule compared single fungicides (metalaxyl, phosphonic acid, dimethomorph and mancozeb) with the formulations of mancozeb with metalaxyl, dimethomorph or phosphonic acid, and metalaxyl + dimethomorph. As formulations including mancozeb were effective in previous experiments, experiment 3 also compared mixes of mancozeb with phosphonic acid or dimethomorph using both a 14 day and eradicant schedule to ascertain the viability of reducing the number of sprays. Seven, four and five fungicide sprays were applied respectively with the 7-10 day, 14 day and eradicant schedules. The eradicant sprays were applied within 48 hours of the first 8 hour leaf wetness period which occurred 10 days or more after the previous spray.

Results

Laboratory studies

Fungicide sensitivity. Sporulation of the metalaxyl sensitive isolate was completely inhibited at 0.01 mg a.i. L^{-1}, whereas concentrations of 0.5 g a.i. L^{-1} and 1 g a.i. L^{-1} did not inhibit sporulation of the other isolates. Dimethomorph inhibited sporulation by over 95% at 0.01 g a.i. L^{-1} and at 0.05 g a.i.L^{-1} sporulation was completely inhibited. At concentrations below 0.05 g a.i.L^{-1} the isolate of *B. lactucae* sensitive to metalaxyl was also more sensitive to dimethomorph (Fig. 1). Phosphonic acid inhibited sporulation by over 73% at 1 g a.i. L^{-1} and completely at 2 g a.i. L^{-1} and there was no obvious variation in sensitivity between the isolates tested.

Insensitivity survey. The sensitive isolate was inhibited 96% and 100% by 0.001 and 0.01 g a.i. L^{-1} metalaxyl respectively, whereas the 12 field isolates showed variable levels of insensitivity to the same concentrations of metalaxyl. None were inhibited completely at 0.01 g a.i. L^{-1} (Table 1).

Glasshouse studies

Experiment 1. Drenches of phosphonic acid at 10 and 20 g a.i. L^{-1} completely controlled downy mildew. At 5 and 2 g a.i. L^{-1}, phosphonic acid significantly reduced the disease, whereas at 1.2 g a.i. L^{-1} downy mildew was not inhibited.

Experiment 2. Foliar sprays of 0.375 g a.i. L^{-1} dimethomorph and 2.4 g a.i. L^{-1} phosphonic acid inhibited sporulation for at least 8 days. Sporulation was inhibited for more than 14 days where 4 g a.i. L^{-1} phosphonic acid was applied as a soil drench (Table 2).

Experiment 3. Dimethomorph at 0.375 g a.i. L^{-1} and phosphonic acid at 2.4 g a.i. L^{-1} completely inhibited sporulation when applied 3 days after inoculation. Phosphonic acid at 1.2 g ai L^{-1} and metalaxyl at 0.25 g a.i. L^{-1} were less effective and reduced the mean disease severity to 0.6 and 1.9 respectively, compared to 3.5 in the unsprayed plants.

Experiments 4, 5. All treatments inhibited sporulation when applied up to 3 days after inoculation, however of the foliar sprays applied 5 days after inoculation 2.4 g a.i. L^{-1} phosphonic acid and 0.25 g a.i. L^{-1} metalaxyl were significantly less effective than 0.375 g a.i. L^{-1} dimethomorph (Fig. 2).

Field evaluation

Experiment 1. Downy mildew developed on 92% of the unsprayed plants and on 96% of those treated with 0.25 g a.i. L^{-1} metalaxyl. The incidence of disease in the 5 treatments of dimethomorph and dimethomorph + metalaxyl combinations were similar, ranging from 69% to 79%. The metalaxyl + mancozeb combination had the lowest incidence of disease with 38% of the plants infected. The disease severity was significantly lower in the 0.25 g a.i. L^{-1} metalaxyl + 2 g a.i. L^{-1} mancozeb treatment compared to all other fungicides (Fig. 3).

Experiment 2. By harvest downy mildew had developed on most plants, but the severity was greatest on those of the unsprayed and metalaxyl treatments (Table 3). All treatments reduced the disease severity which was reflected in a 40 - 72% increase in total yields and a 15 - 60% increase in marketable yields compared to the unsprayed treatment.

Experiment 3. By 66 days after planting, all plants were affected by downy mildew. The disease severity was reduced by all fungicide treatments, with 6 of the 9 treatments providing equivalent control. Metalaxyl, mancozeb and the low rate of dimethomorph (0.3375g a.i. L^{-1}) showed a significant reduction in the severity of downy mildew infection when compared with the formulations of Ridomil MZ (0.25 g a.i. L^{-1} metalaxyl + 2 g a.i. L^{-1} mancozeb) and Acrobat (0.3375 g a.i. L^{-1} dimethomorph + 2.25 g a.i. L^{-1} mancozeb) which provided the best control within the 7-10 day spray schedule. (Table 4). Acrobat was equally effective with both the eradicant and the 7-10 day schedule. The phosphonic acid (2.4 or 4.8 g a.i. L^{-1}) and mancozeb (1.8 g a.i. L^{-1}) mixes were most effective on the 14 day schedule, although there was no significant difference in severity between the three schedules.

Discussion

Strains of *B. lactucae* insensitive to metalaxyl were shown in these studies to be present in the main lettuce growing areas of South Australia. How long the insensitive isolates have been present in the these areas is unknown as testing for sensitivity did not commence until 1991, although cases of poor fungicide control were reported before then. In South Australia, metalaxyl has been widely used on lettuce since its release in 1979. However metalaxyl has only been available in a formulated mixture with mancozeb which may have delayed the development of insensitive strains. By comparison the development of insensitivity to the acylalanine fungicides occurred within 5 years of the release of metalaxyl in the UK and California (Crute, 1987; Schettini *et al.*, 1991). Overall our results have shown that the metalaxyl/mancozeb mixture did not prevent the development of insensitive populations of *B. lactucae*. A similar selection of metalaxyl insensitive isolates of *Peronospora destructor* (Berk.) Caspary resulted from the use of a metalaxyl/mancozeb mixture on onions (O'Brien, 1992).

In our survey it was impractical to test the relative proportion of sensitive and insensitive isolates in the fungal population at each site. Field sampling was biased to plantings where poor fungicide control had been reported and the samples were bulked from each site. Nevertheless isolates insensitive to metalaxyl were detected in all samples, and although the degree of sensitivity varied the results show that insensitive isolates are widespread in South Australia. Although other acylalanine fungicides are also used on lettuce in South Australia, tests for sensitivity to related fungicides was not undertaken as cross-insensitivity has been reported elsewhere (Crute, 1987; O'Brien, 1992).

Our work also confirms earlier studies (Wicks, *et al.*, 1993) that showed the unrelated systemic fungicides dimethomorph and phosphonic acid controlled metalaxyl insensitive isolates of *B. lactucae* and warrant registration for use on lettuce. The good control achieved with these two fungicides was anticipated as phosphonic acid is the active ingredient of Aliette, a fungicide that has controlled downy mildew in lettuce elsewhere (Crute, 1978; Raid, 1991; Raid and Datnoff, 1991) and the efficacy of dimethomorph on downy mildew has been reported on onions (O'Brien 1992) and grapes (Wicks and Hall, 1990). Although O'Brien (1992) found no evidence of cross insensitivity between dimethomorph and metalaxyl on onion downy mildew, a slight decrease in sensitivity to dimethomorph of the metalaxyl sensitive isolate was observed in the dose response experiments at concentrations below 0.005 g a.i. L^{-1} dimethomorph. If dimethomorph becomes widely used on lettuce in the future, regular checks would be warranted to ensure that the observed reduction in sensitivity at low concentrations does not develop into widespread insensitivity. Testing the sensitivity of isolates of *B. lactucae* to any newly developed fungicides would be advisable. Our results indicated that discriminatory doses of 0.001 to 0.05 g a.i. L^{-1} dimethomorph and 0.5 to 2 g a.i. L^{-1} phosphonic acid could be used to indicate shifts in sensitivity. However further dose response experiments need to be conducted with a range of isolates to determine the variations in sensitivity of the fungal populations.

Crute (1987) reported that the rapid spread of the metalaxyl insensitive isolates in the UK may have been attributed to the distribution of transplants. A similar means of spread would have been possible in South Australia as many commercial nurseries produce lettuce seedlings infected with downy mildew. In South Australia many of the nurseries are situated in the main lettuce growing areas. It is likely that during seedling production the seedlings become infected with metalaxyl insensitive strains of *B. lactucae* from surrounding plantings of mature lettuce. In these nurseries the application of acylalanine fungicides for downy mildew control has been discouraged to limit further spread of the insensitive isolates. Drenches or foliar applications of phosphonic acid successfully controlled downy mildew on lettuce seedlings and the use of either should be encouraged in nursery production. Although foliar applications of either phosphonic acid or dimethomorph also controlled downy mildew in the field, a different material should be applied in the field than that used in the nursery. Adopting such a practice should lessen the likelihood of *B. lactucae* populations becoming insensitive to these fungicides.

In addition to testing field isolates of *B. lactucae* for insensitivity to metalaxyl, the presence of insensitivity in S.A. was also confirmed by the high levels of downy mildew that developed in the field plots treated with metalaxyl. In these experiments the severity of downy mildew was significantly less than that in the untreated plots, suggesting that either a proportion of the fungal population was sensitive to the dose of metalaxyl applied or that there was a wide variation in the sensitivity of isolates to metalaxyl. The latter is more likely as isolates from the different localities showed a wide range of sensitivities to metalaxyl. Differences in the levels of metalaxyl sensitivity have been reported between English and Californian isolates (Schettini *et al*, 1991).

The control achieved with the metalaxyl-mancozeb formulation used in these field experiments was unexpected, as neither metalaxyl nor mancozeb applied alone were effective and the same formulation performed poorly in a similar field experiment the previous season (Wicks *et al.* 1993). The control achieved with this formulation in the 1992 experiments is difficult to explain unless the efficacy of the metalaxyl + mancozeb formulation is due to a high level of synergistic action with the mixture. Similar synergistic interactions with fungicide mixtures have been reported (Cohen and Levy, 1990). Our results indicated synergism between phosphonic acid + mancozeb but not with combinations of dimethomorph + metalaxyl or dimethomorph + mancozeb.

As the glasshouse experiments showed that both dimethomorph and phosphonic acid controlled *B. lactucae* when applied up to 3 days after inoculation, we attempted to reduce the frequency of spraying for the control of downy mildew by applying fungicides only after conditions were suitable for infection. Our results showed that at least 2 sprays could be saved using an eradicant schedule rather than a protectant 7 day schedule without reducing the degree of control. However there are difficulties with the scheduling based on leaf wetness as the sensors may not accurately reflect the actual duration of leaf wetness particularly on lettuce when water accumulates in leaf depressions. In addition weather conditions suitable for infection occur frequently in winter crops and on these there may be no advantage in spraying on an eradicant schedule.

The field experiments emphasised the importance of controlling downy mildew on lettuce. In most treatments the incidence of disease was near 100%, but the application of fungicides significantly reduced the disease severity with a corresponding increase in marketable yield.

The glasshouse experiments showed that phosphonic acid drenches protected lettuce seedlings from infection for at least 14 days. Further work to ascertain the full extent of the protection provided by drenches would be advantageous, as application of a soil drench to seedlings before planting could delay the application of field fungicides and further reduce the amount of pesticide applied.

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TABLE 1. Sensitivity (percent inhibition of sporulation) to 0.001 and 0.01 g a.i. L^{-1} metalaxyl of 12 *Bremia lactucae* isolates collected from commercial lettuce plantings in South Australia 1992.

Isolate and locality	Inhibition (%) Metalaxyl g a.i. L^{-1}	
	0.001	0.01
Sensitive[A]	96	100
Adelaide Plains		
Two Wells	3	14
St Kilda	32	68
Angle Vale	0	34
Virginia (1)	40	22
Virginia (2)	0	23
Virginia (3)	2	55
Adelaide Hills		
Carey Gully	0	8
Uraidla (1)	31	12
Uraidla (2)	54	61
Uraidla (3)	0	2
Murray Bridge (1)	40	80
(2)	21	61

[A] Obtained from D. Trimboli, Yates, Narromine, New South Wales.

TABLE 2. Severity of *Bremia lactucae* infection on lettuce seedlings cv Cos Verdi (Yates), treated with fungicides 1, 4, 8 and 14 days before inoculation.

	Severity[C] Days before inoculation				
Treatment and rate (g a.i. L^{-1})	1	4	8	14	l.s.d. (P=0.05)
unsprayed	2.5	2.5	2.5	2.5	-
metalaxyl[A] ,0.25 g	1.4	2.1	-	1.6	n.s.
dimethomorph[A], 0.375 g	0	0	0	1.15	0.61
phosphonic acid[B], 4 g	0	0	0	0	n.s.
phosphonic acid[A], 2.4 g	0.4	0.2	0	1.2	0.53
l.s.d. (P=0.05)	0.68	0.80	0.51	0.90	

A foliar spray

B soil drench

C based on a 0-4 scale, 0, no sporulation; 1, <50% of 1 leaf; 2, 1 complete leaf; 3, <50% of 2 leaves; 4, 2 complete leaves with sporulation

TABLE 3. Fungicide treatments, incidence (percentage of plants infected) and severity of downy mildew and yield of lettuce cv El Toro at Virginia.

Treatment and rate (g a.i. L^{-1})	Incidence	Severity[A]	Yield (g $plant^{-1}$)	
			total	marketable
unsprayed	100	4.8	476	426
metalaxyl, 0.25 g	100	3.6	700	550
metalaxyl, 0.25 g + mancozeb, 2 g	99	1.8	820	680
dimethomorph, 0.3375 g	100	2.7	818	612
dimethomorph, 0.3375 g + metalaxyl, 0.25 g	100	2.7	750	652
dimethomorph, 0.195 g + metalaxyl, 0.15 g	100	2.7	668	490
phosphonic acid, 2 g	100	2.8	706	620
l.s.d. (P=0.05)	-	0.15	189	132

[A] based on a 0-5 scale: 1, 1 to 5; 2, 6 to 10; 3, 11 to 25; 4, 26 to 50; 5 >51% of basal area infected.

TABLE 4. Fungicide treatments and severity of downy mildew on lettuce cv El Toro at Lenswood.

Treatment and rate (g a.i.L^{-1})	Severity[A]		
	7-day	14-day	eradicant[B]
unsprayed	5.0	5.0	5.0
metalaxyl, 0.25 g	4.0	-	-
mancozeb, 1.8 g	3.3	3.8	-
metalaxyl, 0.25 g + mancozeb, 2 g	2.4	-	-
dimethomorph, 0.3375 g	2.9	-	-
dimethomorph, 0.75 g	2.7	-	-
dimethomorph, 0.3375 g + metalaxyl, 0.25 g	2.6	-	-
dimethomorph, 0.3375 g + mancozeb, 2.25 g	2.4	3.0	2.5
phosphonic acid, 2.4 g	2.5	2.8	-
phosphonic acid, 4.8 g	-	-	3.1
phosphonic acid, 2.4 g + mancozeb, 1.8 g	2.5	2.3	-
phosphonic acid, 4.8 g + mancozeb, 1.8 g	-	-	2.4
l.s.d. (P=0.05)	0.48	0.31	0.22

A based on a 0 to 5 scale where 1, 1 to 5; 2, 6 to 10; 3, 11 to 25; 4, 26 to 50; 5 > 51% of basal area infected

B sprayed after the first 8 hours leaf wetness occurring after 10 days from the previous spray

FIG. 1. Inhibition (%) of *Bremia lactucae* sporulation on excised lettuce cotyledons cv Cos Verdi (Yates) sprayed with different rates of dimethomorph, using the metalaxyl sensitive isolate (■) and two metalaxyl insensitive isolates (▲,▼).

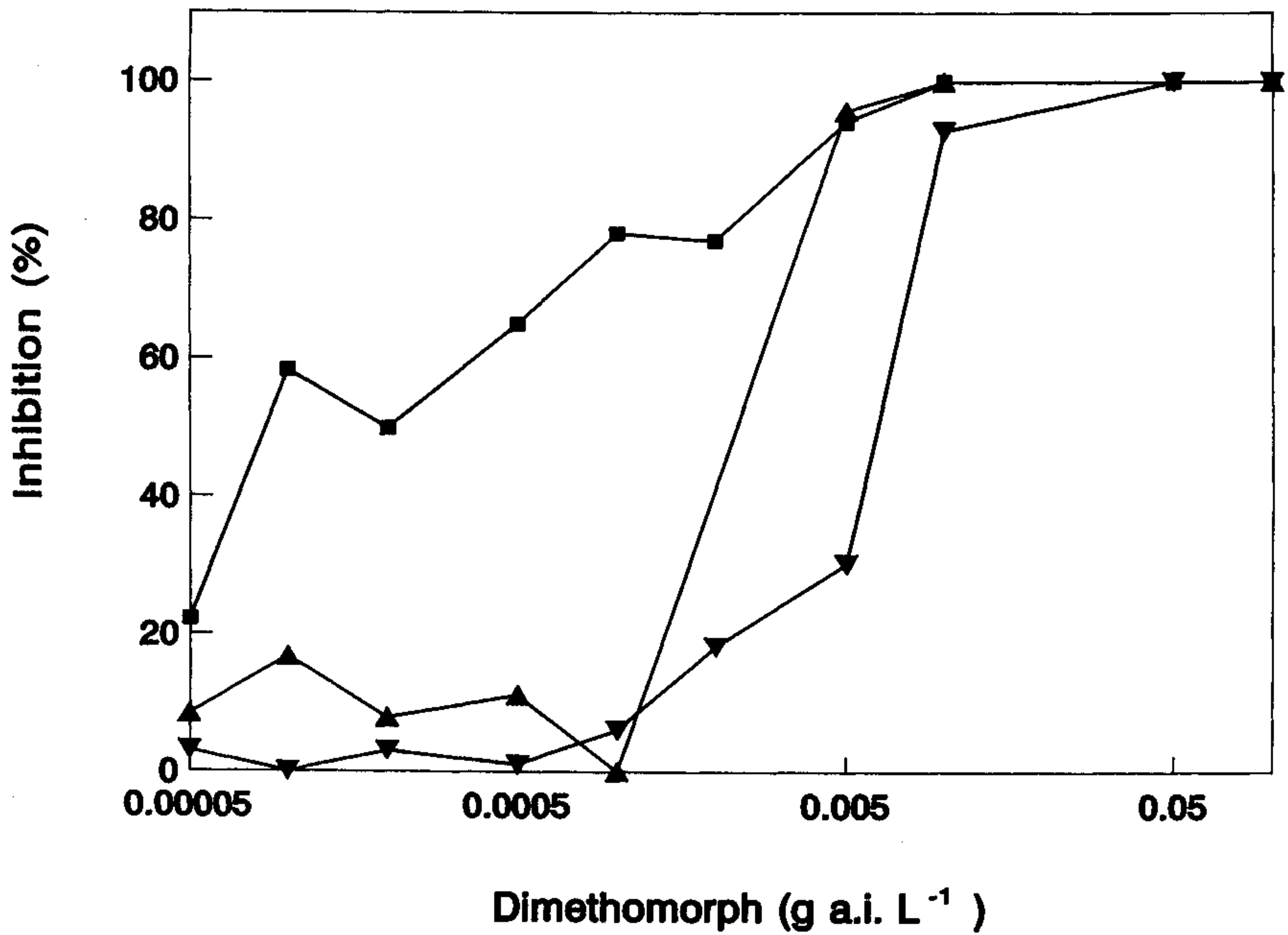
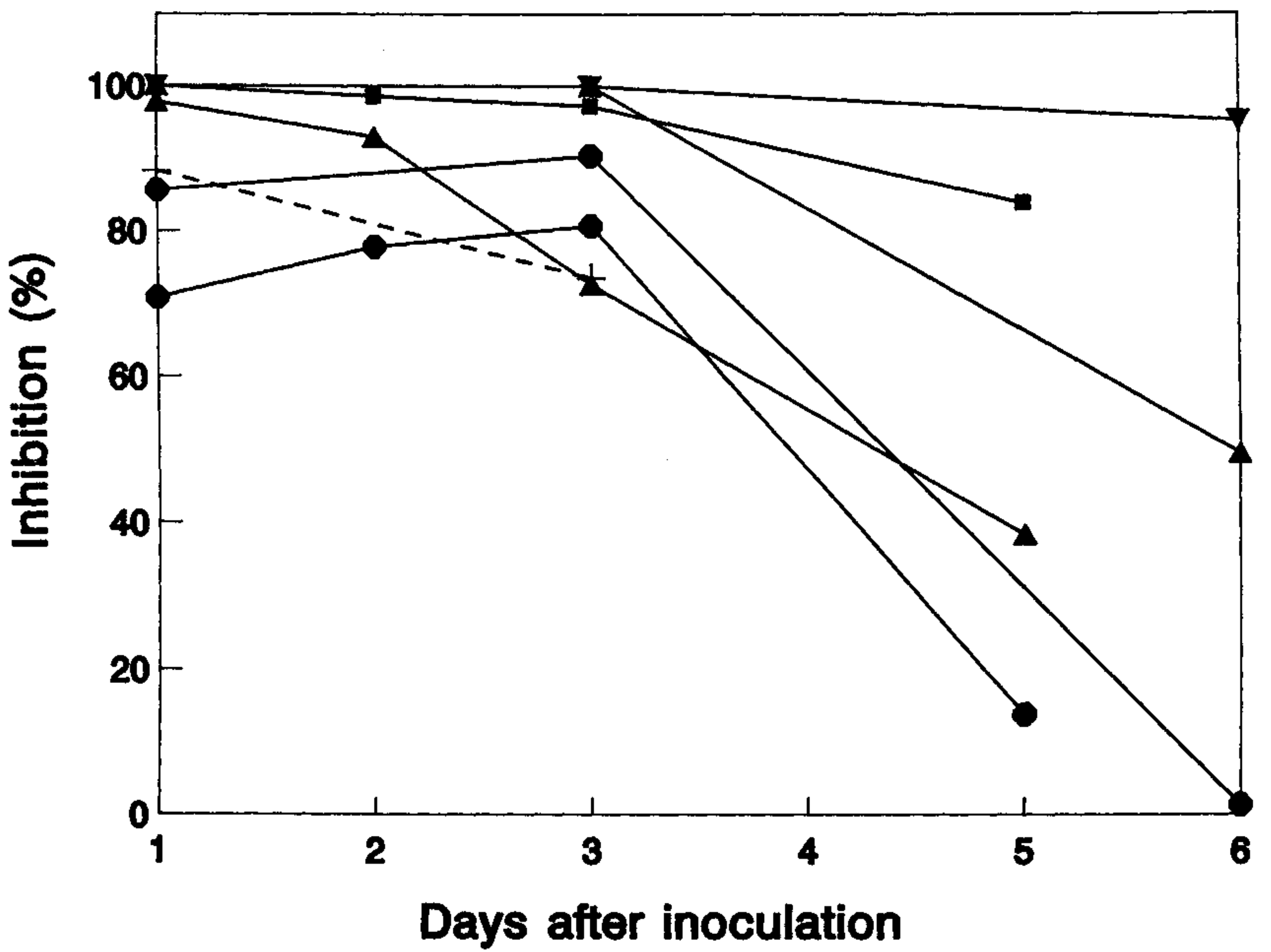
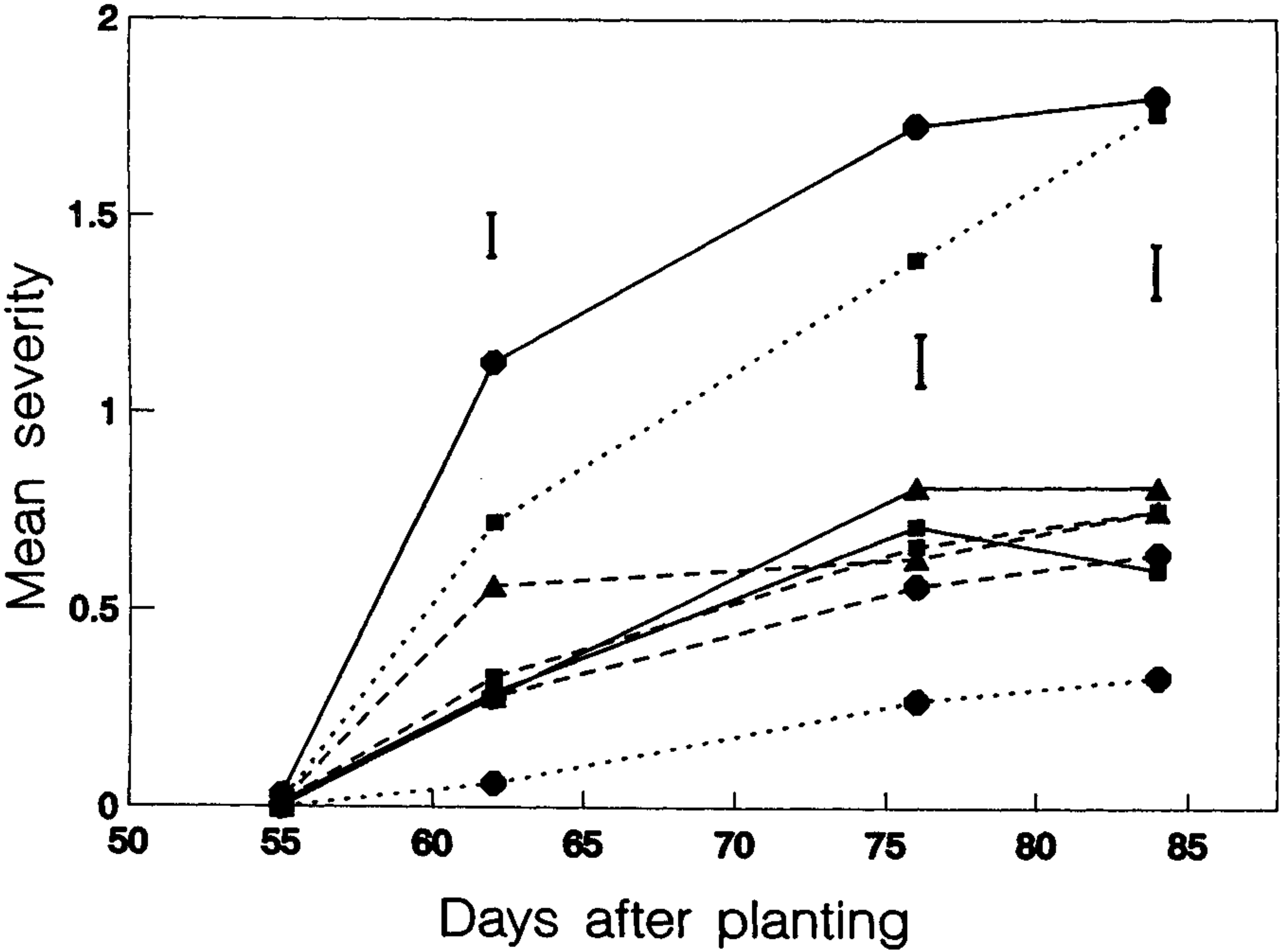

Inhibition (%)
100
80
60
40
20
0
0.00005
0.0005
0.005
0.05
Dimethomorph (g a.i. L -1)

FIG. 2. Inhibition (%) of *Bremia lactucae* sporulation on lettuce cv Cos Verdi (Yates) sprayed or drenched with fungicides at 1 to 6 days after infection. Metalaxyl 0.25 g a.i. L^{-1} (●), dimethomorph 0.375 g a.i. L^{-1} (■) and 0.75 g a.i. L^{-1} (▼) , phosphonic acid 2.4 g a.i. L^{-1} foliar spray (▲) and 4 g a.i. L^{-1} soil drench (+).



100
80
60
40
20
0
Inhibition (%)
1
2
3
4
5
6
Days after inoculation

FIG. 3. Effect of fungicides on development of *Bremia lactucae* on lettuce cv El Toro at Virginia. Unsprayed (● ●); 0.25 g a.i. L^{-1} metalaxyl (■ ■); dimethomorph at 0.3375 g a.i. L^{-1} (● ●), 0.2475 g a.i. L^{-1} (■ ■) and 0.195 g a.i. L^{-1} (▲ ▲); dimethomorph + metalaxyl at 0.3375 g a.i. L^{-1} + 0.25 g a.i. L^{-1} (■ ■) and 0.2475 g a.i. L^{-1} + 0.1875 g a.i. L^{-1}(▲ ▲); 0.25 g a.i. L^{-1} metalaxyl + 2 g a.i. L^{-1} mancozeb (● ●), L.S.D. at P = 0.05 (I).


Mean severity
2
1.5
1
0.5
0
50
55
60
65
70
75
80
85
Days after planting