Project title	Sensitivity of apple powdery mildew (*Podosphaera leucotricha*) populations to triazole, QoI and SDHI fungicides
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AUTHENTICATION

I declare that this work was done under my supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- There are some indications that there is more variability in the mildew response to Systhane (myclobutanil - triazole fungicide) among the orchards tested than to Luna (fluopyram – a succinate dehydrogenase inhibitor (SDHI) fungicide) and Vivid (pyraclostrobin - a quinone outside inhibitor (QoI) fungicide).

Background and expected deliverables

Apple powdery mildew can reduce yield and fruit quality, with levels as low as 8% mildewed leaves reducing yield and quality on sensitive varieties such as Cox. On other varieties, high levels of powdery mildew have been recorded in many commercial orchards but the effect on yield and quality is not as well understood as on Cox.

All those growers and advisors consulted on powdery mildew agree that control is becoming increasingly difficult. On average, some badly affected farms in East Kent have orchards with 50-100% mildew-infected shoots. There are many possible reasons for the poor mildew control including: limited range of effective fungicides, reduced efficacy of triazole or QoI (strobilurin) fungicides due to changes in the sensitivity of the mildew population, change in shoot growth pattern due to climate change, poor spray cover or insufficient monitoring of mildew development.

Good control of powdery mildew during the growing season is the top priority. Triazoles are currently the most effective fungicides against apple powdery mildew and consequently are used intensively in apple orchards as there are few alternative products. This leads to repeated use of fungicides from the same chemical group resulting in a high risk of mildew isolates being selected with reduced sensitivity.

Alternative products for mildew control, including potassium bicarbonate, potassium phosphite, Milsana (knotweed extract) and a biocontrol agent *Ampelomyces quisqualis,* were evaluated as part of a Defra project (HH2502STF). Most had limited efficacy. The Defra funded Horticulture LINK project (HL01109) on chemical control in horticultural crops (SCEPTRE) started in 2011. One of its objectives is to find new fungicides and/or alternative products for controlling powdery mildew on apple. Information on whether triazole (myclobutanil, penconazole) or QoI (kresoxim-methyl, pyraclostrobin) fungicides are less effective due to reduced sensitivity of mildew populations in orchards is important for selecting appropriate fungicides to achieve good control and minimise the risk of insensitivity development. SDHI fungicides have a different mode of action to triazoles and QoI fungicides. New fungicides likely to be approved for use in apples in the future are SDHI fungicides and therefore it is relevant to include this group in this study.

Any new information from this project on the sensitivity of apple powdery mildew to triazole and QoI fungicides and its possible contribution to the current poor mildew control will benefit the industry in the following aspects:

(1) The information generated will complement that generated in the Defra Horticulture LINK project (CP 77, SCEPTRE) in which new fungicides and/or alternative products for controlling powdery mildew will be investigated.

(2) It should provide the industry with a clearer understanding on fungicide control of apple powdery mildew.

(3) It will enable growers to select appropriate products in order to improve control as well as to reduce the risk of development of fungal resistance/insensitivity to fungicides.

Summary of the project and main conclusions

Potted MM106 rootstocks and Malus cv. Bittenfelder seedlings were raised in a mildew-free glasshouse. They were then treated with a range of doses of the test fungicides – Systhane (myclobutanil), Tucana (pyraclostrobin) and Luna Privilege (fluopyram). They were then placed in apple orchards at East Malling Research and various parts of Kent where control of powdery mildew was known to be a problem. Untreated controls were also included. The apple rootstocks / seedlings were exposed to mildew for 48 hours and then collected and returned to the glasshouse to allow any mildew infection to develop. After one week the plants were assessed for mildew. A total of 60 such exposure studies (Plants [rootstock or seedlings] x time x orchards) have been conducted in three years. For some unknown reasons, only 16 out of these 60 exposure studies resulted in sufficient numbers of lesions for statistical analysis. Therefore further studies are necessary to validate conclusions from this project.

The following conclusions have been drawn from the data recorded:

(1) Of the three fungicides, the differences in the dose-response of mildew among sites tested were greatest for Systhane, confirming anecdotal evidence.

(2) Furthermore, the largest within-site variability in the dose-response of mildew at a given site was also greatest for Systhane.

(3) These ***limited*** data suggest possible existence of reduced sensitivities to Systhane and to a lesser extent to Luna and Vivid. There are several orchards that can be used for further studies to confirm this preliminary conclusion.

Financial benefits

Growers can benefit from the project results through selecting the correct fungicide products in spray programmes to control mildew and minimise the establishment and subsequent spread of mildew strains that are insensitive to fungicides.

Action points for growers

- Maintain the use of a good range of fungicides against powdery mildew to achieve effective control.

SCIENCE SECTION

Introduction

After scab and canker, apple powdery mildew is the most important disease of apples in the UK. The disease can reduce yield and fruit quality. Levels of powdery mildew as low as 8% mildewed leaves can reduce yield and quality on susceptible varieties such as Cox. On other varieties high levels of mildew have been recorded in many commercial orchards but the effect on yield and quality is not as well understood as on Cox.

All growers and advisers we have spoken to agree that powdery mildew control is becoming difficult with some badly affected farms in East Kent having on average orchards with 50-100% mildew-infected shoots. All apple varieties appear to be affected, but the worst are Cox, Bramley, Gala, Jonagold and Braeburn. There are many possible reasons for the poor mildew control including:

- Limited range of effective fungicides – sulphur, myclobutanil, penconazole, pyraclostrobin, bupirimate and potassium bicarbonate;
- Reduced efficacy of triazole or quinone outside inhibitor (QoI, including strobilurin) fungicides possibly due to a change in the sensitivity of the mildew population – a previous HDC-funded project at EMR indicated some reduced sensitivity to myclobutanil;
- Change in the shoot growth pattern due to climate change – shoot growth continuing after harvest when spraying has stopped leading to high incidence of primary vegetative mildew the following season;
- Milder winters in some years leading to improved survival of overwintering mildew;
- Very favourable spring and summer weather – warm wet weather promoting shoot growth and mildew development and spread;
- Growers being more concerned with scab control;
- Poor spray cover or insufficient monitoring of mildew development.

Previous HDC-funded research (TF 156) at EMR showed that application of some surfactant products during the winter may significantly reduce the level of overwintering mildew in the buds. However, the rate necessary for achieving this exceeds the maximum concentration permitted. Thus, good control of powdery mildew during the growing season is the top priority.

Triazoles are currently the most effective fungicides against apple powdery mildew and consequently are used intensively in apple orchards as there are few alternative products.

This leads to repeated use of fungicides from the same chemical group, resulting in a high risk of mildew isolates being selected with reduced sensitivity.

Alternative products for mildew control, including potassium bicarbonate, potassium phosphite, Milsana (knotweed extract) and a biocontrol agent *Ampelomyces quisqualis,* were evaluated as part of a Defra project (HH2502STF). Most had limited efficacy. A HortLINK project on chemical control in horticultural crops (CP 77, SCEPTRE) started in 2011 and finishes after the 2014 season. One of its objectives is to find new fungicides and/or alternative products for controlling powdery mildew on apple. Information on whether triazole (myclobutanil, penconazole) or QoI (kresoxim-methyl, pyraclostrobin) fungicides are less effective due to reduced sensitivity of the mildew populations in orchards is important for selecting appropriate fungicides to achieve good control and minimise the risk of insensitivity development. New fungicides likely to be approved for use in apples in the future are succinate dehydrogenase inhibitor (SDHI) fungicides and therefore it is relevant to include this group in the study.

Chemical companies routinely monitor fungicide resistance for the major fungicide chemical groups and the major fungal pathogens including apple scab as part of FRAC (Fungicide Resistance Action Committee). No monitoring of sensitivity of apple powdery mildew to triazoles has been conducted in recent years and there are no standard methods for monitoring apple powdery mildew sensitivity to fungicides published by FRAC. Reduced sensitivity of powdery mildews to triazoles and more recently to QoIs has been reported in other crops, including grape.

Project aim

To develop a sustainable, cost effective system for control of apple powdery mildew (*Podosphaera leucotricha*)

Project objectives

1. To develop a practical method for monitoring the sensitivity of populations of *P. leucotricha* to triazole, QoI and other fungicides
2. To use the method to monitor the sensitivity of mildew populations to triazoles, QoI and other fungicides in apple orchards

Materials and methods

Establishing protocols (Year 1)

Because powdery mildew is an obligate pathogen, meaning that it can only survive on live plant tissues, *in vitro* testing for fungicide insensitivity as used for most pathogens is not practical. Thus, in the first year, we have looked at fungicide concentration and orchard exposure time to develop a protocol for monitoring mildew sensitivity. Initially, we planned to include apple seedlings as a comparison to the use of rootstocks. Unfortunately, we had difficulty in sourcing the *Malus bittenfelder* seeds and so were not able to use them in the first year of the project. Apple seeds have now been obtained and were used in year 2.

Potted rootstocks of MM 106 plants were raised in a mildew-free glasshouse. They were treated with a range of fungicide concentrations of Systhane (myclobutanil), Vivid (pyraclostrobin) and Luna Privilege (fluopyram). Four levels of concentration for each fungicide (Table 1) were used; these levels were determined on the assumption of 1,000 L per ha for spray volume. On the day of the treatment, the first fully unrolled leaf on each shoot was labelled; on each plant, up to five shoots were selected.

Treated plants were then exposed to one of several sources of powdery mildew (these plants were physically placed next to trees/plants with mildew) for 24-48 h before being moved back to a contained glasshouse compartment. There were four or five plants for each combination of fungicide, concentration and inoculum source. In addition, for each inoculum source there were also six plants that did not receive any fungicides. The number of powdery mildew lesions was counted on the tagged leaf and three leaves immediately below (i.e. fully unrolled at the time of fungicide application and hence covered with fungicide).

This exposure treatment was conducted twice, once in July 2011 and once in August 2011. In the July exposure, three orchard sources of inoculum were used: TL161, CW121 and EE190 at EMR. In the August exposure trial, two sources of inoculum were used: CW121 and P1 (tunnel).

Table 1. Concentration of each fungicide product used

Product	a.i. [single a.i. product]	Mode of action	Concentration (g or ml in 1 L water)			
			C1	C2	C3	C4
Systhane	myclobutanil (20%)	DMI	0.0045	0.045	0.225	0.45
Vivid	pyraclostrobin (23.6%)	QoI	0.0044	0.044	0.22	0.44
Luna	fluopyram (50%)	SDHI	0.003	0.030	0.15	0.30

Tests in year 2 and 3

Potted rootstocks of MM 106 plants and apple seedlings cv. *Malus bittenfelder* were raised in a mildew-free glasshouse. Whenever, there was trace mildew on these plants, they were treated immediately with fungicides other than the three test products [these treatments were applied at least 10 days before the experimental treatments (fungicides) were applied]. They were then treated with a range of fungicide concentrations of Systhane, Vivid and Luna. Four levels of concentration for each fungicide (Table 1) were used as established in Year 1.

Treated plants were then exposed to one of several sources [orchards in Kent, Table 2] of powdery mildew (these plants were physically placed next to trees/plants with mildew) for 48 or 72 h before being moved back to a contained glasshouse compartment. These orchards are known to have experienced problems in controlling powdery mildew in the past. There were four or five plants for each combination of fungicide, concentration and location. In addition, for each location, there were also six plants that did not receive any fungicides and these served as a control treatment.

About one week after the exposure the number of powdery mildew lesions was counted on the top four fully unrolled leaves on each shoot; usually there were three shoots per potted plant. For seedlings, only the four youngest unrolled leaves were assessed for powdery mildew.

This exposure treatment was conducted twice in 2012 (May and July) and four times in 2013 (May, June, July and August). Table 2 gives the summary of each exposure treatment.

Table 2. Sites used for rootstock (R) and seedling (S) tests on sensitivity of apple powdery mildew to several fungicide fungicides

SITE	2012				2013							
	21 May		24 July		21-May		17-Jun		08-Jul		06-Aug	
	R	S	R	S	R	S	R	S	R	S	R	S
Blean	✓				✓	✓	✓	✓	✓	✓	✓	
EMR - TL161	✓	✓			✓	✓						
EMR- CW108	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓
EMR- CW121			✓									
EMR – WM132			✓		✓	✓	✓	✓	✓	✓	✓	✓
West Malling	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Pluckley			✓	✓	✓	✓					✓	
Paddock Wood	✓				✓	✓	✓	✓		✓	✓	✓
Chartham			✓								✓	✓
Upchurch	✓		✓	✓							✓	✓

Results

Protocol development in 2011 (Year 1)

In the first exposure experiment, the rootstocks were not exposed to mildew in the orchard for the full 24 hour period planned and were removed early due to a thunderstorm. Nevertheless, the air was quite humid during the exposure period and it was possible that dew might have formed on leaves during this time. We did not observe any mildew lesion on these exposed plants.

The number of mildew lesions varied greatly with fungicides and their concentrations in the second exposure experiment (Figure 1). Overall, the number of mildew lesions on rootstock plants exposed to P1 was about three times as much as those exposed to CW121. The key point is that the range of concentrations (C1-C4 as in Table 1) covered the full range of disease severity (i.e., close to the untreated to nearly 100% control). Thus, these concentrations can be used to estimate ED_{50} values, which can then be used to represent fungal sensitivity.

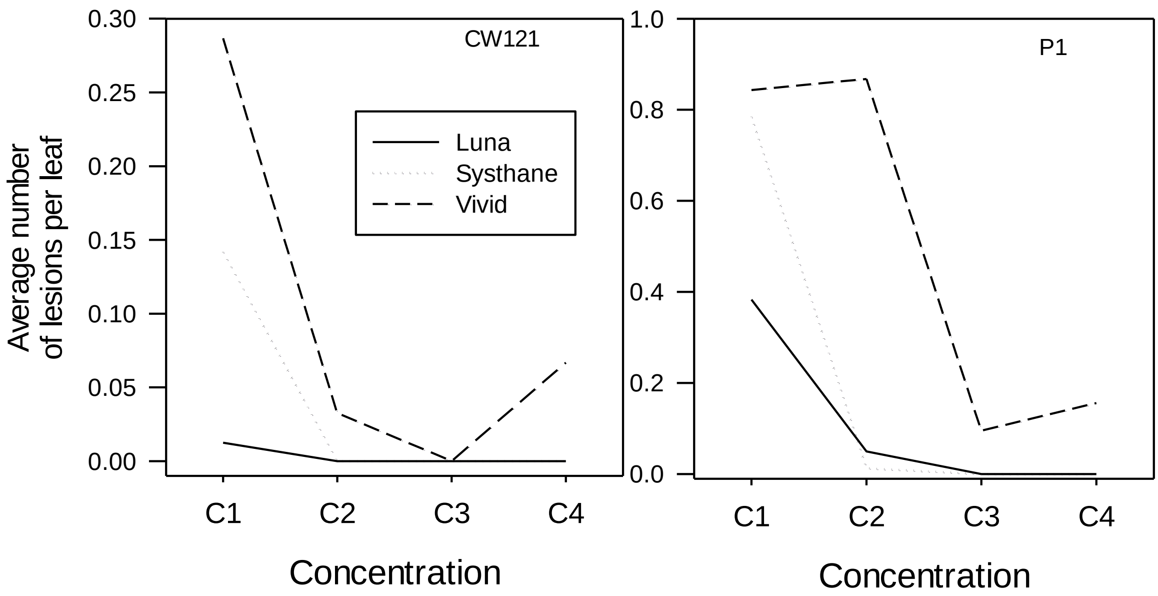

Figure 1. Average number of lesions per leaf on MM. 106 rootstock plants exposed to powdery mildew spores for 24 h at two sites (CW121 & P1) in August 2011. The exact concentration for each fungicide was given in Table 1

Trial results in 2012 **(Year 2)**

A total of 18 combinations (rootstocks/seedlings x site x time) were tested in 2012: 12 combinations for rootstocks (Table 2). In the May exposure experiment the rootstocks were exposed to six sites whereas the seedlings to three sites. For the rootstocks, only at five of the six sites was the level of resulting mildew moderate to high. For the sixth site (the

Upchurch site), most plants stopped growing because of wind damage to the shoot tips during the transportation.

For Luna, there were small differences in the relationship of mildew severity and incidence on seedlings with the dose (Fig. 2). At the C2 concentration, Luna Privilege effectively controlled mildew. For Systhane, there were considerable differences between the three sites in the dose-responsive curves for mildew development, particularly when the incidence of mildewed leaves was considered (Fig. 1). For example, only at one site was mildew effectively controlled at the C3 concentration. For Vivid, the dose-response of mildew development was between those to Luna Privilege and Systhane.

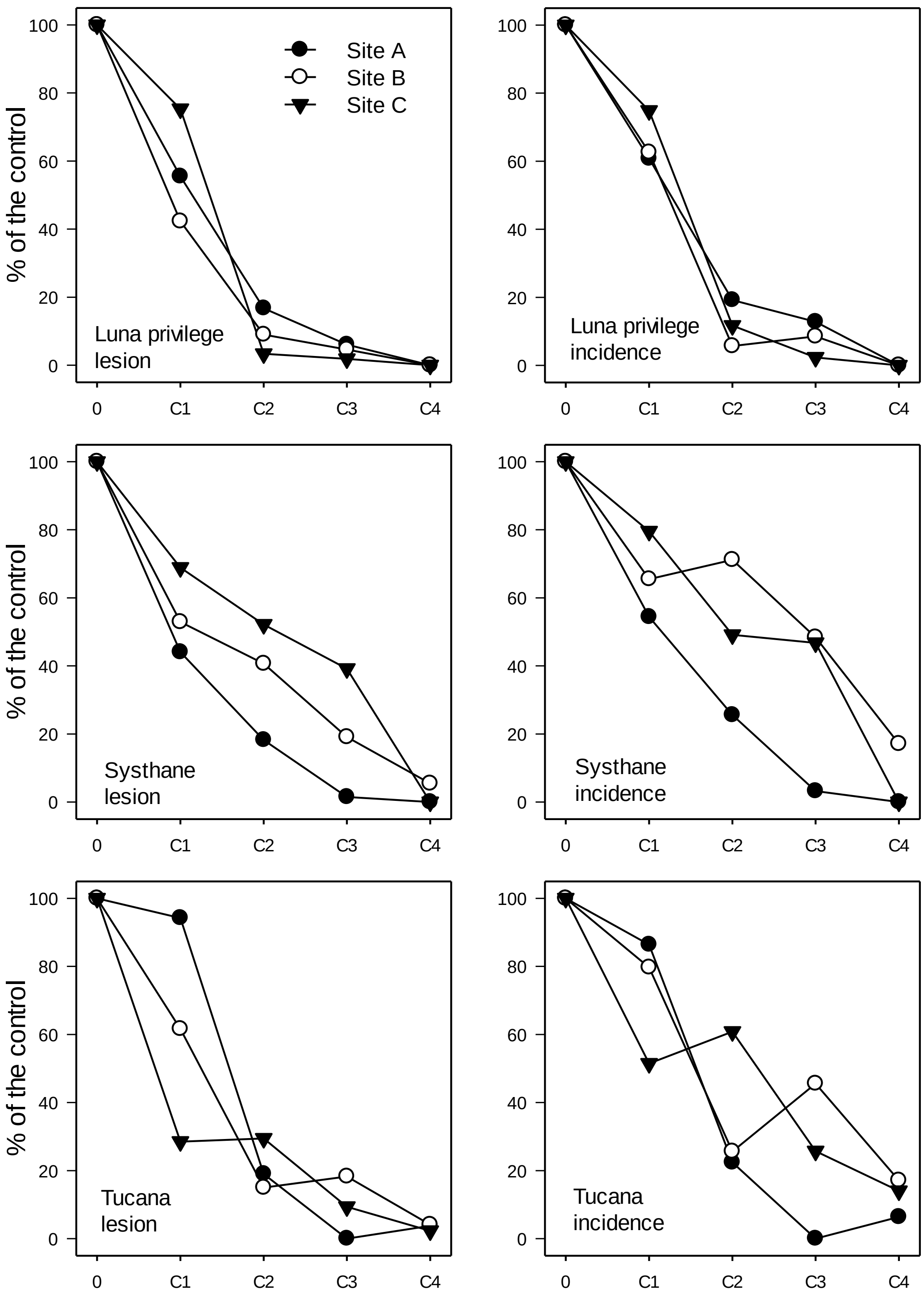

Figure 2. Average number of lesions per leaf and incidence of leaves with mildew on seedlings of cv. *Malus bittenfelder* to powdery mildew for 48-72 h at three sites in May 2012. The exact concentration for each fungicide and location were given in Table 1 and Table 2, respectively.

Similar results were obtained for the rootstock plants, except that differences in the dose-responses among the three fungicides were less than on seedlings (Fig. 3).

For the July exposure experiment, there was only a trace level of mildew on rootstocks at all six sites; hence no useful data were generated on rootstocks. On the seedlings, the level of mildew was moderate on two sites, whereas it was at the trace level at the third site. Again, similar to the seedling exposure in May, there was little difference in the dose-response curves between the two sites for Luna Privilege but large difference for Systhane (Fig. 4).

Trial results in 2013 **(Year 3)**

On four occasions, rootstock plants and/or seedlings were treated and placed in different orchards (Table 2). A total of 36 combinations (rootstocks/seedlings x site x time) were tested in 2013: 20 combinations for rootstocks (Table 2). Unfortunately, a sufficient number of mildew lesions only developed in seven of these combinations despite severe mildew epidemics in those orchards at the time of experimentation. The overall results were similar to those observed in 2012 (Fig. 5-6).

ED_{50} values

Figure 7 shows all the estimated ED_{50} values for 16 exposure experiments against the three fungicides. For Luna Privilege, the ED_{50} value is generally very low, ranging from 0.164 to 13.7 ppm for incidence, except for two exposures (50.0 and 64.0 ppm) (Fig. 7A). The results were similar when disease severity (lesion number) was analysed, except only one exposure had very high ED_{50} value (54.1 ppm). Overall, there was little difference in ED_{50} values between different exposure experiments in the same orchards except the TL161 orchard at EMR. There was highly significant correlation ($r = 0.735$) in ED_{50} values between incidence and severity data (Fig. 7).

For Systhane, the ED_{50} value varied greatly between different exposure experiments at the same sites as well as between sites (Fig. 7B), ranging from 3.0 to 556 ppm for incidence and from 2.1 to 500 ppm for disease severity. For example, the ED_{50} values (incidence) for two exposures at the Blean site were 6.6 ppm and 556 ppm; this large difference between exposures was also observed for the CW108 and TL161 orchards at EMR. There was a highly significant correlation ($r = 0.766$) in ED_{50} values between incidence and severity data (Fig. 7).

For Vivid, the ED_{50} value varied to a lesser extent than for Systhane (Fig. 7C), ranging from 1.8 to 108 ppm for incidence (except for one exposure in TL161 – 218 ppm). As for Luna Privilege and Systhane, there was large variability among the four exposure studies in the

TL161 orchard at EMR (Fig. 7C). There was significant correlation in ED_{50} values between incidence and severity data (Fig. 7), but not as high as for Luna Privilege and Systhane (r = 0.596).

Although there were significant correlation in the ED50 values for Luna Privilege and Vivid (Fig. 8b), this correlation is due to two exposure studies: one at TL161 and the other at the Upper Church site.

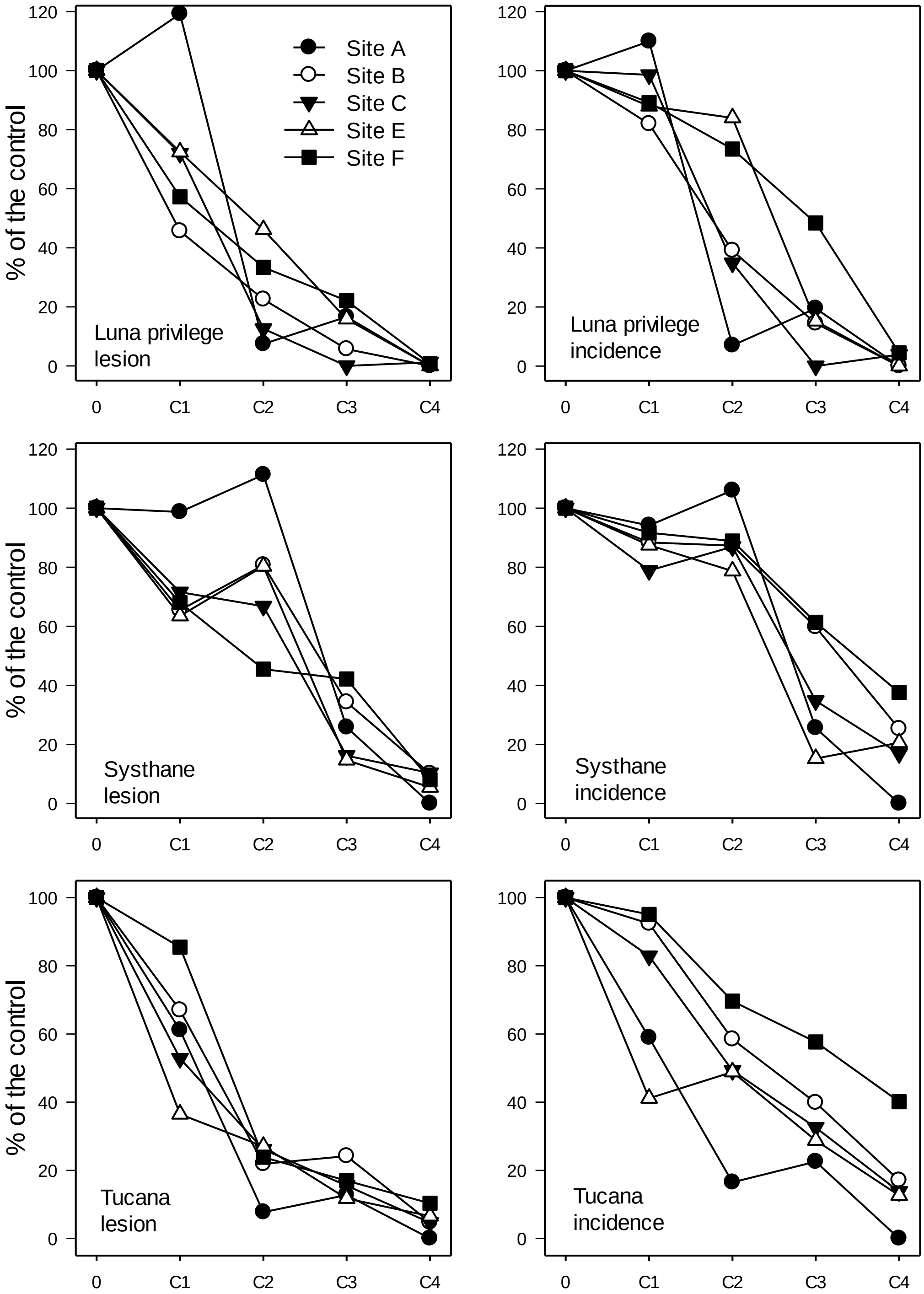

Figure 3. Average number of lesions per leaf and incidence of leaves with mildew on MM106 rootstock plants to powdery mildew for 48-72 h at six sites in May 2012. The exact concentration for each fungicide and location were given in Table 1 and Table 2, respectively. At one site, no mildew lesions were observed because most plants stopped growing due to wind damage to the shoots during the transportation.

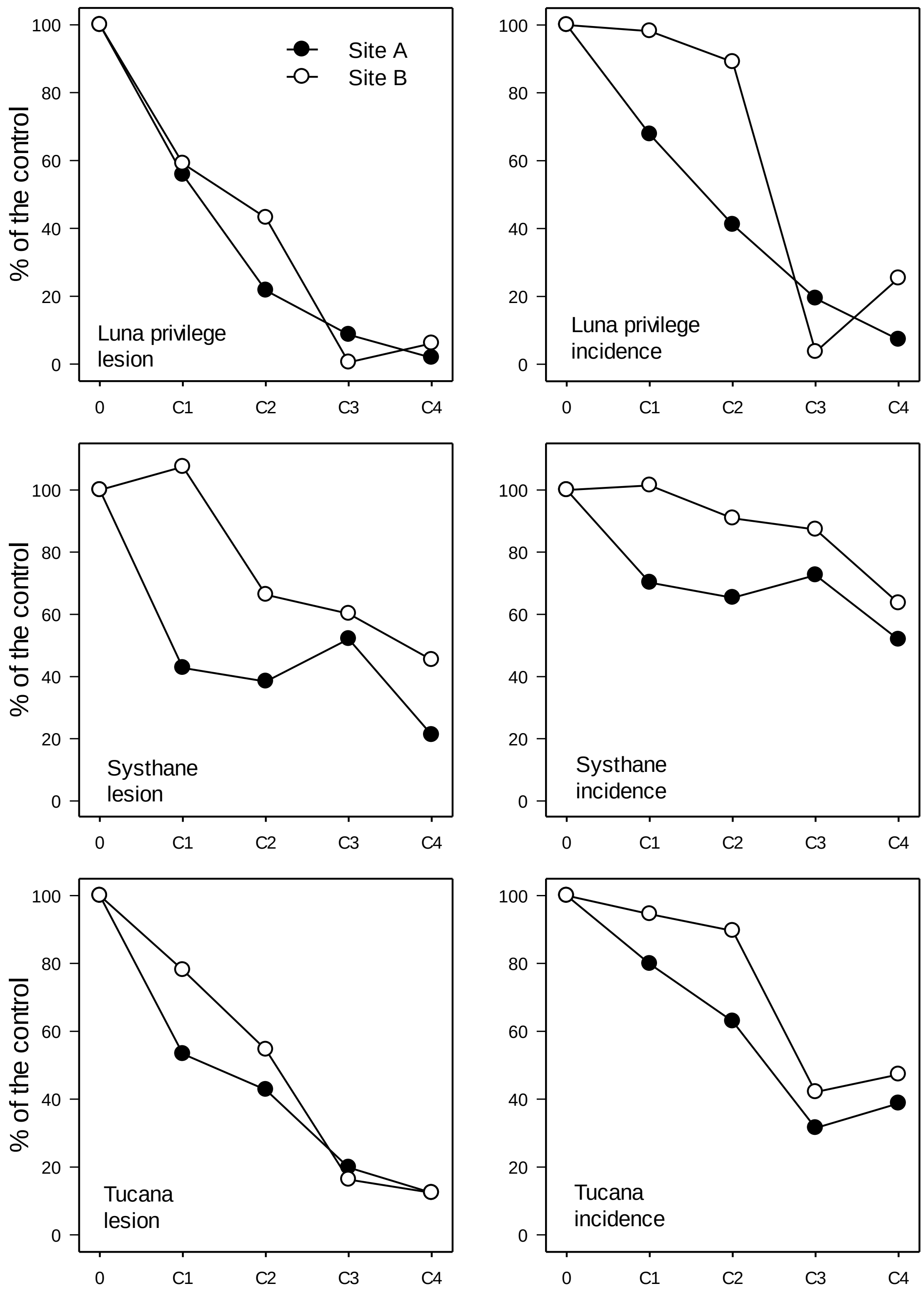

Figure 4. Average number of lesions per leaf and incidence of leaves with mildew on seedlings of cv. *Malus bittenfelder* to powdery mildew for 48-72 h at three

sites in July 2012. The exact concentration for each fungicide and location were given in Table 1 and Table 2, respectively. At two sites, no mildew lesions were observed.

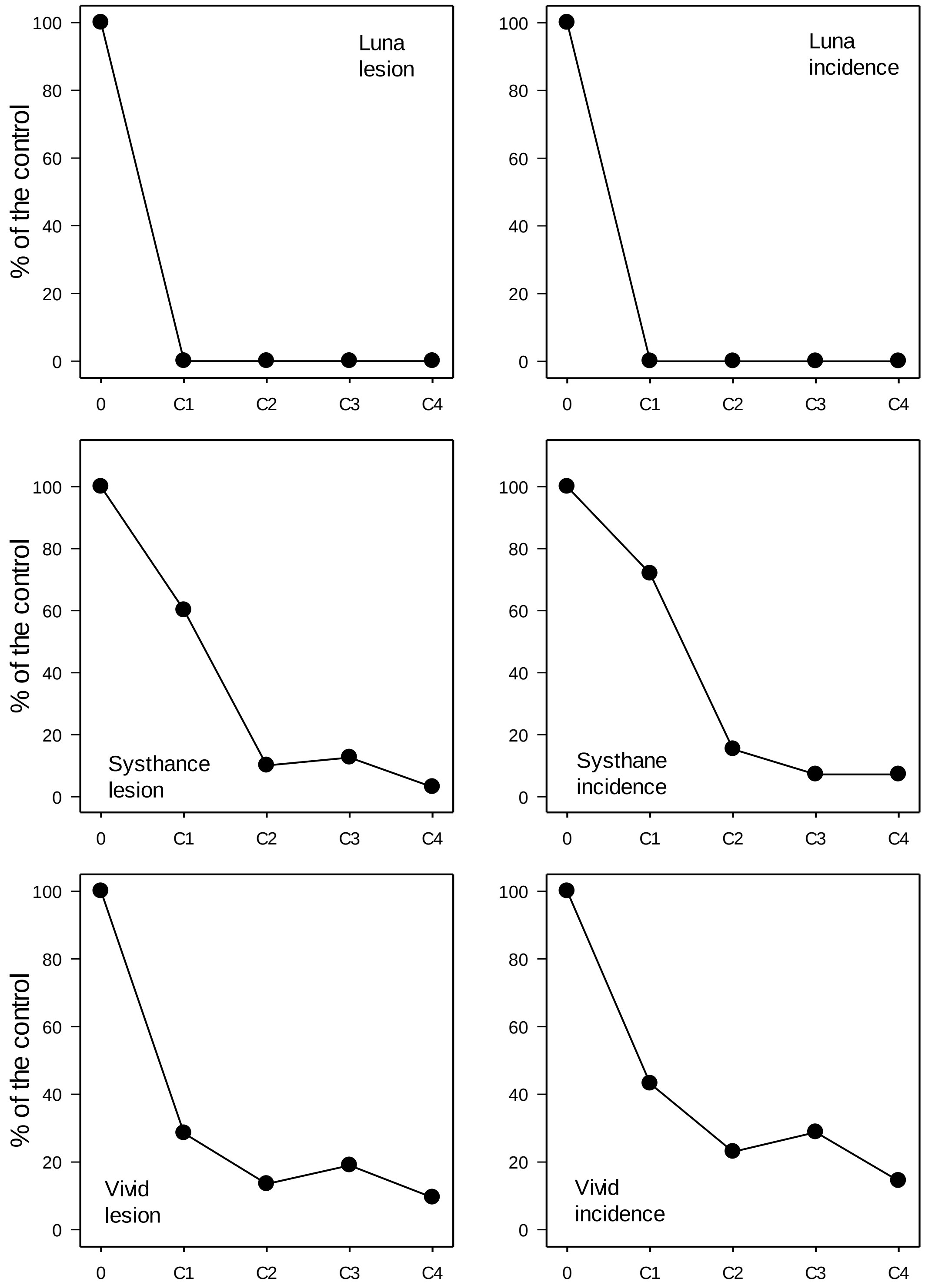

Figure 5. Average number of lesions per leaf and incidence of leaves with mildew on seedlings of cv. *Malus bittenfelder* to powdery mildew for 48-72 h at one site in 2013. The exact concentration for each fungicide and location were given in

Table 1. Only one of the 16 (exposure x site) combinations had sufficient number of mildew lesions.

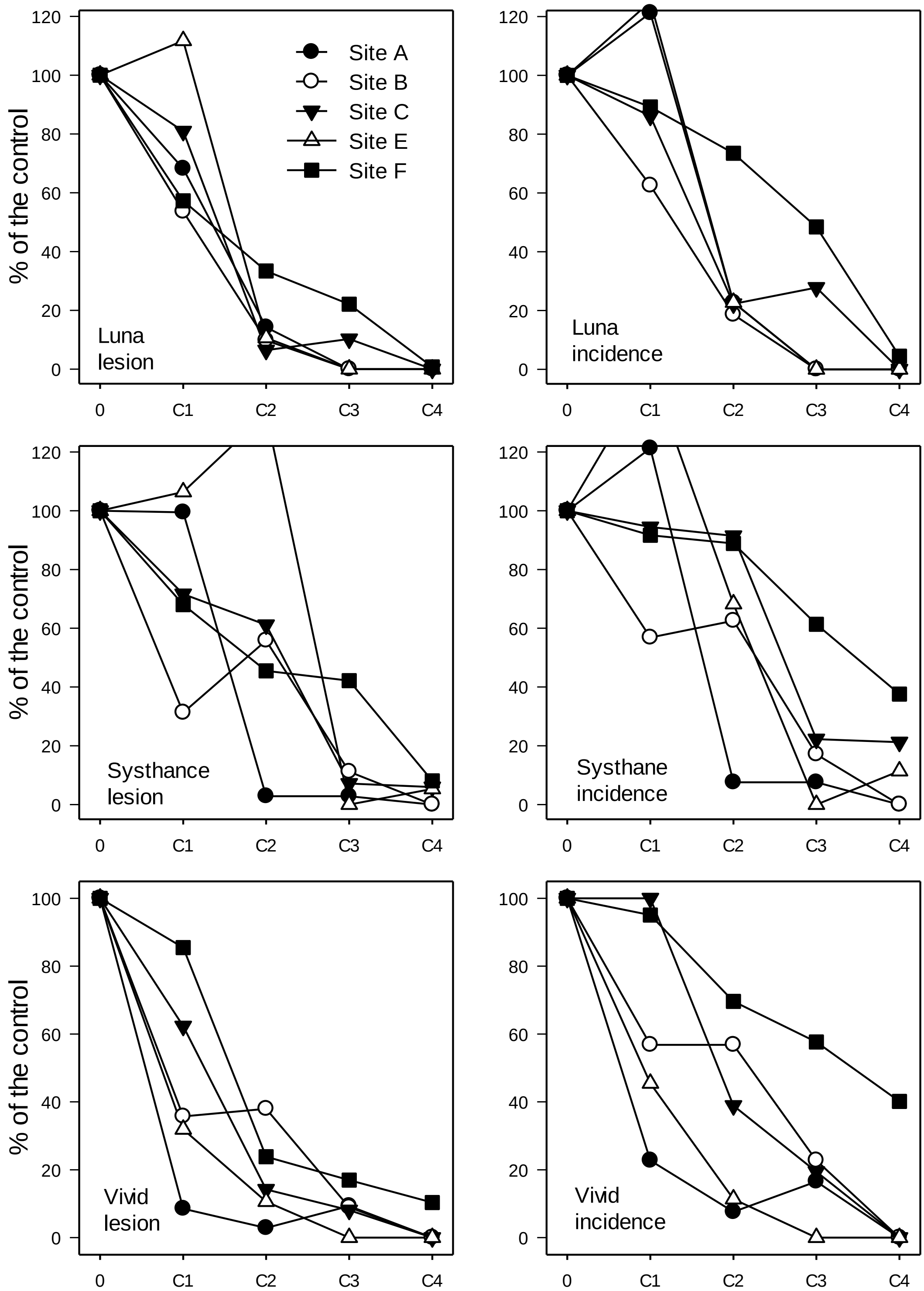

Figure 6. Average number of lesions per leaf and incidence of leaves with mildew on seedlings of cv. *Malus bittenfelder* to powdery mildew for 48-72 h at three sites in May 2013. The exact concentration for each fungicide and location were given in Table 1. Only five exposure x site combinations had sufficient number of mildew lesions.

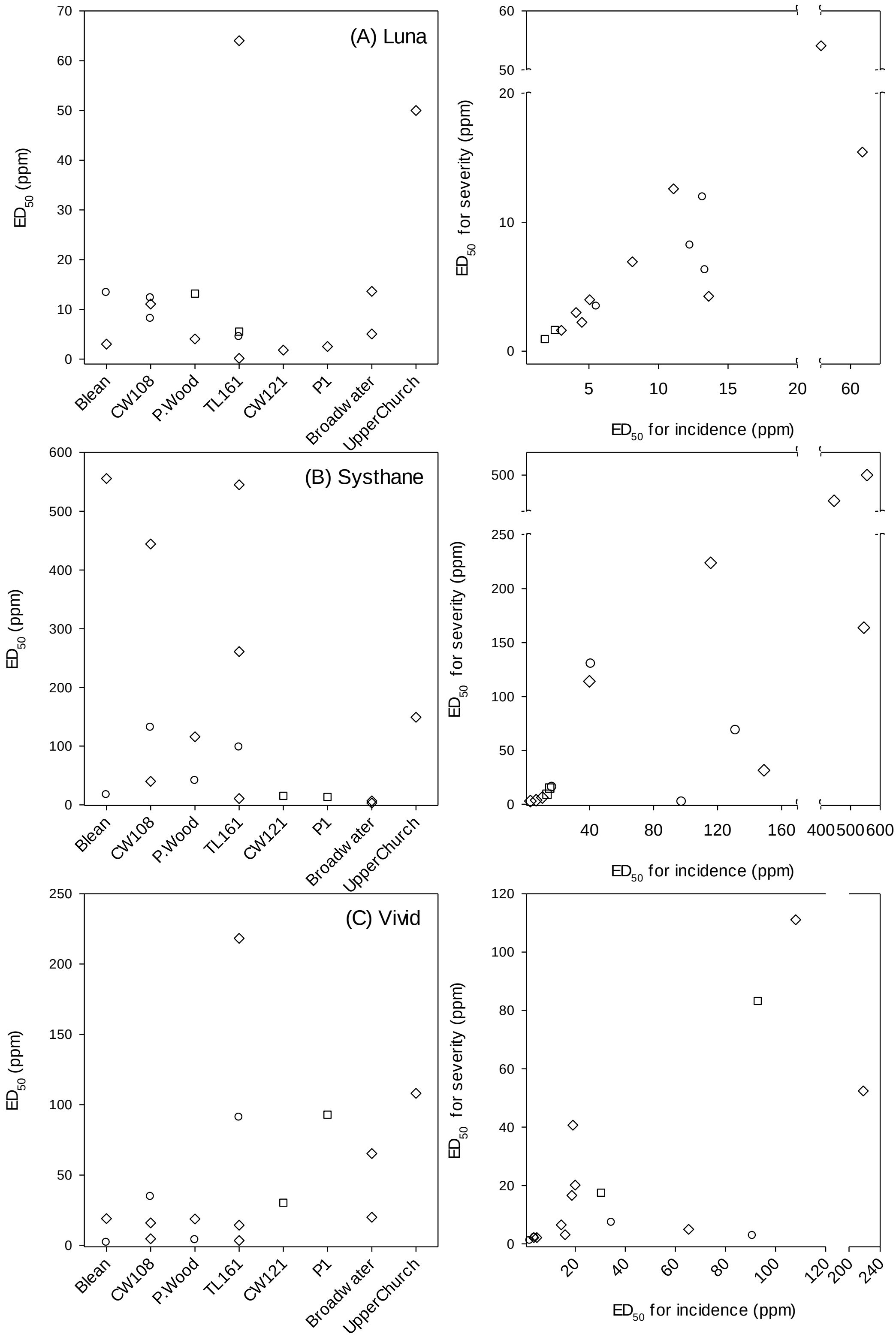

Figure 7. Estimated ED_{50} values for the incidence data from exposure experiments against the three fungicides (Luna Privilege, Systhane and Vivid) across different sites, and the relationship in the estimated ED_{50} values between the incidence and severity data (different symbols represent different exposure experiments).

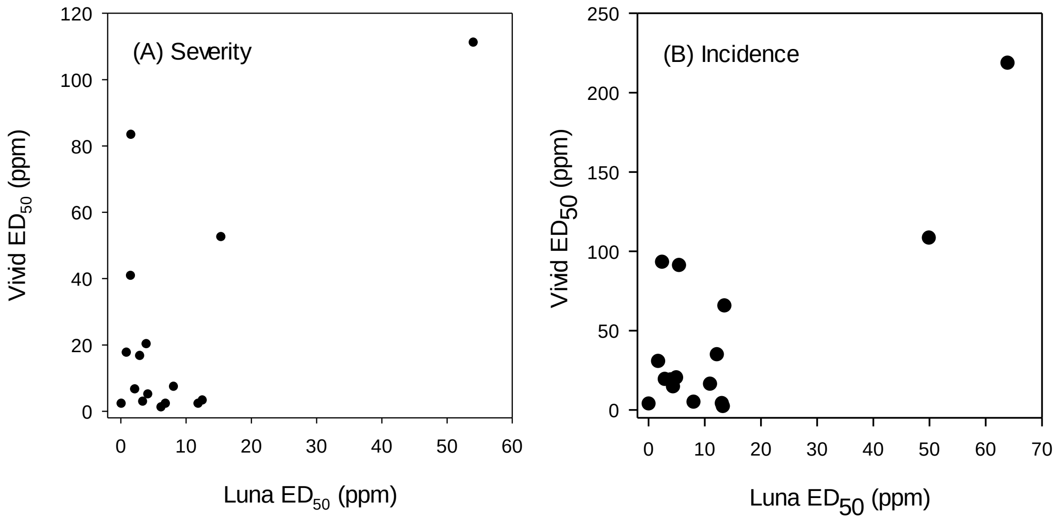

Figure 8. Estimated ED_{50} values for Luna Privilege plotted against those for Vivid: (A) severity data, and (B) incidence data.

Discussion

Through the exposure experiment, we determined the dose-response curves of mildew to Luna Privilege, Systhane and Vivid at several sites in Kent over two years. The ultimate aim was to see whether there is evidence for reduced sensitivities to myclobutanil (Systhane). Unfortunately, despite the huge effort in conducting the exposure experiments, only a small proportion of these experiments yielded meaningful results: 16 out of a total of 60 exposure studies. This is very disappointing, particularly as we cannot find any plausible explanation for this. Rootstocks and seedlings are known to be very susceptible to powdery mildew as shown in the other 16 exposure studies. There were plenty of powdery mildew lesions in the orchards where we placed the plants. Weather conditions were also suitable for mildew infection during the exposure period. This just illustrates the difficulties in conducting such studies for powdery mildew – an obligate pathogen. Indeed, fungicide resistance has been studied for powdery mildew on just the few annual plant species where *in vitro* maintenance of mildew strains on detached leaves is feasible (such as cereal crops).

Of the three fungicides, the ED50 values are the largest for Systhane and least for Luna Privilege. Although there were large differences in the ED50 values for Systhane between different exposures, this large variability on its own cannot be interpreted as evidence for reduced sensitivities. Normally, we would expect that ED50 values should be more consistent among different exposures at the same sites than between sites if reduced sensitivities are present in some orchards. However, the within-site variability in ED50 values is as great as the between-site variability. Unlike for Luna Privilege and Vivid, this large within-site variability is not limited to a single site. This could be due to sampling errors but could also be explained by the fact the reduced sensitivity to myclobutanil is wide spread across Kent (or at least at those sites we have data for multiple exposure studies) but has not completly dominated the mildew population yet at these sites. Thus at these sites, the sampled population of mildew strains at a given exposure depended on time (in relation to past sprays in the same season), fungicides used and possible fitness cost due to reduced insensitivity to fungicide(s).

It must be stressed that further data are needed to assess whether this speculation is valid. Most importantly, we have identified sites that can serve as ideal sites for studying the fungicide resistance problem in apple powdery mildew. One such site is the TL161 orchard, since at this orchard two exposures resulted in very high ED_{50} values against all three fungicides. CW108 at EMR and the orchard in Blean are interesting sites in that the large within-site variability in ED_{50} values is only observed for Systhane. Finally, we have two sites for which ED_{50} values from multiple exposures are low for all three fungicides. These five sites will provide sites for future studies to assess the extent of mildew resistance against one or more fungicides in apple.

Conclusions

- We have established mildew response to a range of concentrations of three fungicides at several orchards
- For some unknown reasons, only 16 out of 60 exposure studies resulted in sufficient number of lesions for statistical analysis
- Of the three fungicides, the differences in the dose-response of mildew among sites tested were greatest for Systhane
- Furthermore, the largest within-site variability in the dose-response of mildew at a given site was also greatest for Systhane
- These **limited** data suggest the possible existence of reduced sensitivities to Systhane and to a lesser extent to Luna Privilege and Vivid. Now we have several orchards that can be used for further studies to confirm this preliminary conclusion

Technology transfer

- We discussed the project work with several consultants and briefly discussed this work during the HDC Agronomists Day at East Malling Research in 2012