Sclerotinia stem rot field nursery for evaluation of resistance and fungicide efficacy

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Abstract

A two year study was initiated to design, construct and make operational a field scale Sclerotinia stem rot (SSR) disease nursery structure, in which infection would not be dependant on the weather, but rather relied upon due to the controlled environment within the nursery. Despite heavily inoculation with highly virulent *Sclerotinia sclerotiorum* ascospores and provision of humidity by misting throughout the latter half of the canola growing period, no SSR symptoms were observed in the nursery in year one (2006) of the study. In light of the disappointing performance of the pilot-scale nursery, the expansion of the nursery planned for year two was cancelled; however improvements to the structure were made to improve the chances of developing infection. The operational data gained in 2006 were valuable and were used to modify nursery design and operation in 2007. This included utilizing a better water source, reducing lateral air movement through the tent, adding another fogging unit to increase relative humidity, and improving inoculation methods. Application of these improved methods in concert with the modifications to the nursery structure, allowed SSR symptoms to develop on canola plants in the nursery in 2007. Despite the improved performance, statistically significant treatment effects could only be measured on plants treated with fungicides because overall disease severity and incidence remained low. Effects of genotype on disease development were either too subtle to be detected, or the genotypes tested were in fact not SSR-resistant. Environmental data collected automatically in 2007 indicated that the nursery structure was able to increase relative humidity substantially, but also showed that the difference in humidity from ambient conditions became very small during the warmest part of each day. This daily dry period may have been sufficient to prevent the SSR epidemic within the nursery structure from building to levels high enough to detect genetic resistance. Given the low levels of SSR over both years of the study, we conclude that the nursery cannot reasonably be used for screening for disease resistance.

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of Sclerotinia stem rot (SSR), can cause severe economic damage to Argentine canola *Brassica napus* L. Estimated yield loss to canola growers caused by SSR is approximately 0.5% (del Río et al, 2007) to 1.3% (Kirkegaard et al, 2006) of the potential yield for every percent increase of SSR incidence; the "rule of thumb" for western Canada is yield loss is approximately one-half of the percentage of symptomatic plants. Economic losses can be estimated by multiplying the estimated yield loss by the estimated (non-diseased) yield and the anticipated sale price. Using this formula and March 2007 prices, this is equal to approximately $3.05 per acre for each percentage point of infected plants

Currently, the most effective methods of managing SSR in canola are rotation with non-host crops and application of foliar fungicides. Because of the persistent nature of sclerotia of *S. sclerotiorum* as well as migration of inoculum from neighbouring fields, crop rotation alone is not always an effective SSR management tool. The efficacy of foliar fungicides is governed in part by environmental conditions, requires application at the correct crop and fungal developmental stages, and is also a significant input cost. For this reason, the economic benefit of fungicide application can be questionable unless disease pressure, canola prices, and yield potential are sufficiently high. If it were available, genetic resistance to SSR would be an attractive option to canola growers, since reliance on fungicides would lessen, and canola production would become more profitable.

An important part of the development of genetic resistance to SSR or any other disease is screening putatively resistant germplasm in order to confirm that resistance genes have been successfully incorporated into breeding lines. Initial testing of SSR resistance can be done in growth chamber or greenhouse tests, but must also be done in field trials to ensure resistance is effective under production conditions and across different locations or environments. Testing of disease resistance in field nurseries is standard practice for canola cultivar development with

respect to several diseases, including Blackleg (*Leptosphaeria maculans*) or Fusarium wilt (*Fusarium oxysporum*). In the case of Sclerotinia stem rot resistance however, the difficulty in reliably producing sufficient disease pressure in field nurseries has been a serious impediment to developing and testing SSR resistant canola varieties; for this reason, reports of successful screening of germplasm for SSR resistance in field nurseries are somewhat rare, and results are variable ((Li et al, 2003;Li et al, 2006)). Even though genetic resistance in *B. napus* have been generated using several different approaches ((Hennin et al, 2001;Zhao &Meng, 2003;Zhao &Meng, 2003;Liu et al, 2005a;Liu et al, 2005b;Zhao et al, 2006;Zou et al, 2007;Jin et al, 2007)) , and despite existence of laboratory or greenhouse-based methods for confirming introgression of resistance genes (e.g. (Zhao et al, 2004;Bradley et al, 2006)), the lack of a reliable method for evaluating putative resistance under field conditions remains a serious impediment to bringing SSR resistance into commercial use.

ARC has had success in building and running SSR field nurseries. A small-scale nursery constructed in 2004 was able to produce severe epidemics in canola in 2004 and 2005; like some other Sclerotinia nurseries, this nursery is equipped with overhead misting nozzles, but plants are also covered with a tent structure during the inoculation and disease development phases of nursery trials. The plants in the nursery were inoculated with laboratory-produced *Sclerotinia sclerotiorum* ascospores. Unfortunately, the small size of this nursery limited the number of entries that could be tested to a few dozen in short, single-row plots. The small size also and precludes use of the nursery for fungicide testing, since collection of yield data are often a requirement of such tests. An additional problem with the existing nursery was the excessively high stem rot disease severities attained in the nursery in 2004. The disease exceeded the ability of currently-available stem rot resistance genes to reduce symptom development, and as a result no statistically significant genotype effects could be measured. This situation was partially remedied in 2005, when disease severity was sufficiently high to obtain useful resistance evaluation data, but no so high that expression of resistance was overwhelmed by the disease epidemic. This more optimal level was obtained by reducing the inoculum load in the nursery, and by reducing the number of inoculations. The price for reduced disease pressure was decreased uniformity of infection across the nursery.

We set out apply past experience gained with the small SSR nursery structure to construct a larger structure that would allow larger plot sizes (and therefore large and more statistically robust data sets), and to design a system that could be expanded to accommodate a commercially viable number of entries. The objectives of this project were:

1. Construct and evaluate a pilot-scale SSR nursery, and use the nursery to determine construction parameters, experimental design and sample size required for a full size nursery. Design to be based on information and experience gained from AARI Project 2002A103R (Introgression and Molecular Characterization of Multiple Disease Resistance Traits from *Brassica carinata* into *B. napus*). A sub-objective will be to determine suitability of the facility for fungicide evaluation.

2. Build a full scale nursery using information gathered in Objective 1, and use the nursery for cultivar (and fungicide) evaluation.

Materials and Methods

Design and Construction

The 1680 square meter nursery consisted of 14′x 6″x6″ pressure treated timber posts inserted 4′ in the ground spaced 5 to 7 meters apart. To form walls and a ceiling, the top and bottom of the posts on the perimeter of the nursery and the tops of all other posts were joined by 3/16″ steel rope, to which secured was secured a shade-cloth (Green-Tek, Edgerton, WI, USA, 50% black) using grommets and 3/16″ spring hooks spaced at 2′ intervals. The final height of the ceiling was 10′ high in order to accommodate plot equipment (Figures 1 to 4). Detailed construction drawings are available from the Alberta Research Council on request.

The entire nursery, enclosed with shade cloth, was humidified with three large capacity, oscillating misting foggers (Aquafog TurboXE, Jaybird Manufacturing Inc., State College, PA USA) supplied with water from a nearby pond. Metal stands were constructed in order to mount the foggers two meters from the ground. Electricity was generated by a large capacity diesel powered generator. The foggers and oscillating motors operated on 220 volts AC, however the generator also provided 120volts to power the water pump that supplied the foggers with 60psi of pond water pressure. Two 4500 l holding tanks located near the generator were periodically filled with water from the pond by a 3.5 hp transfer pump.

Climate data was collected by a portable data logger that recorded temperature and relative humidity in the nursery structure, confirmed by daily manual measurements inside and outside of the nursery structure.

Figure 1 Aerial view of the SSR disease nursery at the Alberta Research Council research farm in Vegreville**,** AB. The image was made in 2006, and shows the shade cloth structure, water supply tanks, and generator.

Plot establishment and Inoculation in 2006

In each year of the study, only ½ of the nursery was used for canola plots to prevent contamination of plots in the second year with canola volunteers. Two experiments were seeded n half of the nursery structure used in 2006; a fungicide experiment to test the inoculation efficiency of the nursery structure environment, and a test of forty canola genotypes with putative stem rot resistance. All plots consisted of four 3.5m-long rows spaced 20cm apart, and were placed in a randomized complete block design. Fungicide treatments were i) an non-inoculated treatment sprayed with water only (no fungicide), ii) an inoculated, water only, treatment, iii) inoculated plots sprayed with Boscalid as Lance 70% WDG at 142 g product per acre at the 20% flowering stage, iv) inoculated plots sprayed with Iprodione (Rovral Flo 50% WP, 1253 ml product per acre) applied at 50% flowering, v) inoculated plots sprayed with Lance and Rovral at 20% and 50% flowering, respectively, and vi) inoculated plots sprayed with Lance at first flower, plus the two fungicide sprays applied in the previously-listed treatment.. The unused half of the nursery was seeded with peas as a rotation crop. Prior land use for the nursery location was an established mixed grass hay field. Glyphosate application followed by multiple disc and rototill operations ensured a good seedbed.

Prior to seeding, the entire nursery structure was fertilized with a Gandy broadcast spreader and incorporated with a rototiller. Plots were seeded on June 13 with a ten row double disc cone seeder which also applied 30lbs./acre 11-51-0 fertilizer with the seed. Fertilizer rates were determined using soil tests. All equipment used in the nursery structure nursery was carefully considered due to height constraints imposed by the steel rope supports for the ceiling, which was furled until a few days prior to inoculation with *S. sclerotiorum* ascospores. Emergence and establishment of the crop were uniform (Figure 2).

Figure 2 Rosette-stage canola plots established in the nursery structure in 2006. Note the furled ceiling along the tops of the supporting posts, and wire rope ceiling supports.

Ascospore inoculum was produced using the protocol given in Appendix 1. Inoculation of the canola with a previously proven virulent isolate of *Sclerotinia sclerotium* at $1x10^5$ ascospores•ml^{-1} occurred in the evenings during the 20-100% flowering stage on . The inoculum was applied with a CO^2 back-pack sprayer, with a two nozzle wand (spacing 50cm) using Lurmark 01-F110 nozzles operating at 20psi. The initial inoculation was performed on 1 August, followed by a second inoculation, fourteen days after the first. For the second inoculation, ascospores were only applied to one half of each plot to allow evaluation of the effect of the second inoculation.

Immediately prior to each inoculation, Petri-dishes filled with potato dextrose agar were set out in the upper, mid and lower canopy of a randomly-selected plot, then retrieved, covered and incubated at room temperature on a laboratory bench until fungal growth became visible. Viable inoculum was considered to have reached the plants if *S. sclerotiorum* mycelium could be identified on the sprayed plates. Application of inoculum within the canopy was deemed to be evenly distributed if all three plates produced *S. sclerotiorum* mycelium.

Weed populations were very low in 2006, allowing hand weeding of plots with no herbicide treatments being necessary.

To test the effects of water-borne precipitates deposited on leaves by the misters, 500 µl of sterile distilled water (SDW) were placed onto one of two large *B. napus* leaf collected on 23 August, 2006. The leaves were heavily covered with the fine grayish-white precipitate, but were otherwise healthy. Once placed on the leaf, the droplet rubbed with a rubber policeman, and the droplet then transferred to the second leaf. The procedure was repeated on the second leaf, and the fluid transferred to two Petri-plates filled with potato-dextrose agar (PDA) which had been evenly

inoculated with a suspension of $1 \times 10^5 \cdot ml^{-1}$ *S. sclerotiorum* ascospores in SDW. Control plates were inoculated with the ascospore suspension but not the leaf wash fluid. Plates were incubated on a laboratory bench for 14 days before observations for growth of *S. sclerotiorum* and other organisms were made.

The water source used for misting the nursery in 2006 was also tested for effects of viability of *S. sclerotiorum* by suspending ascospores ($1 \times 10^5 \cdot ml^{-1}$) in water collected from the source (pH7.73), municipal (tap) water, or SDW. A 100 µl droplet of each suspension was spread over PDA in each of three Petri plates, which were then incubated for 56 days on a laboratory bench.

Design modifications in 2007

In year two, lateral air movement was reduced significantly by replacing the shade cloth walls with a durable polyfabric (Inland Plastics, Drumheller, AB. #1455 U.V.I). Ten foot long 2"x6" boards were screwed upright to the posts in order to help retain the tarpaulin in the wind. The shade cloth ceiling remained unmodified (Figure 3).

A fourth misting fogger was added, enabling placement of a fogger in each corner of the nursery. Unlike year one, only the portion of the nursery structure with trials was enclosed, reducing the total enclosed space to be misted by 50%. Municipal water from the nearest source was supplied by an above ground line which eliminated the need to use pond water.

Plot establishment and Inoculation in 2007

The seeding and fertilizing methods used in 2007 were identical those used in 2006. The half of the nursery structure that had been seeded to peas in the previous year was used for three canola trials in 2007. All trials were arranged as four-replicate RCBDs, and seeded on 17 May. Plot length and row spacing were the same as those used in 2006. The first experiment was a fungicide trial, with all plots of the same canola variety. A total of ten fungicide treatments were applied, plus a water control. Plots were four rows wide. The second trial was a comparison of experimental lines with and without the protection of a fungicide (Lance) applied at the same rate as in 2006 at 20% bloom; six rows were seeded per plot, The third trial compared varietal lines with no fungicide component; each plot had four rows.

Seed germination and seedling growth were excellent; herbicide application (Lontrel and Muster as a tank mix applied at label rates and timing) was necessary and was applied a CO^2 back-pack sprayer, with a two nozzle wand (spacing 50cm) using Lurmark 01-F110 nozzles operating at 40psi.

The surfactant (Tween 80, Croda International Plc, UK) was added to the inoculum to help distribute ascospores evenly across plant surfaces. A preliminary laboratory spray trial was conducted to confirm the efficacy of surfactants as well as their lack of toxicity to *S. sclerotiorum* ascospores. Three pots with one *B. napus* plant per pot were sprayed with a 5×10^5 ascospore$\cdot ml^{-1}$ suspension in water alone or suspended in water amended with 0.05% Tween 80 or 0.05% SilWet L-77 (GE Silicones, Friendly WV, USA). The plants were allowed to dry, then sprayed again in the same manner to simulate two field inoculations. The same sprayer, nozzles and spray pressure used for field inoculation in 2006 were used. When the plants had dried, five flowers (twenty petals) were collected from each plant, re-immersed in 1ml of 0.01% Tween 80 and vortexed vigorously to remove ascospores from petal surfaces. After resuspension, the spore suspensions were centrifuged at 3000 rpm for one minute to concentrate ascospores, and fluid volume reduced to 150µl. Each ascospores in each tube were then re-suspended by vortexing, and ascospore counts taken using a haemocytometer. The suspensions were used within four hours to inoculate PDA plates to confirm ascospore viability. Unused suspensions were kept on

the lab countertop at room temperature for three days, then examined macro- and microscopically for effects of the surfactant treatments.

Figure 3 Modifications to the nursery design in 2007. Upper left photo shows the solid walls that replaced the permeable shade cloth side walls used in 2006. Photos on the upper right and lower left are interior view of the nursery showing the solid walls and the additional misting unit installed in 2007; Campbell Scientific instrumentation is also visible in the latter image. The diesel generator used to power the nursery in seen in the lower right image; water tanks were not necessary in 2007 as the nursery was supplied directly from municipal water supply.

In 2007, each canola trials was inoculated on 9 July, 12 July, and 18 July with the same *S. sclerotiorum* isolate used in 2007. Spore density of the suspension was fixed at 1×10^5 ascospores•ml^{-1} amended with 0.05% Tween 80. Inoculations took place in the evening at the 20-100% flowering stage of canola (different *B. napus* genotypes varied widely with respect to growth stage), with the misting foggers turned off during inoculation and the following night. Fogger operation was started on 10 July, and turned off on 20 August, and run continuously except for inoculation and brief maintenance intervals.

Trials were evaluated according to the scales given in Table 1. The Henson scale was used for the genotype trials, and Kutcher scale used for the fungicide experiments, at the request of the co-operating companies. For the fungicide experiment, five leaves were also collected from each plot and digitally photographed on a blue background. Assess Image Analysis Software (©2002 The American Phytopathological Society) was used to determine the percentage of each leaf that exhibited lesions from *S. sclerotiorum*. Software settings for leaf and lesion thresholds were Hue (31-206) and Hue (0-129), respectively.

All data were analysed using PROC GLM of SAS version 9.1 (SAS Institute, Cary NC, USA).

Table 1 SSR severity rating scales used in this study.

Score	Henson	Kutcher
0	No symptoms of disease	No symptoms of disease on plant
1	Main stem with small non-girdling lesion <30 mm OR girdling of primary branch OR secondary branch dead	A few pods affected
2	Main stem with large non-girdling lesion >30 mm OR 1-2 primary branches dead	One major branch girdled; ¼ of the plant affected
3	Main stem at least 50% girdling OR more than 2 primary branches dead	Two major branches of the plant affected; ½ of the plant affected
4	Main stem fully girdled but plant still alive OR a number of primary branches dead	Three of more major branches girdled; ¾ of the plant affected
5	Prematurely ripened or dead plant	Main stem lesion affecting entire plant

Results and Discussion

Year 1 (2006)

No SSR symptoms were observed in the nursery in 2006. Environmental conditions coinciding with the flowering stage were hot and dry which did not favor infection. Figure 5 illustrates how high humidity was obtained during the evenings, night, and morning. However, the nursery was unable to maintain high humidity during the afternoon and early evening, even with the three humidifying foggers running at constantly at full capacity (Figure 5).

Approximately 7500 liters of pond water were dispensed by the foggers every 24 hours. After several days of fogging, a precipitate from the pond water was visible on plant leaves and the fogging equipment. Growth of *S. sclerotiorum* mycelium from ascospores suspended in this water was not different from mycelium originating from ascospores suspended in tap water or SDW. Ascospores suspended in water rinsed from leaves covered with the precipitate formed after more than two months of misting were no less viable than ascospores suspended in SDW. We therefore conclude that the water-source or the precipitates originating from this water source were not detrimental to the viability of the ascospore inoculum.

Growth of *S. sclerotiorum* mycelium in Petri-dishes set out in the plant canopy during inoculation indicated that the inoculum was viable when applied, and had penetrated into the lower canopy during the application process.

Fourteen days after the first inoculation, plant sections were taken from *B. napus* stems collected in the nursery that appeared to be developing early symptoms of SSR. Surface sterilization and incubation of the collected stem sections revealed that the stems were not *S. sclerotiorum*, but with *Fusarium* spp. and *Alternaria* spp.

Lodging was observed in many plots in 2006, however the lodging was not due to stem rot as no stem rot symptoms other than lodging were observed. Individual plots were subjectively evaluated for lodging using a subjective 0 – 10 scale where a value of 10 was used to indicate complete lodging of all plants in the plot (Table 2). Analysis of variance indicated that lodging scores were not affected by plant genotype (P=0.5197), although the some entries were far more prone to lodging than adjacent border plots or to plot in the fungicide experiment (all affected plots were *B. napus* cv. 717RR). Plotting of lodging scores in space (Figure 11) showed that lodging became more common in blocks distant from the north side of the nursery (i.e. away from the outside edge of the nursery), suggesting that lodging was at least partially caused by

environmental factors. In addition, lodging in some plots may have encouraged lodging in adjacent plots, since racemes of neighbouring canola plants are usually interwoven with each other, causing lodged plants to pull their neighbours to the ground.

Table 2 Crop lodging in an experimental trial in year one among putative SSR-resistant canola breeding lines, 23 August 2006. Values of 0 represent plots with all plants standing, while values of 10 represent plots where no plants were standing.

	Lodging score (1 – 10)				
Entry	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
1	0	1	0	10	2.8
2	0	0	1	10	2.8
3	2	1	2	7	3.0
4	5	6	10	3	6.0
5	1	0	8	10	4.8
6	2	7	1	3	3.3
7	0	2	9	2	3.3
8	0	4	10	8	5.5
9	0	2	5	7	3.5
10	0	0	0	6	1.5
11	0	9	6	8	5.8
12	2	7	9	10	7.0
13	1	0	1	1	0.8
14	5	7	1	9	5.5
15	3	2	10	5	5.0
16	0	10	2	9	5.3
17	0	10	5	1	4.0
18	0	5	9	3	4.3
19	0	0	9	1	2.5
20	0	2	5	3	2.5
21	0	0	0	0	0.0

Replicate 1		Replicate 2		Replicate 3		Replicate 4	
Lodging Score	Entry	Lodging Score	Entry	Lodging score	Entry	Lodging Score	Entry
0	7	0	2	1	2	2	7
0	2	0	13	10	15	6	10
0	21	4	8	10	4	10	2
5	4	9	11	9	19	10	12
0	11	7	12	6	11	5	15
0	18	6	4	1	14	3	6
0	17	2	15	1	13	3	18
2	3	7	14	10	8	10	1
1	13	10	17	9	7	9	14
0	1	0	21	5	20	8	11
2	6	7	6	1	6	7	9
3	15	1	3	5	17	0	21
0	9	0	19	9	18	1	13
2	12	1	1	5	9	7	3
5	14	5	18	8	5	9	16
0	10	10	16	9	12	8	8
0	16	2	9	2	3	3	20
0	19	0	10	0	10	1	19
0	8	2	7	0	21	3	4
1	5	2	20	2	16	10	5
0	20	0	5	0	1	1	17

Figure 4 Distribution of lodged plots in the Sclerotinia nursery in 2006. Each box represents a single plot, numbers in boxes indicate the lodging score (0 – 10, where 10 is completely lodged) and entry. Plots are colour-coded to indicate severe (red), moderate (grey) or no lodging (no fill). North is to the left.

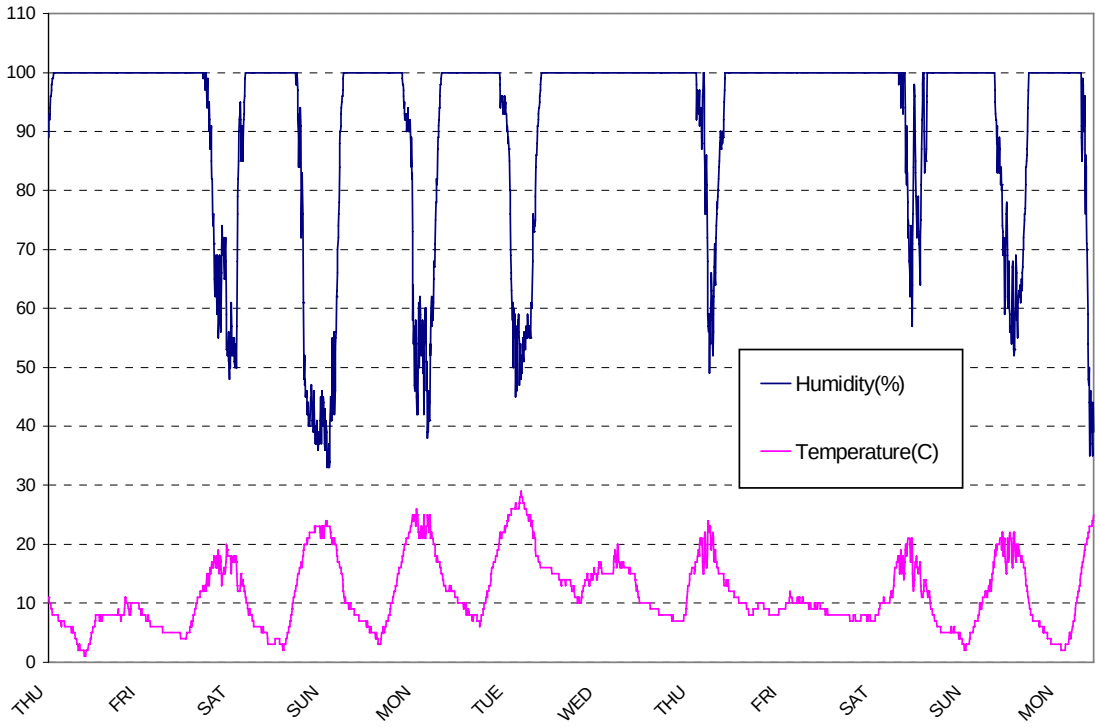

Figure 5 Example of relative humidity and temperature in the nursery over a ten-day period during the 2006 growing season.

Figure 6 Example of mist generated by fogger units in 2006. Note mist escaping from the open-mesh nursery walls. This image was made in the early morning; mist was not visible from mid-morning until early evening.

Year 2 (2007)

The number of ascospores recovered from suspensions amended with Silwet were greater than those suspended in either Tween 80 or water (**Error! Reference source not found.**). Neverthess, we concluded that Tween 80 was the optimal surfactant to use for field inoculations because Silwet appeared to affect water imbibition for the ascospores. After 48 hours in the surfactant solution, the ascospores in the SilWet suspension did not appear to have imbibed as they had in the water or Tween 80 suspensions. The potential toxic effect of Silwet was considered particularly important in cases where prepared inoculum suspensions could not be used immediately, for example if spraying were to be delayed by rain or other contingencies.

In the second year of the study, comparisons of temperatures inside and outside the nursery structure showed that temperatures within the nursery remained close to ambient (Figure 11). Temperatures within the structure varied within a range of approximately 1.5 °C cooler to 1.25 °C warmer than temperatures recorded outside the structure. Over the measurement period, the nursery tended to be cooler than ambient prior to 20 August, and warmer than ambient temperature after that date.

Average measured wind speed inside the structure was only 1.5 km/h below the ambient mean wind speed of 2.1 km/h, suggesting that wind speed inside and outside the structure did not usually differ by a substantial amount. However, the structure did appear to be effective in reducing extreme wind gusts significantly. The maximum 15-minute wind speed recorded outside the structure during the measurement period was 27.5 km/h, while the maximum wind speed inside the structure was 4.3 km/h.

On average, relative humidity inside the structure was 7.6% higher than ambient humidity (Figure 9). Daily mean relative humidity inside the structure was greater than ambient prior to 20 August, when the foggers were shut off. The mean relative humidity for the period of 12 July to August 20 was 12.2% higher than ambient, but was only 2.7% higher than ambient afterwards, indicating that humidity from the foggers was responsible for the greatest part of the increase over ambient. When these observations are taken together, it appears that factors humidity increases created by the structure's walls and roof, such as wind speed reductions, moisture renursery structureion, reduced evaporation due to shading or renursery structureion of humid air created by evaporation of moisture from soil were small in comparison to direct humidification via the foggers.

When averaged over periods longer than a day, it appeared that the nursery structure and foggers were able to create elevated relative humidity (Figure 9). Examination of Figure 10, however, will show that relative humidity inside the structure decreased to near-ambient in the warmest part of the day. Thus, the nursery was effective in maintaining elevated relative humidity only in the morning, evening and night, creating a relatively dry period during the day. This dry period may have important epidemiological consequences – daytime drying may be sufficient to reduce infection of canola plants by increasing ascospore mortality.

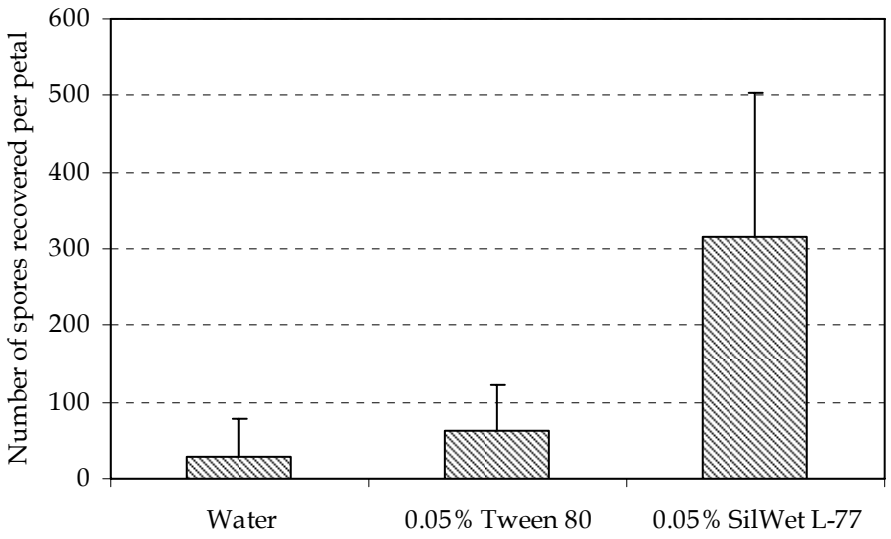

Figure 7 Ascospores recovered from *B. napus* petals sprayed with ascospores suspended in two surfactants. Error bars indicate standard deviation.

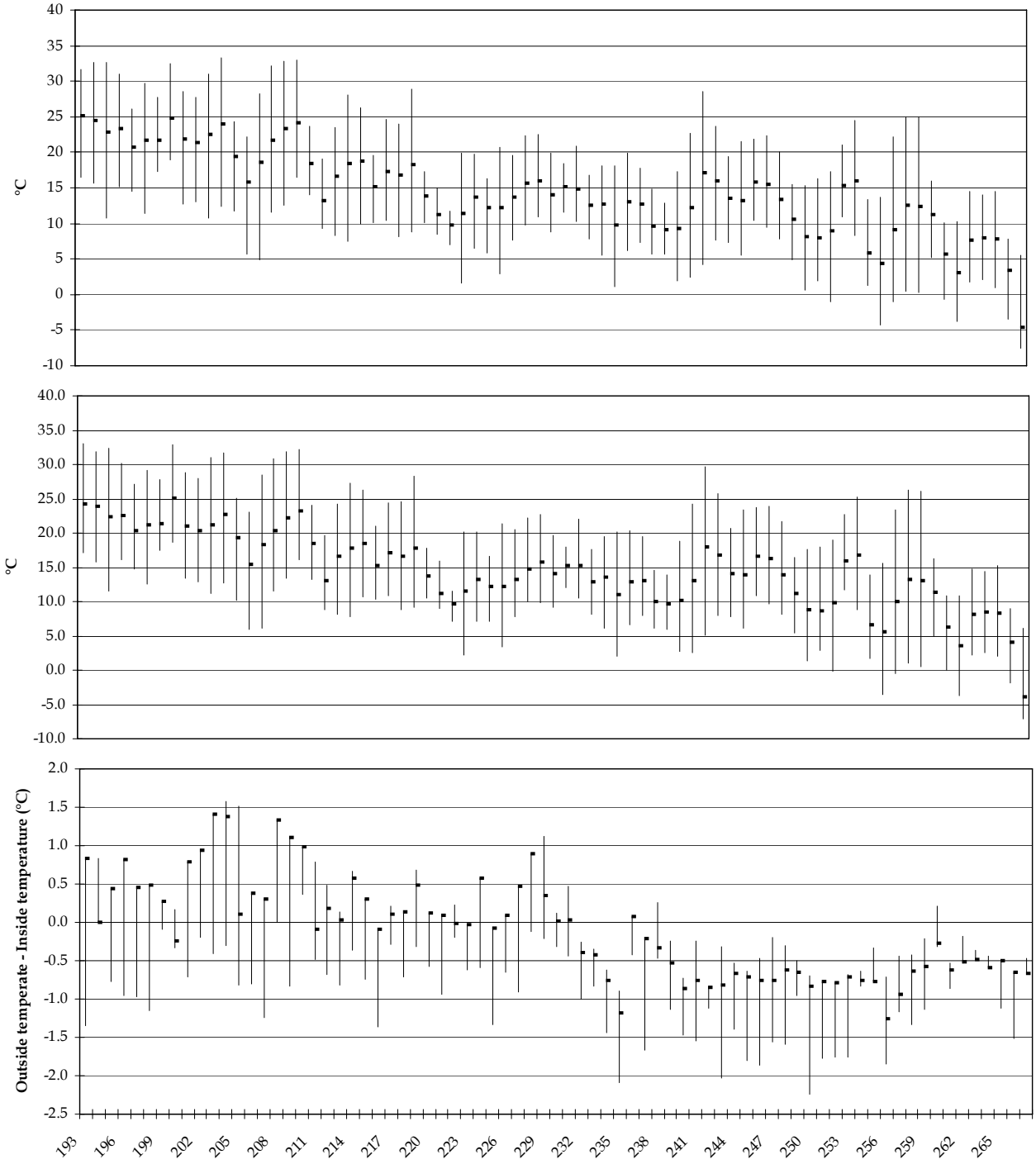

Figure 8 Daily average, minimum, and maximum temperatures outside (top) and inside (middle) the nursery structure between 12 July (Day 193) to 24 September (Day 267), 2007. The bottom chart gives the difference between daily average outside and inside temperatures.

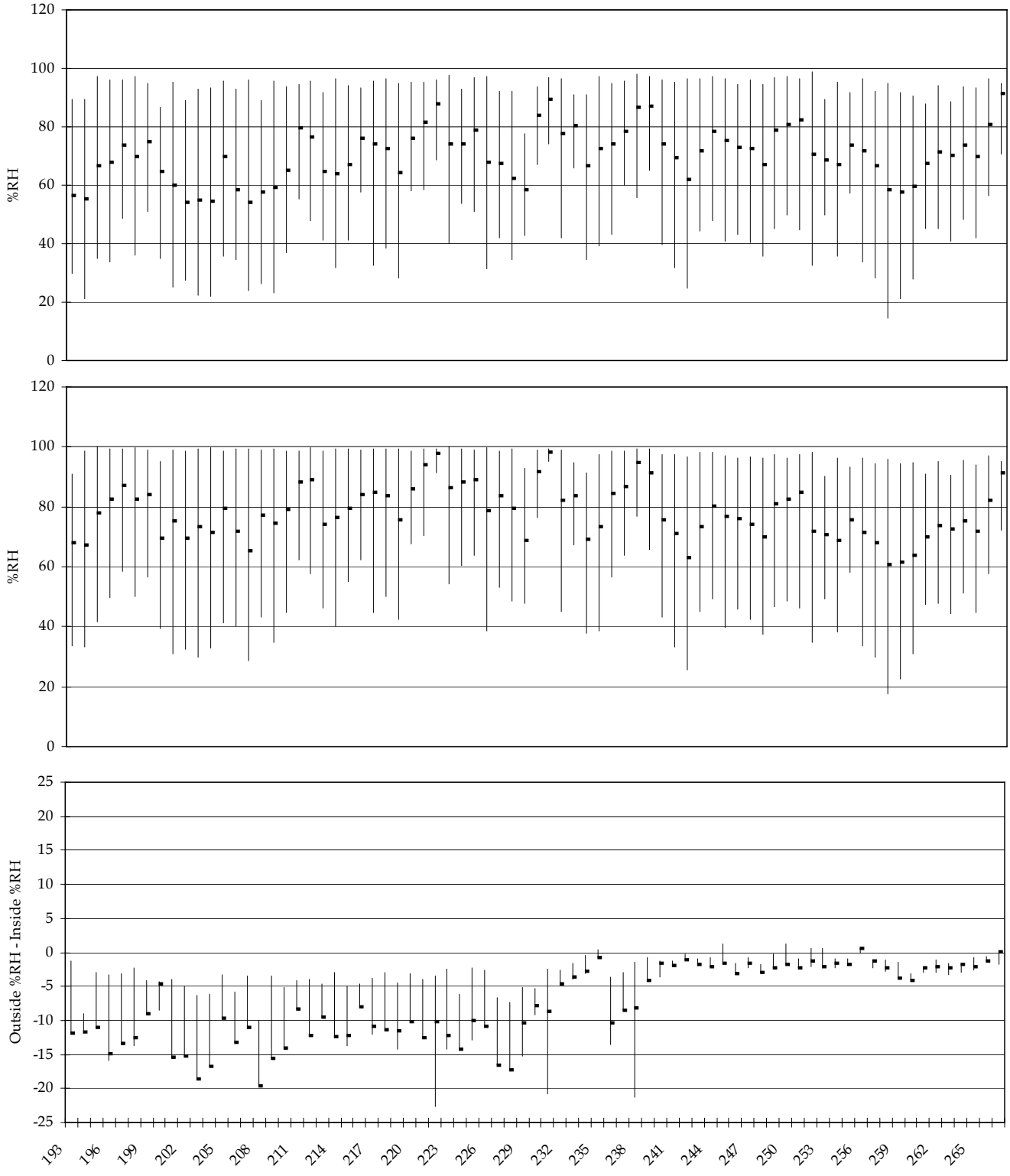

Figure 9 Daily average, minimum, and maximum relative humidity outside (top) and inside (middle) the nursery structure between 12 July (Day 193) to 24 September (Day 267), 2007. The bottom chart gives the difference between daily average outside and inside %RH

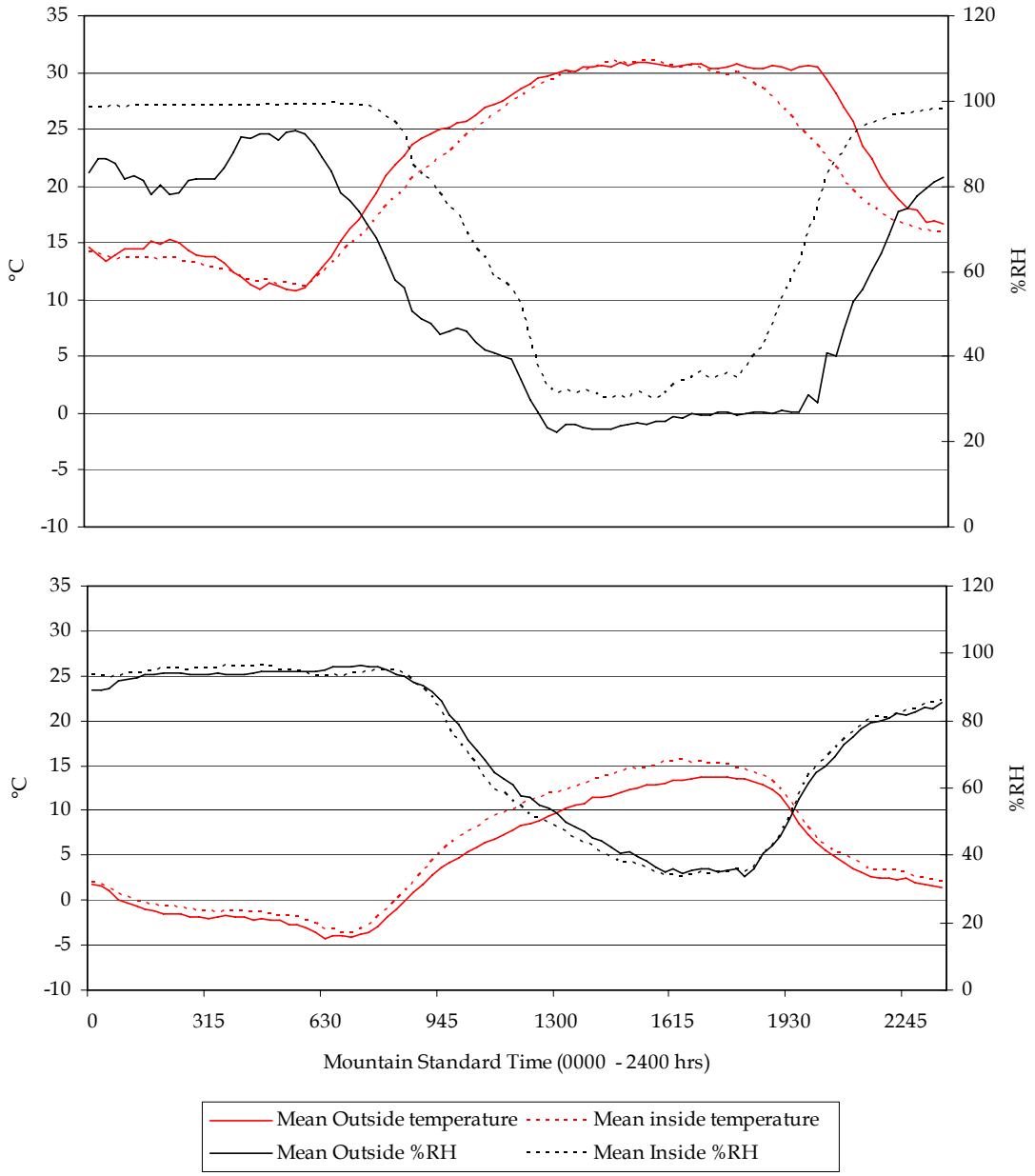

Figure 10 Temperature and relative humidity inside and outside the nursery structure over two 24 h periods. Data presented are hourly means of values recorded at 15-minute intervals on 22 July and 13 September, 2007.

Analysis of variance of the interaction between putative genetic resistance data collected in 2007 revealed no significant effects of genotype, or fungicide by genotype interactions (Table 3). The lack of genotype or interaction effects may have been due to absence of genetic resistance in the genotypes tested. The effect of fungicide on severity and incidence on stems was significant, and also had a significant effect ($P < 0.0001$) on leaf lesion size when non-significant treatment effects (genotype and the interaction effect) were pooled.

Figure 11 and Figure 12 show the response of SSR severity or incidence on stems and leaves, respectively. Overall disease severity and incidence were low; for example, maximum disease severity for any genotype was well below 0.5 on the 0 – 5 severity scale, and incidence less than

6% of all stems examined Figure 11. Experimental error accounted for a large portion of the observed variation among treatments, evidenced by the large standard deviation values recorded (Figure 11 and Figure 12) and by the high coefficients of variation observed for severity and incidence on stems (51.0% and 50.5%, respectively) and on leaves (128.6%). Low disease pressure resulted in few stems or leaves with lesions; i.e. observation of symptoms in the sampled stems and leaves were somewhat rare events, resulting in non-gaussian distribution of the observed data. Occurrence of a small number stems or leaves with lesions in a plot had a relatively large effect on the treatment mean and on variability among observations (and calculated standard deviations and coefficients of variation). Much of this problematic variation would have been avoided if disease pressure, and thus the proportion of symptomatic plants, had been greater.

In contrast, no significant effects of fungicide treatment were observed in the single genotype fungicide efficacy trial conducted on a single *B. napus* cultivar (Table 4). The plot size of this second experiment was smaller than that used in the fungicide × genotype experiment (four vs. six rows) although the number of plants analyzed per plot was the same (100). Since significant fungicide effects were noted in the former, but not the latter experiment, we conclude that the additional two rows may have been important for detecting fungicide effects. The use of the Henson rating scale in the fungicide × genotype experiment and the Kutcher scale for the fungicide-only experiment An alternative explanation may be the use of different rating scales in the two experiments (each at the request of the co-operating companies, see Table 1); the Henson scale used for the fungicide × genotype experiment may be more sensitive in situations when disease pressure is low.

Table 3 Analysis of variance of severity and incidence of SSR data recorded on stems and leaves of three putatively stem rot-resistant *Brassica napus* genotypes in 2007.

	Disease severity and incidence on stems, P>F			Disease severity on leaves determined by image analysis, P>F	
Source	DF	Severity	Incidence	DF	% Lesioned leaf area
Replicate	3	0.4898	0.7677	3	0.2940
Fungicide	1	0.0351	0.0469	1	0.0662
Genotype	2	0.6240	0.5402	2	0.9641
Fungicide × Genotype	2	0.1179	.0663	2	0.2209
Error	15	-	-	111	-
Total	23	-	-	119	-

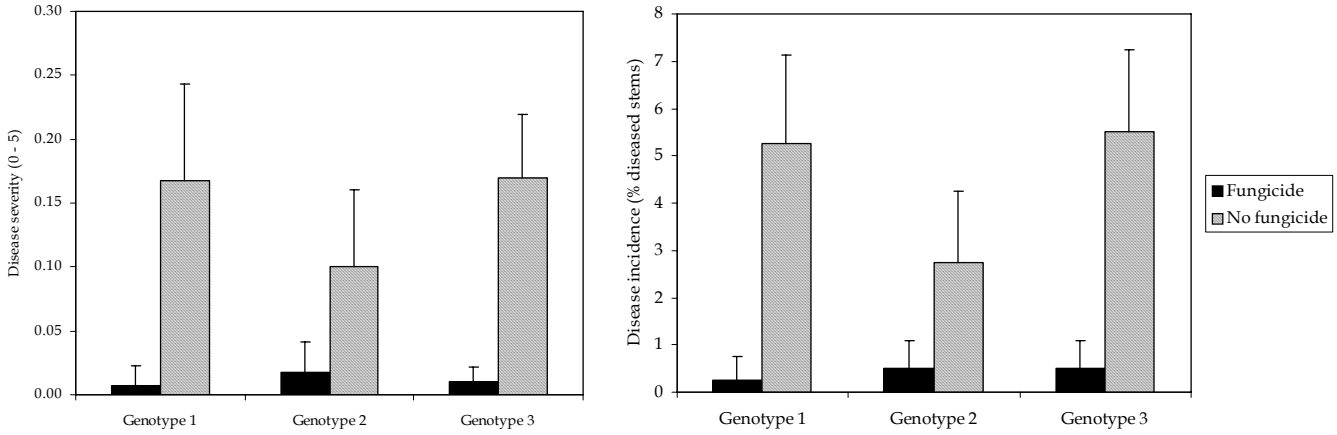

Figure 11 Effect of fungicide application on severity and incidence of SSR of three putative resistant *Brassica napus* genotypes in 2007. Error bars indicate standard deviation.

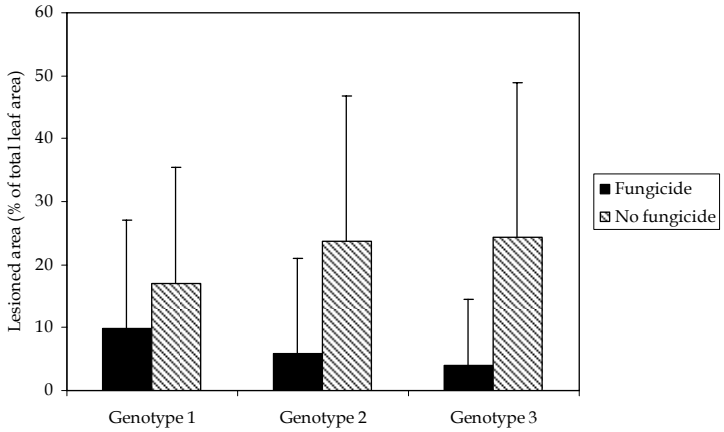

Figure 12 Effect of fungicide application on severity of SSR on leaves of three putative resistant *Brassica napus* genotypes in 2007. Error bars indicate standard deviation.

Table 4 Analysis of variance of yield and SSR disease severity and incidence on *Brassica napus* in 2007.

		P>F		
Source	DF	Severity	Incidence	Yield
Replicate	3	0.4826	0.3424	0.2940
Fungicide	10	0.1353	0.0850	0.0662
Error	30	-	-	-
Total	43	-	-	-

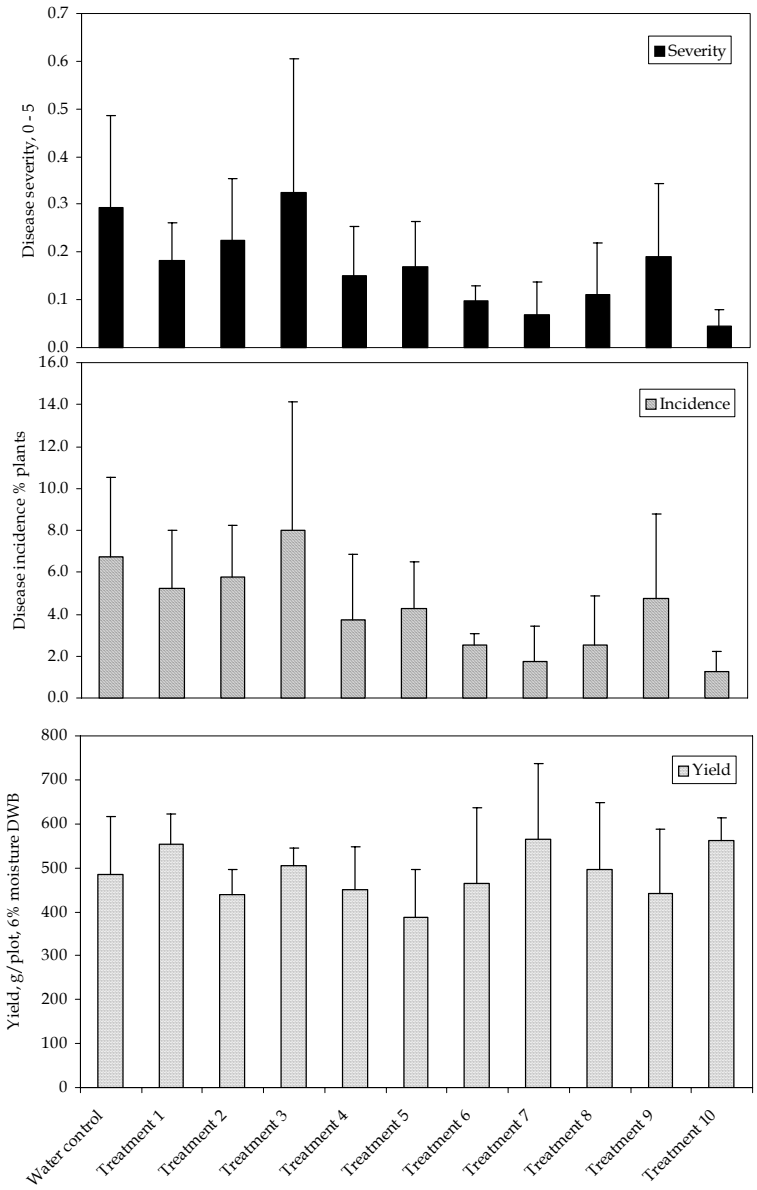

Figure 13 Effect of fungicide application on severity and incidence of SSR, and yield of *Brassica napus* in 2007. Error bars indicate standard deviation.

Conclusions

Our previous experience with the small nursery had been that disease pressure had been too high to be practically useful. This nursery was completely enclosed by solid walls and ceiling. Reducing disease pressure in the small nursery by reducing inoculum density and inoculation frequency was only partially successful; disease pressure was reduced, but low inoculum pressure meant that SSR symptom development was patchy. Given the small plot size (one row), this patchiness was problematic, since it meant that some plots were subjected to adequate pressure, while others had no diseased plants at all. This made it difficult to separate plants that were not infected due to genetic resistance from those that had simply been missed by the SSR epidemic.

The strategy we adopted for the present project was to encourage an evenly-distributed epidemic by apply an adequate amount of a highly infectious inoculum (ascospores), but also prevent excessive symptom development by allowing more air circulation in the nursery, thereby reducing relative humidity, an important driver of SSR epidemics ((Huang et al, 1998;Kora et al, 2005;Mcdonald et al, 2008)). We accomplished this by using shade cloth, on the assumption that reduced evaporation and wind speeds would be sufficient to reduce humidity, while still maintaining %RH levels higher than those in the fields surrounding the nursery. We found that this assumption was on the whole correct, but the lack of strong SSR epidemics either in 2006 or 2007 suggests that we reduced %RH too far.

In comparison to normal values for Vegreville, 2006 and 2007 were exceptionally dry, resulting in lower than normal %RH in the summer months of both years (REF). It is therefore possible that the nursery may function perfectly well in most years. The operating cost of the nursery, exclusive of construction costs, building materials, scientist salaries, or overhead was $24,700, or approximately $60 for a single 3.5m row. In comparison, this is at least four times the cost of operating a blackleg disease nursery. Given the high operating costs, we feel that operating a SSR nursery that only returns useful results in average or wet years is not justified. It would be a better use of resources to operate multiple non-misted field nurseries, perhaps with supplemental ascospore inoculum. Another alternative would be to repeat the work done for this study at a location when higher humidity is the norm, for example in the Red River Valley of Manitoba.

Appendix 1. Procedure for production of *Sclerotinia sclerotiorum* ascospores

1. Inoculate Potato Dextrose (PD) broth (100 ml) with isolate #184 and place on a shaker for 7-14 days.
2. Chop 25 pounds of carrots using continuous slicing feature on food processor (approximately 0.5 cm thick) and distribute into about 10 20cm × 45 cm patch bags Western Biological Ltd, Aldergrove BC, Canada).
3. Autoclave bags at 121°C with 45 min. exposure time. Place bags in 4°C cooler overnight or until the inoculum is ready.
4. Cut or snap the tip of a 10 ml pipette off so that mycelium or agar doesn't plug the tip. Add approximately 10 ml of inoculum to patch bags and shake slightly to mix.
5. Incubate bags on a laboratory bench at room temperature.
6. After about 4-5 weeks, sclerotia can be washed from the mycelium and carrot tissue, which will have become macerated. Alternatively, the bags can be stored in a cooler at 4°C if washing must be delayed. To wash, place the bag contents into a No. 12 soil sieve and spray water over the carrots from the tap into the sink.
7. After washing, surface sterilized the sclerotia in 1.5 parts household bleach to 2.5 parts distilled water for 2 minutes, followed by 3 rinses with distilled water.
8. Spread sclerotia onto paper towel on a lab bench, cover with a layer of paper towel and leave overnight. Place sclerotia into a sealed jar or container and store in a -20°C freezer.
9. Prepare a 1:4 sterile water and sand mixture in a clean container. Alternating layers, fill a 1L Mason jar with sand and sclerotia, mixing in between with large tweezers or a scoop. Cover with a layer of moist sand (approx. 1cm). Once full, cover with plastic wrap and an elastic band and store in darkness at 4°C. Store until stipes form. This should take approx. 5-6 weeks.
10. Once stipes form, empty the sand and sclerotia onto a clean surface or dish. Using forceps, place sclerotia into fresh 1:4 sand mixture in small brown glass jars, so that stipes project up out of sand and sclerotia are mostly buried. Note that the stipes are very fragile. Cover jar again with plastic wrap secured with an elastic band. Place jars into an incubator set at 18°C and constant fluorescent and black (near-UV) light.
11. Once mature apothecia are formed (approx. 2-6 weeks), place 0.65 micron, 47mm paper into in a Buchner funnel, and connect the narrow end of the funnel to a vacuum source with a vacuum hose.
12. Collect spores by carefully removing elastic without disrupting the air pressure in jar. A slight change in pressure can cause spores to release prematurely. Remove plastic and apply vacuum in one motion. When apothecia are mature, a "puff" of spores will be visible. Remove vacuum after a few seconds, replace plastic and rubber band, and proceed to the next jar. Once the spores are visible on the filter paper (slightly yellow to tan), change the paper. The best time to collect spores is late in the afternoon, after the apothecia have had exposure to the lights all day. They seem to release more spores at this time.
13. Store papers with ascospores at 4°C in a dessicator jar, and return jars with apothecia to the incubator.
14. Repeat steps 12-13 until no more spores are produced, or until the jars become contaminated, then dispose appropriately.

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