The effect of Zumsil and MicroSoil on the growth of *Sclerotinia sclerotiorum* and *Leptosphaeria maculans*

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A “poisoned plate” technique was used to determine effect of two products, Zumsil and MicroSoil, on the growth (*in vitro*) of the fungal plant pathogens *Sclerotinia sclerotiorum* and *Leptosphaeria maculans*.

Fungal isolates were obtained from existing collections at Charles Sturt University and represent isolations taken from canola crops in the Riverina region. Cultures were established on full strength potato dextrose agar (PDA) and incubated for 7-14 days to establish active mycelial growth, and in the case of *S. sclerotiorum*, fresh sclerotia.

Poisoned plate media was prepared using ½ strength PDA containing no product (control), a 1:50 final dilution and a 1:100 final dilution of Zumsil, or a 1:50 final dilution of MicroSoil. Plates were then inoculated with a $5mm^2$ plug of actively growing mycelium. For *S. sclerotiorum* individual sclerotia of approximately 2mm were also used as inoculum. All plates were incubated at 25°C in darkness. Each treatment was conducted in triplicate.

Plates inoculated with *S. sclerotiorum were* assessed 6 days post inoculation with visual examination and measurement of colony diameter. Each of the treatments resulted in inhibition of fungal colony growth compared to the no-product control (Figure 1; Table 1). Plates inoculated with *L. maculans* were assessed at 6-days (Figure 2) and 28 days post inoculation (Figure 3; Table 1). Zumsil completely inhibited colony growth, while fungal colonies established on the MicroSoil plates, but failed to expand substantially.

An additional assessment was made for plates inoculated with *S. sclerotiorum* at 28 days post inoculation. Zumsil and MicroSoil Plates inoculated with active mycelium of the pathogen remained completely inhibited. However, for those plates inoculated with sclerotia of *S. sclerotiorum* germination and colony expansion of the pathogen occurred at the 1:100 dilution (42.7mm colony diameter; Figure 4). While inhibited compared to the control, this would suggest that sclerotia are able to tolerate exposure to Zumsil, and with the potential diffusion of volatile compounds, able to germinate and active mycelium emerge to form a pathogen colony.

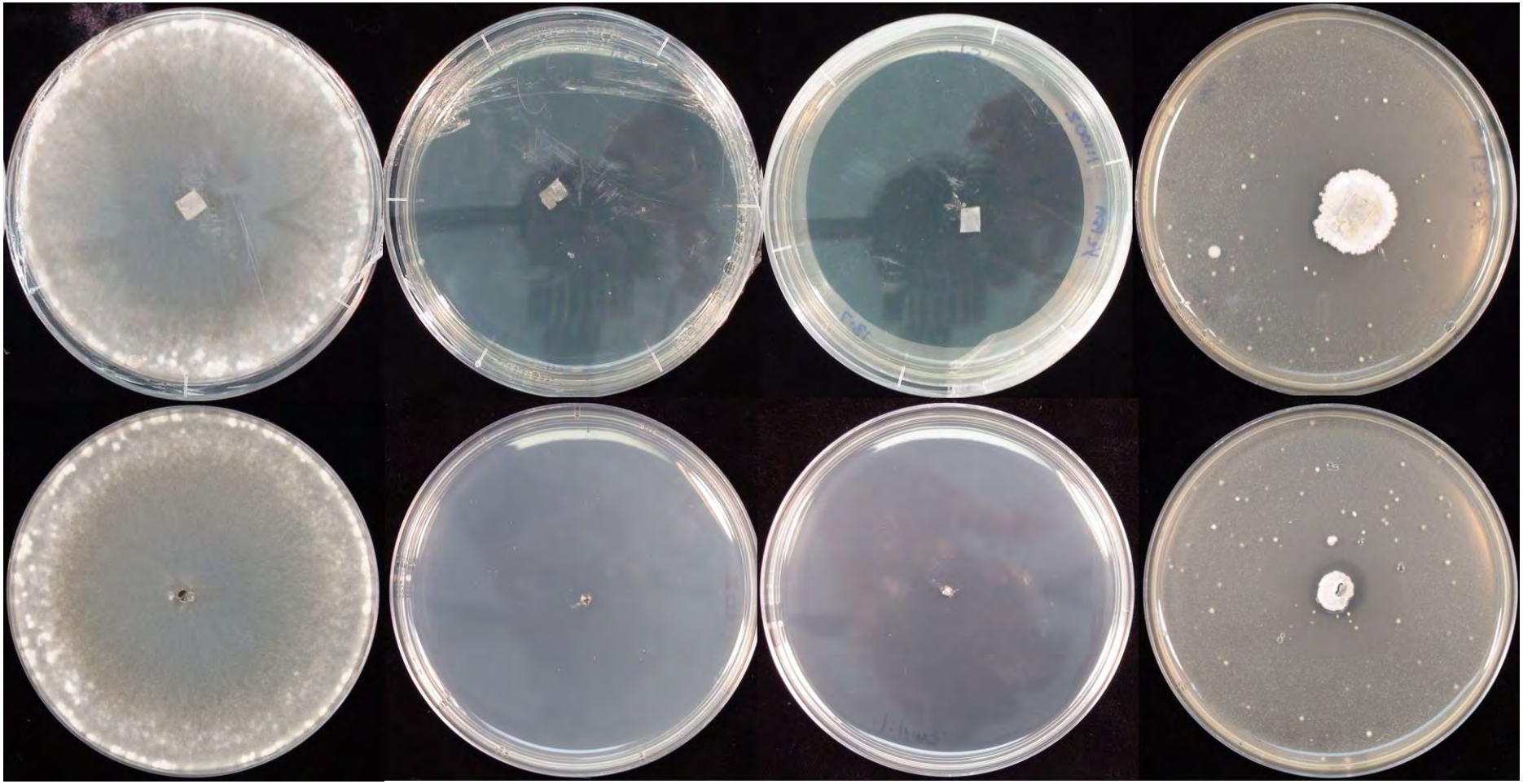

Figure 1: *Sclerotinia sclerotiorum* 6 days post inoculation on potato dextrose agar (PDA) infused with Zumsil or MicroSoil

Top row – inoculation with 5mm^2 agar plug of active mycelium. Bottom row – inoculation with sclerotia.

From left – PDA only; PDA + 1:50 Zumsil; PDA + 1:100 Zumsil; PDA + 1:50 MicroSoil.

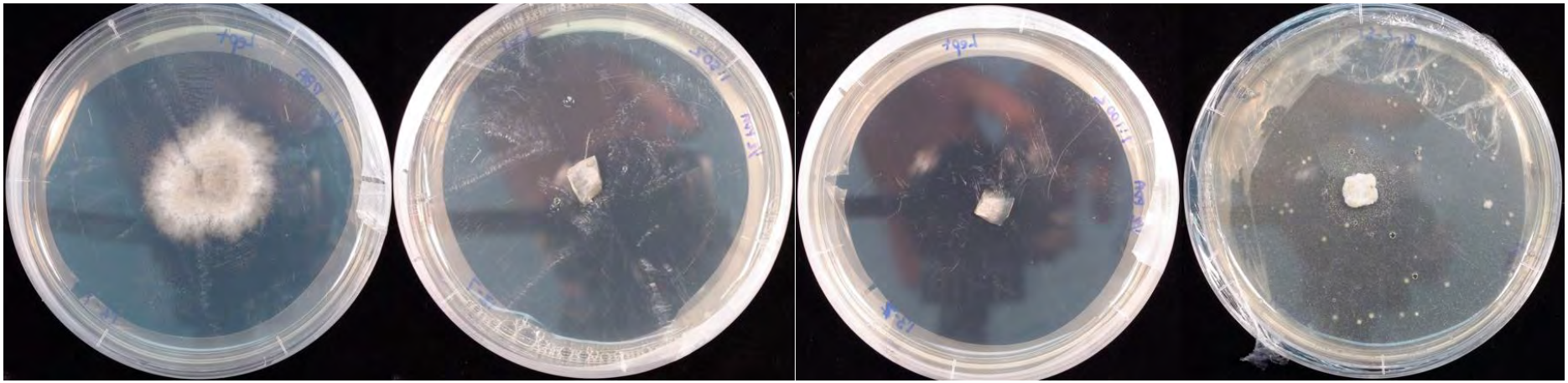

Figure 2: *Leptosphaeria maculans* 6 days post inoculation on potato dextrose agar (PDA) infused with Zumsil or MicroSoil

From left – PDA only; PDA + 1:50 Zumsil; PDA + 1:100 Zumsil; PDA + 1:50 MicroSoil.

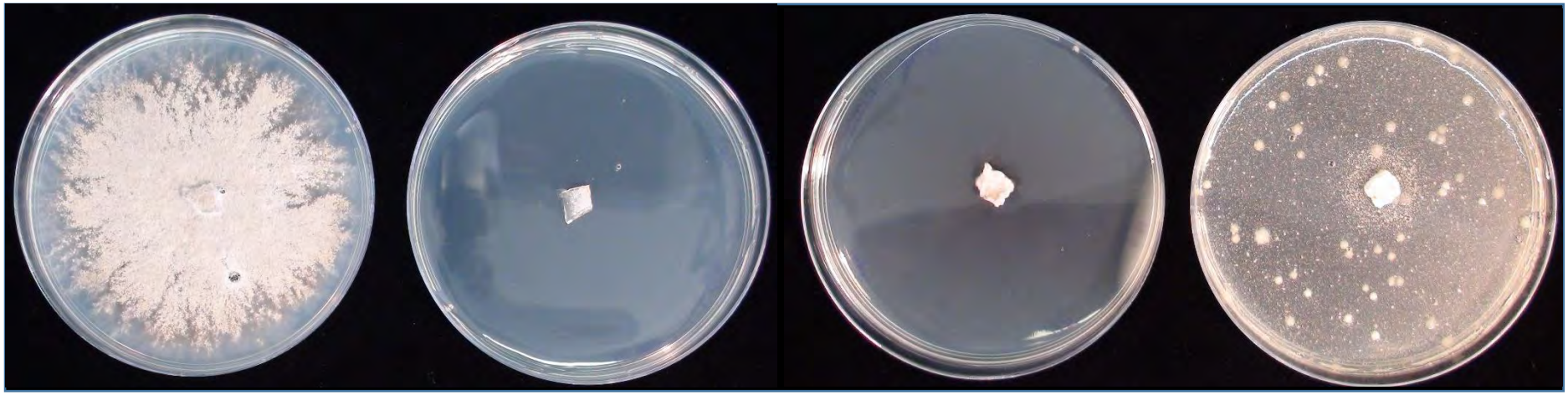

Figure 3: *Leptosphaeria maculans* 28 days post inoculation on potato dextrose agar (PDA) infused with Zumsil or MicroSoil

From left – PDA only; PDA + 1:50 Zumsil; PDA + 1:100 Zumsil; PDA + 1:50 MicroSoil.

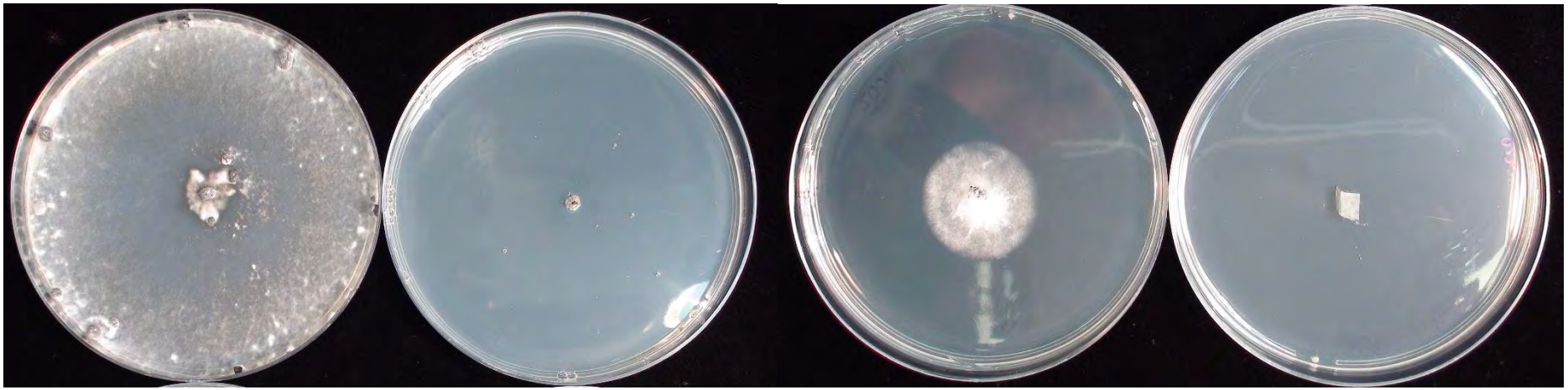

Figure 4: **:** *Sclerotinia sclerotiorum*28 days post inoculation on potato dextrose agar (PDA) infused with Zumsil.

From left – PDA only; PDA + 1:50 Zumsil; PDA + 1:100 Zumsil; PDA + 1:100 Zumsil inoculated with agar plug (active mycelium of pathogen).

Table 1: Diameter of colony growth (mm) for each species cultured on ½ strength PDA infused with either Zumsil or MicroSoil. Measurements were taken on two axis, minus the inoculum size (5mm for plug; 2mm for sclerotia). *Sclerotinia* recorded 6 days post inoculation; *Leptosphaeria* recorded 28 days post inoculation.

Fungus	Treatment	Rep1	Rep2	Rep3	Average diameter (mm)
***Sclerotinia sclerotiorum* mycelia**	½ PDA	85	85	85	**85.0**
	½ PDA+1:50 Zumsil	0	0	0	**0**
	½ PDA+1:100 Zumsil	0	0	0	**0**
	½ PDA+1:50 MicroSoil	10	8	12	**10.0**
***Sclerotinia sclerotiorum* sclerotia**	½ PDA	87	87	87	**87.0**
	½ PDA+1:50 Zumsil	0	0	0	**0**
	½ PDA+1:100 Zumsil	<1	<1	<1	**<1**
	½ PDA+1:50 MicroSoil	10	12	10	**10.66**
***Leptosphaeria maculans* mycelia**	½ PDA	80	79	81	**80.00**
	½ PDA+1:50 Zumsil	0	0	0	**0**
	½ PDA+1:100 Zumsil	<1	<1	<1	**<1**
	½ PDA+1:50 MicroSoil	<1	<1	<1	**<1**

<1 represents emergence of new mycelia from the inoculation source however colony measurement was not possible.

An additional assessment was undertaken with the MicroSoil product in which the bacterial component was isolated from the supplied formulation. A bacterial suspension was used to inoculate both Nutrient Agar (favouring bacterial growth) and ½ strength Potato Dextrose Agar (favouring fungal growth) with a heavy inoculum load applied to one side of the plate. Agar plugs carrying active mycelium of *S. sclerotium* were placed on the opposite side of the plate. Plates were sealed and incubated at 23°C for 7 days.

Following incubation, *S. sclerotiorum* was observed to be somewhat inhibited by the bacterial inoculant when grown on nutrient agar. In this instance, the agar used would favour bacterial growth. On potato dextrose agar, however, the development of *S. sclerotiorum* was not inhibited by the bacterial inoculant (Figure 5). It was also noted that the different media used influenced the development of the bacterial inoculant, which may in turn have impacted on the fungal pathogen. It must be noted that at this point in time these data are strictly observational.

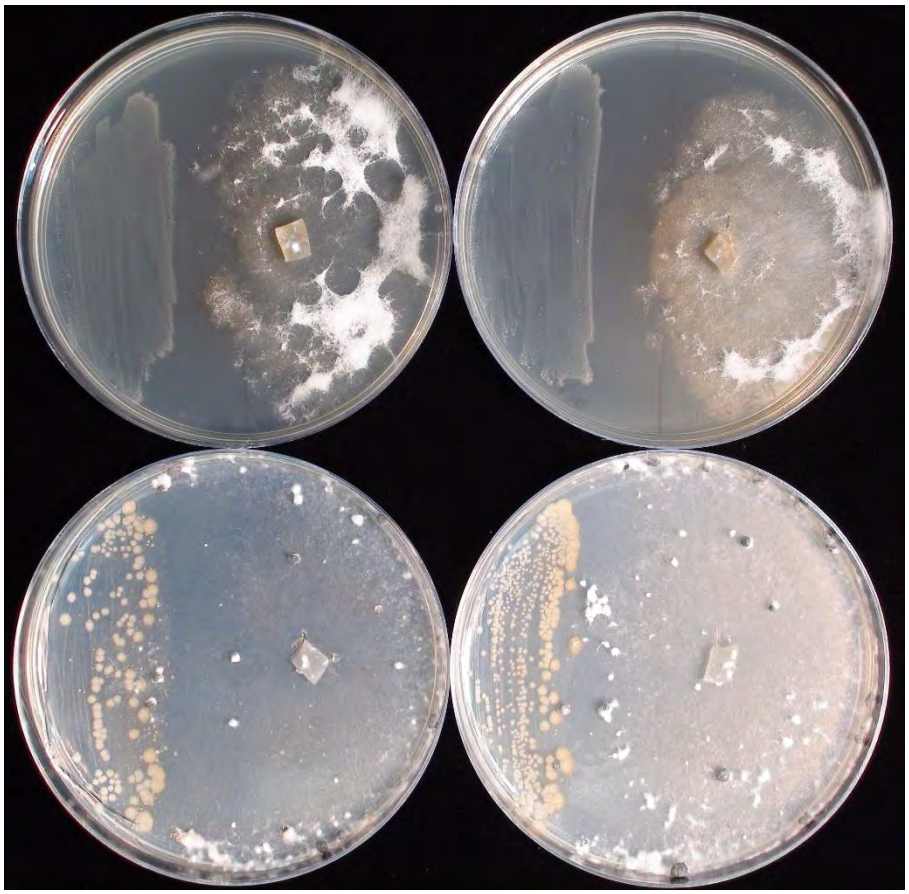

Figure 5: *Sclerotinia sclerotiorum 7* days post inoculation on Nutrient Agar (top) and Potato Dextrose Agar (bottom). Plates were co-inoculated with a bacterial suspension isolated from the MicroSoil product.

General notes:

Sclerotinia

Zumsil at 1:50 inhibited growth of active mycelia and the emergence of mycelia from sclerotium. At the 1:100 concentration, active mycelia remained inhibited, however mycelia were observable from the sclerotium. These however did not progress to a measurable colony.

From the inoculation technique used there is potentially an effect of volatile production causing the inhibition, as active fungal mycelium were not in contact with the infused agar medium.

MicroSoil inhibited colony growth for both the active mycelium and sclerotia inoculation. A clear zone of inhibition was present around the fungal growth in which bacterial colonies were not present.

Leptosphaeria

As for the *Sclerotinia* active mycelium were inhibited at both concentrations of Zumsil examined. All cultures will be examined again on the 26th of July, due to the slower colony expansion dynamics of the fungus.

From the inoculation technique used there is potentially an effect of volatile production causing the inhibition, as active fungal mycelium were not in contact with the infused agar medium.

Microsoil inhibited colony growth with mycelia restricted to the agar plug used for inoculation. Unlike the case for *Sclerotinia*, no clear inhibition zone was observable. In this case, bacterial colonies appeared to have increased in density surrounding the fungal plug. An additional assessment will take place on 26th July.