

## Supplementary Information

### Mass Spectrometry Imaging of Secondary Metabolites Directly on Fungal Cultures

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**Figure S1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of phomopsinone A (**1**)

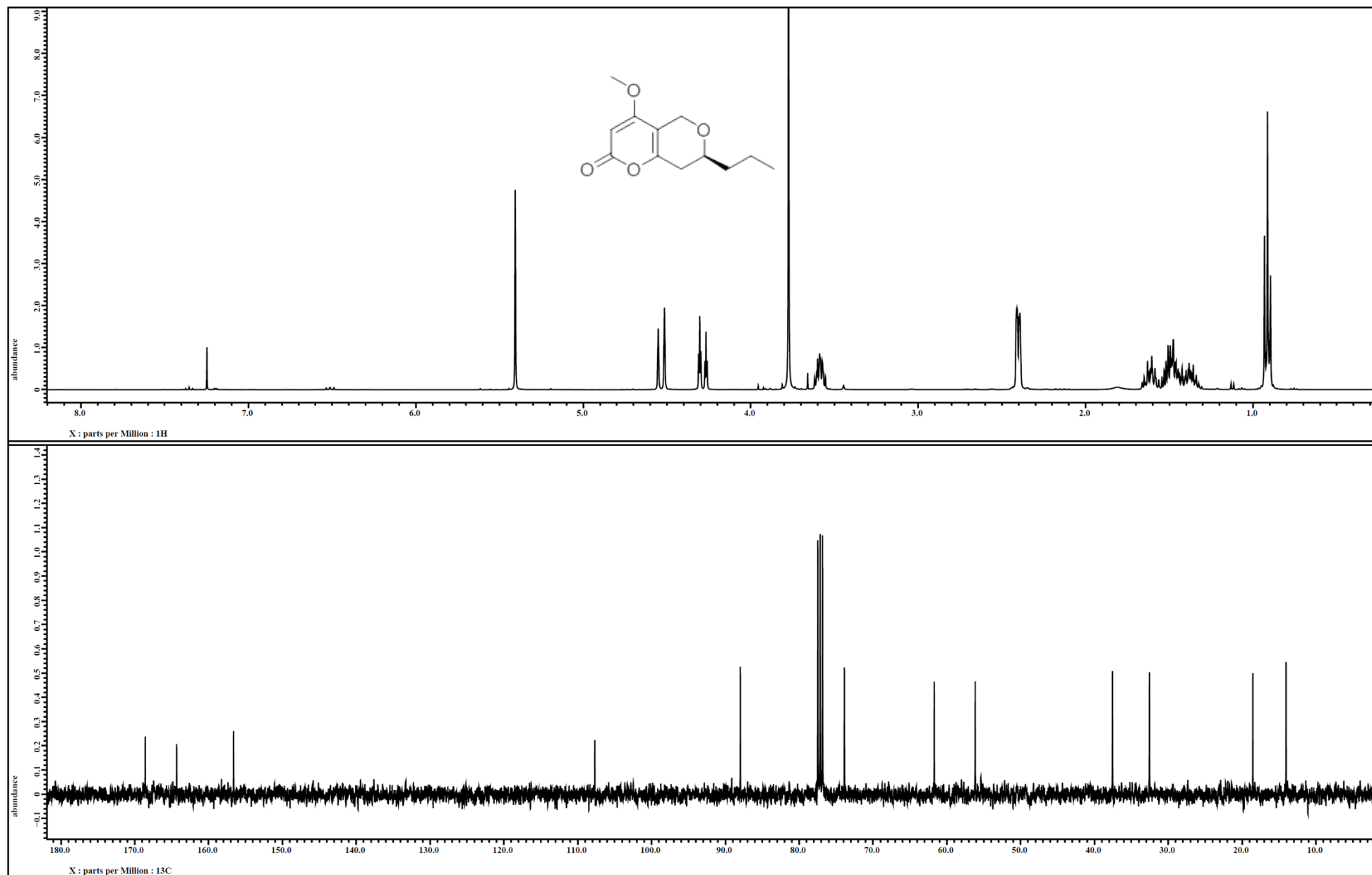
**Figure S2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of T-2 toxin (**2**)

**Figure S3.** Examples of the inconsistent growth patterns of G100 on balsa wood such as a raised, bulbous fungal growth (A) and a thin, transparent fungal growth (B).

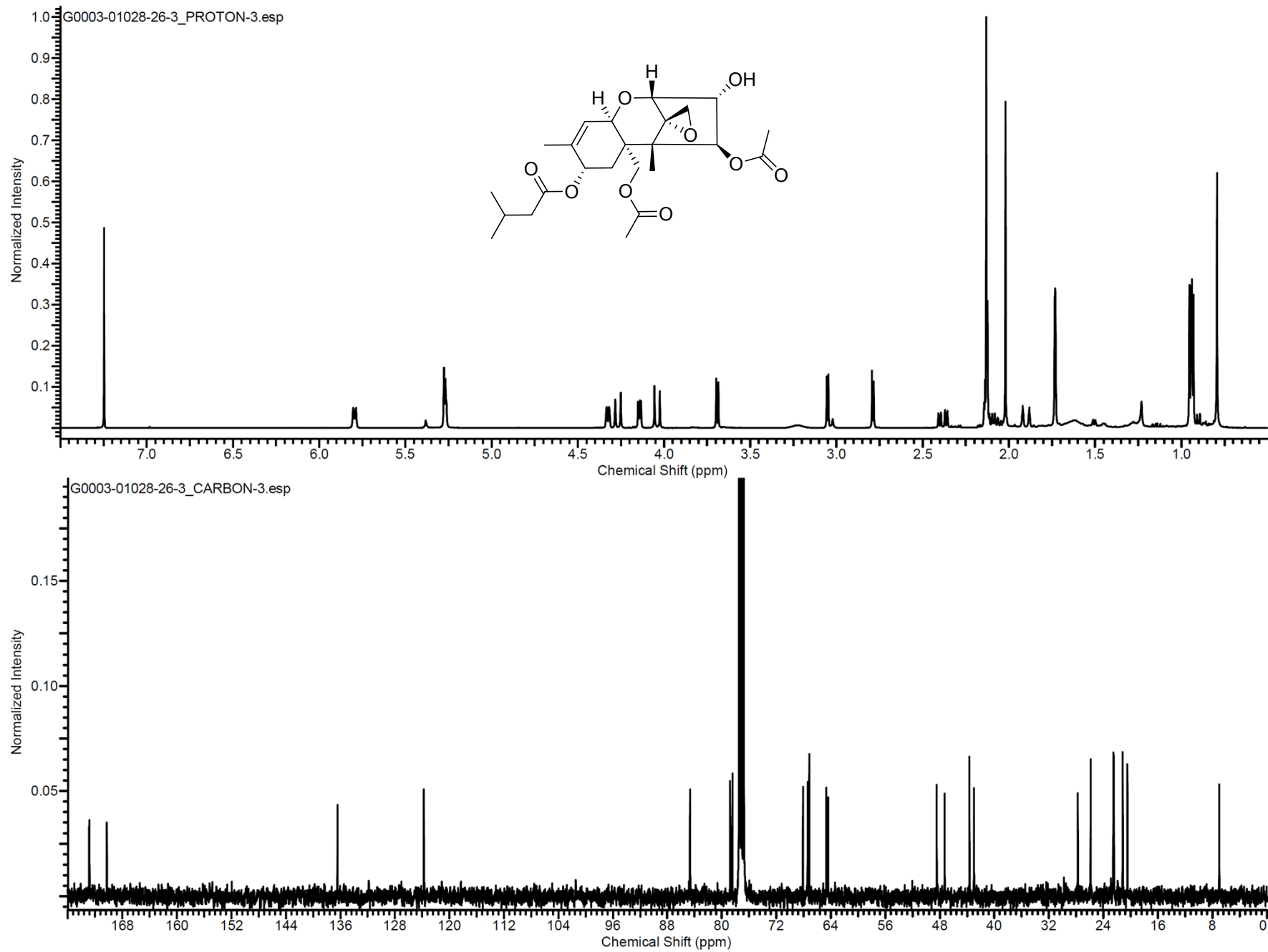
**Figure S4.** Photographs of the challenges observed when inoculating an insert in liquid media.

**Figure S5.** (A) G100 (left) on agar grown against G3 (right) on agar. (B) G100 (left) on cardboard grown against G3 (right) on agar.

**Figure S6.** Photographs of the cardboard inserts.

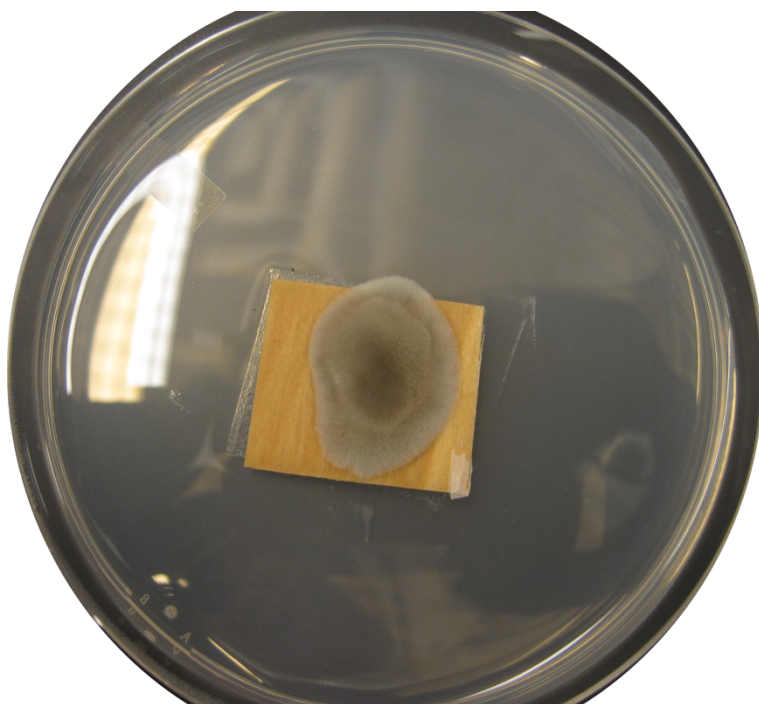


**Fig. S1**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of phomopsinone A (1) [400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$ ;  $\text{CDCl}_3$ ].

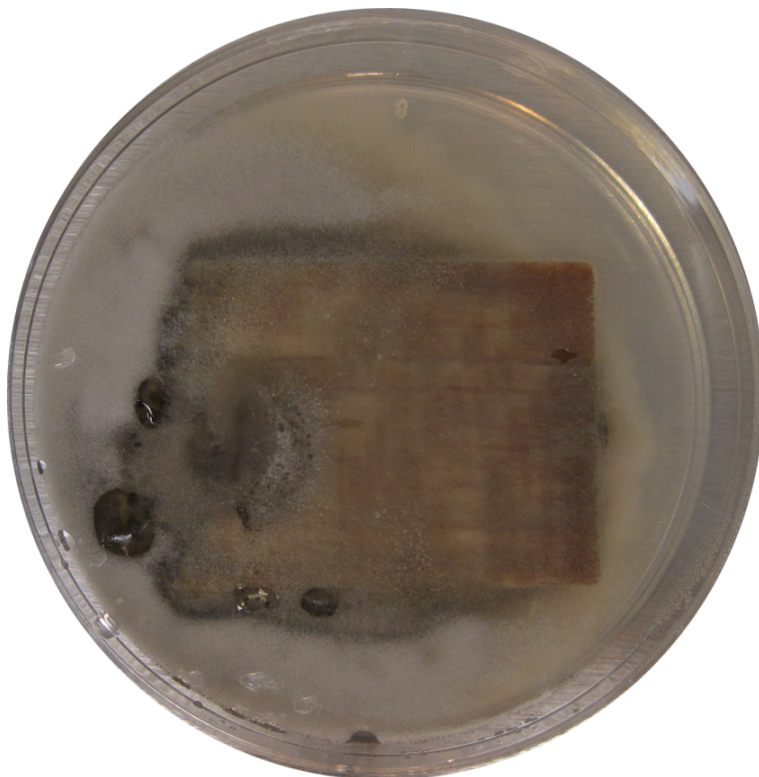


**Fig. S2**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of T-2 toxin (**2**) [400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$ ;  $\text{CDCl}_3$ ].

**A**

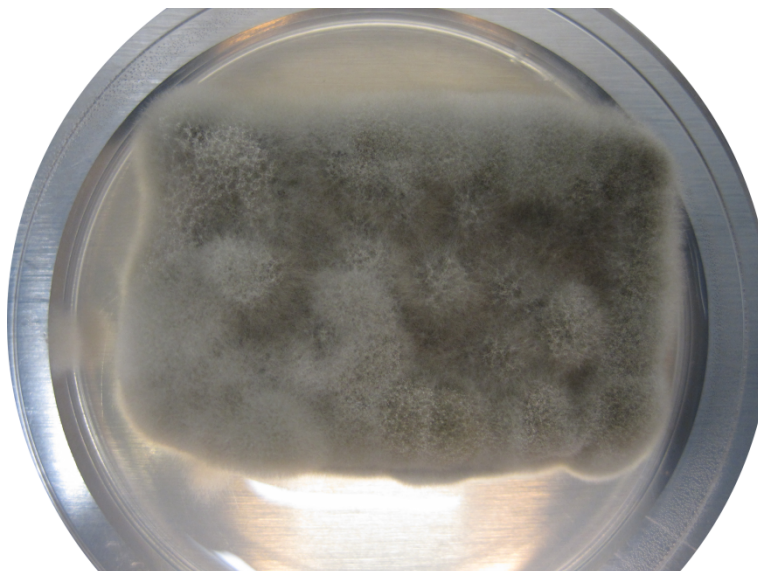
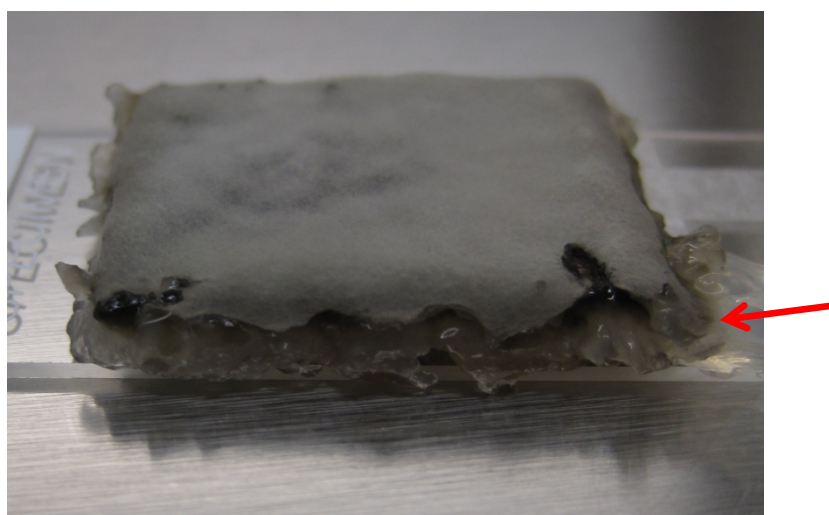


**B**



**Fig. S3** Examples of the inconsistent growth patterns of G100 on balsa wood such as a raised, bulbous fungal growth (A) and a thin, transparent fungal growth (B).



**A****B**

**Fig. S4** Photographs of the challenges observed when inoculating an insert in liquid media. In this example, a cardboard insert was inoculated in liquid media and then placed on agar in a Petri dish. After three weeks of growth (A), the insert was removed from the Petri dish and placed on a microscope slide (B) in preparation for DESI-MS analysis. The uneven nature of the growth across the insert is apparent in both photographs, while the agar layer between the fungus the insert is noted with an arrow in B.

**A**

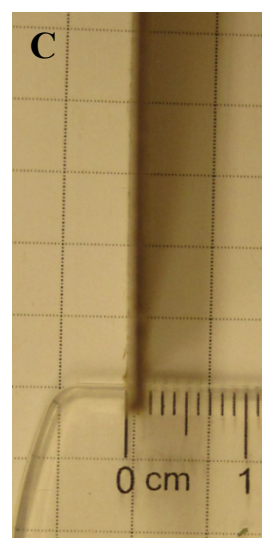
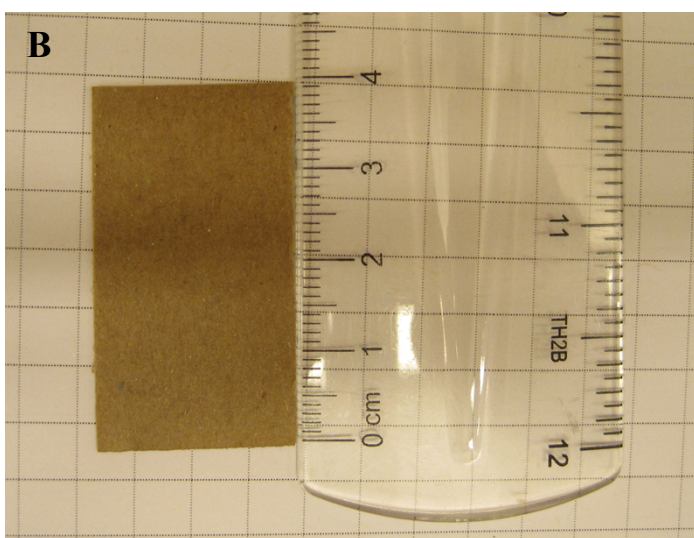
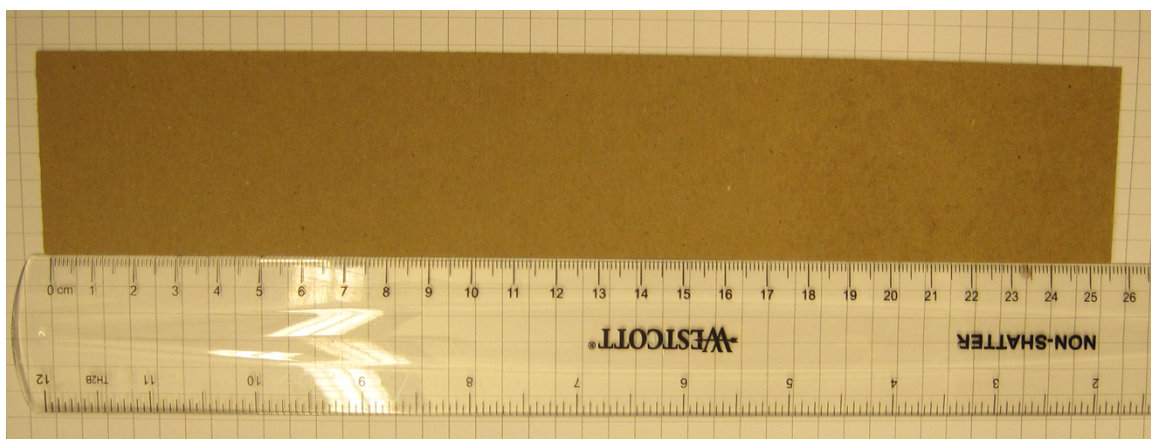


**B**



**Fig. S5** (A) G100 (left) on agar grown against G3 (right) on agar. (B) G100 (left) on cardboard grown against G3 (right) on agar. In both cases, when the two cultures were inoculated simultaneously, G3 (white/yellow mycelium) rapidly overgrew the plates.

**A**



**Fig. S6** Photographs of the cardboard inserts. (A) The cardboard inserts were from the reinforcing cardboard bottom inserts for the Pendaflex® Box-Bottom Hanging File Folders purchased from Staples (item # 521401). The strips were cut to 40 mm × 20 mm

pieces (B). The cardboard was 1 mm thick and non-corrugated (C).