

Minicell-based fungal RNAi delivery for sustainable crop protection

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Table S1. RNA production data from four different bioreactors

	Analysis Average (ng)	Volume Analyzed (μL)	Total Volume (mL)	RNA Yield (ng/mL)	Total RNA Yield (mg)
Bioreactor 1	3342.92	41	1310	81534.63	106.81
Bioreactor 2	5424.15	38	1317	142740.82	187.99
Bioreactor 3	6119.88	44	1329	139088.21	184.85
Bioreactor 4	4552.50	42	1319	108392.92	142.97

Table S2. Specific primers used for qRT-PCR analysis

Target gene	Gene Bank Number	Forward sequence	Reverse sequence
<i>Chs3a</i>	AF494188.1	5'-ACTGCTACCCCATCATCA-3'	5'-GGATCCCTTAGGCCGATTA-3'
<i>Chs3b</i>	AF529208.1	5'-AAGGCCCTGCTGAGTTTCT-3'	5'-ACATGCGACCGAAGTGAAT-3'
<i>DCL1</i>	XM_024696562.1	5'-TTCAAACGGCAATCAAGGT-3'	5'-CCTTAAAGGAGGCCCAAAG-3'
<i>DCL2</i>	XM_024697207.1	5'-CTTCGCGTTTGAATGATCC-3'	5'-CAGACGTTCCCATGCTCTC-3'
<i>β-actin</i>	AJ000335.1	5'-GATTCCGGTGACGGAGTTA-3'	5'-AAATCACGACCAGCCATGT-3'
<i>Tubulin</i>	XM_001555875.2	5'-CTACCCAGCACCAAAACC-3'	5'-ACAGTCGGAGTGCTCAAGG-3'

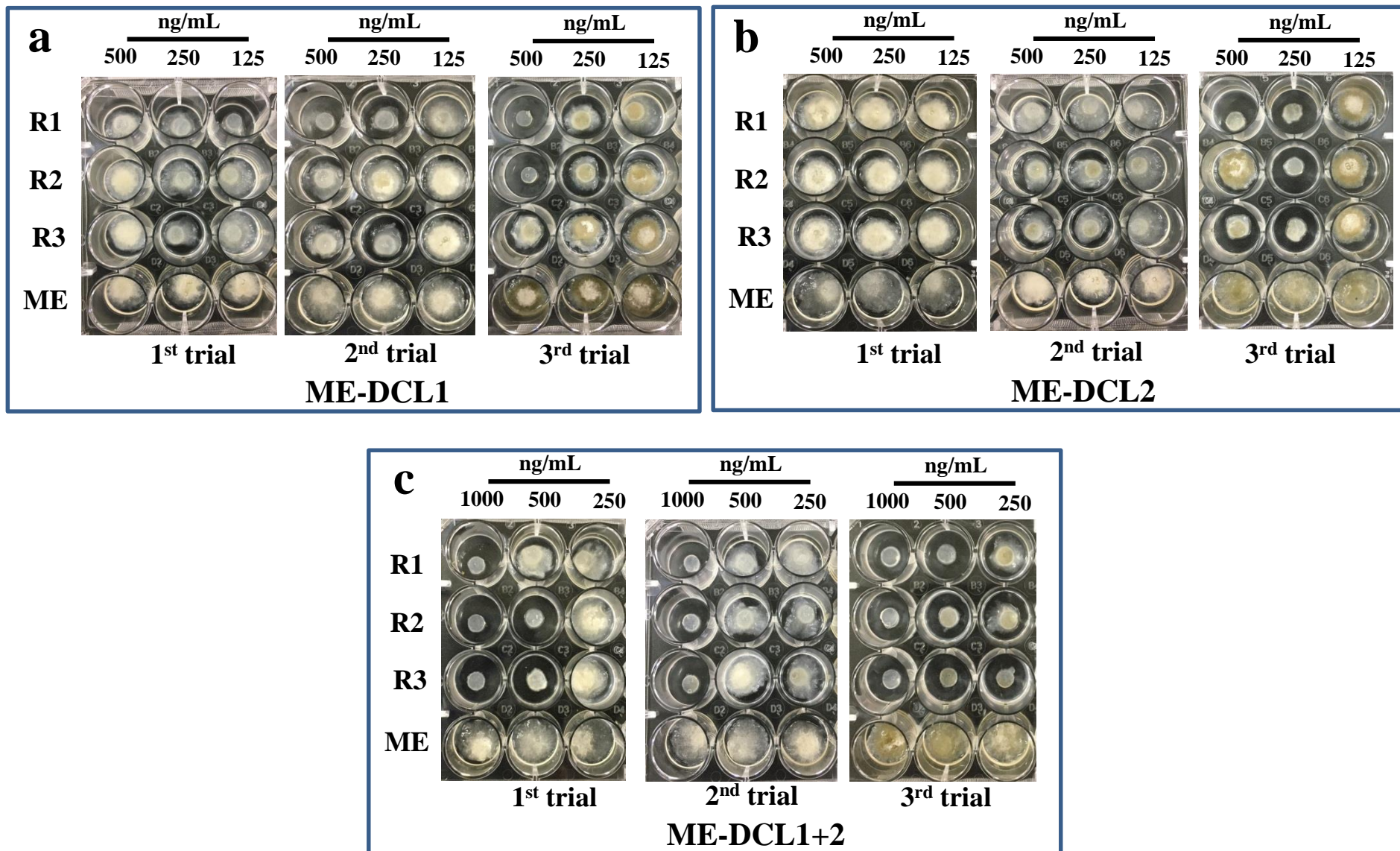


Fig. S1. The *Botryotinia fuckeliana* mycelial growth inhibitory activity of the minicells-encapsulated dsRNAs targeting *DCL1* and *DCL2* genes of *B. fuckeliana* at 72 hours after treatment. (a) ME-DCL1, (b) ME-DCL2, and (c) ME-DCL1+2. R: Biological replication; ME: empty-Minicells.

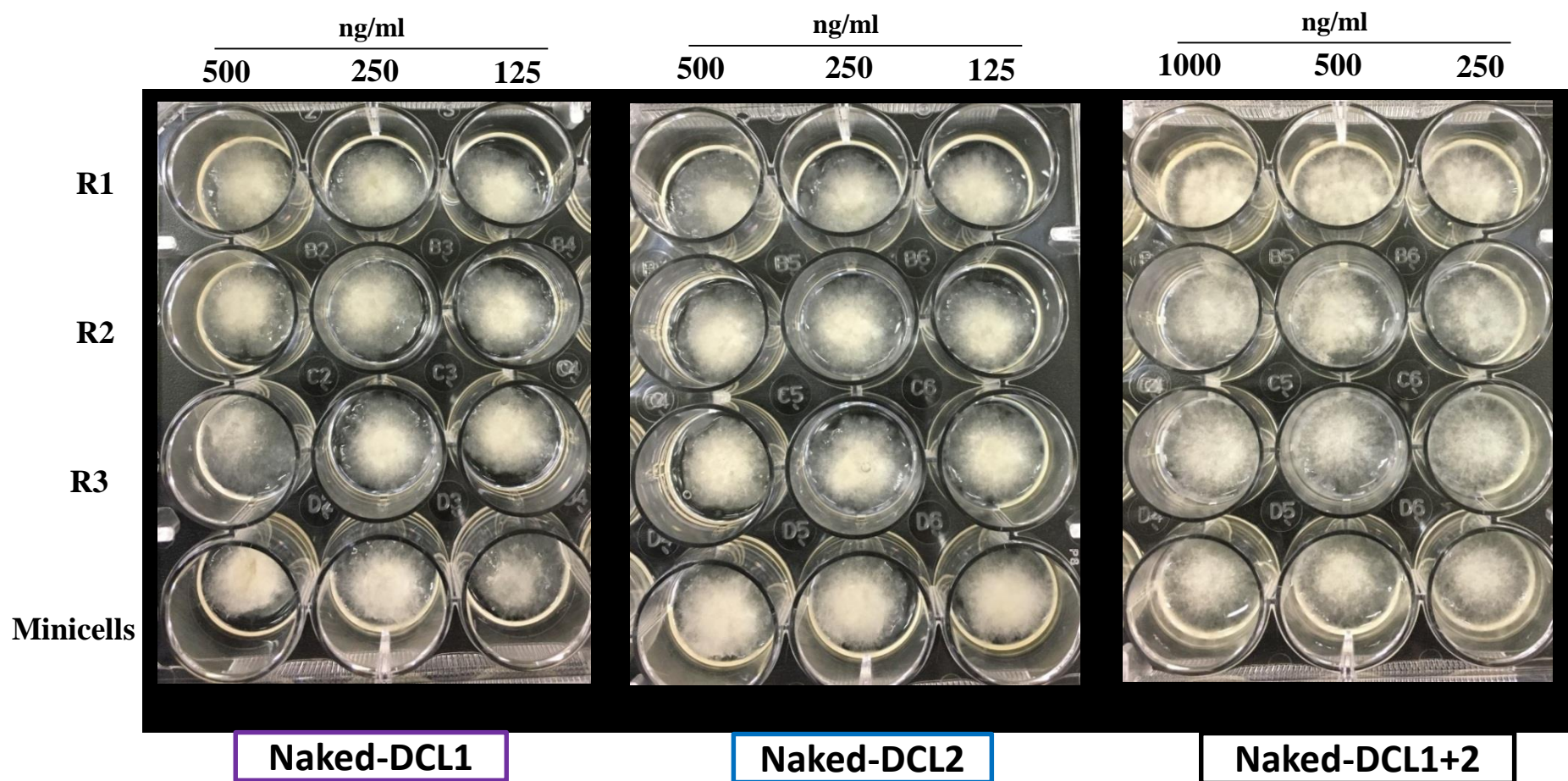


Fig. S2. The *Botryotinia fuckeliana* mycelial growth inhibitory activity of the minicells-encapsulated dsRNAs (ME-dsRNAs; ME-DCL1 and ME-DCL2) targeting both *DCL1* and *DCL2* genes of *B. fuckeliana* at 72 hours after treatment. R: Biological replication.

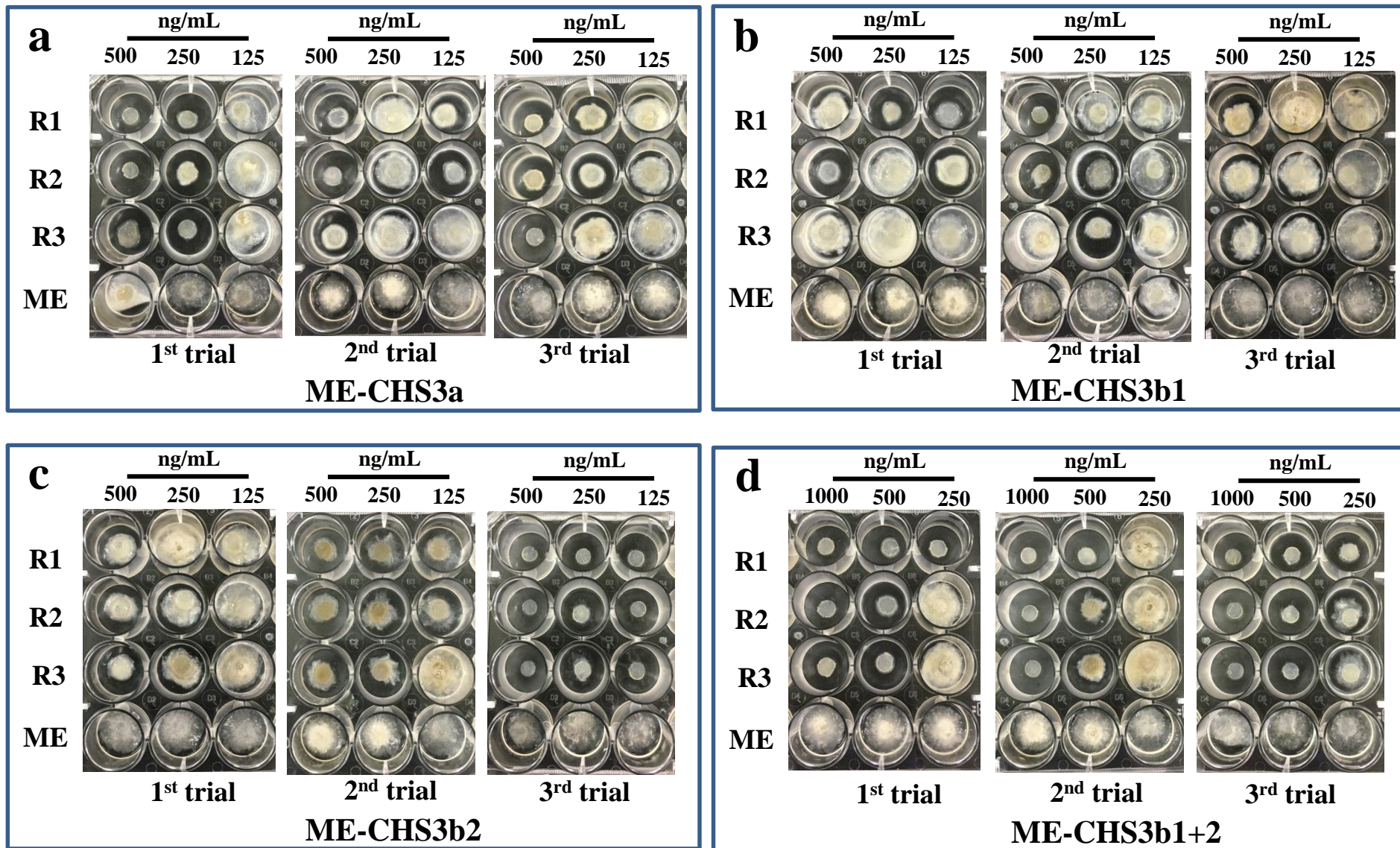


Fig. S3. The *Botryotinia fuckeliana* mycelial growth inhibitory activity of the minicells-encapsulated dsRNAs targeting *Chs3a* and *Chs3b* genes of *B. fuckeliana* at 72 hours after treatment. (a) ME-CHS3a, (b) ME-CHS3b1, (c) ME-CHS3b2, and (d) ME-CHS3b1+2. R: Biological replication; ME: empty-Minicells.

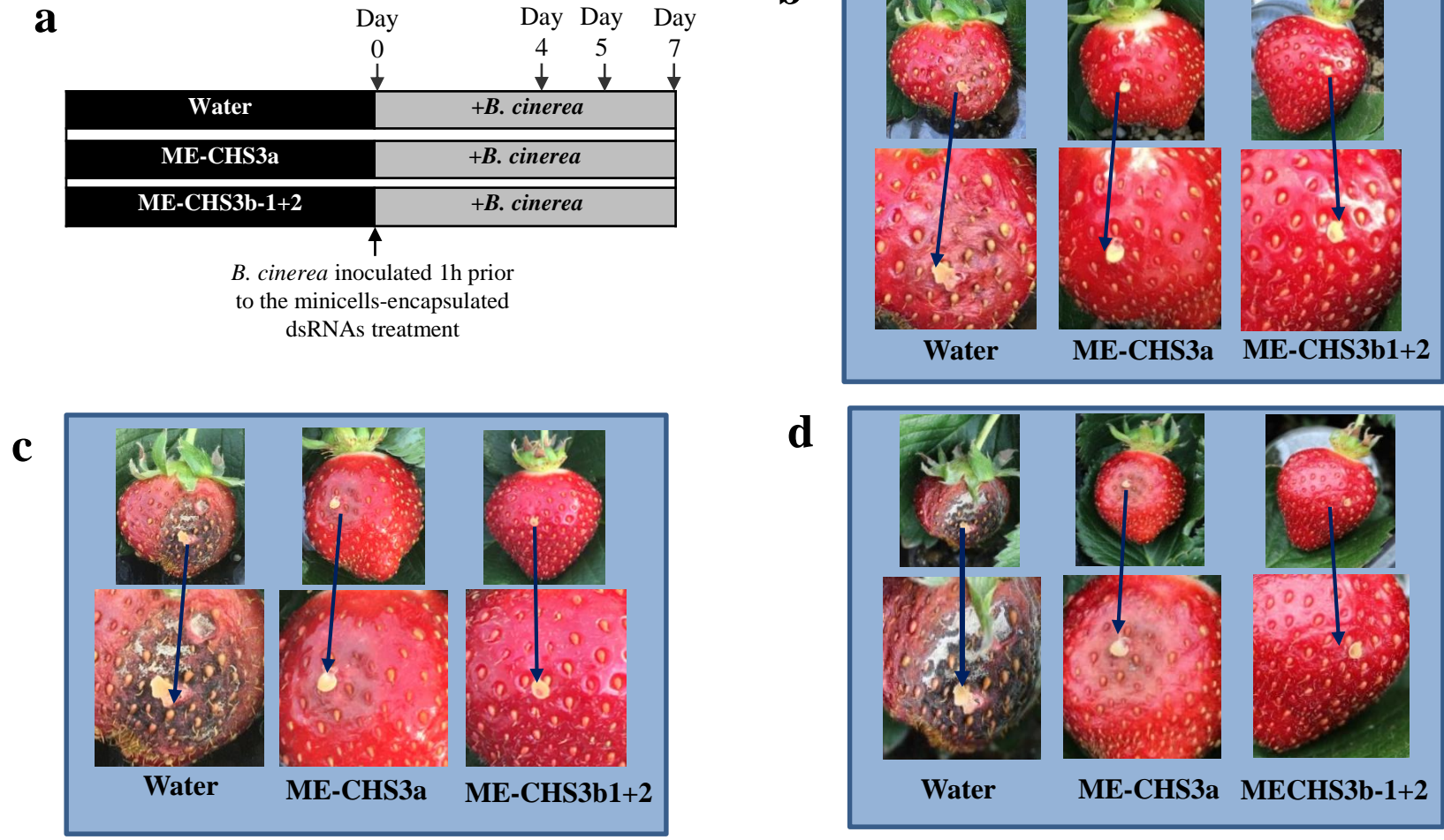


Fig. S4. Topically applied minicells-encapsulated dsRNAs (ME-dsRNAs; ME-CHS3a) targeting chitin synthase 3a (*Chs3a*) and combination of two ME-dsRNAs (ME-CHS2b1+ME-CHS3b2) targeting chitin synthase 3b (*Chs3b*) of *Botryotinia fuckeliana* on the strawberry fruits inhibited the gray mold disease progression. (a) Schematic representation of the ME-dsRNAs on strawberry fruit 1 hour prior to inoculation. The gray mold diseases symptom development at (b) 4 days post inoculation (dpi), (c) 5dpi, and (d) 7dpi. In order to determine the gray mold disease symptom development following ME-dsRNAs treatment. ME-CHS3a applied at dose of 500 ng/mL and the combination of ME-CHS3b-1 and 2 used at 1000 ng/ml (500 μ l/fruit).



Minicells



ME-CHS3a



ME-CHS3b-1+2



ME-DCL1+2



Naked-CHS3b-1+2

Fig. S5. Gray mold diseases symptom development on minicells-encapsulated dsRNAs (ME-dsRNAs) or naked-dsRNA targeting *Botryotinia fuckeliana* genes sprayed fruits when challenged with the *B. fuckeliana* 1h post treatment (1st trial). In first trail we used the minicells, ME-CHS3a (targeting *Chs3a*), ME-CHS3b1+2 (targeting *Chs3b*), ME-DCL1+2 (targeting *DCL1* and *DCL2*), and naked-CHS3b1+2 (*Chs3b*).



Minicells



ME-DCL1+2



Naked-DCL1+2

Fig. S6. Gray mold diseases symptom development on minicells-encapsulated dsRNAs (ME-dsRNAs) or naked-dsRNA targeting *Botryotinia fuckeliana* genes sprayed fruits when challenged with the *B. fuckeliana* 1h post treatment (2nd trial). In 2nd trail we used the minicells, minicells-encapsulated dsRNAs (ME-DCL1+2) and naked-DCL1+2 (targeting *DCL1* and *DCL2*) treatments.



Minicells



ME-CHS3b-1+2



Naked-CHS3b-1+2

Fig. S7. Minicells-encapsulated dsRNAs (ME-dsRNAs) for sustained protection against *Botryotinia fuckeliana* (1st trial). Plants were sprayed on day 0 with minicells, ME-CHS3b1+2 or naked-CHS3b1+2 (targeting *B. fuckeliana* *Chs3b* gene) and inoculated with *B. fuckeliana* on sprayed fruits at 7 days post treatment. Gray mold disease severity was observed at 5 days post inoculation (dpi).



Minicells



ME-DCL1+2



Naked-DCL1+2

Fig. S8. Minicells-encapsulated dsRNAs (ME-dsRNAs) for sustained protection against *Botryotinia fuckeliana* (2nd trial). Plants were sprayed on day 0 with minicells, ME-DCL1+2 or naked-DCL1+2 (targeting *B. fuckeliana* *DCL1* and *DCL2* genes) and inoculated with *B. fuckeliana* on sprayed fruits at 7 days post treatment. Gray mold disease severity was observed at 5 days post inoculation (dpi).