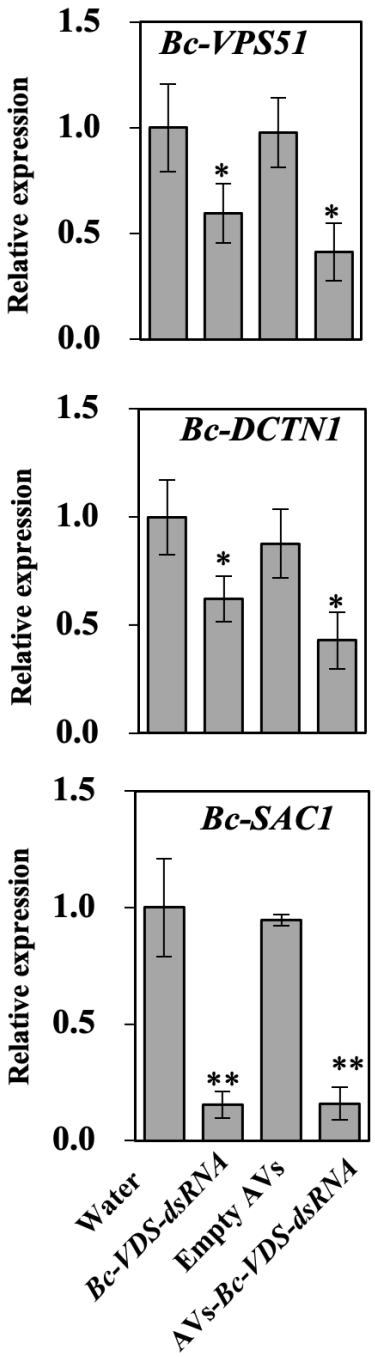
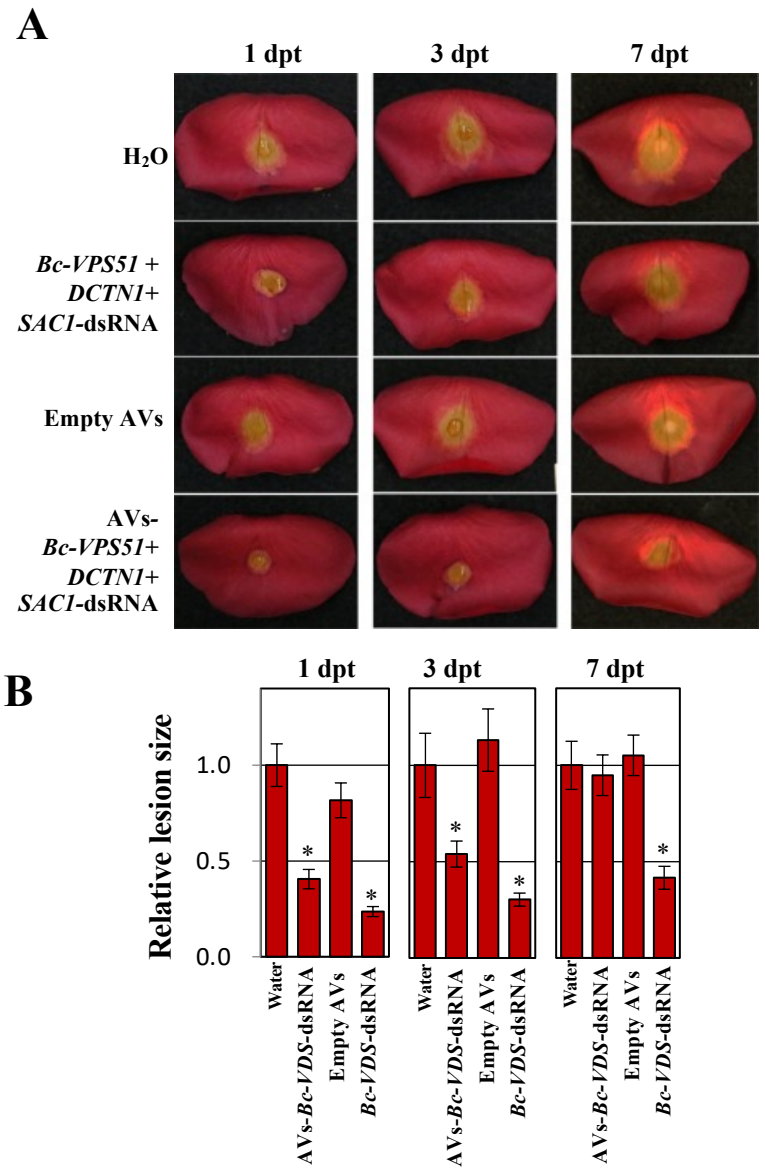
Supplementary Figures

Supplemental Figure 1



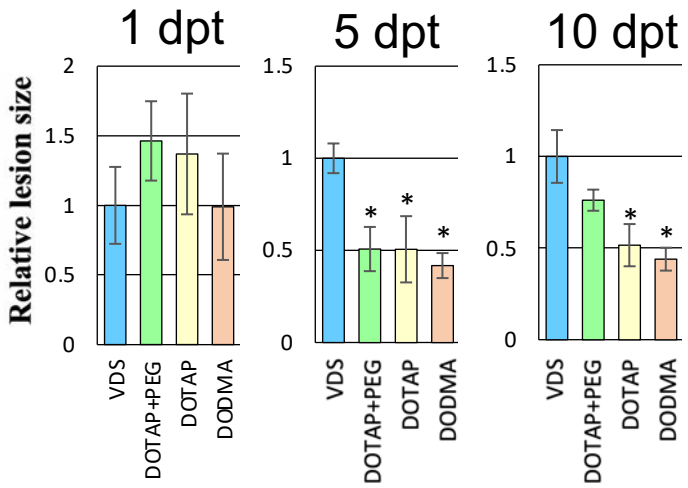
Supplementary Figure 1. Genes targeted through SIGS-applied dsRNAs are downregulated in *Botrytis cinerea.* The expression of the *B. cinerea* target genes were down regulated as measured by RT-PCR.

Supplemental Figure 2



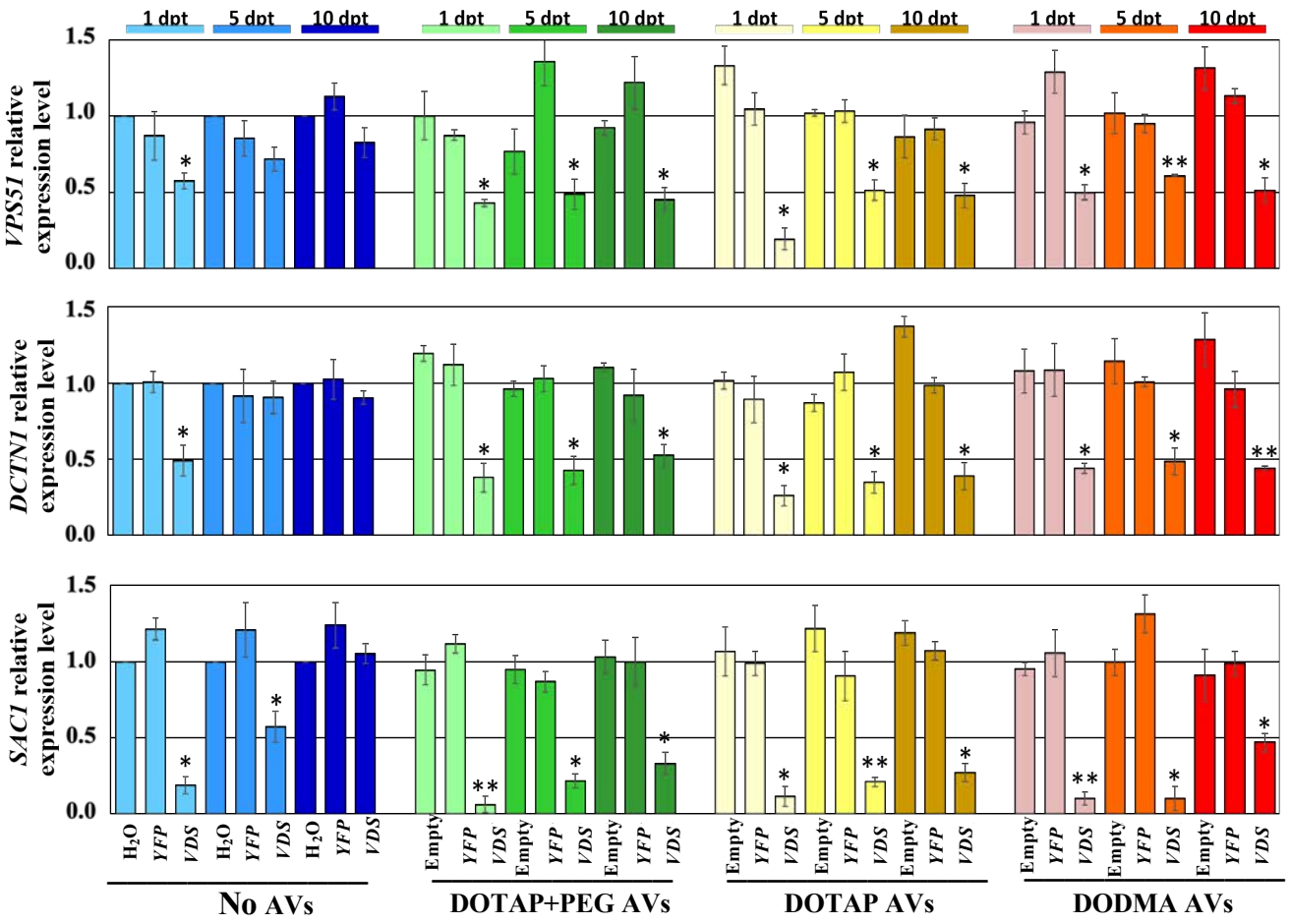
Supplementary Figure 2. Treatment with AV-dsRNA provides prolonged protection against *B. cinerea* in rose petals. (A) Rose petals were pre-treated with naked- or AV-*Bc-VDS*-dsRNA, for 1, 3, and 7 days, then inoculated with *B. cinerea.* Pictures were taken at 3 dpi. **(B)** The relative lesion sizes were measured with the help of ImageJ software. Error bars indicate the SD. Statistical significance compared to water (Student's t-test): *, $P < 0.05$.

Supplemental Figure 3



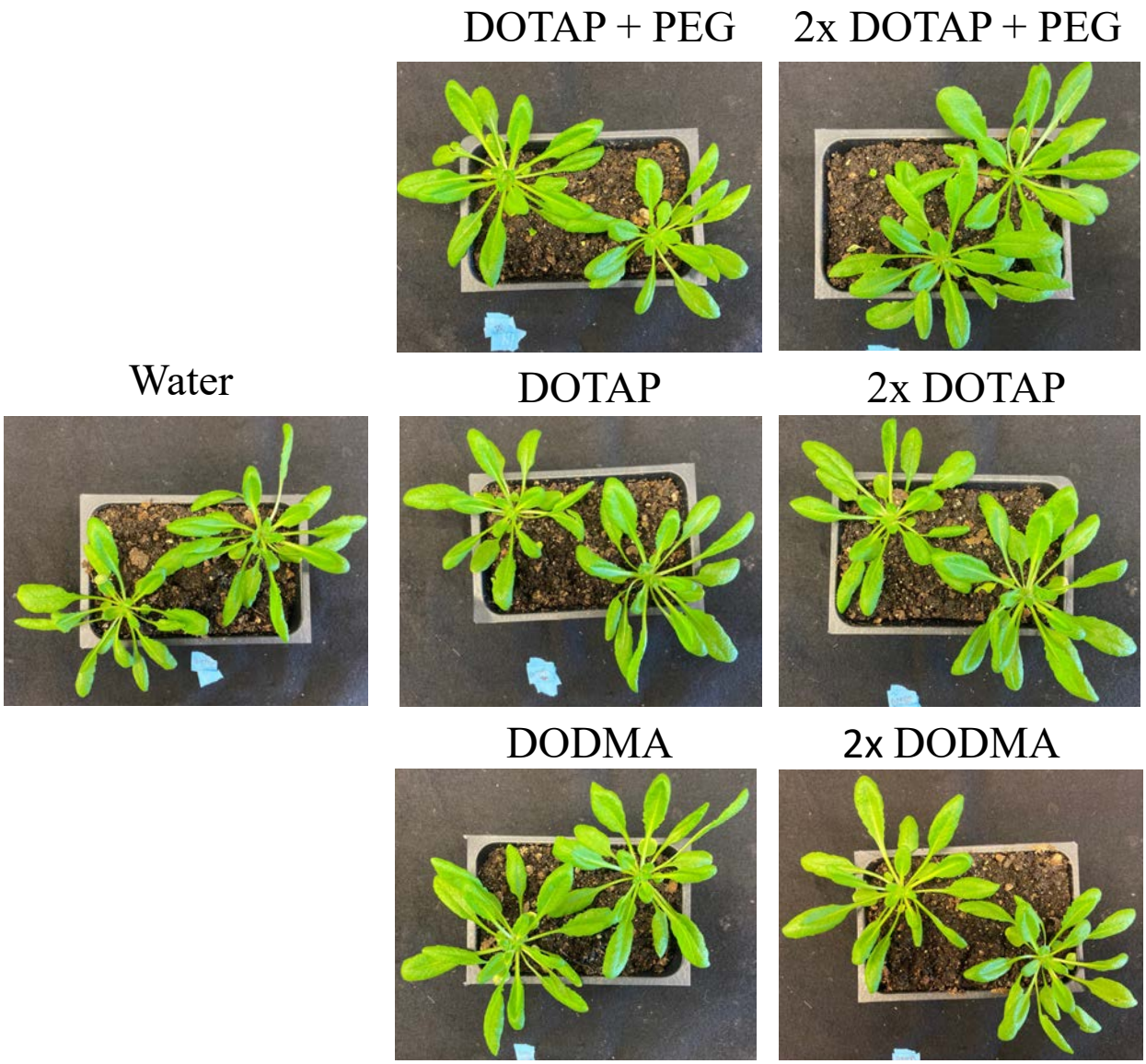
Supplementary Figure 3. Direct comparison of AV formulation performance with dsRNA performance. Tomato fruits were pre-treated with naked- or AV(DOTAP+PEG)-*Bc-VDS*-dsRNA, AV(DOTAP)-*Bc-VDS*-dsRNA and AV(DODMA)-*Bc-VDS*-dsRNA, for 1, 5, and 10 days, then inoculated with *B. cinerea.* Relative lesion sizes were measured with the help of ImageJ software. Error bars indicate the SD of the 3 biological replicates. Statistical significance compared to naked-*Bc-VDS* (Student's t-test): *, $P < 0.05$.

Supplemental Figure 4



Supplementary Figure 4. Treatment with AV-dsRNAs reduces expression of targeted genes in *B. cinerea*. From tomato treatments shown in Figure 6, relative gene expression of the three targeted genes in *Botrytis*, *VPS51, DCTN1,* and *SAC1* was quantified by qPCR using RNA extracted from the infected fruits at 5 dpi. Statistical significance compared to water (Student's t-test): *, $P < 0.05$; **, $P < 0.01$.

Supplemental Figure 5



Supplementary Figure 5. AV formulations display no signs of phytotoxicity on *Arabidopsis* plants. 5-week-old *Arabidopsis* plants were treated with a 20 μL suspension on each leaf of either water, DOTAP+PEG-, DOTAP-, or DODMA-AVs. AV treatments were performed at working concentrations or at two times the working concentration. Working concentration is determined by the amount of lipid needed to encapsulate dsRNA at a concentration of 20ng/μL.

Supplementary Tables

Supplemental Table 1: Size distribution of unloaded and dsRNA-loaded AV formulations

Mixing protocol	N/P	Z-avg (d.nm)	PDI	Zeta potential (mV)
Empty DOTAP:Chol:DSPE-PEG2000 (2:1:0.1)	N/A	120.3	0.06	44.32
VDS-dsRNA+DOTAP:Chol:DSPE-PEG2000 (2:1:0.1)	(4:1)	365.3	0.455	47.17
Empty DOTAP:Chol (2:1)	N/A	123.4	0.06	57.59
VDS-dsRNA+DOTAP:Chol (2:1)	(4:1)	259.5	0.368	47.23
Empty DODMA:Chol (2:1)	N/A	298.3	0.337	38.77
VDS-dsRNA+DODMA:Chol (2:1)	(4:1)	393.6	0.385	-9.55

Supplemental Table 2: Primers used in this study

Primer Name	Primer Sequence (5'-3')	Primer Description
YFP-dsRNA-T7-F	TAATACGACTCACTATAGGGAGAATGGTG AGCAAGGGCGAGGA	Template DNA for in vitro YFP-dsRNA synthesis
YFP-dsRNA-T7-R	TAATACGACTCACTATAGGGAGATTACTT GTACAGCTCGTCCATGC	
BcDCL1-dsRNA-T7-F	TAATACGACTCACTATAGGGAGATGCGGA AGAACTTGAAGGTTTGCTACA	Template DNA for In vitro Bc-DCL1/2 synthesis
BcDCL1-dsRNA-T7-R	GCAGCAAATGGCATCCGTCCAGATCTGGT CAACACACCAAG	
BcDCL2-dsRNA-T7-F	CTTGGTGTGTTGACCAGATCTGGACGGAT GCCATTTGCTGC	
BcDCL2-dsRNA-T7-R	TAATACGACTCACTATAGGGAGAACTCTT GAGTACTTTCGCCAGCTCAC	
BcVPS51-dsRNA-T7-F	TAATACGACTCACTATAGGGAGATTCGTT CCAGGAGTTACACG	Template DNA for In vitro Bc-VPS51+DCTN1+SAC1 synthesis
BcVPS51-dsRNA-T7-R	CTTTCGACGAGAGCACGAATGATGAGAC AAGTGAGAGTCCA	
BcDCTN1-dsRNA-T7-F	TGGACTCTCACTTGTCTCATCATTCGTGCT CTCGTCGAAAG	
BcDCTN1-dsRNA-T7-R	CACTGCACTTTGAACAACGTCCAGCTTAC AACTGTGCTCT	
BcSAC1-dsRNA-T7-F	AGAGCACAGTTGTAAGCTGGACGTTGTTC AAAGTGCAGTG	
BcSAC1-dsRNA-T7-R	TAATACGACTCACTATAGGGAGA CCTTCAATGCTGCTGTAGAAG	
BcVPS51-qRT-F	GTTAGGCAAAGTAGGAGAAGG	qRT-PCR BcVPS51, BcDCTN1, and BcSAC1 expression
BcVPS51-qRT-R	CATCCATTCGCCCCAATACC	
BcDCTN1-qRT-F	GCTTCCCTTACCTCGAATTC	
BcDCTN1-qRT-R	CAACCTCTAGTTCTTCCGGTC	
BcSAC1-qRT-F	CGAAAAGTTTCGAGGCATTTG	
BcSAC1-qRT-R	GAACTTAATGGGGTCAAAGCC	
Tomato-tubulin-qRT-F	GATTTGCCCACTAACCTCTCGT	qRT-PCR for relative fungal biomass assays
Tomato-tubulin-qRT-R	ACCTCCTTTGTGCTCATCTTACCC	
Bc-actin-qRT-F	TGCTCCAGAAGCTTTGTTCCAA	
Bc-actin-qRT-R	TCGGAGATACCTGGGTACATAG	