

## Supplemental

### **Double-stranded RNA targeting ergosterol biosynthesis pathway control *Botrytis cinerea* and postharvest gray mold**

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**Short title:** Control of *Botrytis* by dsRNA

**Keywords:** Double-strand RNA; ergosterol biosynthesis; *Botrytis cinerea*; gray mold; postharvest.

## Methods S1

### ERG construct

ERG construct combined sequences of 3 chosen essential genes from the ergosterol biosynthesis pathways (ERG13, ERG11=CYP51, and ERG1) in *Botrytis cinerea* B05.10, a total of 751bp

GAATTC - EcoRI restriction site

TAATACGACTCACTATAGGGAGA - T7 promoter sequence(sense)

#### ERG11=CYP51-307bp

CTGTTTTGACAACCCCGTATTTGGCAAAGATGTAGTTTACGACTGCCCAAATGCGAAGTTGATGGA  
GCAAAAGAAGTTCATGAAAATTGGCTTGCTACAGAAGCTTCCGATCCTACGTCCCAATCATACAAA  
TGGAGGTGGAAAACCTTATGAAGCGTTCCTCGGCGTTCAAAGGTCCAAAGGGAAGTCTGACATTG  
GTCCCGCTATGGCTGAAATCACCATCTACACTGCTTCGCACACTCTGCAAGGAAAGGAAGTCCGCGA  
TCGATTCGATACCTCCTTGCCTCTCTCTACCACGACCT

#### ERG1-210bp

AGAAGCAGATTGTTATTTGGCCATCTCACCATCATCGCAGATGGATATGCCTCCAAATTCCGCAAGC  
AATACATCAACAAAACCTCCATTGTCAAAAGTAAATTCTACGCTCTAGAATTAATAGATTGTCCCATG  
CCAGTCCCAATCATGGAATCGTAGTCCTCTCGGACGTCTCCCCAGTTCTCCTCTATCAAATCGGTACC  
CACGA

#### ERG13-234bp

ATGCTACGGTGGTACCAACGCCGTTTTCAACGCTGTCAACTGGGTAGAATCATCTGCATGGGATGGA  
AGAGACGCCATTGTCGTTGCTGGAGATATTGCTCTATATGCCAAGGGTGCTGCACGTCCAACTGGAG  
GTGCTGGAGCTGTTGCCATGTTGATTGGACCAAATGCTCCAGTTGTTGTCGAGCCTGGTCTTCGCGG  
ATCCTACATGCAACATGCCTACGATTTCTACAA

### Dicer construct

The DCL1/DCL2 dicer was cloned in a similar manner between the T7 transcriptional promoters using the following sequences for DCL1 and DCL2, respectively:

#### DCL1 - 252bp

TGCGGAAGAAGTGAAGGTTTGCTACACAGTCAAATATGTAAGTGCAGAAGATCCCAGCTTGCTGCAG  
TACTCAATCAAAGGTAAACCTGAGACTCTTGCCTACTATGATCCCTTGGGCCCGAAATTCAATACTCC

TCTTTATCTTCAAATGCTCCCGCTTCTAAAAGACAATCCTATCTTTCGGAAGCCATTTGTATTTGGGAC  
AGAAGCCAGTAGAACTCTAGGATCTTGGTGTGTTGACCAGATCTGGAC

DCL2 -238bp

GGATGCCATTTGCTGCACGCCAAAAATACATCGAGCAGATCTTCGCCTTCGAGTAAAGCTACCACTTC  
TATCTATTATCTACTATACCCAGAGTCAAATATCATCGTGACGAAAAGTGTGGCGAGCCTGAGAAA  
GATTGTGCAAAGTCTCAACATTTTCGAAGACCCCTACGTTTTGACACTAAAAGGAGTGATAGCGAA  
AAAAGTCAACGTGAGCTGGCGAAAGTACTCAAGAGT

## Results

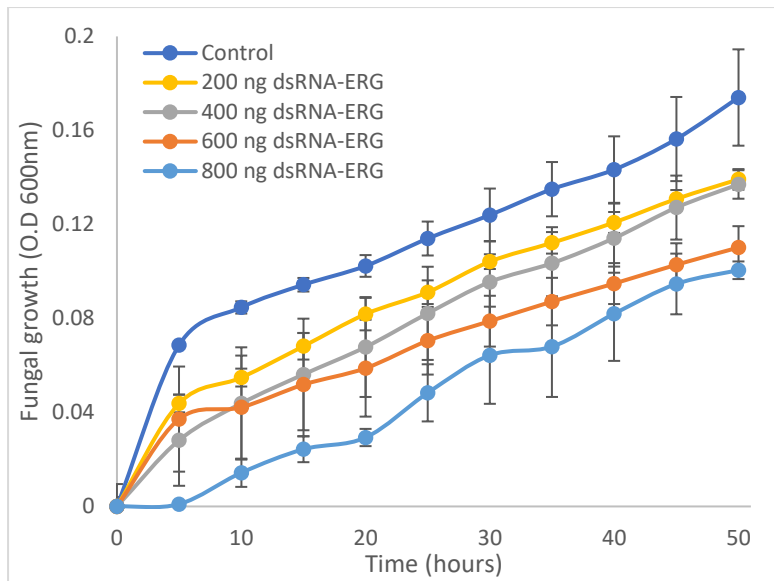
**Table S1.** Coefficient treatment interaction and growth inhibition by dsRNA and fungicides.

CNT			<sup>1</sup> Final growth level (O.D 600nm)				<sup>2</sup> Growth inhibition (%)		
	0		0.32±0.04				0		
	dsRNA- ERG (ng)	Fungicides (ppb)	dsRNA- ERG Only	Fungicide only	dsRNA-ERG + Fungicides	<sup>3</sup> Coefficient treatment interaction	dsRNA- ERG only	Fungicide only	dsRNA-ERG + Fungicides
Prochloraz		0	0.08±0.02				75.41		
	800	1		0.31±0.02	0.07±0.02	0.9		5.82	77.89
		10		0.30±0.03	0.06±0.01	0.8		6.96	82.53
		100		0.25±0.02	0.05±0.00	0.8		21.42	85.93
		1000		0.10±0.00	0.04±0.00	1.6		68.07	86.24
Fludioxonil		0	0.08±0.02				75.41		
	800	1		0.10±0.00	0.04±0.00	1.6		68.66	89.07
		10		0.10±0.01	0.03±0.00	1.2		69.38	89.79
		100		0.10±0.00	0.04±0.00	1.6		67.99	88.25
		1000		0.10±0.00	0.03±0.00	1.2		67.76	89.64
CNT	0		0.22±0.03				0		
Fludioxonil low concentrations		0	0.14±0.01				31.75		
	800	0.01		0.18±0.01	0.15±0.01	1.3		16.61	28.73
		0.1		0.16±0.02	0.14±0.01	1.3		27.80	36.96
		1		0.15±0.03	0.13±0.01	1.2		29.66	40.84

<sup>1</sup>The final growth level is the final O.D measurement during the kinetics of fungal growth.

<sup>2</sup>Growth inhibition percentage was calculated by  
(O.D in control well – O.D in treatment well)/ O.D in control well X100

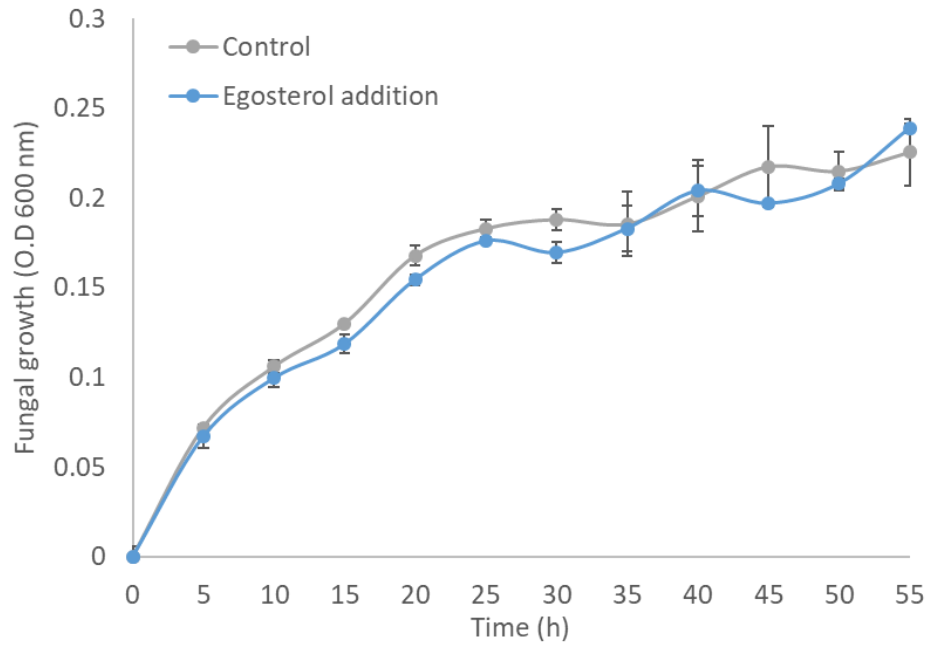
<sup>3</sup>Coefficient treatment interaction (CTI) was calculated as follows:  $CTI = AB / (A \times B)$ . AB is the ratio of the treatment combination to control; A or B is the ratio of the single treatment to control.  $CTI < 1$  is considered a synergistic interaction.



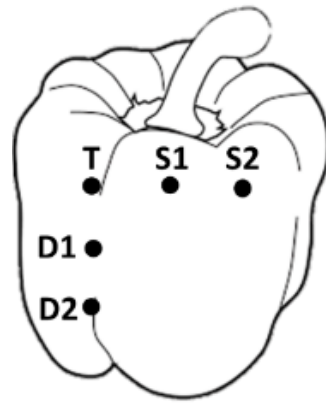
**Figure S1.** Dose effect of dsRNA-ERG on fungal growth. *B. cinerea* conidia were grown at room temperature in 1% MSB, or 1% MSB supplemented with various concentrations of dsRNA-ERG. O.D. measurements at 600 nm were taken every hour. The presented data are mean and standard errors.



**Figure S2.** No wounding infection. Tomato fruit was treated with water (control), Bc-DCL1/2-dsRNA (dsRNA-Dicer), or Bc-ERG1/13/11-dsRNA (dsRNA-ERG) following inoculation by *B. cinerea*. No decay was observed. Representative pictures were taken three days post-inoculation.

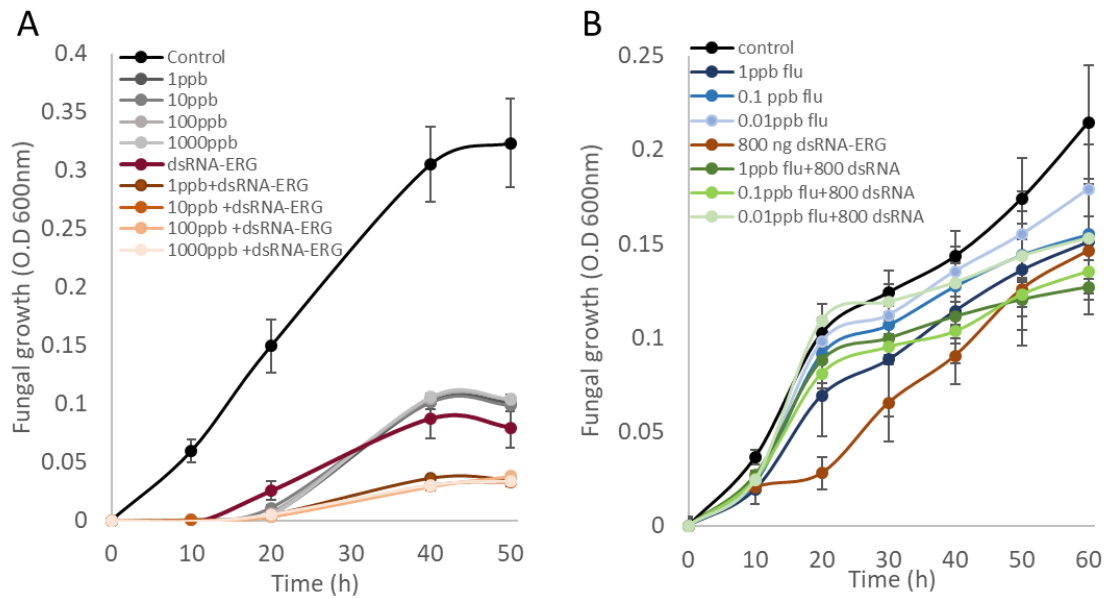


**Figure S3.** Fungal growth with external ergosterol addition. *B. cinerea* conidia were grown at room temperature in 1% MSB, or 1% MSB supplemented with ergosterol. O.D measurements at 600 nm were taken every 5 hours. The presented data are mean and standard errors.



**Figure S4.** Illustration of systemic evaluation experiment set-up. dsRNA-ERG was applied at the top left corner, marked with T, following inoculation of *B. cinerea*. S1, S2, D1, and D2 are *B. cinerea* inoculation points located 1.5 and 3 cm (in tomatoes) or 2 and 4 cm (in bell peppers) distance from the treatment point.





**Figure S5.** dsRNA-ERG application reduces fungicide-needed concentration for growth inhibition of *B. cinerea*. *B. cinerea* conidia were incubated at room temperature in 1% SMB, or 1% SMB supplemented with various concentrations of fludioxonil with or without dsRNA-ERG. O.D measurements at 600 nm were taken every 10 hours. A. Fludioxonil was applied at concentrations of 1-1000 ppb. B. Fludioxonil was applied at lower concentrations (0.01-1 ppb). The presented data are mean and standard errors.