Boxwood blight: cultivar testing

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Valley Laboratory

Connecticut - 2011

	Precipitation	Days w/ precip.
August	10.9” (7”> ave)	19 days
September	9.7” (> 6”)	17 days
October	6.5” (> 2”)	15 days

Disease first recognized in September 2011.

Disease is favored 1° by wet, warm conditions, likely present for some time, no outbreak - smoldering

Initial epidemic - fall 2011

Field grown nursery – plants should all be green

Boxwood blight

Landscape positives:

2011 - 8

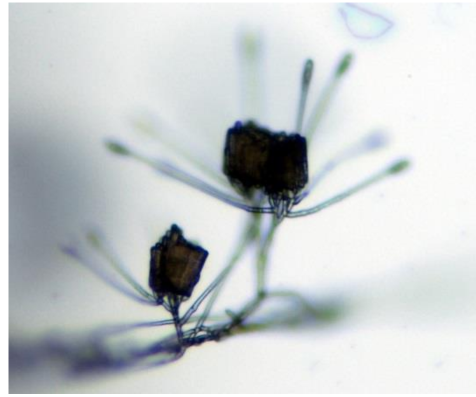
2018 - >400 (192 in October)

2018 was the second wettest year and most humid year ever in CT.

Aug-Oct: 19” rain over 37 days.

RH >90 for 77 days.

- *Calonectria pseudonaviculata* causes disease on all ornamental species of the *Buxaceae* in North America.   No complete resistance has been described in any genus.
- *Buxus* spp and cultivars differ in susceptibility.
- No comprehensive study to date.
- Fungicide efficacy being defined, fungicide resistance has been reported from Europe.
- How best do we manage this pathogen? Can we breed for disease resistance?



- Plant resistance is often the most efficient and successful way to manage disease. Differences in plant susceptibility to boxwood blight have been well documented, however, results have not always been consistent.

Why inconsistent results?

Experiments conducted with whole plants, stem cuttings, or detached leaves.

Pathogen isolates and virulence may have differed.

Environmental conditions may have differed between experiments and plant/leaf age or growth stage differed.

The type of data collected also may have differed.

Susceptibility of *Buxus* from the U.S. National Arboretum collection to boxwood blight caused by *Calonectria pseudonaviculata*.

Buxus species	# accessions	Lesions/plant	Normalized by size
B. bodinieri	1	28.1 AB	26.3 B
B. harlandii	1	7.4 A	7.0 A
B. microphylla koreana x *sempervirens*	6	48.6 B	24.2 B
B. microphylla	7	31.1 AB	16.7 A
B. sempervirens	20	69.6 C	22.9 B
B. sinica	3	11.1 A	11.1 A
Buxus sp.	1	21.9 AB	7.1 A
B. wallichiana	1	160.1 D	37.3 B
	P =	0.00001	0.0001

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B. wallichiana	1	160.1 D	37.3 B
	P =	0.00001	0.0001

Susceptibility of boxwood accessions (best and worst of 42 total)

Accession	*Buxus* species/cultivar	Lesions	Normalized
60705*H	*B. sinica* var. *aemulans*	2.4 A	2.3 A
36365*J	*B. sempervirens*	4.6 AB	2.6 AB
18834*H	*B. harlandii*	7.4 ABC	7.0 ABC
9548*H	*B. sempervirens* 'Scupi'	14.4 ABCD	14.4 ABCDEFG
4899*CH	*B. microphylla* 'Compacta'	14.5 ABCD	18.0 BCDEFGHI
57953*H	*B. sempervirens* 'Arborescens'	16.3 ABCDE	16.3 BCDEFGHI
29703*H	*B. sempervirens* 'Suffruticosa'	91.4 H	45.5 LM
59820*H	*B. sempervirens* 'Pendula'	93.0 H	26.1 DEFGHIJK
69558*H	*B. sempervirens* 'Ohio'	107.0 H	27.1 EFGHIJK
51910*H	*B. sempervirens* 'Northland'	110.6 H	27.7 FGHIJK
31793*H	*B. sempervirens* 'Arborescens'	159.5 H	39.6 KL
51896*H	*B. wallichiana*	160.1 H	37.3 IJKL

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Susceptibility of detached leaves of Buxus from the U.S. National Arboretum collection to boxwood blight caused by *Calonectria pseudonaviculata*.

Buxus species	% w lesions	% symptomatic	% sporulating
B. bodinieri	68.6 B	90.0 B	25.0 AB
B. harlandii	26.3 A	72.0 A	20.0 A
Buxus microphylla koreana x *sempervirens*	68.4 B	74.0 AB	40.7 AB
B. microphylla	67.5 B	71.6 AB	35.8 AB
B. sempervirens	73.9 B	84.4 B	44.6 B
B. sinica	62.4 B	78.6 AB	48.2 B
Buxus sp.	54.3 AB	72.0 A	44.0 AB
B. wallichiana	91.8 C	76.0 AB	76.0 C
P =	0.00001	0.01	0.001

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B. microphylla	67.5 B	71.6 AB	35.8 AB
B. sempervirens	73.9 B	84.4 B	44.6 B
B. sinica	62.4 B	78.6 AB	48.2 B
Buxus sp.	54.3 AB	72.0 A	44.0 AB
B. wallichiana	91.8 C	76.0 AB	76.0 C
P =	0.00001	0.01	0.001

Boxwood cultivars: Ranked High to low susceptibility in experiments:
True Dwarf, Common, Green Velvet, Winter Green, Green Mountain, Korean.

Green Gem, Green Velvet, Tide Hill

B. sempervirens, *B. sempervirens* x *B. sinica*, *B. sinica* var *insularis*

No strong resistance – about 30% disease (23 affected leaves versus 80)

We are continuing to screen germplasm

Conclusions:

- *Buxus* species, cultivars and accessions differed in susceptibility to boxwood blight.
- *B. harlandii* (zone 7-9) most resistant, *B. sinica* > *B. microphylla* > hybrids > *B. sempervirens*.
- *B. wallichiana* most susceptible.
- Some accessions had different rankings with detached leaf assays.
- Genetic potential for resistance breeding. Differences within species is promising.

The HRI project:

Whole plants, liner sized, range of suspected susceptibility.
Inoculated with multiple isolates, virulence maintained, up to 10^6 spores per ml (4 sprays per plant to 4 sides under the same very conducive environmental conditions.

% leaf area infected, AUDPC and # lesions over time.

We will define 4 levels of susceptibility over all stages and environments.

Fall 2018 Boxwood Blight Variety Evaluation

Variety	# plants	# lesions	Percent infected	AUDPC	Overall Rank
Little Missy	15	9.3 (2)	0.3 (1)	43 (2)	1
Winter Gem	15	6.7 (1)	2.5 (4)	76 (5)	2
Franklin's Gem	15	14.7 (5)	1.0 (3)	46 (3)	3
Nana	15	56.3 (10)	0.5 (2)	32 (1)	4
Green Mountain	15	12.5 (3)	2.8 (6)	70 (4)	4
Green Beauty	15	14.4 (4)	2.5 (5)	103 (7)	6
NewGen Ind.	15	22.1 (7)	3.7 (8)	101 (6)	7
NewGen Free.	15	17.6 (6)	3.5 (7)	112 (8)	7
Green Gem	15	23.7 (8)	3.8 (9)	209 (10)	9
sempervirens	15	78.7 (12)	5.2 (10)	184 (9)	10
Dee Runk	15	33.1 (9)	12.3 (13)	251 (11)	11
Green Velvet	15	115.7 (14)	8.0 (12)	409 (12)	12
Justin Brouwers	15	91.5 (13)	6.9 (11)	559 (14)	12
Vardar Valley	15	67.5 (11)	16.7 (14)	490 (13)	12
Cranberry Creek	12	452.7 (16)	18.8 (15)	1098 (15)	15
Calgary	12	1216.3 (17)	32.5 (16)	1280 (16)	16
English/suffruticosa		258.1 (15)	87.3 (18)	2163 (18)	17
Sprinter	12	2953.3 (18)	47.9 (17)	1542 (17)	18

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Spring 2019 Blight Evaluation	Spring 2019 Rank			Overall Rank	
Variety	# lesions	% infected	AUDPC	2019	2018
John Baldwin	1	1	1	1	
Golden Dream	2	11	6	5	
J. Stauffer	3	5.5	9	4	
Little Missy	9	8	8	8	1
Winter Gem	5	2	2	2	2
Franklin's Gem	17	14	15	16	3
Green Mountain	8	4	3	3	4
Green Beauty	NT				6
NewGen Independence	4	12	13	10	7
NewGen Freedom	11	9.5	11	11	7
Green Gem	NT				9
sempervirens	NT				10
Dee Runk	10	15	16	13	11
Richard	12	16	14	14	
Green Velvet	13	3	5	7	12
Justin Brouwers	14	17	17	17	12
Vardar Valley	7	5.5	7	6	12
Cranberry Creek	16	9.5	10	12	15
Calgary	18	13	12	15	16
English/ suffruticosa	20	18	18	18	17
Sprinter	15	7	4	9	18

Spring 2019 Blight Evaluation			Spring 2019 Rank		Overall Rank
Variety	# lesions	% infected	AUDPC	2019	2018
John Baldwin	**1**	**1**	**1**	**1**	
Golden Dream	**2**	**11**	**6**	**5**	
J. Stauffer	**3**	**5.5**	**9**	**4**	
Little Missy	9	8	8	8	1
Winter Gem	5	2	2	2	2
Franklin's Gem	17	14	15	16	3
Green Mountain	8	4	3	3	4
Green Beauty	NT				6
NewGen Independence	4	12	13	10	7
NewGen Freedom	11	9.5	11	11	7
Green Gem	NT				9
sempervirens	NT				10
Dee Runk	10	15	16	13	11
Richard	12	16	14	14	
Green Velvet	13	3	5	7	12
Justin Brouwers	14	17	17	17	12
Vardar Valley	7	5.5	7	6	12
Cranberry Creek	16	9.5	10	12	15
Calgary	18	13	12	15	16
English/ suffruticosa	20	18	18	18	17
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Golden Dream	**2**	**11**	**6**	**5**	
J. Stauffer	**3**	**5.5**	**9**	**4**	
Little Missy	**9**	**8**	**8**	**8**	**1**
Winter Gem	**5**	**2**	**2**	**2**	**2**
Franklin's Gem	17	14	15	16	3
Green Mountain	**8**	**4**	**3**	**3**	**4**
Green Beauty	NT				6
NewGen Independence	4	12	13	10	7
NewGen Freedom	11	9.5	11	11	7
Green Gem	NT				9
sempervirens	NT				10
Dee Runk	10	15	16	13	11
Richard	12	16	14	14	
Green Velvet	13	3	5	7	12
Justin Brouwers	**14**	**17**	**17**	**17**	**12**
Vardar Valley	7	5.5	7	6	12
Cranberry Creek	**16**	**9.5**	**10**	**12**	**15**
Calgary	**18**	**13**	**12**	**15**	**16**
English/ suffruticosa	**20**	**18**	**18**	**18**	**17**
Sprinter	15	7	4	9	18

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Golden Dream	2	11		6	5	
J. Stauffer	3	5.5		9	4	
Little Missy	**9**	**8**	**inconsistent?**	**8**	**8**	**1**
Winter Gem	5	2		2	2	2
Franklin's Gem	**17**	**14**	**inconsistent?**	**15**	**16**	**3**
Green Mountain	8	4		3	3	4
Green Beauty	NT					6
NewGen Independence	4	12		13	10	7
NewGen Freedom	11	9.5		11	11	7
Green Gem	NT					9
sempervirens	NT					10
Dee Runk	10	15		16	13	11
Richard	12	16	***B. harlandii***	14	14	
Green Velvet	13	3		5	7	12
Justin Brouwers	14	17		17	17	12
Vardar Valley	**7**	**5.5**	**inconsistent?**	**7**	**6**	**12**
Cranberry Creek	16	9.5		10	12	15
Calgary	18	13		12	15	16
English/ suffruticosa	20	18		18	18	17
Sprinter	**15**	**7**	**inconsistent?**	**4**	**9**	**18**

Differences between Fall 2018 and Spring 2019 may be due to leaf age and susceptibility or to environmental conditions.

The different data each tells us a part of the story.

lesions: infection success from the initial inoculation, same # spores and conditions.

% leaf area/ foliage infected: accounts for plant size.

AUDPC and # lesions over time: epidemic development, number of secondary spores, time to develop, how long spores are produced etc.

We will follow the disease for a full year to evaluate susceptibility over all stages and a range of environments.

We will also follow up with detached leaf experiments to confirm differences in sporulation over time, effects of leaf age and other questions raised by the differences in whole plant evaluations.

DLA Blight Evaluation – lesion size

Variety	Area (mm2)
Green Mountain	94.4
NewGen Independence	43.8
Green Velvet	42.4
John Baldwin	40.3
English/ suffruticosa	38.3
Dee Runk	38.1
Justin Brouwers	12.4
NewGen Freedom	8.8
Calgary	7.6
Little Missy	5.0
Winter Gem	2.0
Grace	0.2

DLA Blight Evaluation – lesion size

Variety	Area (mm2)
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Little Missy	5.0
Winter Gem	2.0
Grace	0.2

Resistance may best be evaluated on whole plants over a full year

Management of boxwood blight

Fungicide activity: *in vitro* testing

Incorporate fungicides into artificial growth media at a range of concentrations: 50 ppm to 0.01 ppm. Twenty active ingredients from 13 FRAC groups.

- Against germination of conidia (germ tube growth)
- Against mycelial growth

Fungicide activity: *in planta* testing

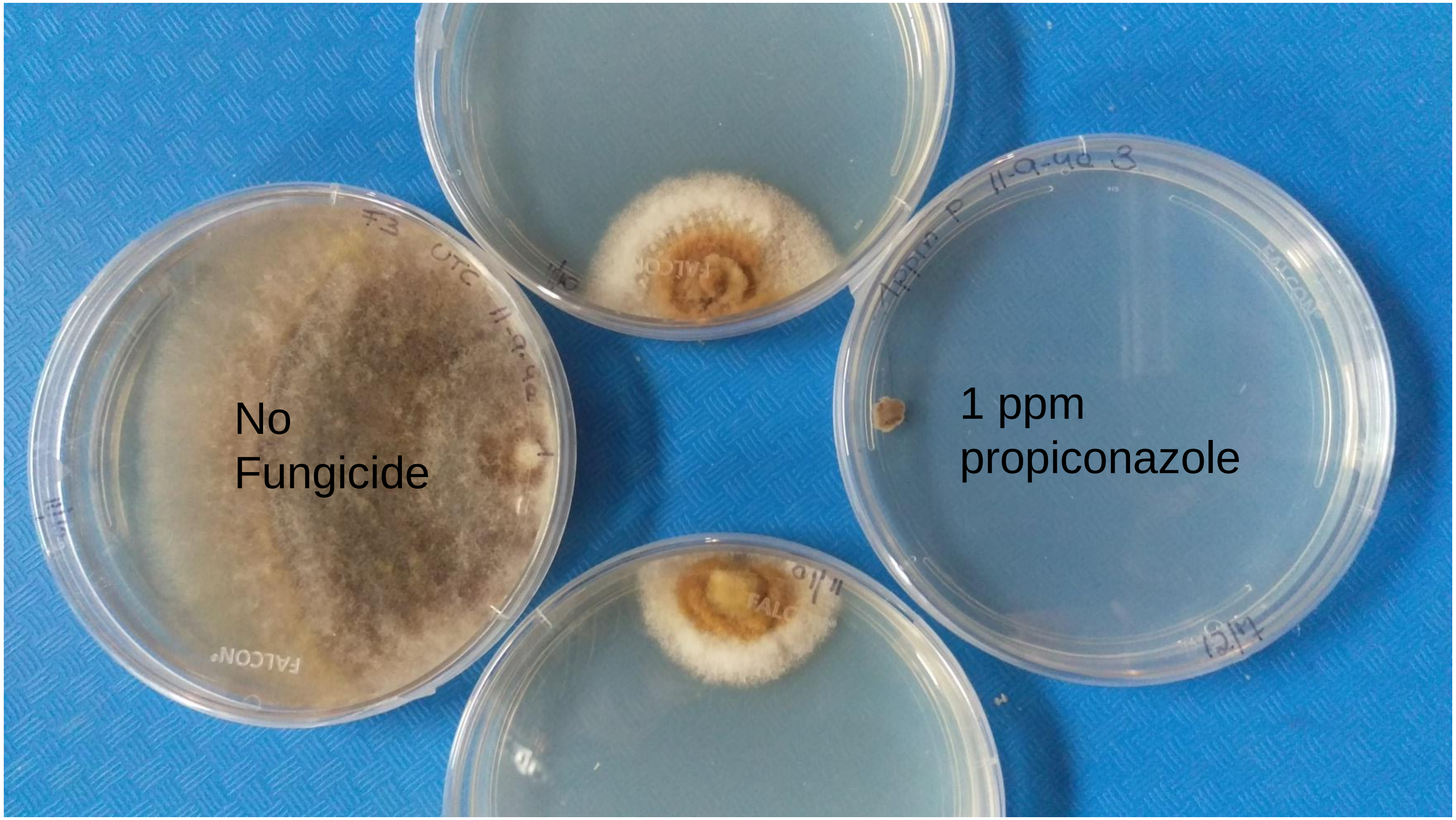
Apply fungicides to liners and 3-gal pots, inoculate

- Activity against disease development
- Curative activity in plant tissue
- Phytoxicity?

Efficacious Fungicides: in vitro, in planta

- Thiophanate methyl (FRAC 1)
- Propiconazole (FRAC 3) other DMIs
- Fludioxonil (FRAC 12)
- Benzovindiflupyr (FRAC 7)
- Fluxapyroxad (FRAC 7)
- Kresoxim-methyl (FRAC 11)
- Pyraclostrobin (FRAC 11)
- Chlorothalonil (FRAC M5)

In vitro fungicide screens



In vitro testing:

Growth from plugs removed from fungicide-amended media

Conc (ppm)	T-M	K-M	Teb	Prop	Triflumizole
5	2/5	9/10	1/6	0/6	0/6
10	9/12	8/9	0/6	0/6	0/6
50	1/10	7/7	0/6	0/6	0/6

DMI fungicides – potential for curative activity?

Curative fungicide activity?

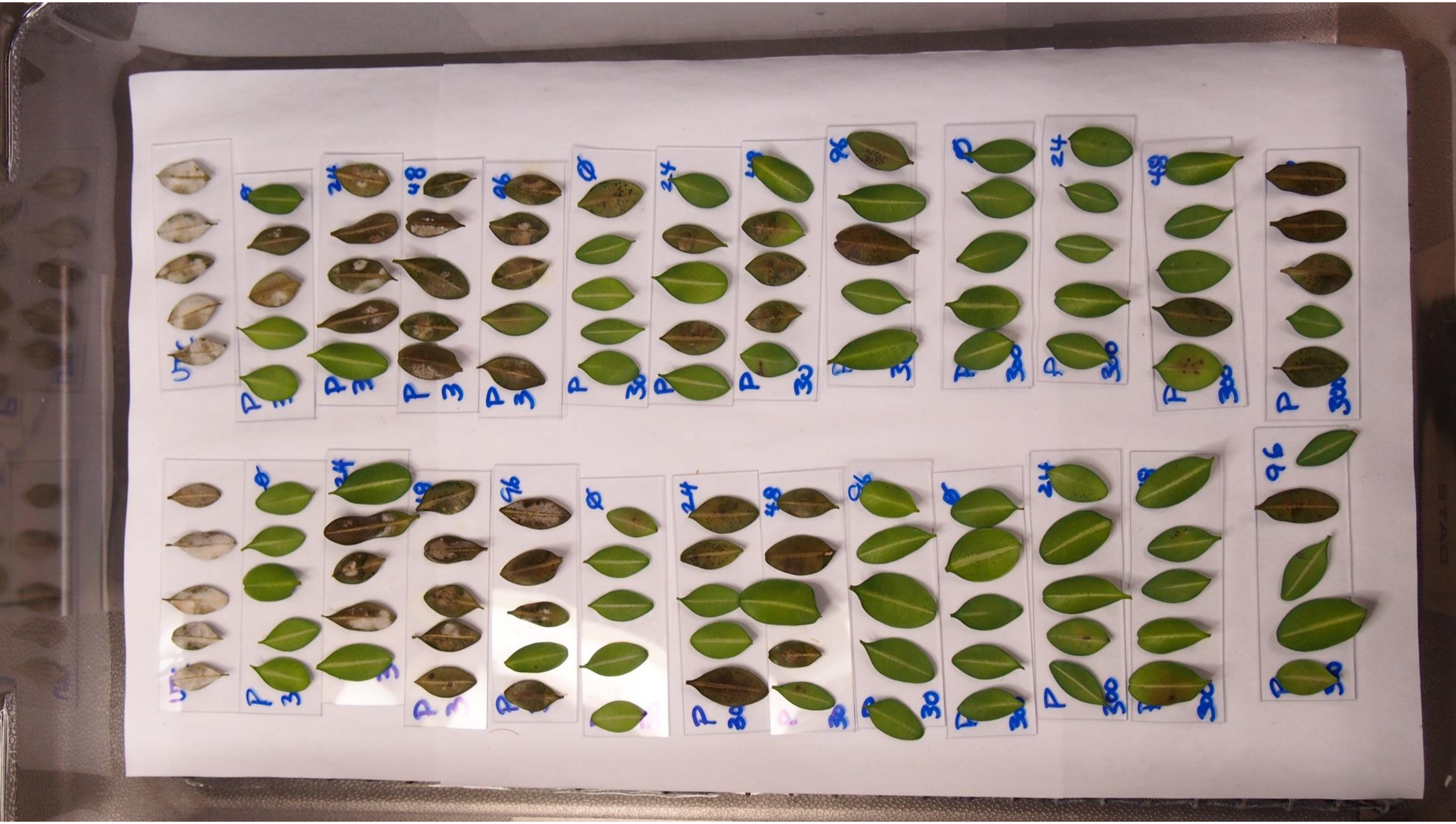
Two approaches: **detached leaves**; whole plant

- Pretreat or **curative** treatments
- Inoculate Green Velvet leaves with 200 conidia
- 24, 48, or 96 h after inoculation, apply 3, 30 or 300 ppm of selected fungicides
- Count infected leaves, % area and sporulation
- Re-isolate from visible lesions

200 Conidia added in a single drop per leaf

Infection through stomates by 24 h

Detached leaf assay - propiconazole



Curative fungicide activity

Leaf Disease - Incidence

Different from the UTC (Fishers Exact Test)

	PreTrt	24 h	48 h	96 h
Propiconazole	3, 30, 300	30, 300	--	--
Tebuconazole	3, 30, 300	--	--	--
Kresoxim-methyl	3, 30, 300	--	--	--
Thioph-methyl	3, 30, 300	--	--	--

Curative fungicide activity

Sporulation Incidence

Different from the UTC (Fishers Exact Test)

	PreTrt	24 h	48 h	96 h
Propiconazole	3; 30; 300	30;300	30;300	300
Tebuconazole	3; 30; 300	30;300	30;300	30;300
Kresoxim-methyl	3; 30; 300	--	--	--

Curative fungicide activity?

Two approaches: **whole plant**; detached leaves

- Inoculated liners with the pathogen
- 48 h after inoculation, apply fungicides
- Count the lesions that developed
- Re-isolate from visible lesions

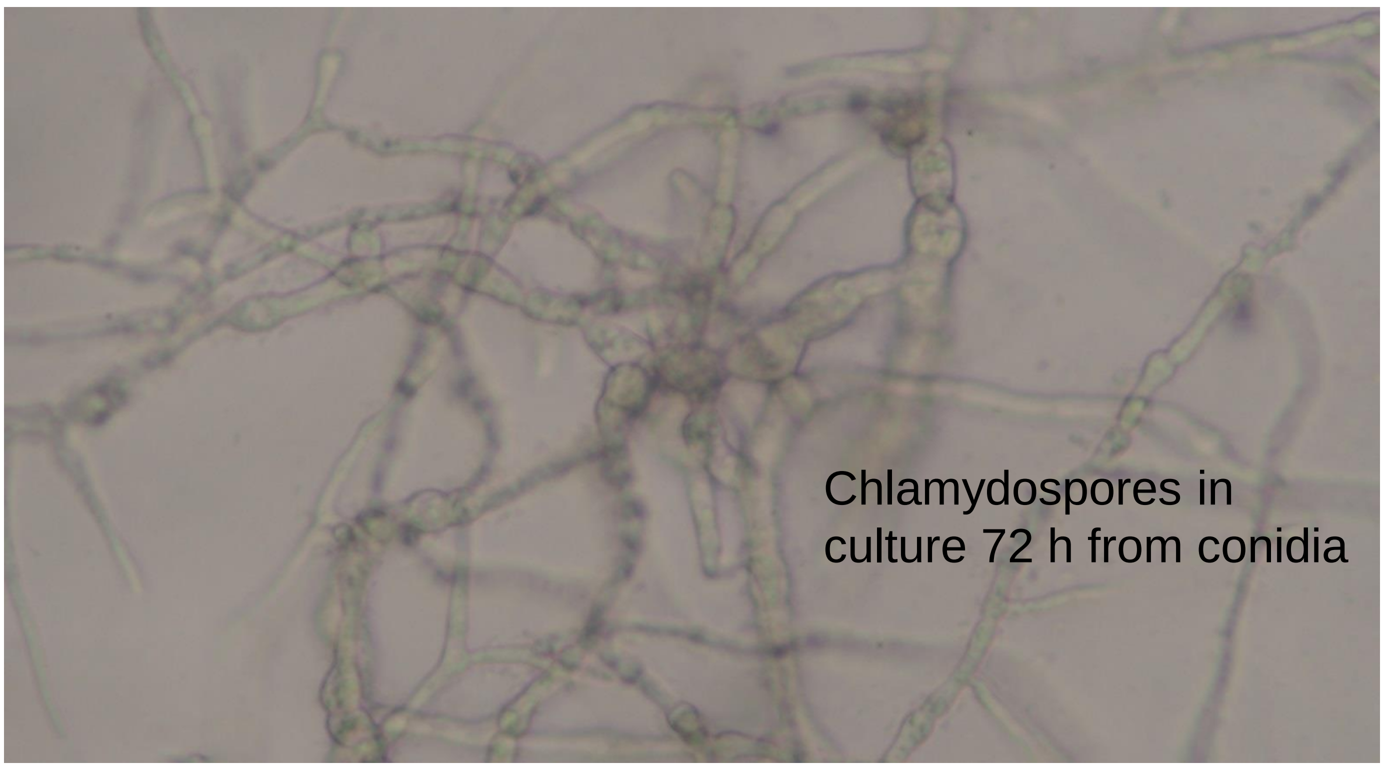
Curative fungicide activity?

Fungicide	Diseased	Percent re-isolation
Untreated	45.3 a	46.7 a
thiophanate	51.0 a	66.7 a
pyraclostrobin	40.0 a	43.3 a
propiconazole	27.7 b	10.0 b
	P= 0.01	p= 0.04

- Post-infection sprays reduced lesion numbers and ability to re-isolate the pathogen.

Why no complete curative fungicide activity?

- Testing against hyphae. Chlamydospores and microsclerotia form in leaves. How quickly? Testing fungicides vs microsclerotia.



Chlamydospores in culture 72 h from conidia

Microsclerotia 120 h from conidia on media

Microsclerotia exposed to >300 ppm thiophanate-methyl or kresoxim-methyl for 96 h survived and grew on media.

Only propiconazole reduced survival and colony diameter.

Normalized viability of MS after exposure to propiconazole.

Fungicide ppm	Hours of exposure 24	48	72	96
3.16	0.79	0.78	0.91	0.78
10	0.79	0.49	0.33	0.19
31.6	0.10	0.12	0.07	0.03
100	0.22	0.11	0.00	0.02
316	0.00	0.04	0.00	0.00

Conclusions:

- Initial epidemic disease pressure too high for good fungicide efficacy with late applications.
- A number of efficacious fungicides have activity against spore germination and growth.
- Blight management on plants can be > 95%.
- DMI fungicides had sporulation inhibition and some curative activity prior to chlamydospore and microsclerotia development.
- Demonstrated reduced QoI fungicide sensitivity, fungicide resistance management needed.

Boxwood Blight Research Supported By:

AmericanHort™

The IR-4 Project

HRI
Horticultural Research Institute

Connecticut Nursery & Landscape Association

2008 Farm Bill

2012, 2013 & 2014 Farm Bills: Section 10201

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