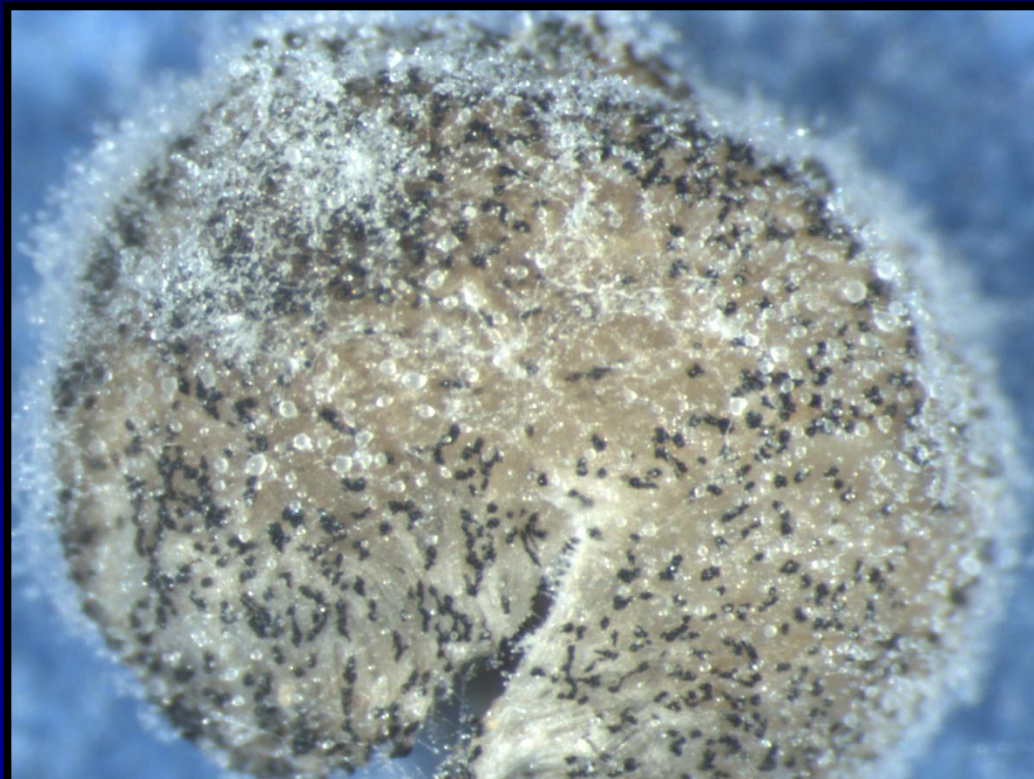


Selecting a Spinach Seed Assay for *Verticillium*: Preliminary Results of an ISHI Ring Test

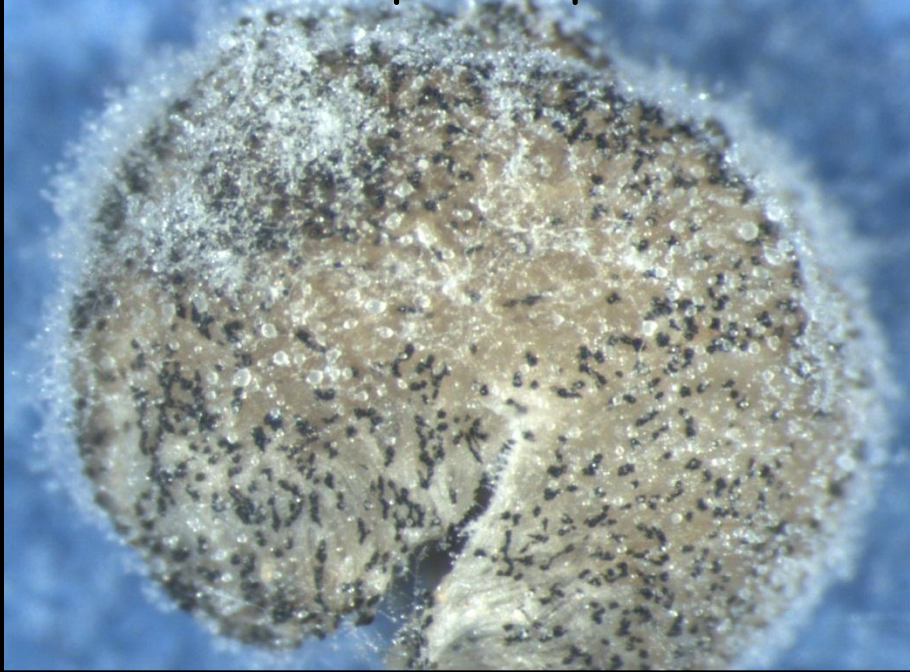


2011 International Spinach Conf., Amsterdam, Holland

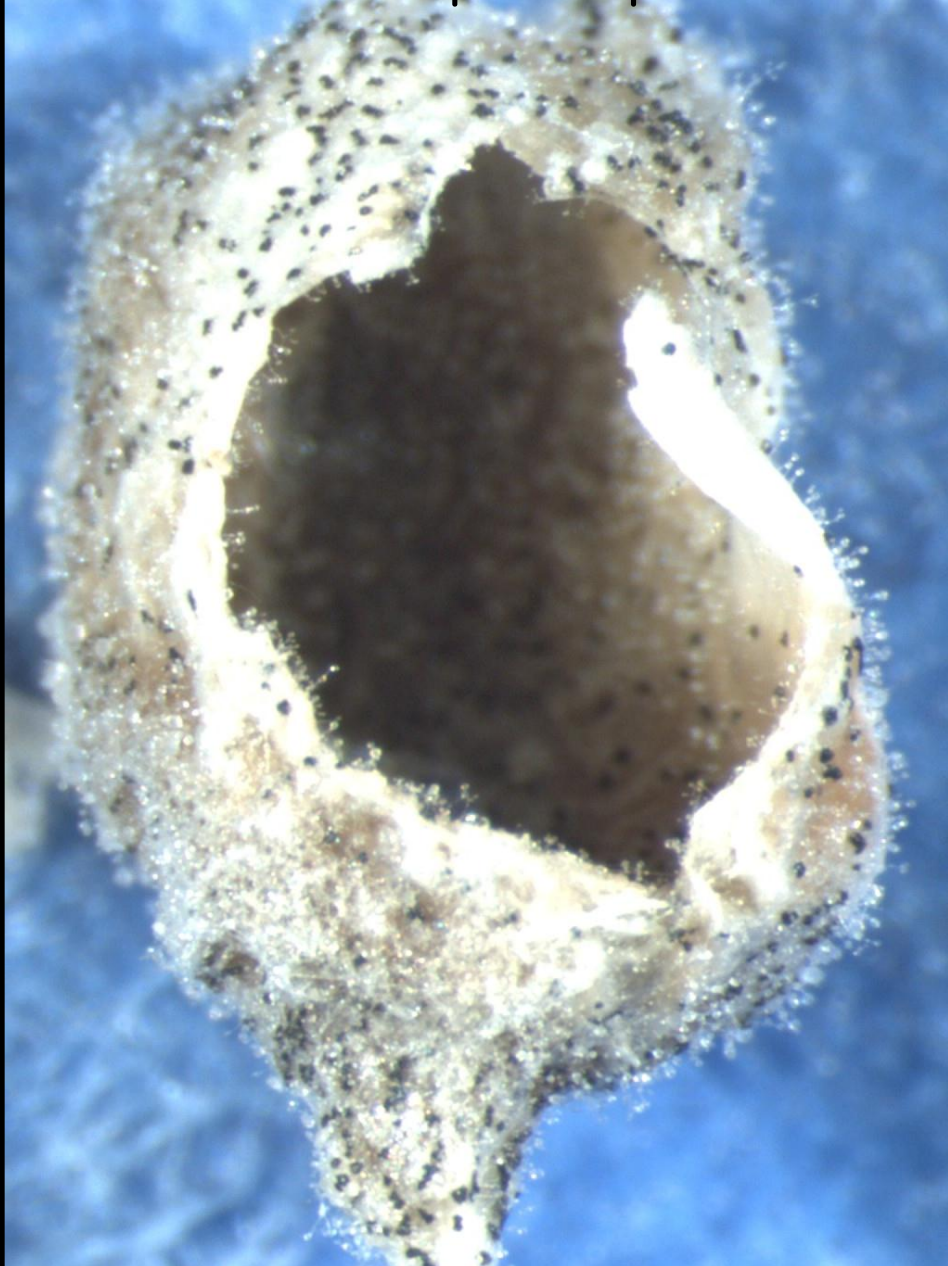
Lindsey du Toit, Washington State University

Cooperators: Philip Brown, Victoria Hallowell, Gerbert Hiddink, Harrie Koenraad, Matt May, Yim So, Parm Randhawa, Margreet Asma

Non-sterilized pericarp



30 sec sterilized pericarp

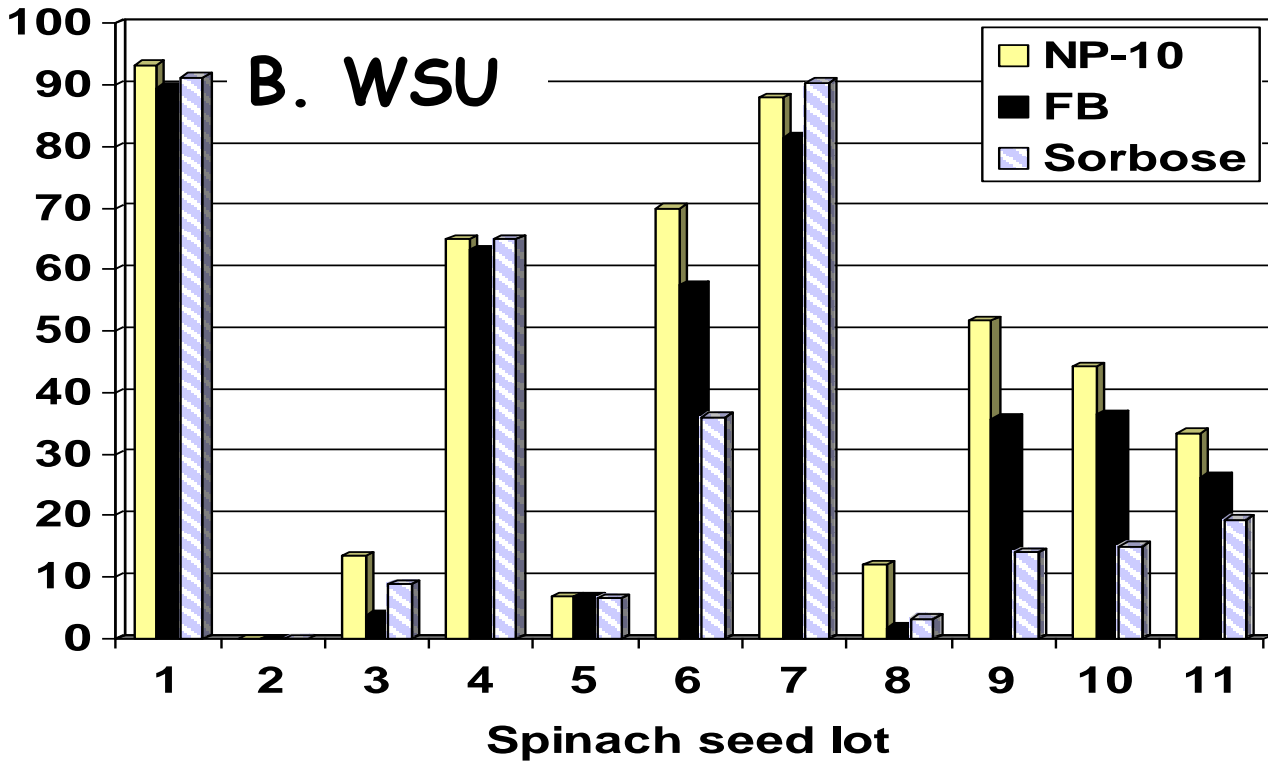
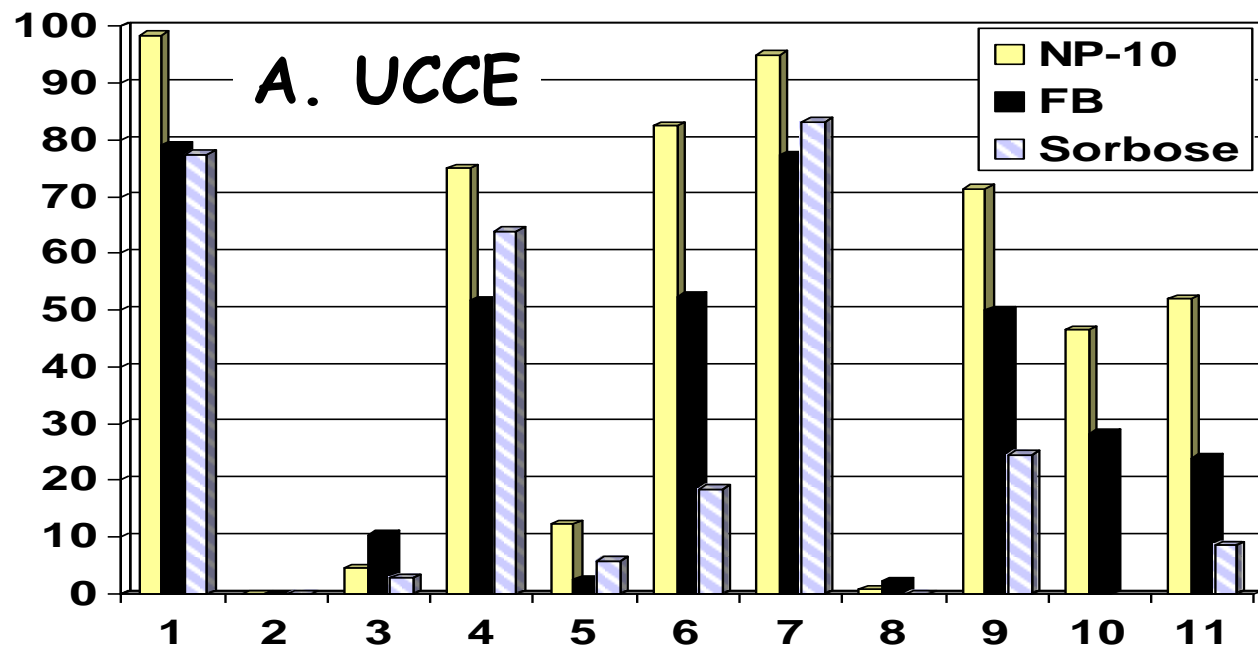


30 sec sterilized embryo



(du Toit et al., 2009. Plant Dis. Mgmt Rep. 3:ST020)

Incidence (%) of seed with *Verticillium* spp.



Detection of *Verticillium* spp. using FB, NP10, & sorbose agar assays

Means:
WSU = 37.3
UCCE = 37.5

NP-10: A
FB: B
Sorbose: C

(du Toit & Koike, 2009)

Seed assay parameters to be standardized

- Type of assay: FB, NP-10 agar, sorbose agar
- Parameters:
 - Container: Petri plates, acrylic boxes, other
 - Number of seeds assayed: 200 or 400/lot
 - Seed preparation: seed rinsed, surface-sterilized, or neither
 - sterilant type, concentration, duration of treatment, rinse
 - Incubation temperature: 20 or 24°C
 - Incubation duration: 9-14 days, longer for treated seed
 - Dark or day/night cycle: 12 h/12 h, if the latter, near-UV only or NUV + cool white fluorescent light
 - Number of times seeds examined, duration of assay
- FB assay:
 - Type of blotters, volume of water added to blotters
 - Duration of imbibing (24-25 hours) in dark
 - Duration of freezing at -20°C (24-25 hours)
- **May not be 1 optimum protocol for all seed industry needs**
 - Biological seed treatments: problem with antibiotics in NP10 agar
 - Effect of fungicide seed treatments
- **COMMUNICATION OF LABS WITH USERS OF RESULTS!**

Objectives of ISHI spinach seed ring test for *Verticillium*

- Compare freeze-blotter (FB) vs. NP-10 agar (NP10) assays for detection of *Verticillium* spp. on spinach seed
- Assess effects of fungicide seed treatments on FB & NP10 assays, including duration of assay
- FB vs. NP10 assays for quantitative detection of other seedborne fungal pathogens of spinach
- FB vs. NP10 assay consistency among labs
- Select a standardized, internationally accepted spinach seed health assay for *Verticillium*

Materials and methods

- Freeze blotter (FB) vs. NP-10 agar (NP10) assays
- 10 participating labs (4 in EU, 6 in USA)
- 3 commercial spinach seed lots (A, B, C)
- Fungicide-treated (metalaxyl + thiram) vs. non-treated seeds of each lot
- Protocol
 - 4 reps of 100 seeds/treatment combination
 - Surface-sterilized for 60 s (1.2% NaOCl), rinsed 3x
 - FB: seeds imbibed 25 h, frozen (-20°C) for 25 h
 - 24°C incubation (except 2 labs: 20°C)
 - 12 h/12 h day/night cycle (NUV + CWF)
 - Examination 5, 9, & 14 d; treated seeds also 21 d
 - Recorded: total *Verticillium* spp., other fungi (e.g, *Stemphylium*)
- Analyses of variance/means comparisons

Materials and methods

Participating labs

- Alf Christianson Seed Co., WA, USA (Philip Brown)
- Bejo Zaden BV (Margreet Asma)
- California Seed & Plant Lab, CA, USA (Parm Randhawa)
- Enza Zaden, The Netherlands (Gerbert Hiddink)
- Eurofins STA Laboratories, CO, USA (Vikki Hallowell)
- Germain's Technology Group NA, Inc., USA (Yim So)
- Monsanto Vegetable Seeds, CA, USA (Matt May)
- Naktuinbouw, The Netherlands (Harrie Koenraad)
- Rijk Zwaan, The Netherlands (Eelco Gilijamse)
- Washington State University, USA (Lindsey du Toit)

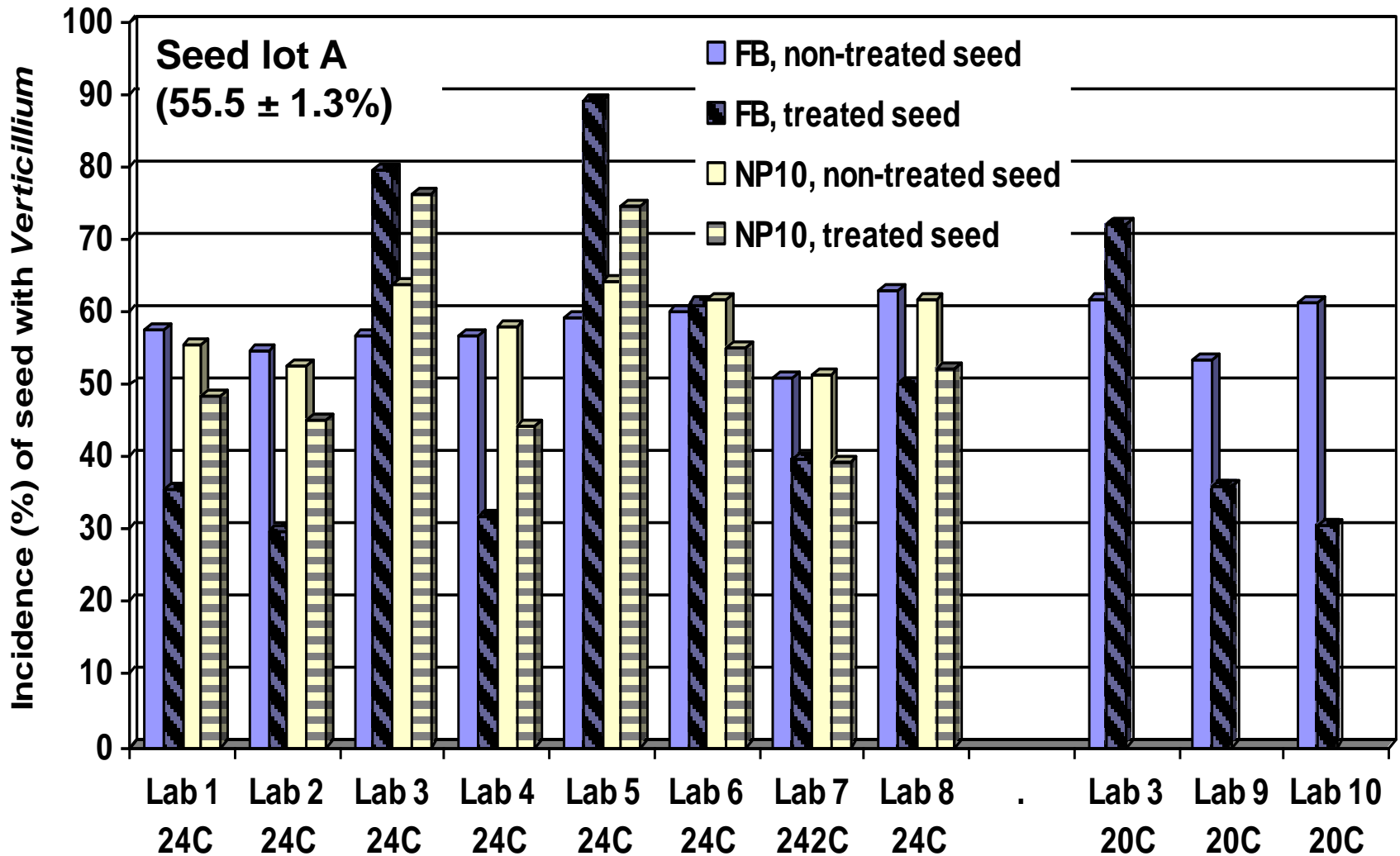
Results: ANOVA

Source of variation	Pr > F	
	<i>Verticillium</i> (final reading)	<i>Stemphylium</i> (final reading)
Lab	<0.0001 **	<0.0001 **
Rep(lab)	-	-
Seed assay	0.0001 **	<0.0001 **
Lab * assay	<0.0001 **	<0.0001 **
Seed lot	<0.0001 **	<0.0001 **
Lab * seed lot	<0.0001 **	<0.0001 **
Fungicide seed treatment	0.1196 NS	<0.0001 **
Lab * seed treatment	<0.0001 **	<0.0001 **
Assay * seed lot	0.4079 NS	<0.0001 **
Assay * seed treatment	0.1123 NS	<0.0001 **
Seed lot * seed treatment	<0.0001 **	<0.0001 **
Assay * seed lot * seed treatment	0.8320 NS	<0.0001 **
Lab * assay * seed lot	0.4652 NS	<0.0001 **
Lab * assay * seed treatment	0.0005 **	0.0065 **
Lab * seed lot * seed treatment	<0.0001 **	0.0217 *
Lab * assay * seed lot * seed treatment	0.3648 NS	0.0006 **

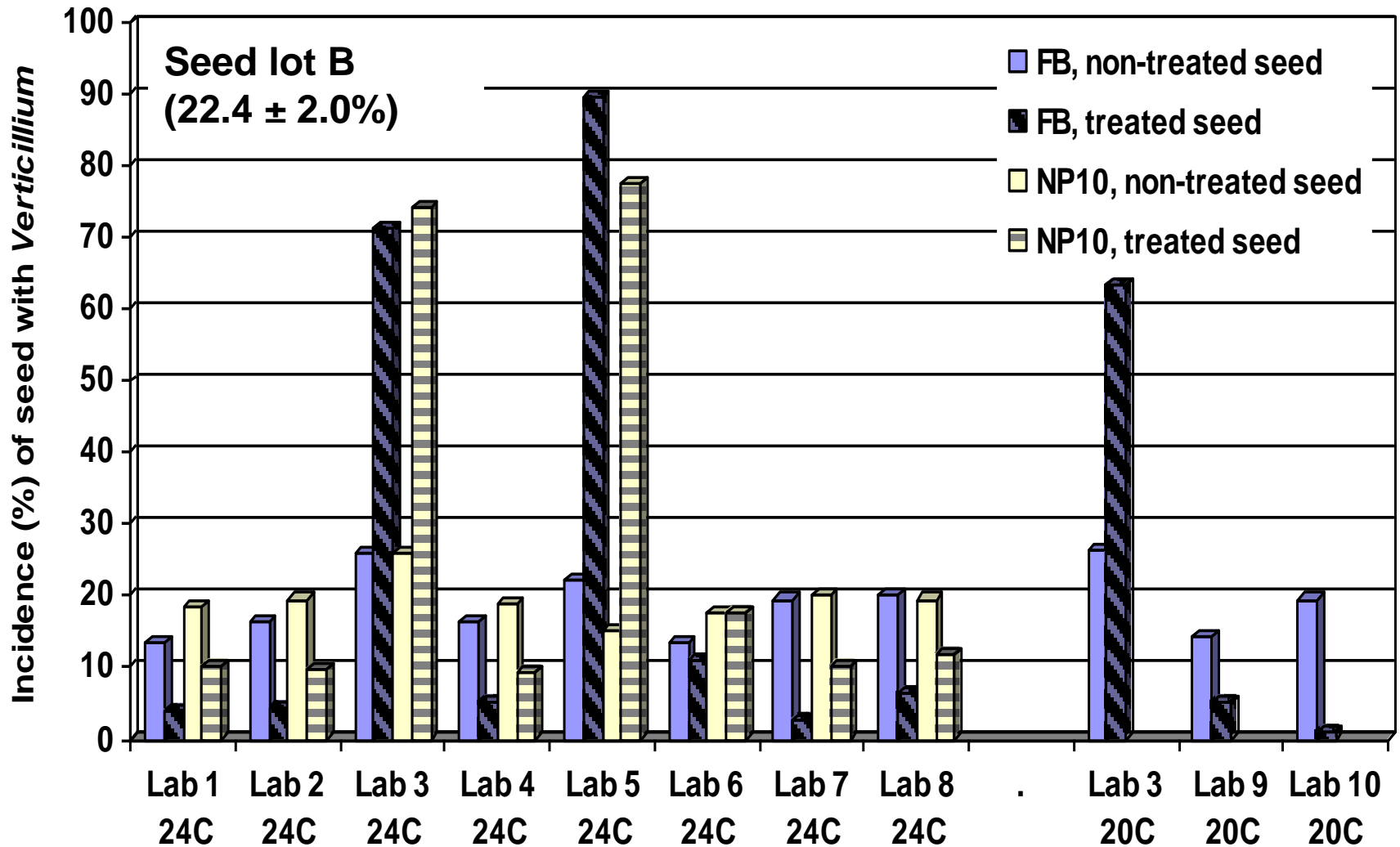
Results: *Verticillium* ANOVA

Source of variation	Pr > F		
	Seed lot A	Seed lot B	Seed lot C
Lab	<0.0001 **	<0.0001 **	<0.0001 **
Rep(lab)	-	-	-
Seed assay	0.2055 NS	0.0635 NS	<0.0001 **
Lab * seed assay	0.3792 NS	0.0374 *	<0.0001 **
Seed treatment	0.0014 **	<0.0001 **	0.3683 NS
Lab * seed treatment	<0.0001 **	<0.0001 **	<0.0001 **
Seed assay * seed treatment	0.7255 NS	0.2808 NS	0.1273 NS
Lab * seed assay * seed treatment	0.0150 *	0.9467 NS	0.0224 *

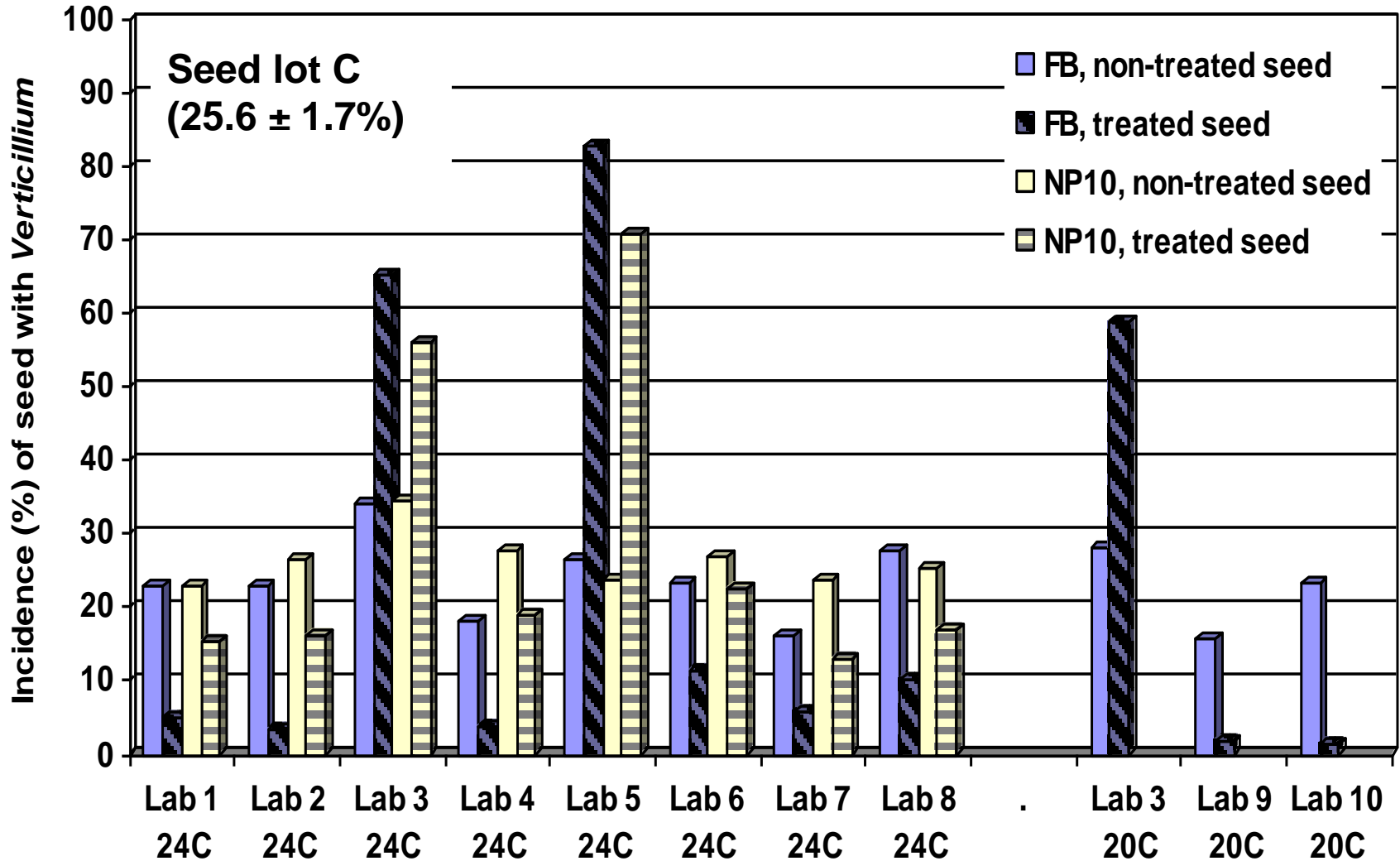
Results: *Verticillium* on seed lot A



Results: *Verticillium* on seed lot B



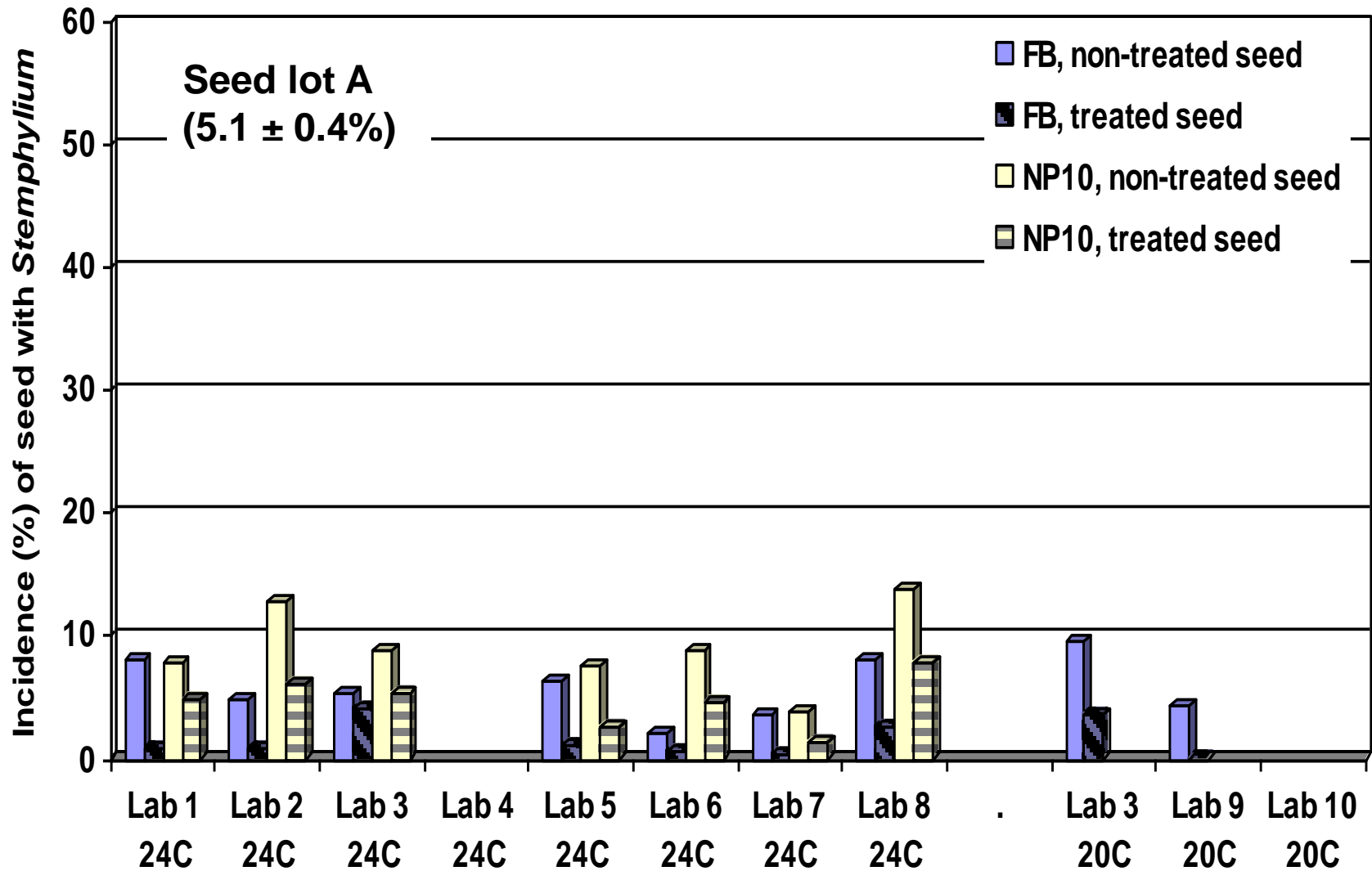
Results: *Verticillium* on seed lot C



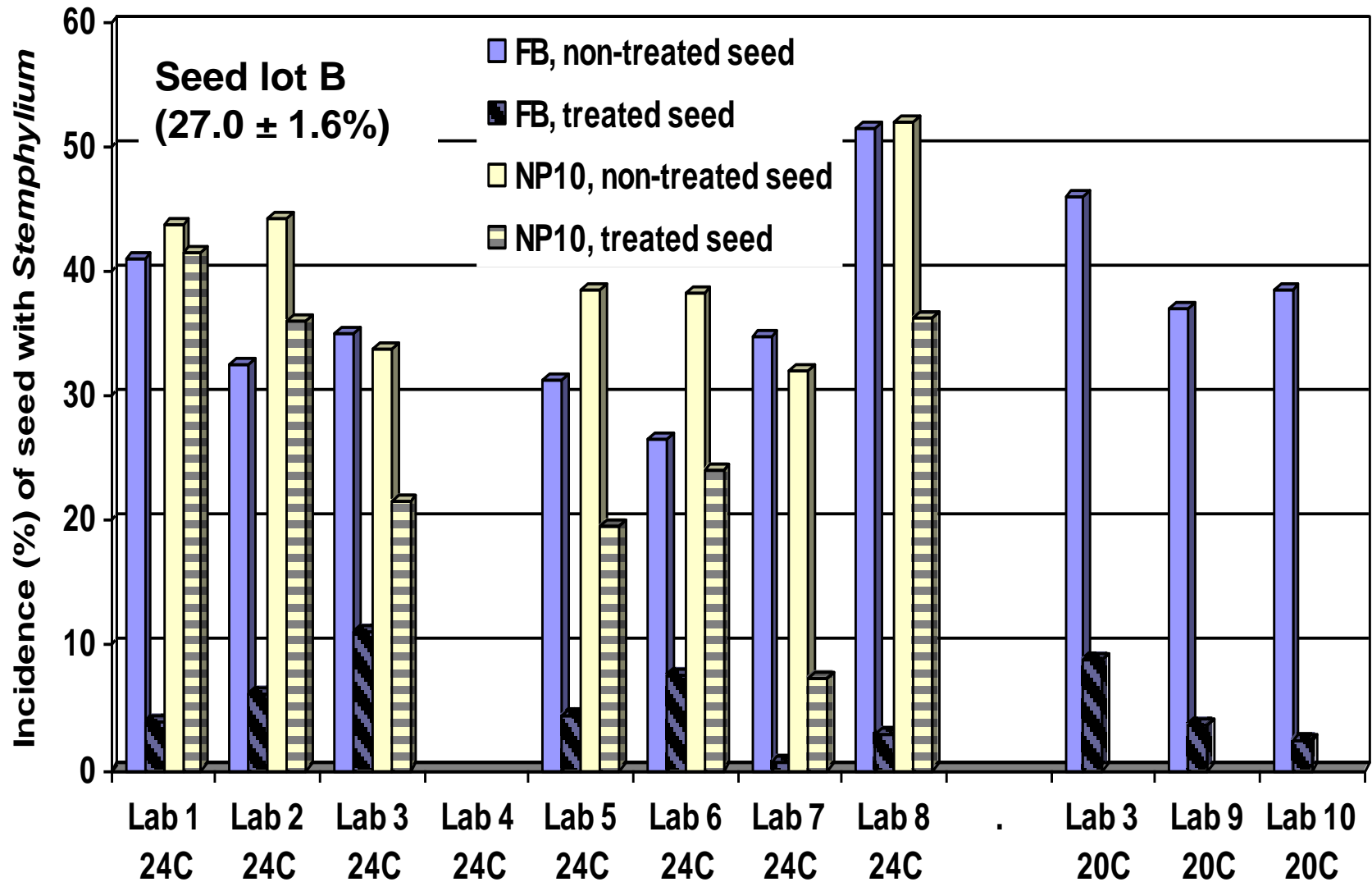
Results: *Stemphylium* ANOVA

Source of variation	Pr > F		
	Seed lot A ^b	Seed lot B	Seed lot C
Lab	<0.0001 **	<0.0001 **	<0.0001 **
Rep(lab)	-	-	-
Seed assay	<0.0001 **	<0.0001 **	<0.0001 **
Lab * seed assay	0.0099 **	<0.0001 **	0.8433 NS
Seed treatment	<0.0001 **	<0.0001 **	<0.0001 **
Lab * seed treatment	0.3460 NS	0.0003 **	0.0178 **
Seed assay * seed treatment	0.2799 NS	<0.0001 **	0.0039 **
Lab * seed assay * seed treatment	0.6358 NS	0.0005 **	0.2749 NS

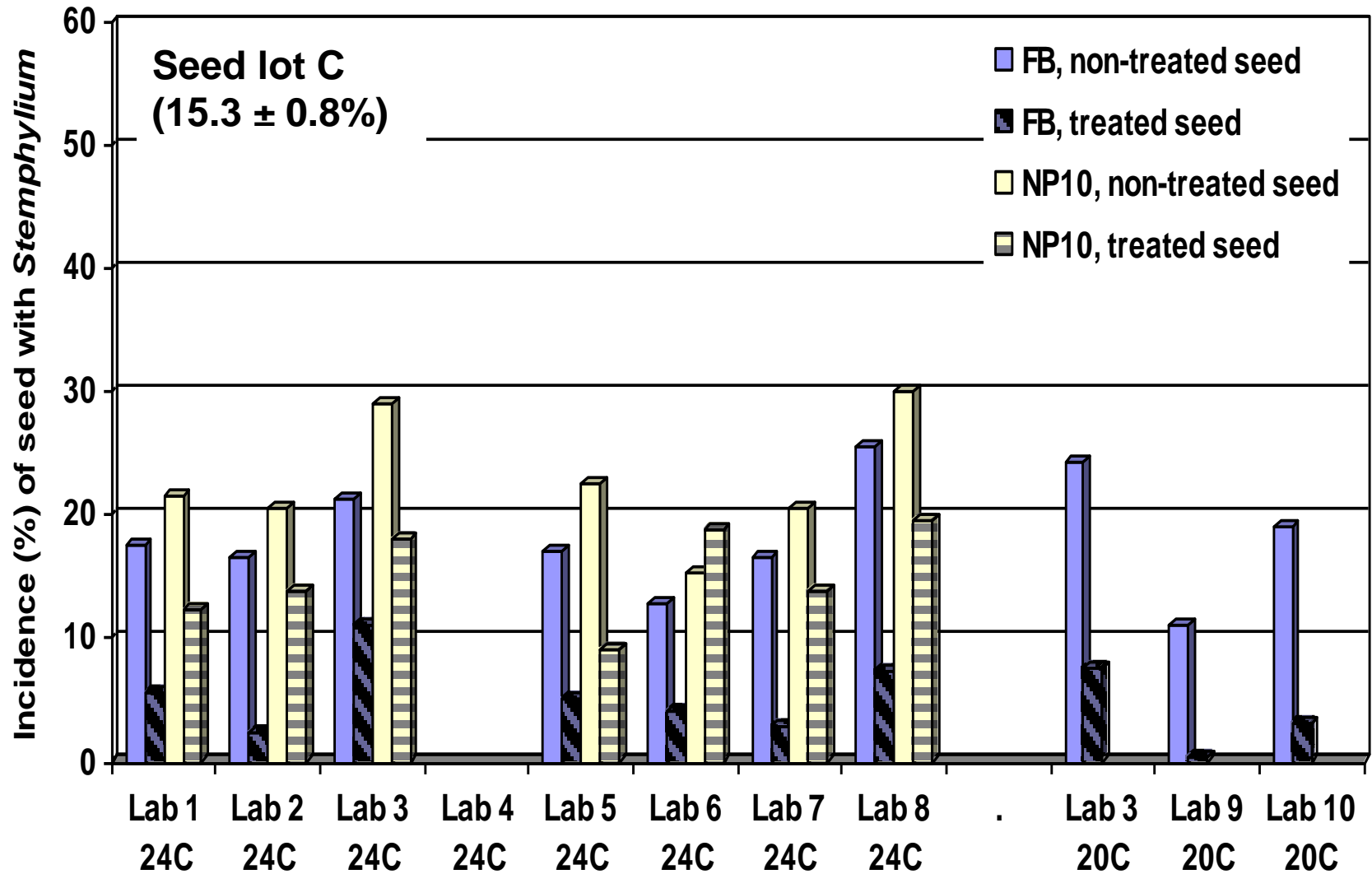
Results: *Stemphylium* on lot A



Results: *Stemphylium* on lot B



Results: *Stemphylium* on lot C

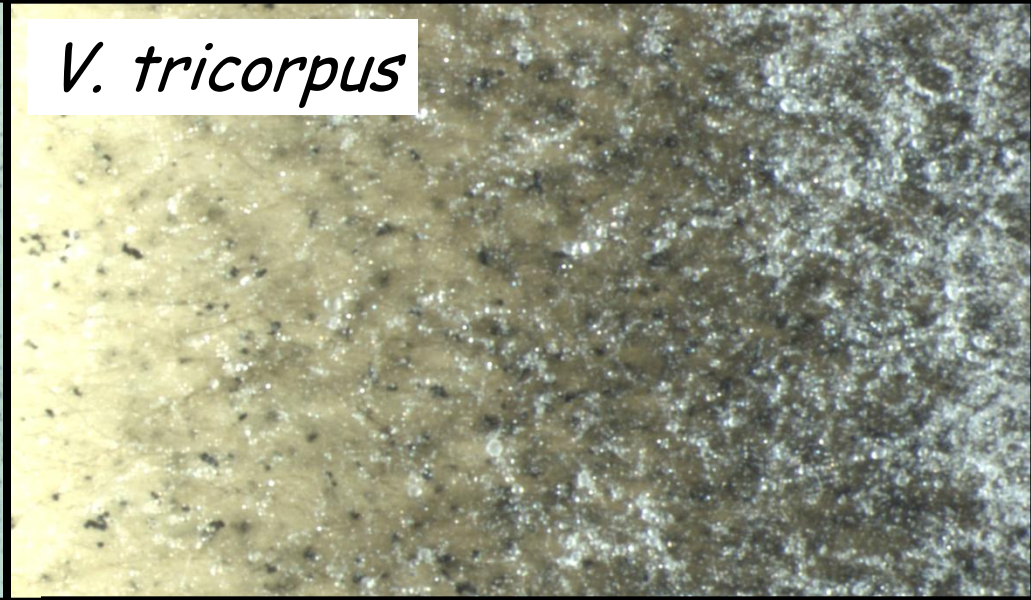


NP-10 agar assay: *Verticillium* spp.

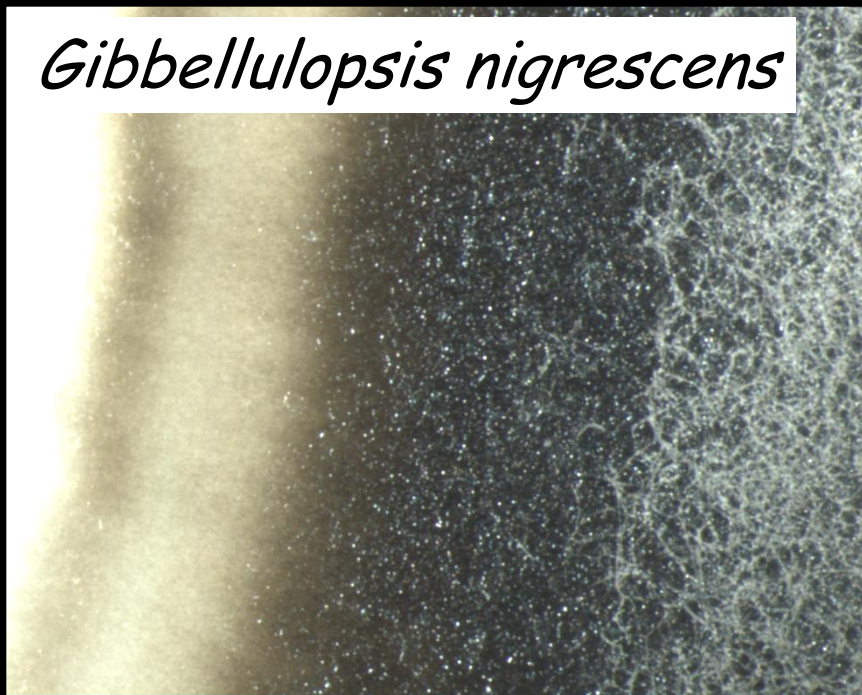
V. dahliae



V. tricorpus



Gibbellulopsis nigrescens



Multiple species?



Discussion

- For 6 of 8 labs with relatively consistent results at 24°C, NP10 & FB assays detected similar incidences of *Verticillium*, but NP10 assay detected significantly more *Stemphylium* than the FB assay
- Fungicide seed treatment significantly reduced incidence of fungi detected compared to non-treated seeds
- FB & NP10: extend assays to 21 d for treated-seeds vs. 14 d for non-treated seeds
- NP10 assay: fungicides dissipate into agar medium? Affects efficacy of seed treatment against *Verticillium* and *Stemphylium*? Implications for assaying treated seeds?
- NP10 assay: not appropriate for lots with biological seed treatments (antibiotics)
- Inconsistency among labs: technical expertise needed
- NP-10 agar: facilitates potential differentiation of *Verticillium* species more than FB assay
- Both assays facilitate quantification of other fungi: *Stemphylium botryosum*, *Cladosporium variabile*

Conclusions

- ISHI RBLV-ITG members: use results to make decisions on an appropriate assay(s) to test spinach seed for *Verticillium*
- May need a different assay for treated vs. non-treated seed lots
- Spinach seed with biological seed treatments should not be tested with NP10 assay (antibiotics in agar medium)
- Clear communication between seed testing labs & users of end results
 - Is the seed treated? With what? Organic or conventional?
 - Do you want to know the potential efficacy of the seed treatment?

Acknowledgements

International Seed Health Initiative - Root, Bulb &
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