

Alliance



Characterisation of Morphology
and Disease Resistance
in *Phaseolus* hybrids to guide crossing

Basic characterization to support climate-ready breeding

- Disease resistance
- Root angle
- Frequency of stomata
- Improve characterization of the hybrid complex
- Identify useful variation, develop F1





Sclerotinia (white mold) – widespread and severe.



Rhizoctonia – encouraged by warming climate.

Foliar and **Root** infecting forms.



Colletotrichum (Anthracnose) widespread and severe.

Disease	Planting location	Assay	Inoculation
Sclerotinia	Glasshouse	Detached leaf	Growth room
Web blight	Glasshouse	Detached leaf	Growth room
Anthracnose	Glasshouse	Detached leaf	Growth room
Root rot	Growth room	Seedling test	Growth room

Glasshouse layout planting into pots



Glasshouse layout planting direct into soil





Detached leaf inoculations: White Mold and Web blight



Growth room inoculations: Detached leaf

4rd to 5th Primary leaves harvested.

Leaves arranged in corning bioassay trays containing wetted filter paper and plastic mesh.



Three sclerotinia isolates; two pea sclerotinia isolates (PS1 and PS2, NIAB) and one Phaseolus sclerotinia (isolate Ph).
Web blight isolates obtained from CIAT, Colombia.

5mm mycelial plug from a 5 day old plate transferred to adaxial side of each leaflet just off centre.

Drop of water added to prevent agar disc drying out.

Both sclerotinia and web blight isolates grown on PDA.



Disease screening in growth rooms

Sclerotinia inoculated leaves incubated at 22°C day 18°C night, 16hr day.

Web blight inoculated leaves incubated at 25-27°C, 9hr day.

- Measure lesions at 3, 5 and 7 days.
- Photograph each accession for digital imaging analysis.



Disease Screening: *Sclerotinia sclerotiorum* controls



G51684
Resistor

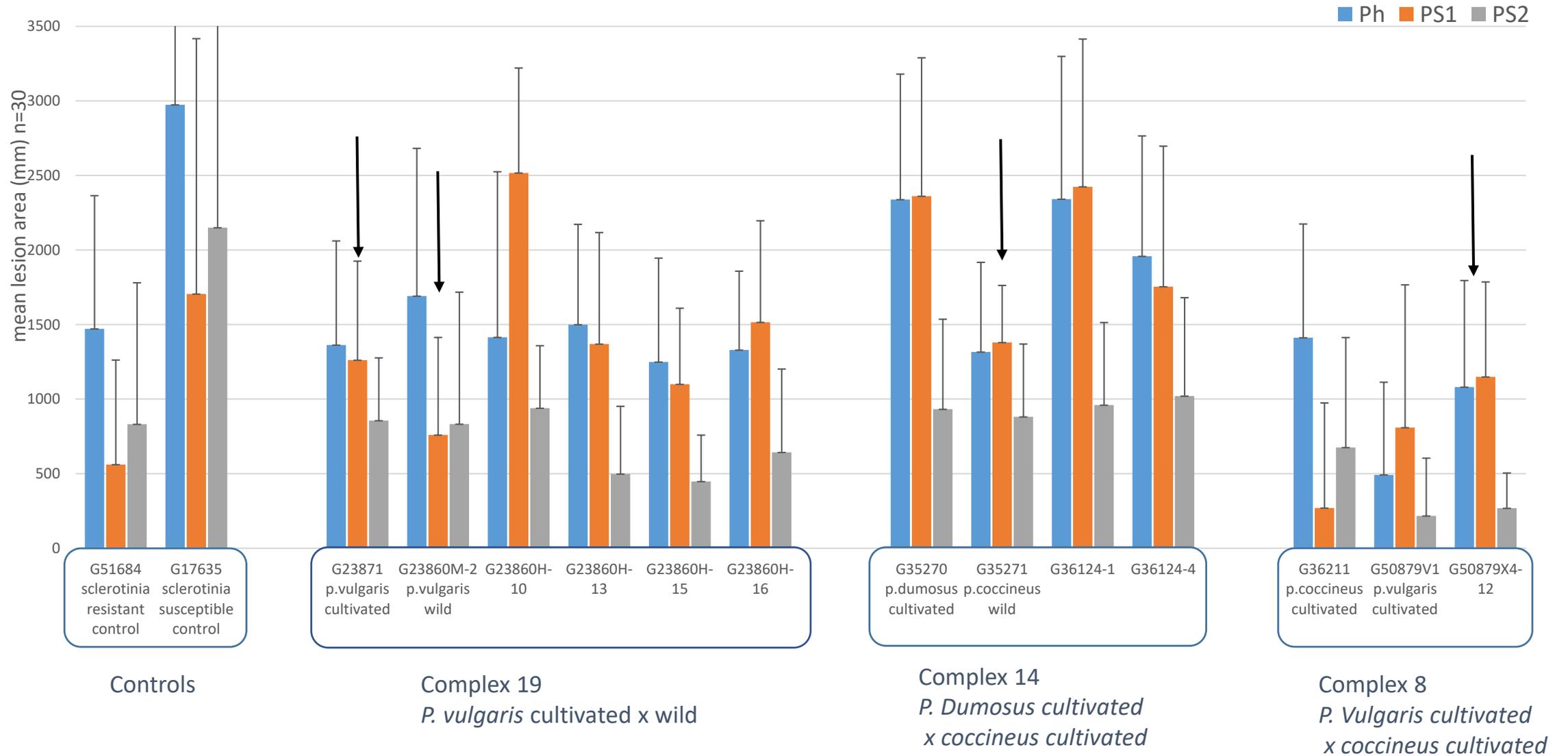
Mild disease symptoms on resistant plants



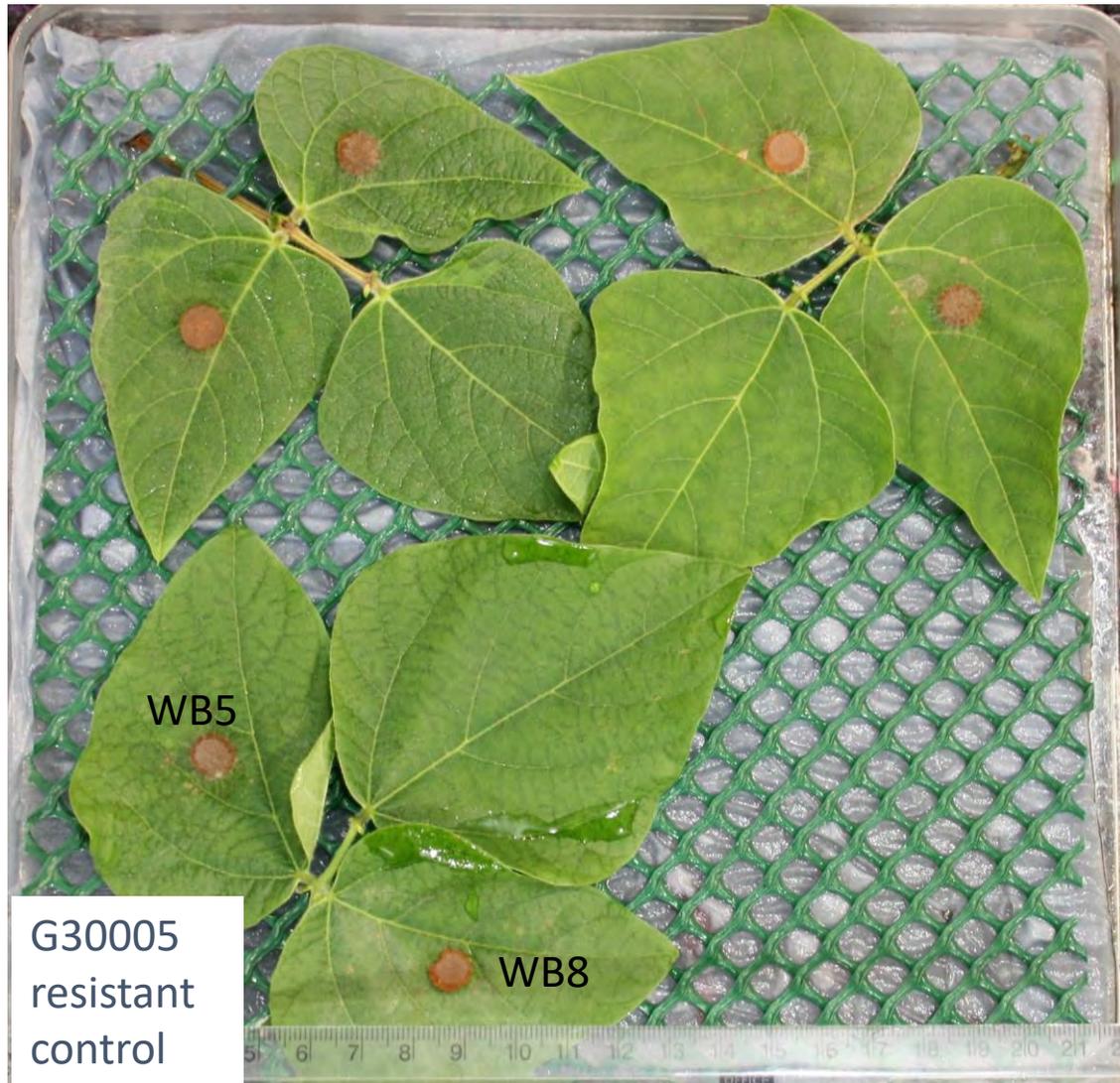
G17635
Susceptible

Severe disease symptoms on susceptible plants

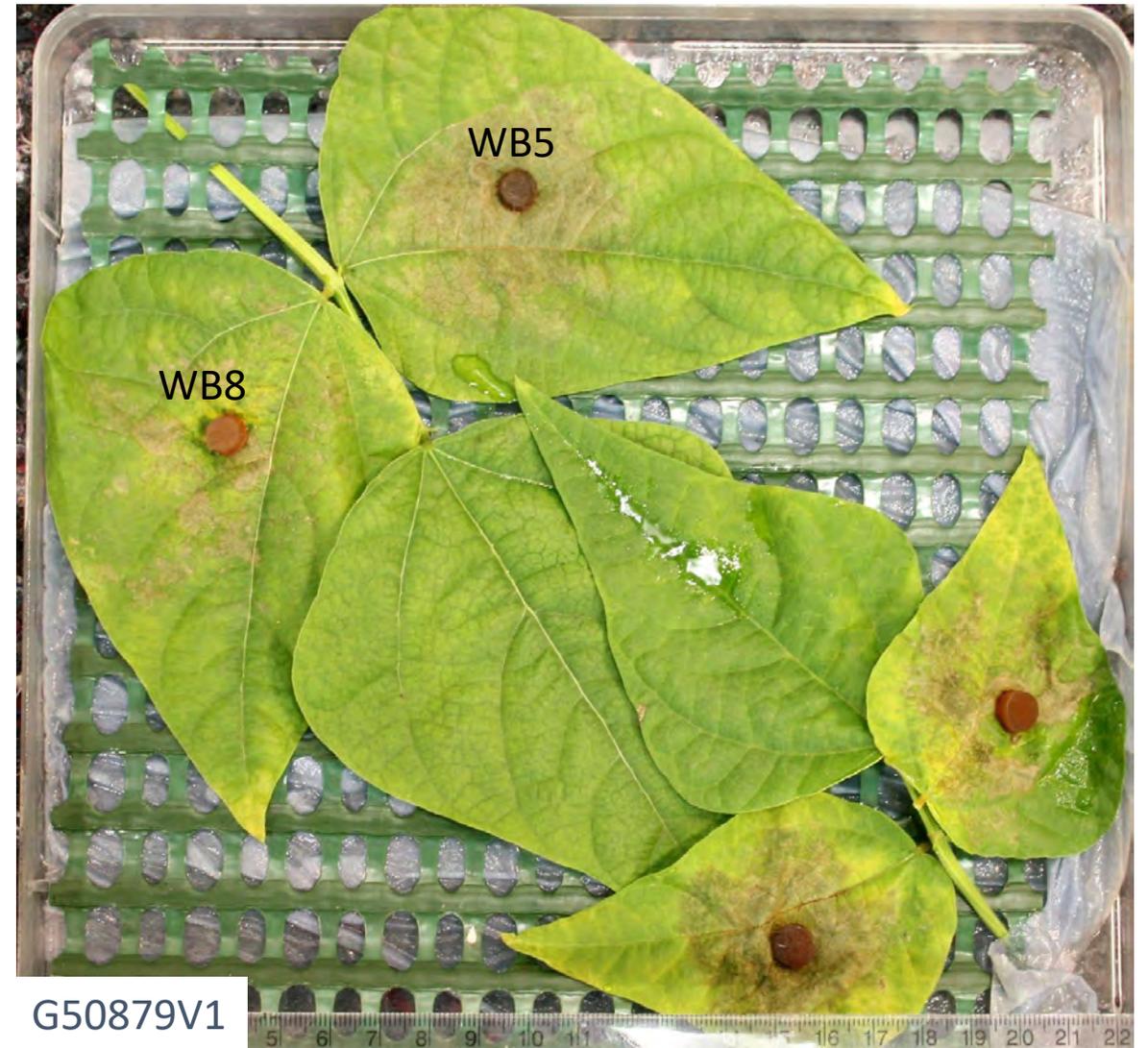
Disease Screening: White Mold



Disease Screening: Web blight symptoms.

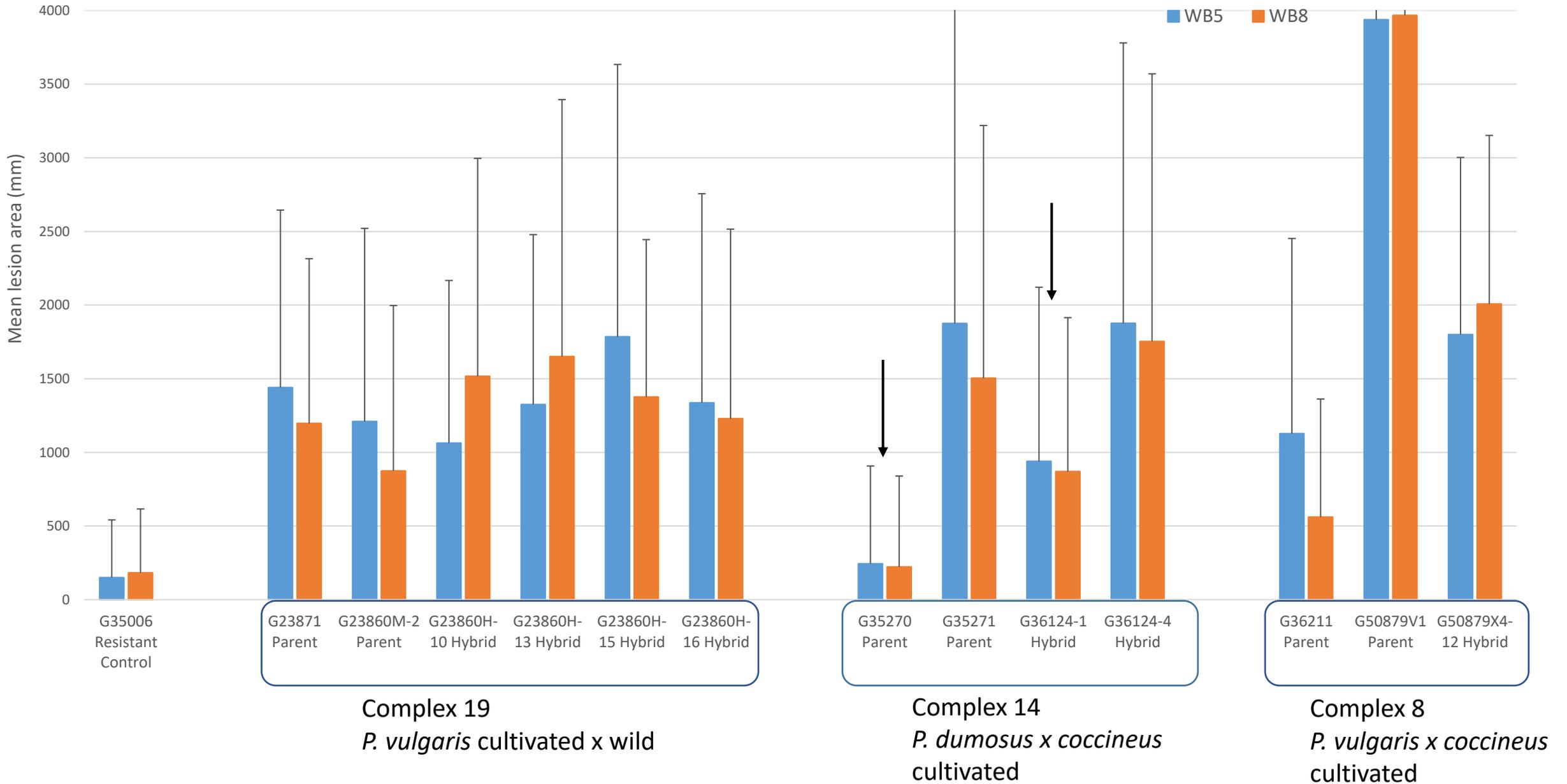


Moderate Web blight symptoms



Severe Web blight symptoms

Disease Screening: Web Blight



Disease screening: Anthracnose

Colletotrichum lindemuthianum isolates

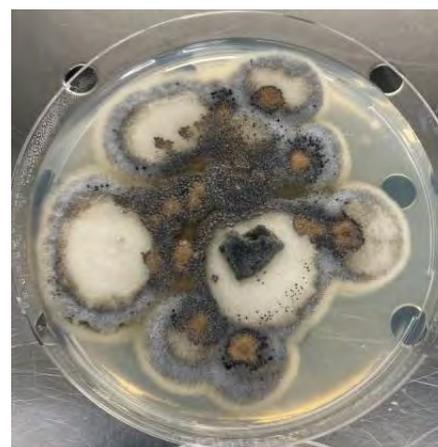
Isolate	Origin	Sporulation on PDA	Typical Spore concentration	Media
CL638	Colombia	Yes	$5 \times 10^6 - 10^7$	PDA/MM
KIS02	Uganda	Yes	$1-5 \times 10^6$	PDA/MM
RA-9B	Uganda	Yes	$1-5 \times 10^6$	PDA/MM
KB011	Uganda	Poor	5×10^6	MM/PBA



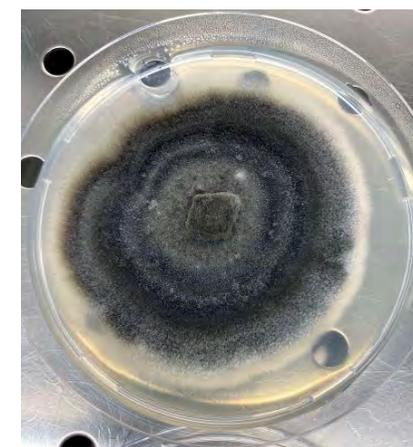
CL638



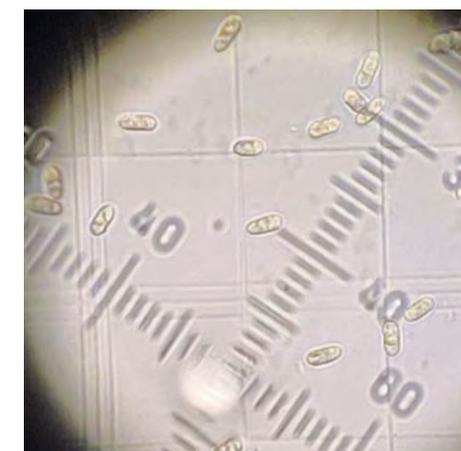
KIS02



RA-9B



KB011



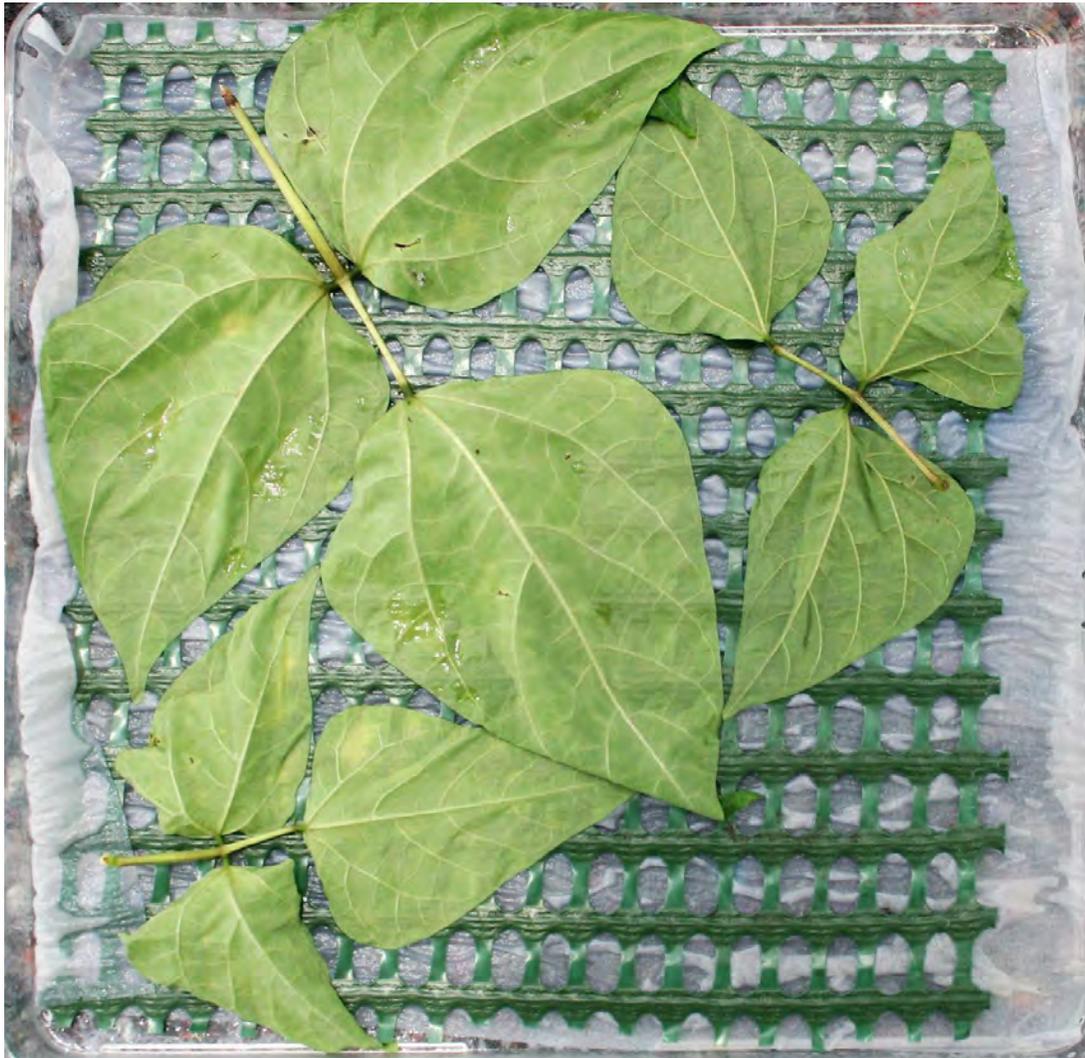
Conidia

Detached leaf inoculations: Anthracnose

- Spray Inoculation
- Harvest leaves; fully open, 3rd -5th trifoliolate, 10 plants per accession per Rep.
- Set up in Corning boxes with filter paper and mesh.
- Scrape 20-30 plates of *colletotrichum* isolate with distilled H²O.
- Count spores in a haemocytometer.
- Add a drop of Tween 20 and inoculate each tray with 5ml inoculum at 1×10^6 - 1×10^7 spores/ml
- Close lids and cover with plastic.
- Water matting.
- Growth room temperature set at 22oC, 9hr day/15hr night.
- Assess leaves 7 dai or when symptoms appear.



Anthracnose disease symptoms



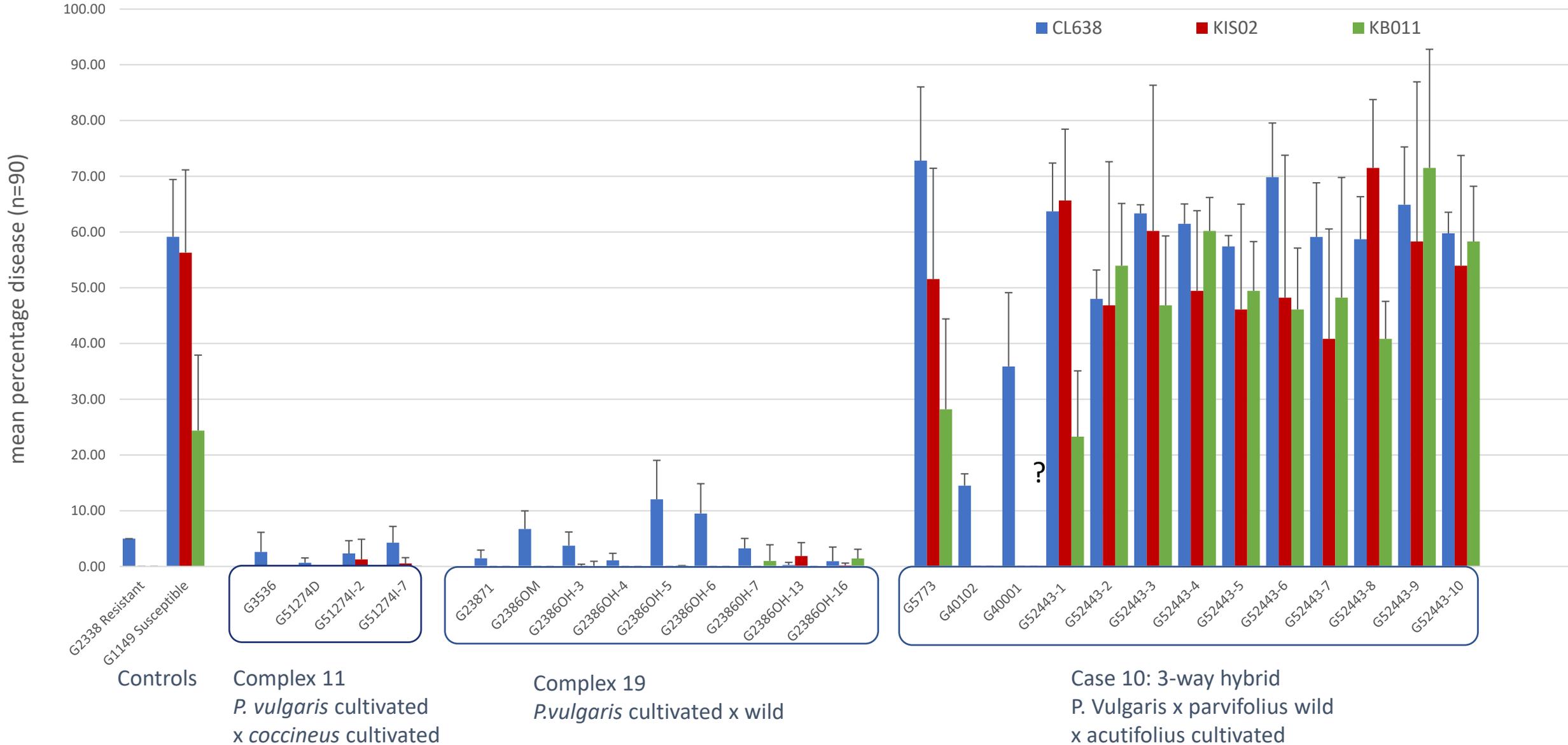
G2338 Resistant control



G1149 Susceptible control

Disease Screening: Anthracnose

Isolate RA-9B no symptoms



Disease screening: Rhizoctonia root rot

Two Rhizoctonia root rot isolates (Colombia): Rh-5 and Rh-17.

Two reps per isolate.

10 seeds per tray (accession).

Plant into A5 size trays in M2 compost.

20 seeds per accessions per isolate.

Inoculate M2 compost 24hr prior to planting

1.5% (w/w by soil volume) inoculum

One isolate per bench.

Growth room temperature set to 25°C day, 17°C night, 16hr day.

Keep trays moist and cover 24hr with plastic.

Sow 10 seeds per tray per accession.

1st assessment; counts

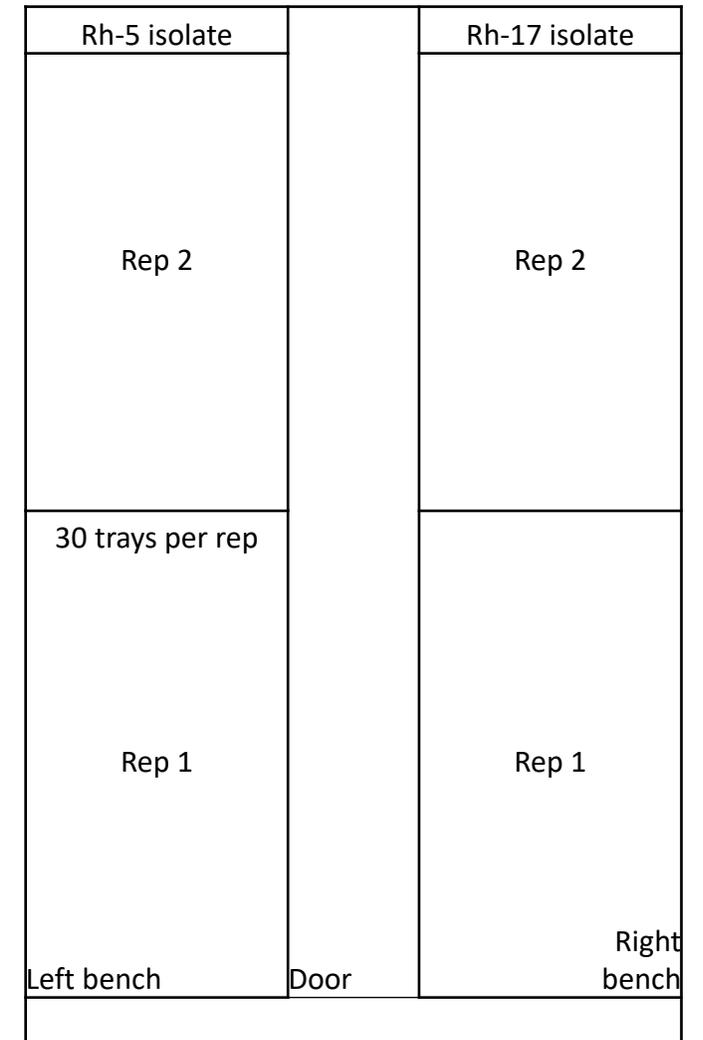
2nd assessment; disease scores

Three resistant *Phaseolus vulgaris* controls (cultivated landraces);

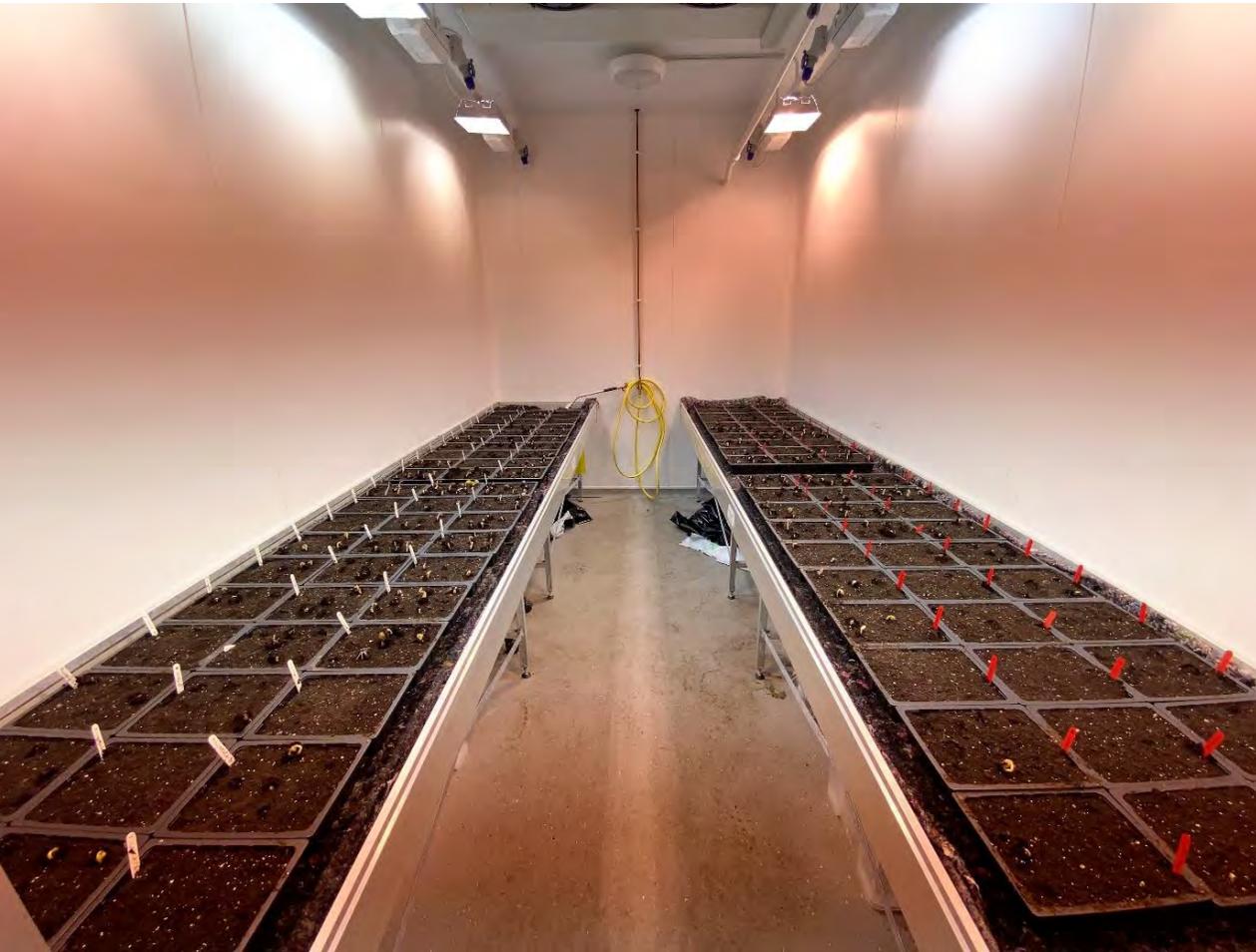
G881 (Mexico)

G1540 (Sweden)

G3151 (Guatemala)



Rhizoctonia root rot: growth room layout



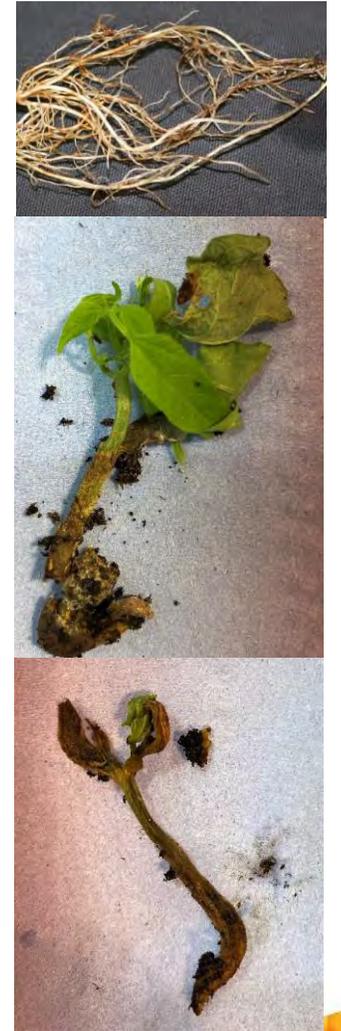
7 days start counting emergence



18 and 25 days assess disease



Rhizoctonia root and stem lesions surrounding the hypocotyl



P.Vulgaris
G881 Resistant

Disease index		Symptoms
0	No disease	No symptoms present
1	<5%	Lesion appearing
2	15%	Lesion present
3	25%	Superficial disease, plant still healthy
4	50%	Disease not penetrating stem
5		Disease penetrating stem, some stunting
6	>75	Disease lesion significant around and within stem
7		Lesions severe, plants stunted
8	>90%,	Withered and dying
9		Plant dead

Disease screening: *Rhizoctonia* root rot. Control variety resistant to root rot growing in substrate inoculated with two isolates of *Rhizoctonia solani*.



Rh-5



Rh-17

Disease screening: *Phaseolus vulgaris* x *cockineus* hybrid susceptible to both isolates of rhizoctonia root.



Rh-5



Rh-17

Disease screening: Wild *phaseolus parvifolius* parent (G40102) native to southern Mexico highly susceptible to rhizoctonia root rot.



Rh-5



Rh-17

Disease screening: Cultivated *phaseolus acutifolius* parent (G40001) origin southern Mexico, highly susceptible to rhizoctonia root rot.



Rh-5



Rh-17

Disease screening: Cultivated *Phaseolus vulgaris* parent (G5773) – showing resistance to both isolates of *rhizoctonia solani* root rot.



Rh-5



Rh-17

Disease screening: *Phaseolus* 3-way hybrid resistant to both isolates of root rot.

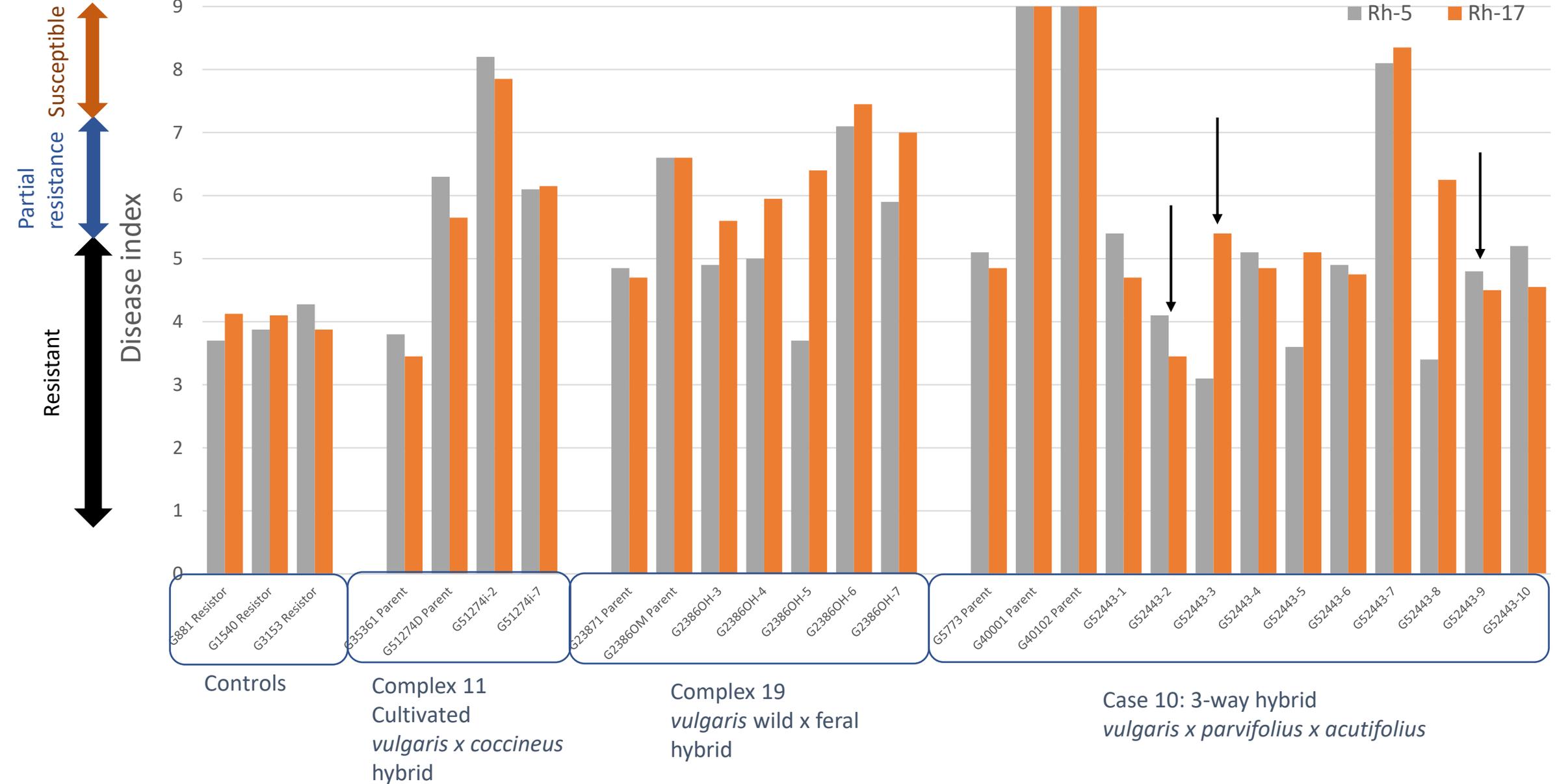


Rh-5



Rh-17

Disease screening: Rhizoctonia root rot



Disease Phenotyping

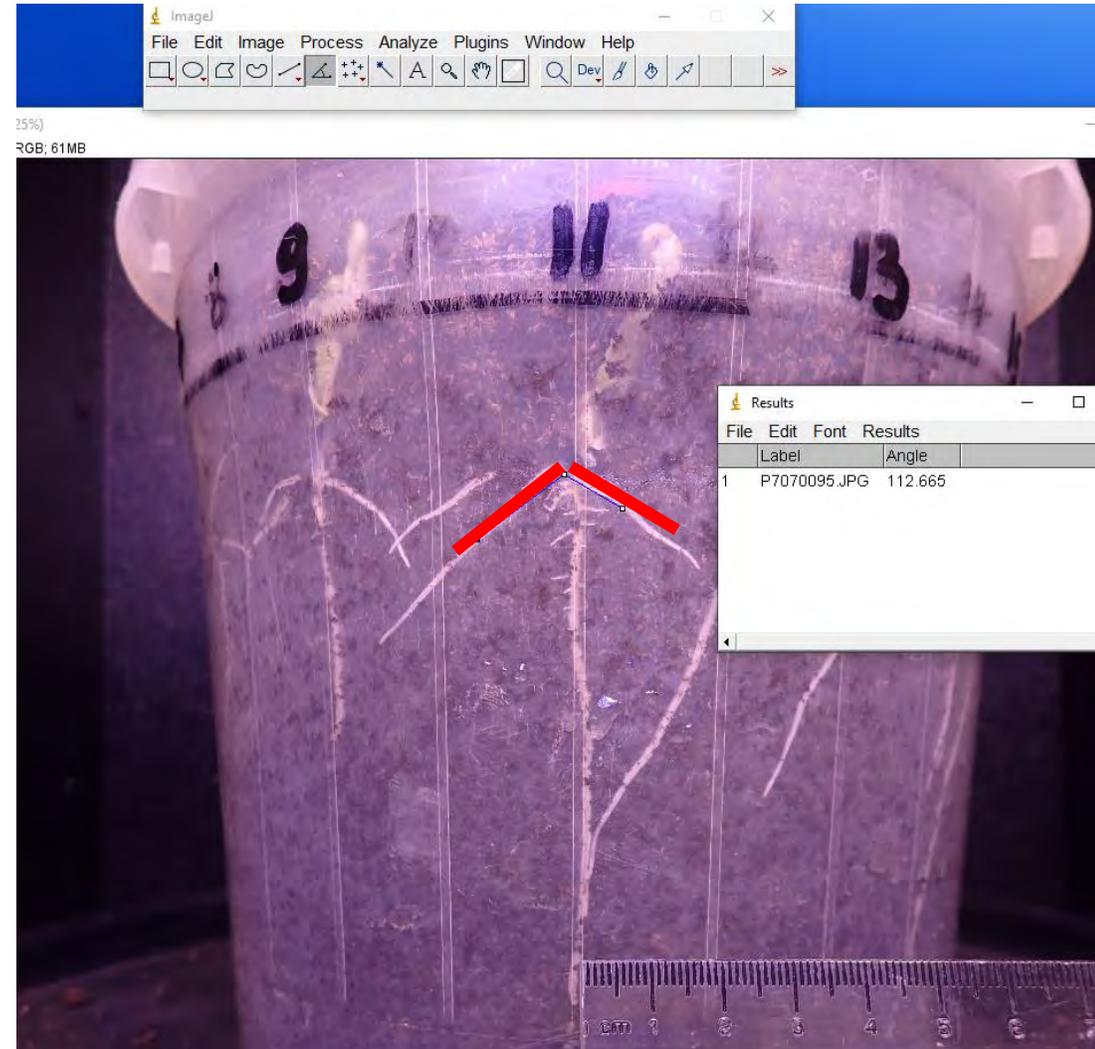
Hybrids	Parent 1	Parent 2	Parent 3	Number of genotypes received	Sclerotinia sclerotiorum	Rhizoctonia Solani Web blight	Rhizoctonia Solani Root rot	Anthracnose	Sclerotinia stem inoculations
Complex 11	<i>P.vulgaris</i> cultivated	<i>P.coccineus</i> cultivated	–	2	✓	✓	✓	✓	✗
Complex 14	<i>P.dumosus</i> cultivated	<i>P.coccineus</i> cultivated	–	2	✓	✓	✗	✗	✗
Case 10	<i>P.vulgaris</i> cultivated	<i>P.parvifolius</i> wild	<i>P.acutifolius</i> cultivated	10	✓	✓	✓	✓	✗
Complex 19	<i>P.vulgaris</i> cultivated	<i>P.vulgaris</i> wild	–	9	✓	✓	✓/✗	✓/✗	✗
Complex 8	<i>P.vulgaris</i> cultivated	<i>P.coccineus</i> cultivated	–	2	✗	✗	✗	✓/✗	✗
Complex 14	<i>P.dumosus</i> cultivated	<i>P.coccineus</i> cultivated	–	4	✓	✓	✗	✓/✗	✗
Complex 3	<i>P.dumosus</i> cultivated	<i>P.vulgaris</i> feral	–	1	✗	✗	✗	✗	✗
Complex 7	<i>P.vulgaris</i> cultivated	<i>P.coccineus</i> cultivated		2	✗	✗	✗	✗	✗

Rooting Angle Characterization

- Root phenotype can contribute towards fitness and resilience towards abiotic stress
- Assess angle of first seminal roots of bean hybrids
 - Wider angles associated with drought resistance in wheat (Pinto & Reynolds, 2015)
- Root whorl number (establishment, phosphorous acquisition)
 - No. basal, adventitious roots associated w/ higher yield (Strock *et al.*, 2019)

Rooting Angle Characterization

- Transparent pots, randomized sowing
- Imaged approximately 7 days after sowing
- The angle between the first pair of seminal roots was measured using ImageJ software
- 30 seed per accession





G40001 Case 10
Phaseolus acutifolius



G35361 Complex 11
Phaseolus coccineus

Phaseolus acutifolius

- Mesoamerican, Case 10 Parent line,
- Desert/semi-desert, $>30^{\circ}\text{C}$ day and $>20^{\circ}\text{C}$ night,
- Short flowering time, fast seed production
- Less investment in producing large root system
- Resistant to drought
- Wide root angle, shallow profile



G40001 parent

Phaseolus acutifolius, Mexico,
Veracruz, Cordoba, Cultivated

Phaseolus coccineus, *Phaseolus dumosus*

- Andean, mountain forest habitat, shade, humidity
- 12-22 °C day, 12-14°C night (16-22°C, *P. dumosus*)
- Tall as 10m- (*P. dumosus*)
- Vigorous establishment, competitive
- Strong root system, low P foraging
- *P. coccineus* - short perennial vine 1-2 years
- *P. dumosus* - 4-6 years growth, polycarpic plant
- Low light requirements
- Tuberous root system which allows them to regrow



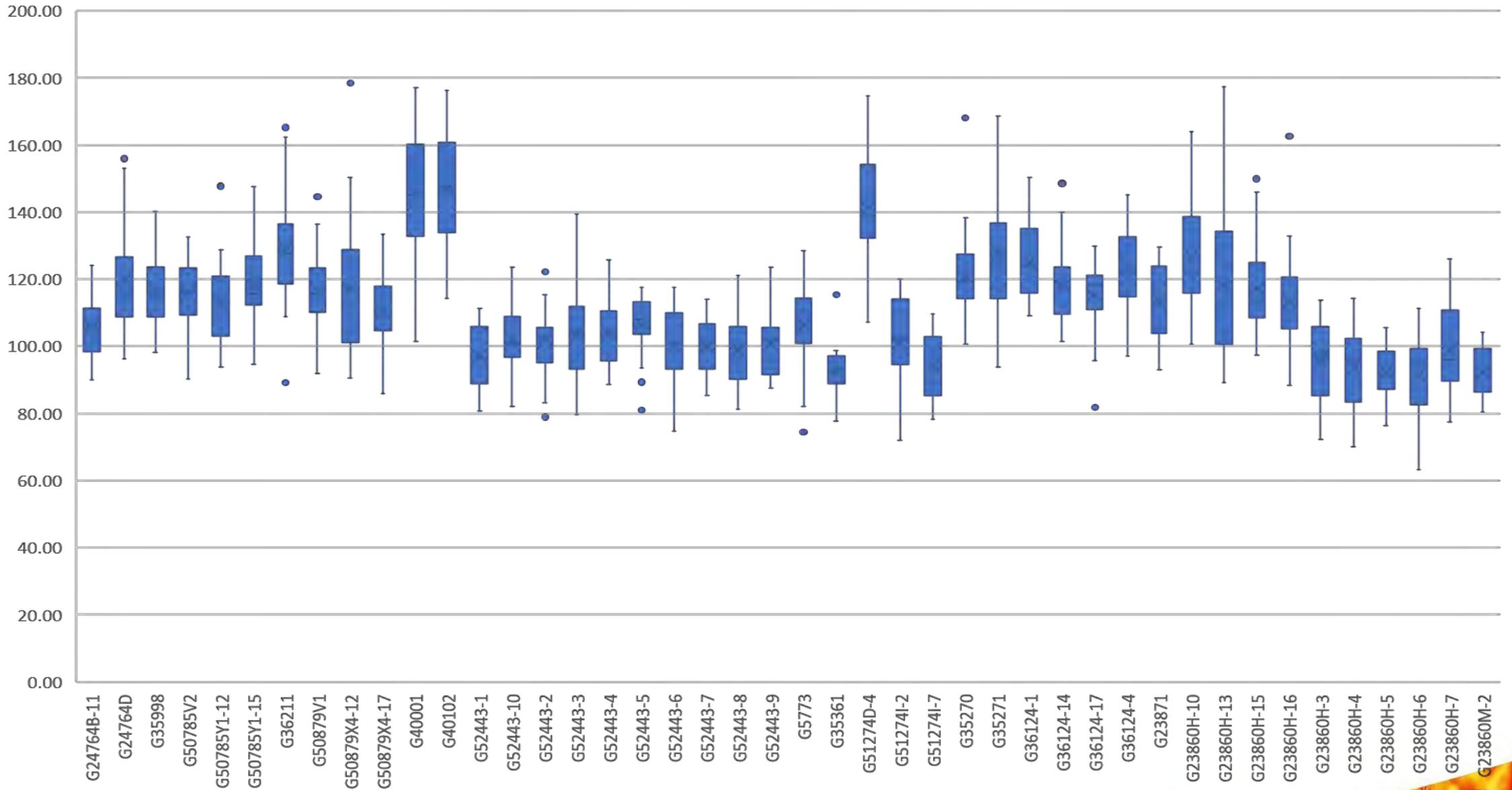
G35271 parent
Phaseolus coccineus, Colombia,
Putumayo, San Francisco , cultivated



G35270 parent
Phaseolus dumosus, Colombia,
Putumayo, San Francisco cultivated



Range of root angles observed in hybrid accessions



Complex 3

4

8

10

11

14

19



world-class experience,
skills and resources

- Considerable morphological differences in primary root angle
- Adaptive trait, numerous assessments
 - *P. acutifolius*/*P. parvifolius* compared with *P. vulgaris* parent
 - Mean root angle of Case 10 hybrids progeny were on average more similar to the values observed in *P. vulgaris* parental line
- Wider root angle observed in the cultivated *P. vulgaris*

Assessing for diversity in the number of stomata

- Plants transpire through stomata in their leaves
- Leaf cooling, higher temperatures can lead to greater transpiration and hence water-loss
- Decrease stomatal conductance, increase in number at high temp (Sinclair *et al.*, 2017;)
- Characterise stomatal density in hybrid complexes
- Case 10 – high temp tolerance, increased stomata?

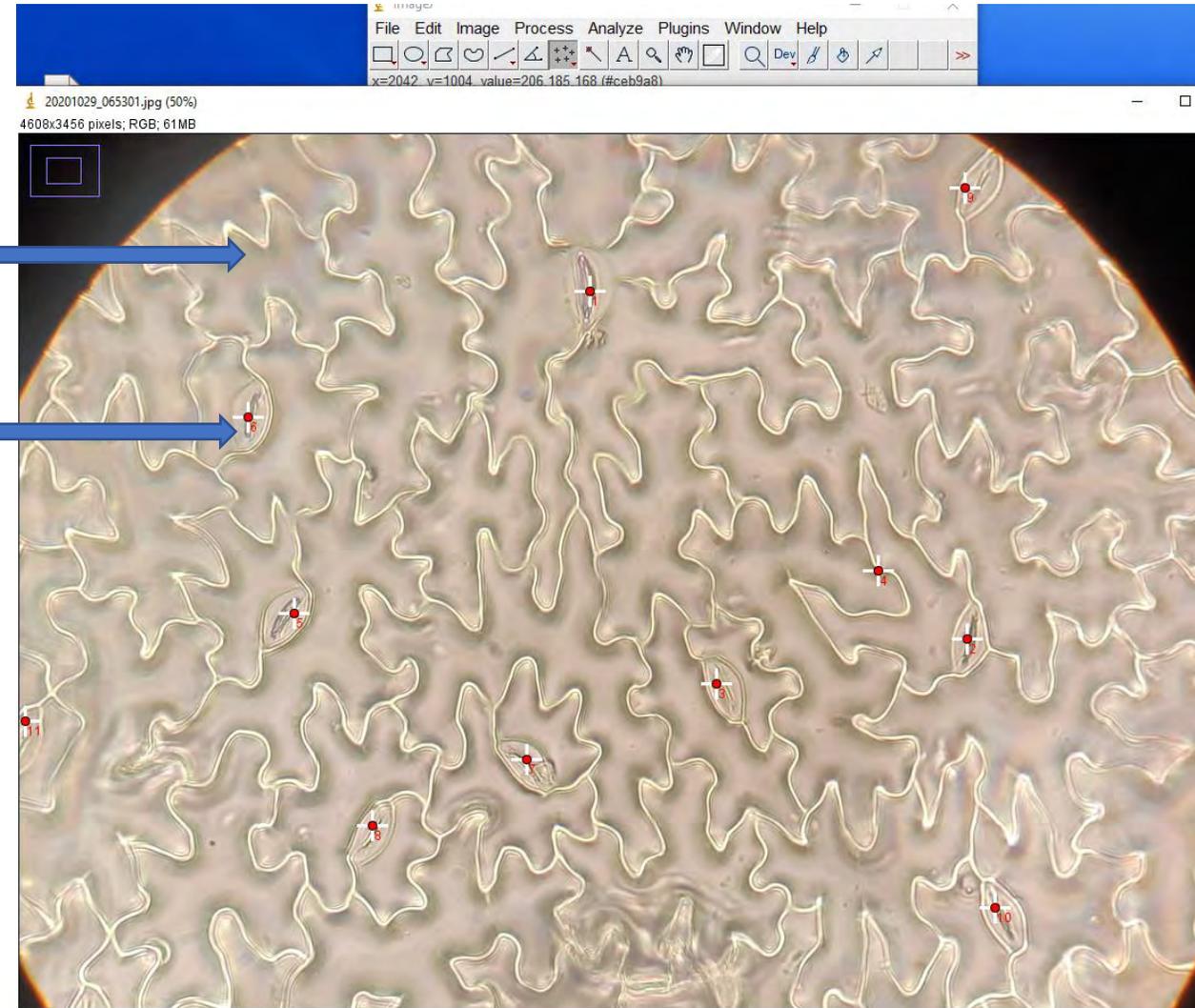
Assessing for diversity in the number of stomata

- 10 leaves per plant (light/shade)
- Abaxial imprint (nail-gloss and tape)
- Photographed 40 x
- Count cells in five random areas
- Converted in stomatal index

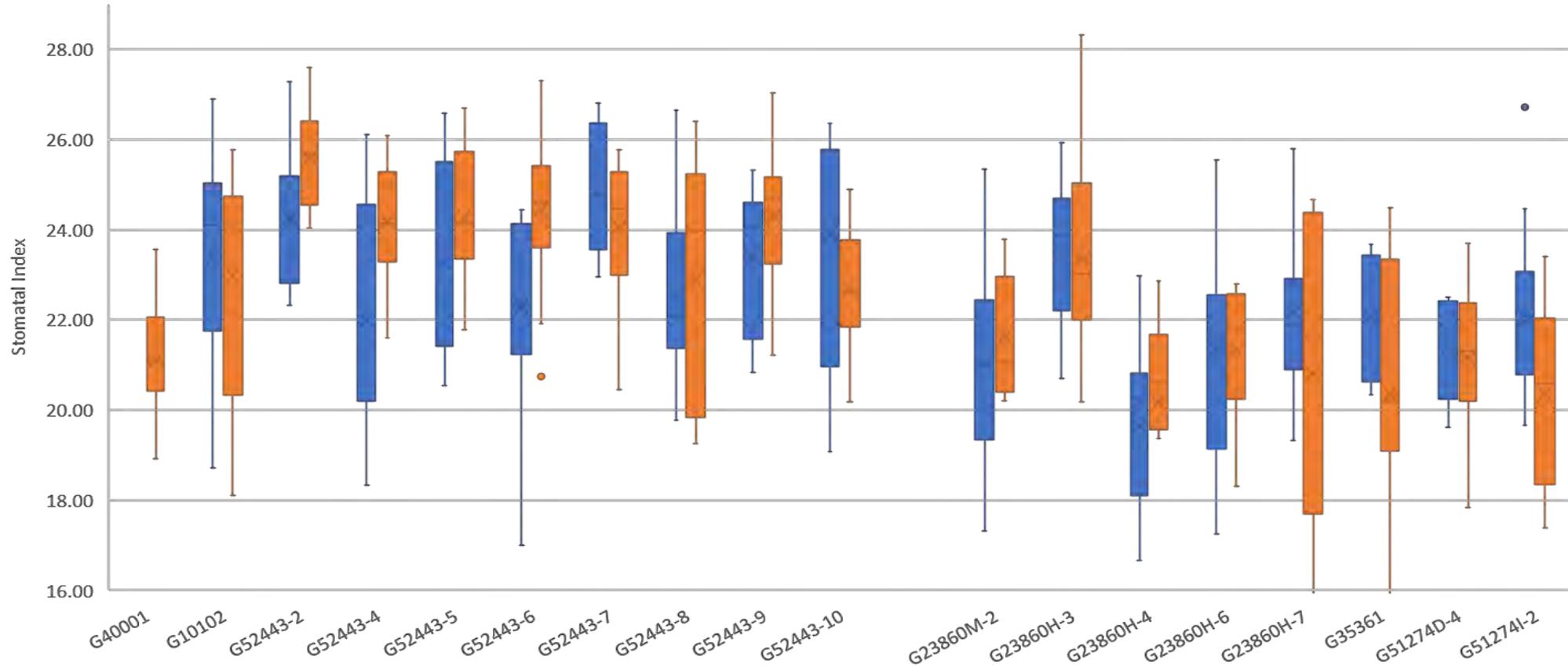
$$\text{No. Stomata} \times 100 / (\text{No. epidermal} + \text{no. stomata})$$

Epidermal cell

Stomata



■ Shade ■ Light



Case 10

Complex 19

Complex 11

- 18 selected accessions
- 360 leaves, ~2160 Stomatal Index calculated
- Case 10 hybrids showed significant variation between accessions, light/shade, leaf number.
- Complex 19, G23860H-4 has <7% less stomata
- G23860H-3 hybrid has >10% stomata
- Complex 11 hybrid line, G51274I-2 and parental line G51274D-4 (*P. vulgaris*) had <2% higher stomatal density compared with G35361 (*P. coccineus*) parent

Accession	group	Accession	Shade	Light	Grand Total
G23860M-2	Complex 19	Parent	21.03	21.64	21.28
G23860H-3	Complex 19	Hybrid	23.47	23.36	23.42
G23860H-4	Complex 19	Hybrid	19.63	20.17	19.85
G23860H-6	Complex 19	Hybrid	21.36	21.33	21.34
G23860H-7	Complex 19	Hybrid	22.17	20.82	21.43
G35361	Complex 11	Parent	22.06	20.36	20.84
G51274D-4	Complex 11	Parent	21.37	21.15	21.21
G51274I-2	Complex 11	Hybrid	22.12	20.34	21.23
G10102	Case 10	Parent	23.35	23.00	23.19
G40001	Case 10	Parent		21.11	21.11
G52443-2	Case 10	Hybrid	24.27	25.60	24.94
G52443-4	Case 10	Hybrid	22.00	24.18	23.09
G52443-5	Case 10	Hybrid	23.26	24.27	23.80
G52443-6	Case 10	Hybrid	22.29	24.46	23.43
G52443-7	Case 10	Hybrid	24.89	24.06	24.43
G52443-8	Case 10	Hybrid	22.55	22.90	22.72
G52443-9	Case 10	Hybrid	23.37	24.30	23.83
G52443-10	Case 10	Hybrid	23.92	22.66	23.29

F1 Crossing program

- Introgress useful hybrid diversity
- Prioritizing crossing of novel traits
- (G51698) as maternal line - fast maturing, HI, drought resistant
- Resistant accessions from Case 10, Complex 11, 19
 - Anthracnose (G52443-2, G51274I-2,-7, G2386OH-4,-13)
 - Sclerotinia (G52443-6)
 - Web blight (G52443-3)
 - Sclerotinia
- SNC 11, MAC 44 selected for anthracnose/web blight resistance
 - Market classes for East Africa



Summary

- Basic characterization on hybrid complexes
- Sources of potential disease resistance
- Characterized root angle, whorl number
- Conducted stomatal counts (abaxial)
- Implementing F1 crossing program