Foliar Nutrient Uptake (Translocation) in HLB Affected Leaves

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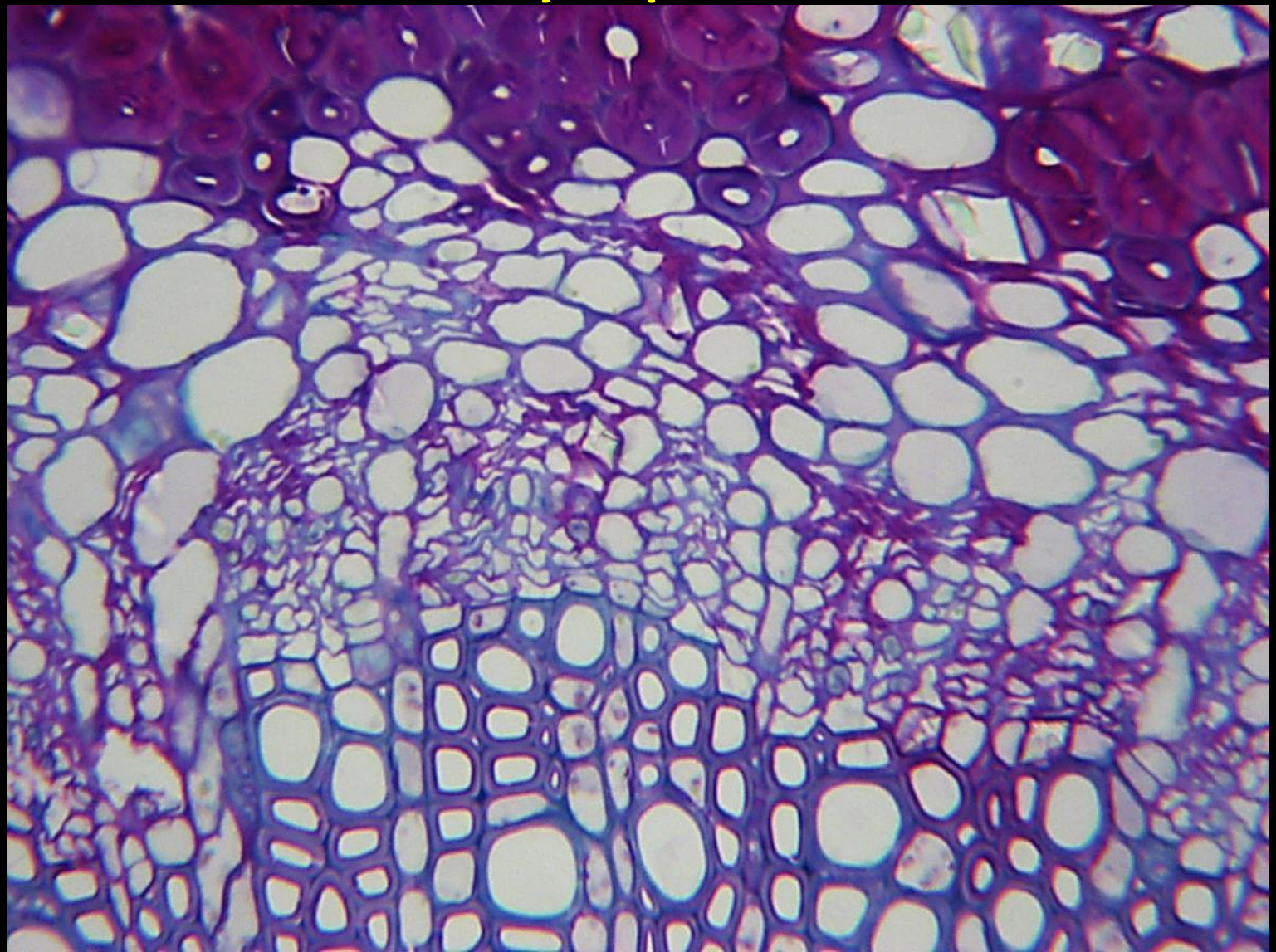
Why does this HLB Tree Look Good?

Samples Taken

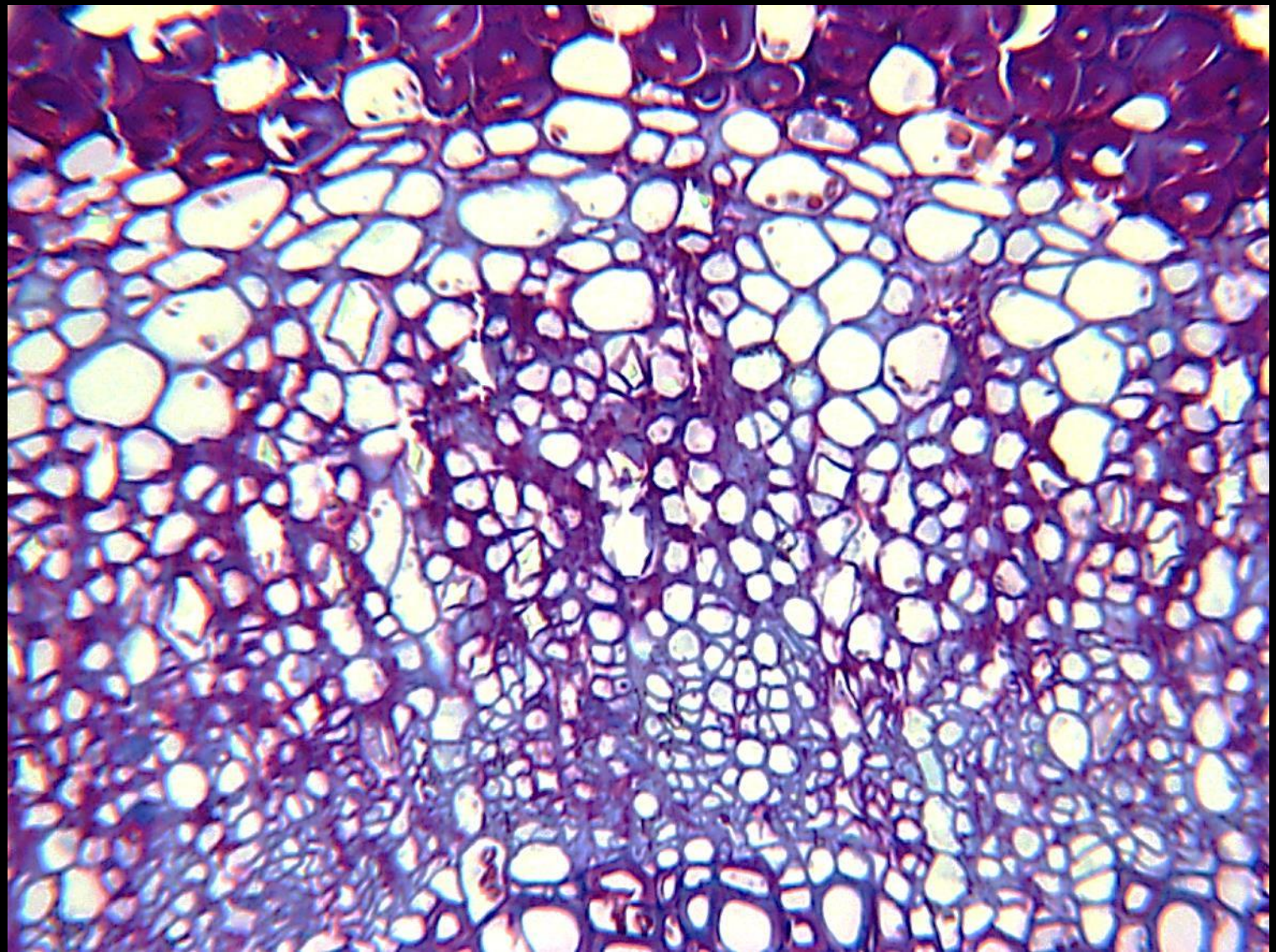
- Locations: Foliar Nutrient treatments at Orange Hammock Grove, Felda, Bob Rouse- SWREC, Immokalee and CREC plots at Lake Placid
- Sampling dates: September 2010, February 2011, July 2011
- Types of samples: leaf petioles, midribs, fruit bearing stems, and fruit columellas
- Fixed, embedded, sectioned and stained for light microscopy and for phloem translocation studies
- Photographs taken, analyzed and compared

Orange Hammock- All trees treated with Nutrient Cocktail

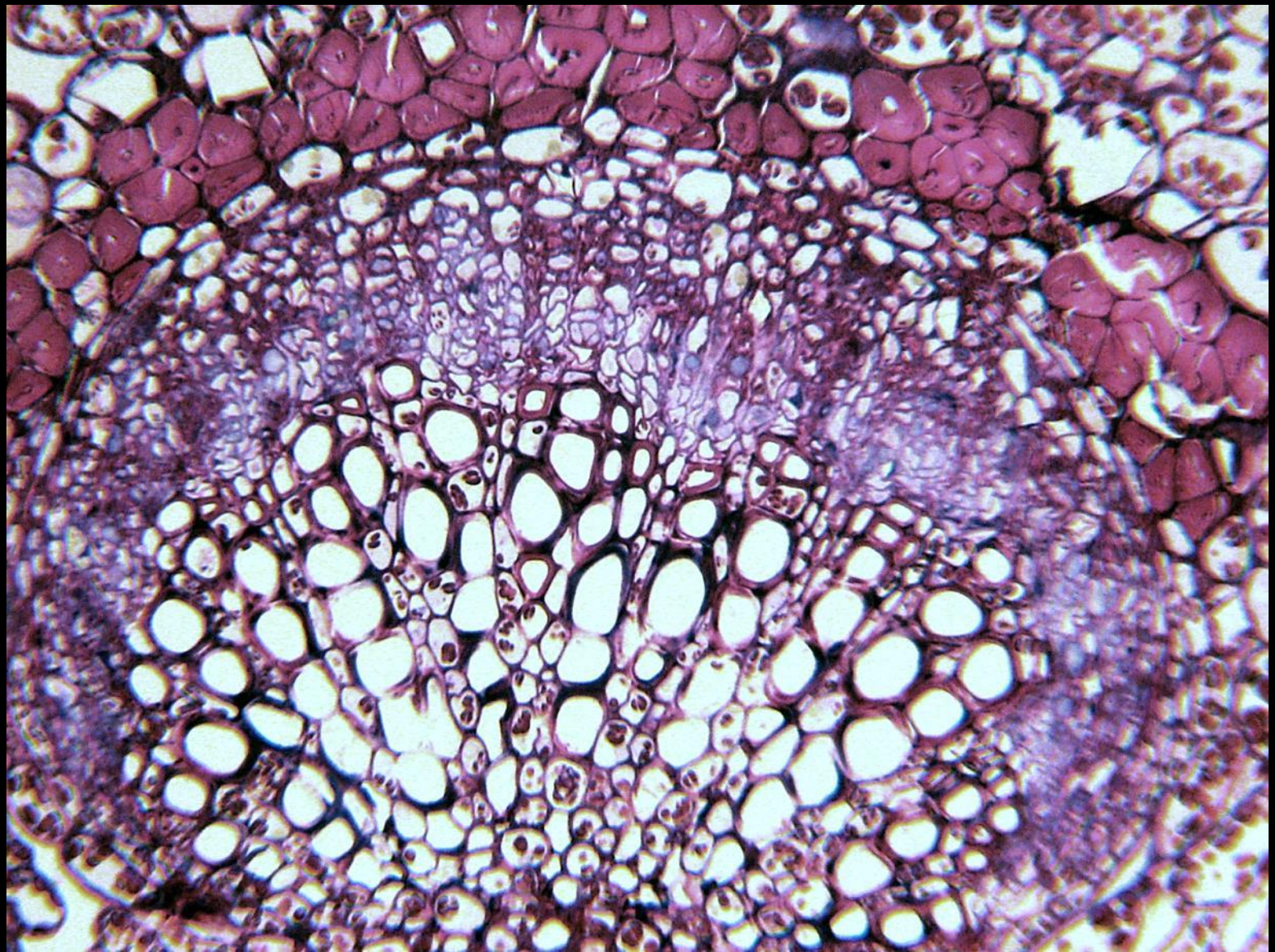
HLB Petioles Val/Swg-Treated -2010-
No Symptoms



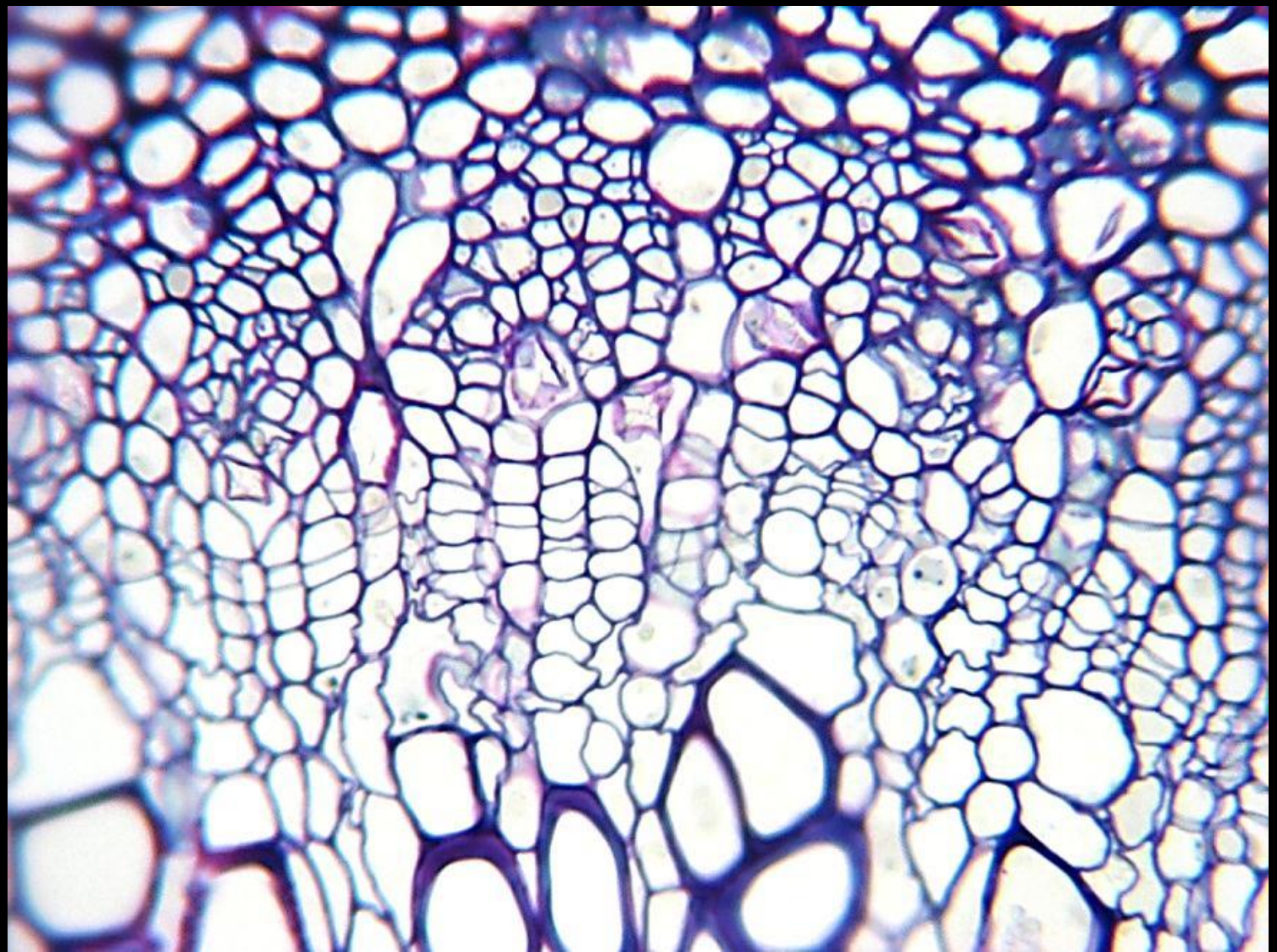
HLB Petioles Val/Car-Treated-2010-With Symptoms



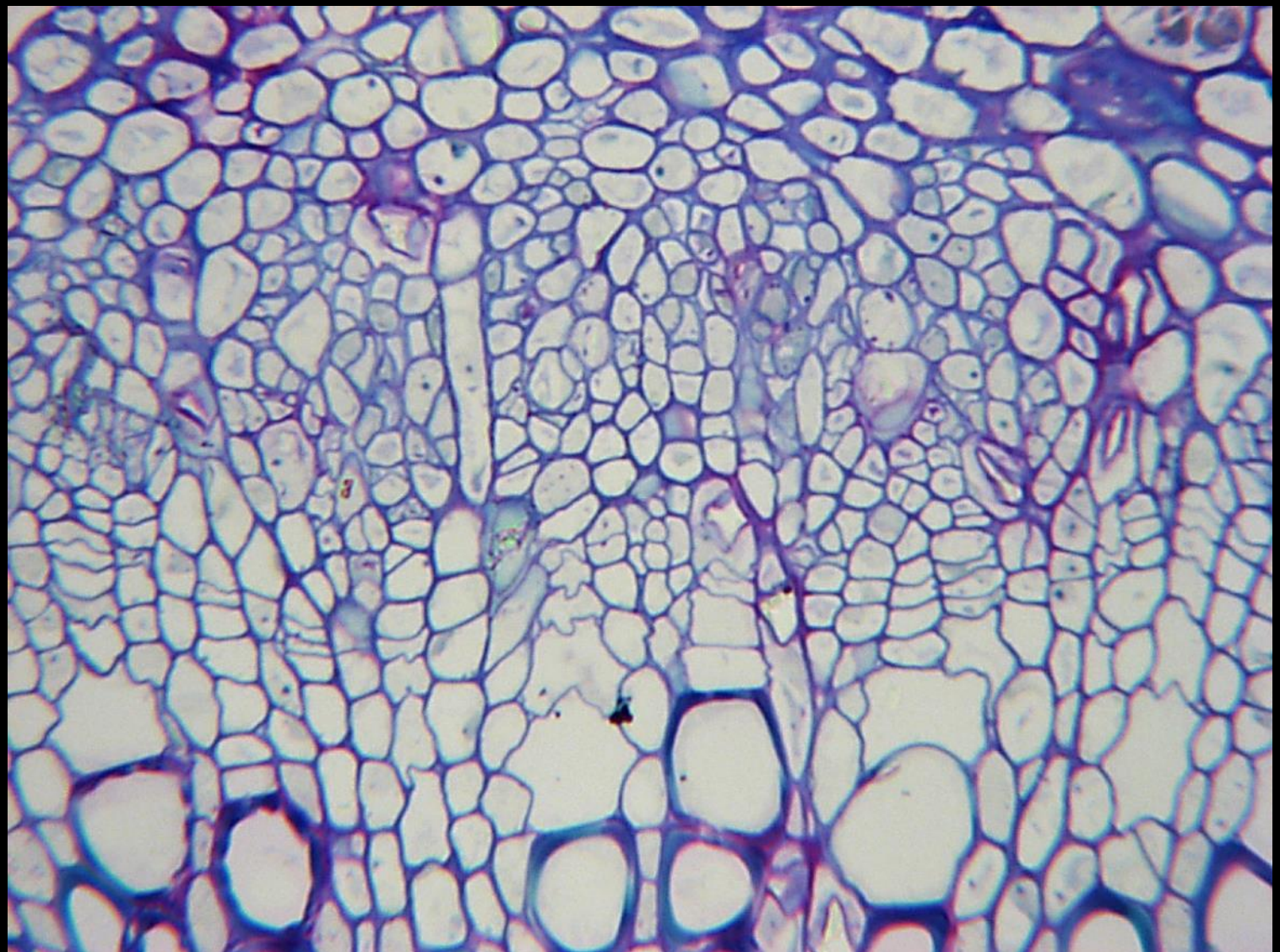
HLB Affected - Untreated



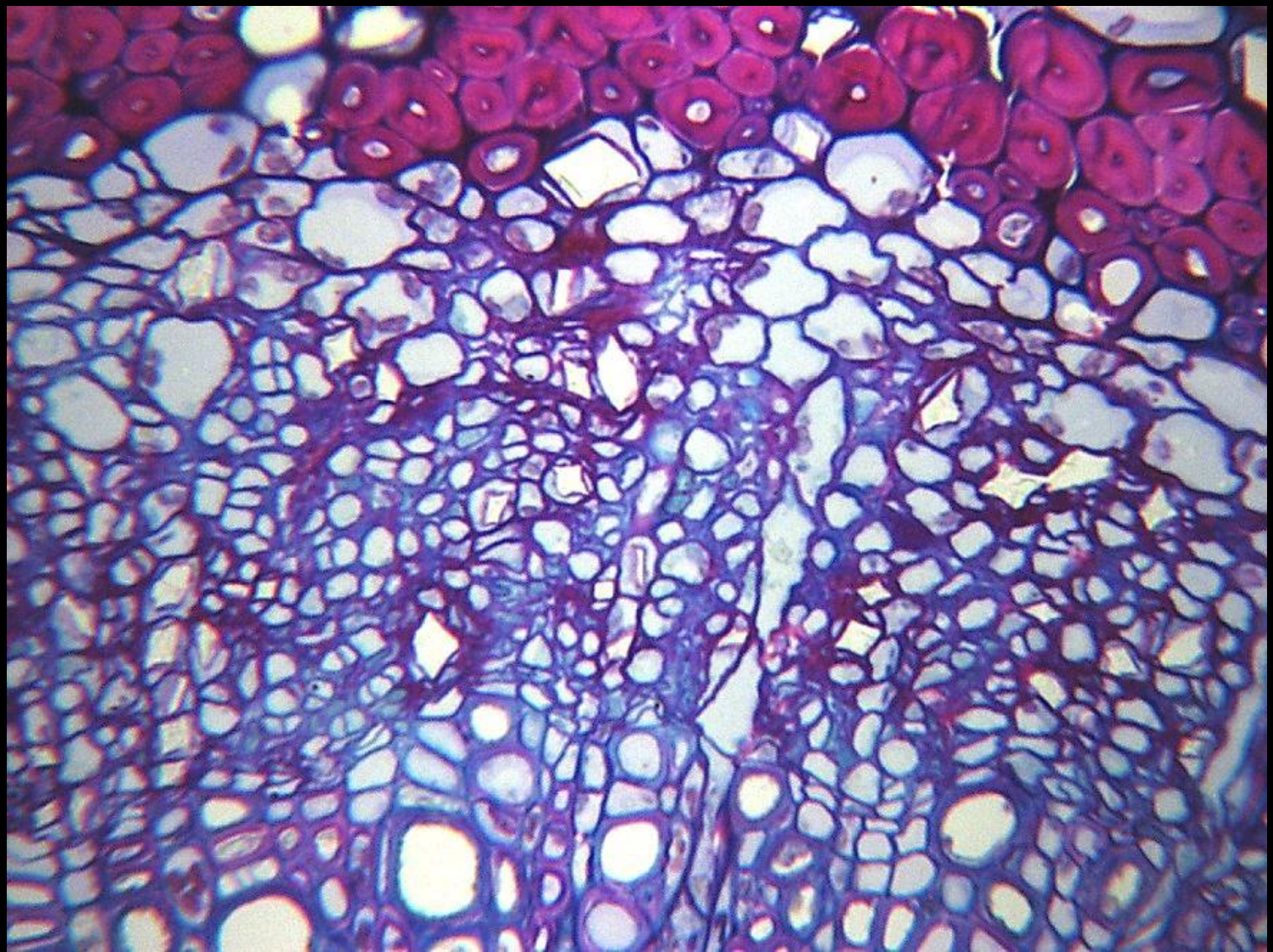
HLB Petioles Young Val/Swg-Treated 2010 - Flush No Symptoms PCR +



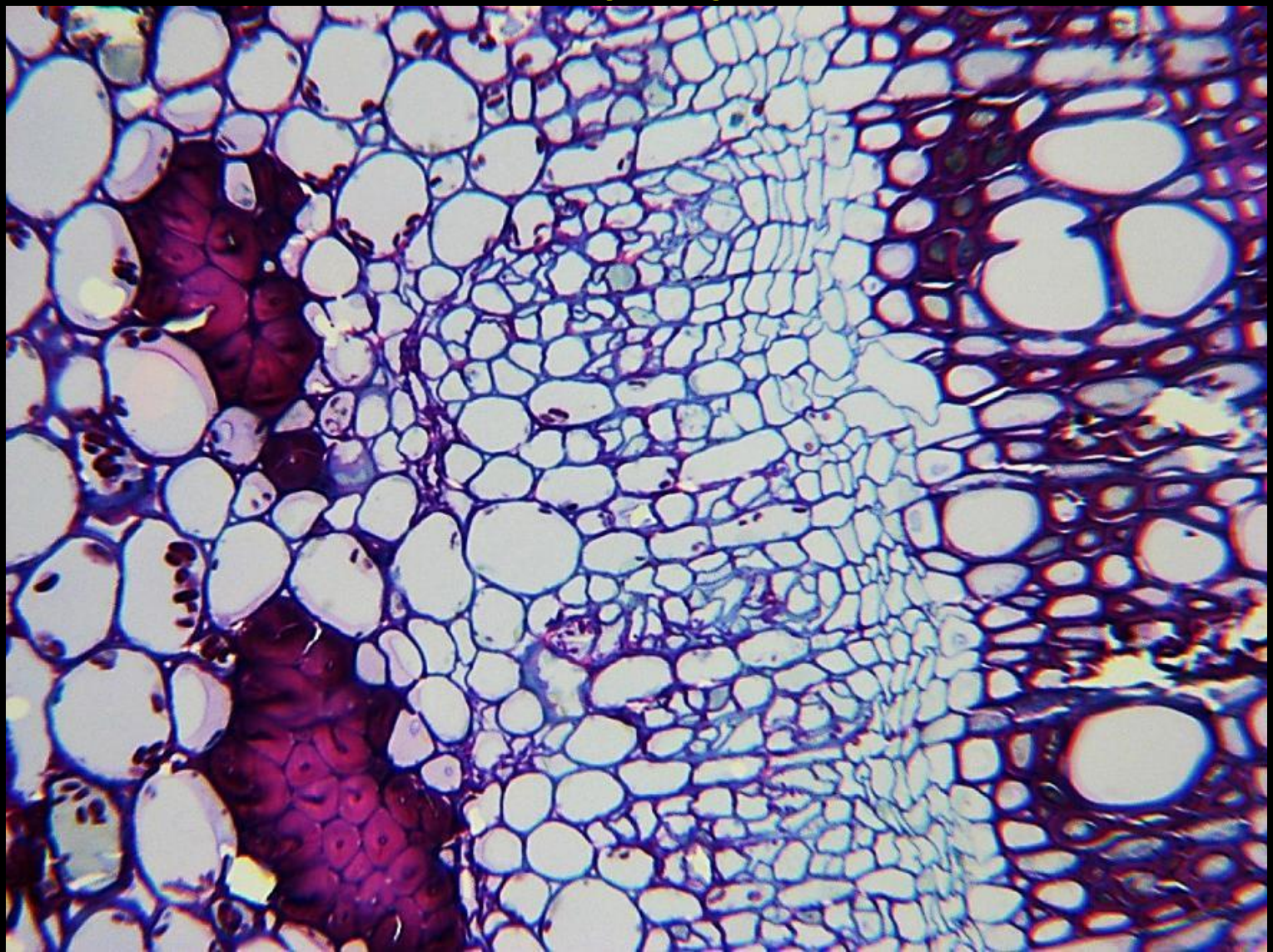
HLB Petioles Young Val/Swg-Treated - 2010 - Minor Symptoms



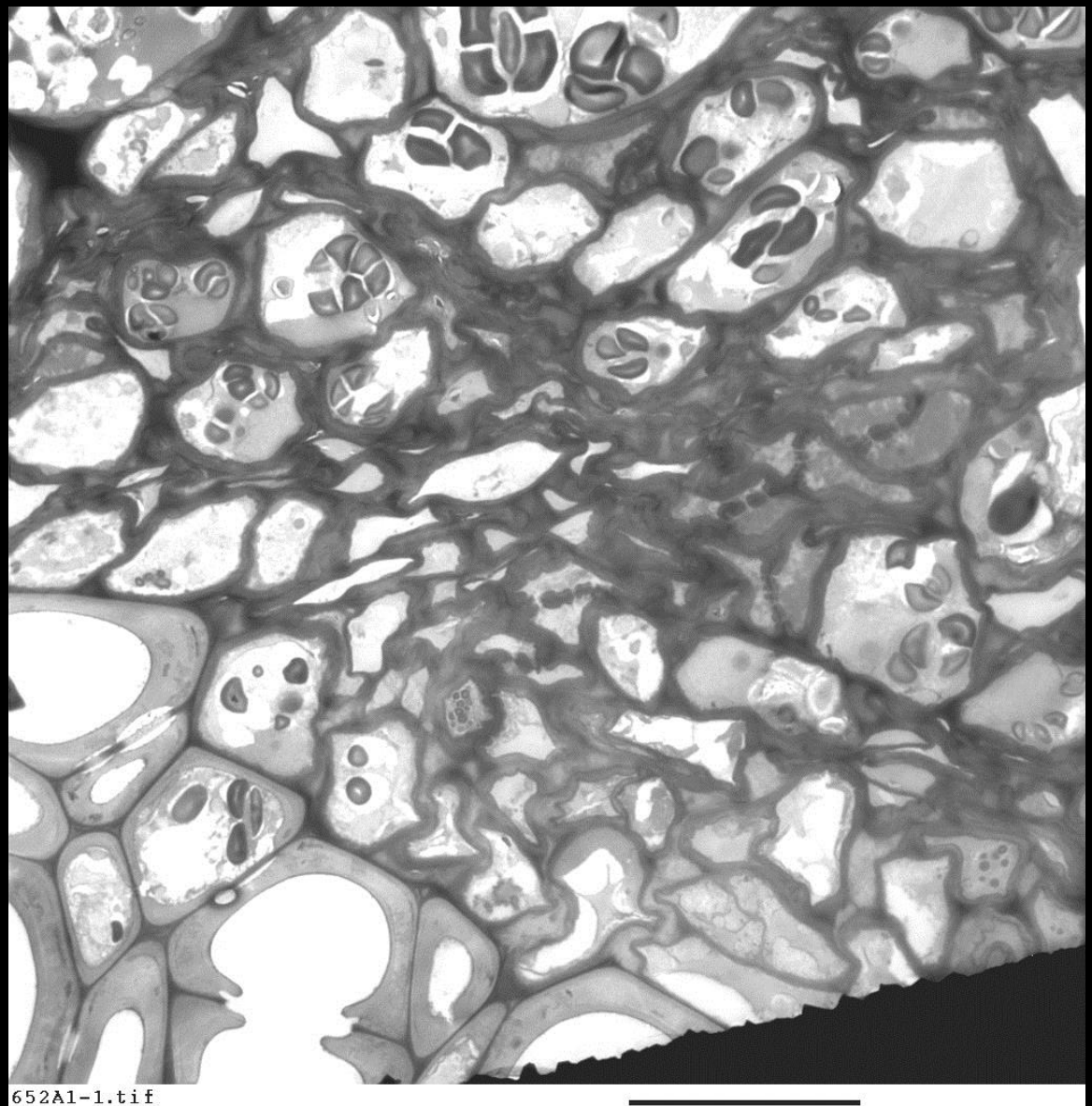
HLB Petioles Val/Swg-Treated-2011-With Symptoms



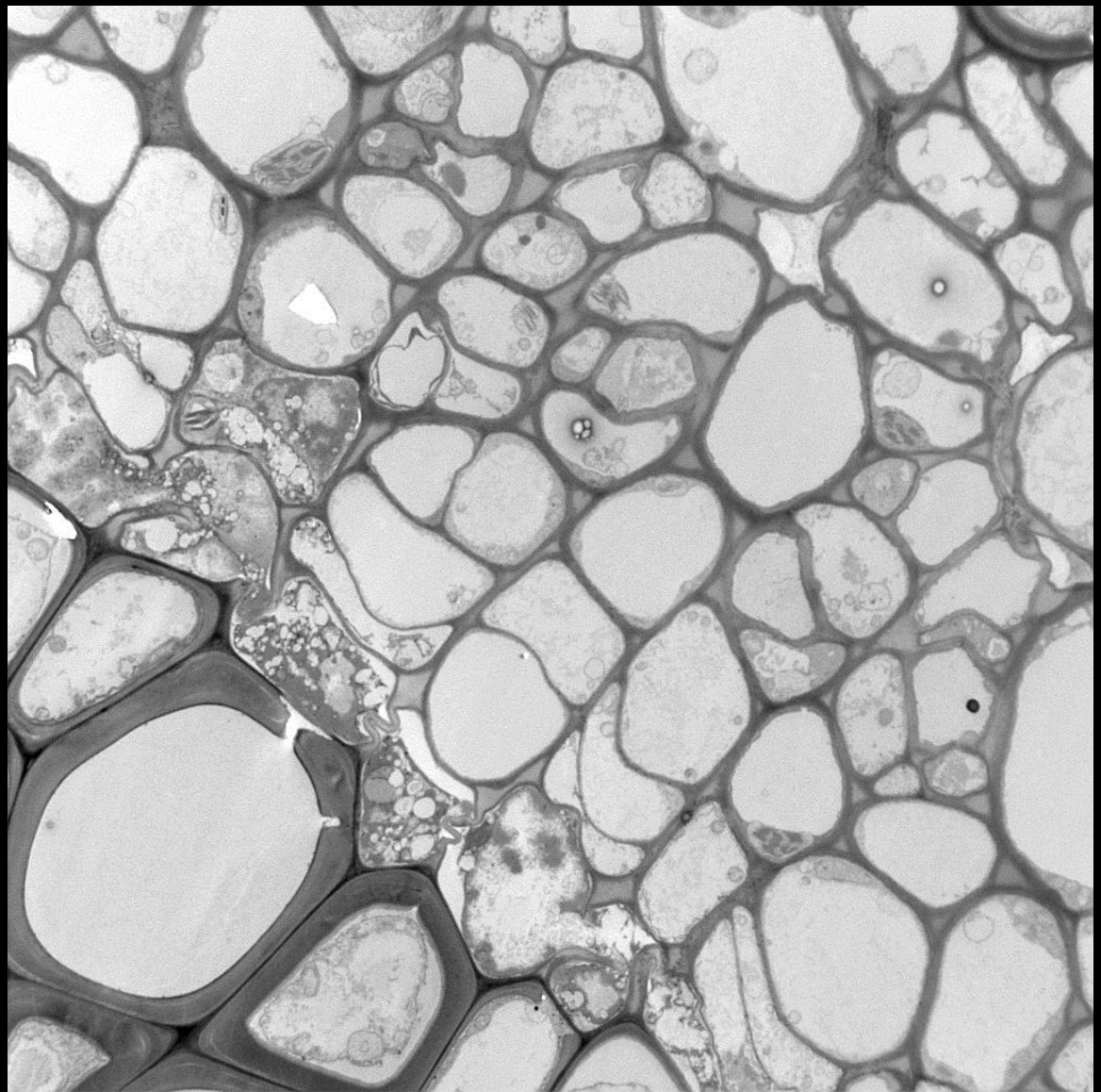
HLB Stems Val/Swg-Treated-2011-With Symptoms



HLB Infected- Untreated

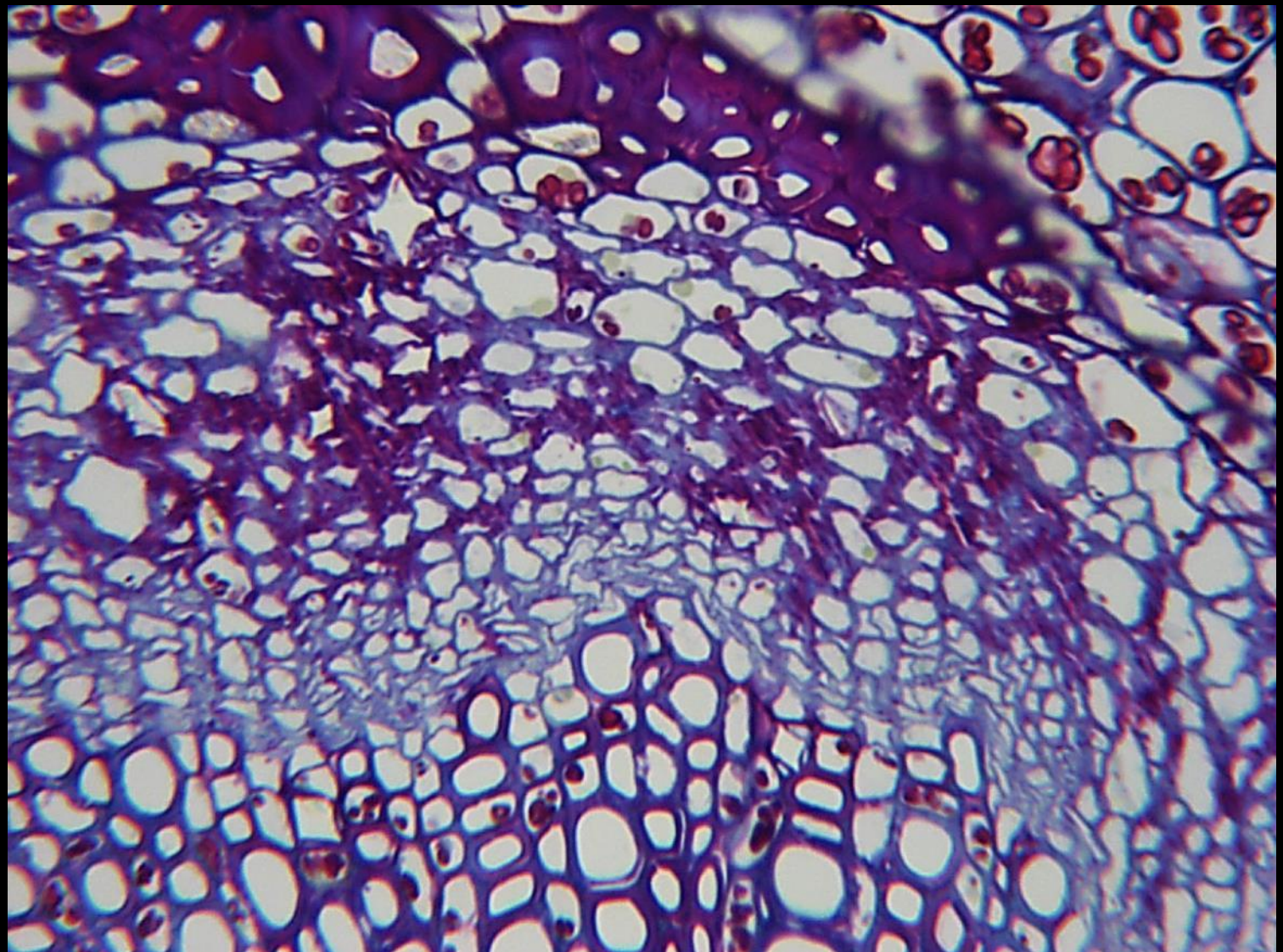


Treated

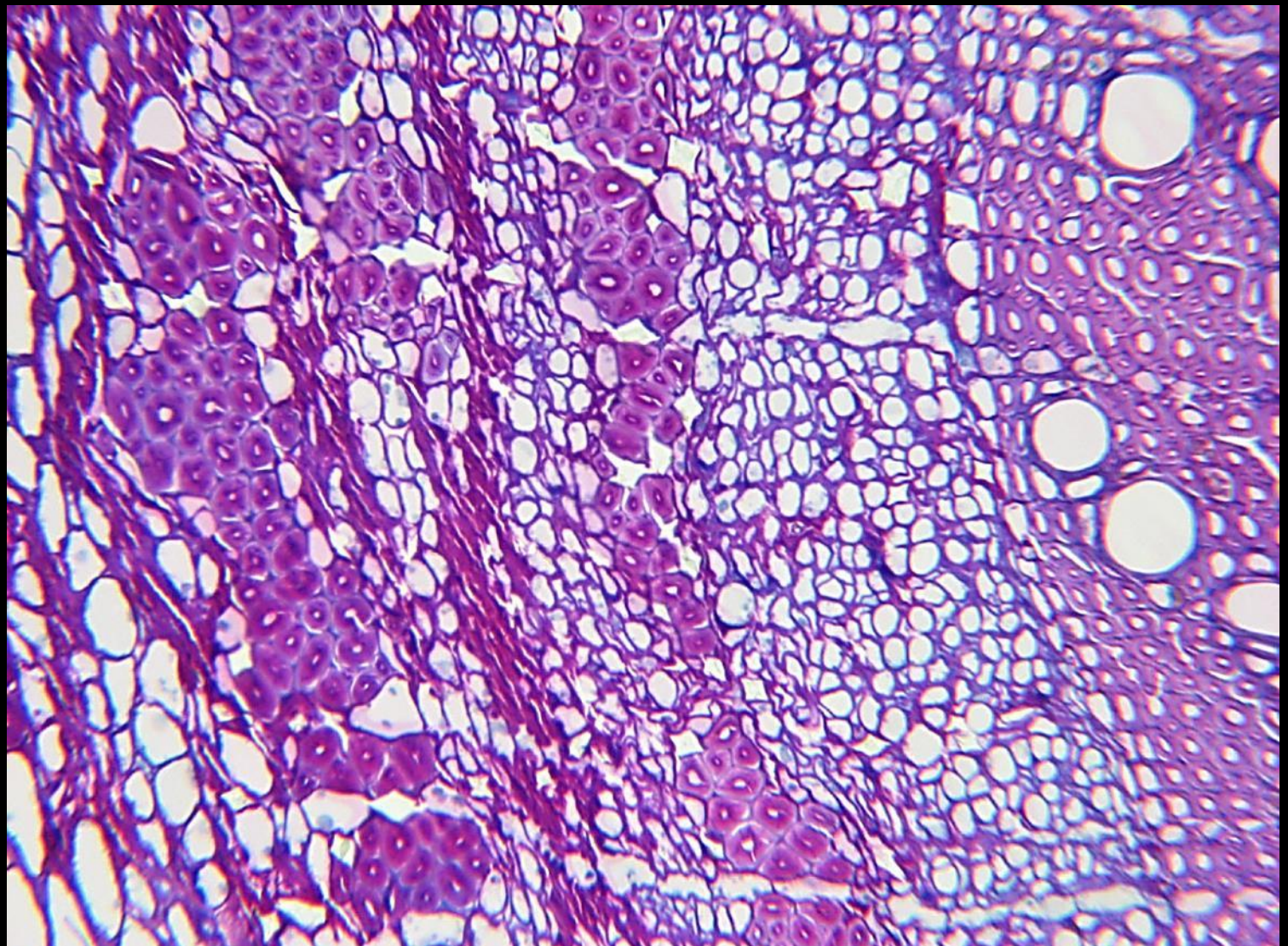


SWREC: Untreated,Row 6, Tree 18

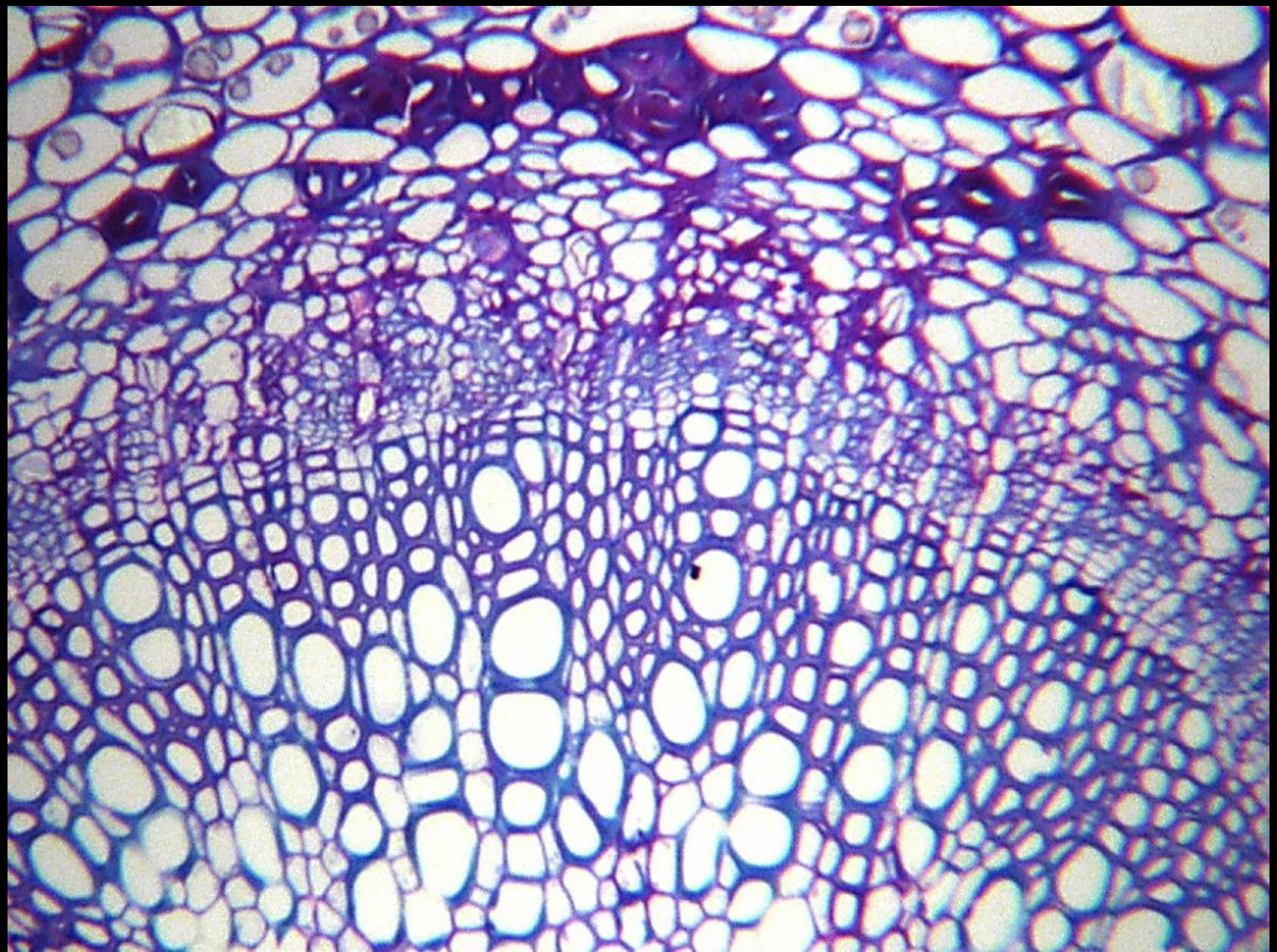
HLB Petioles Untreated B.R.-2010



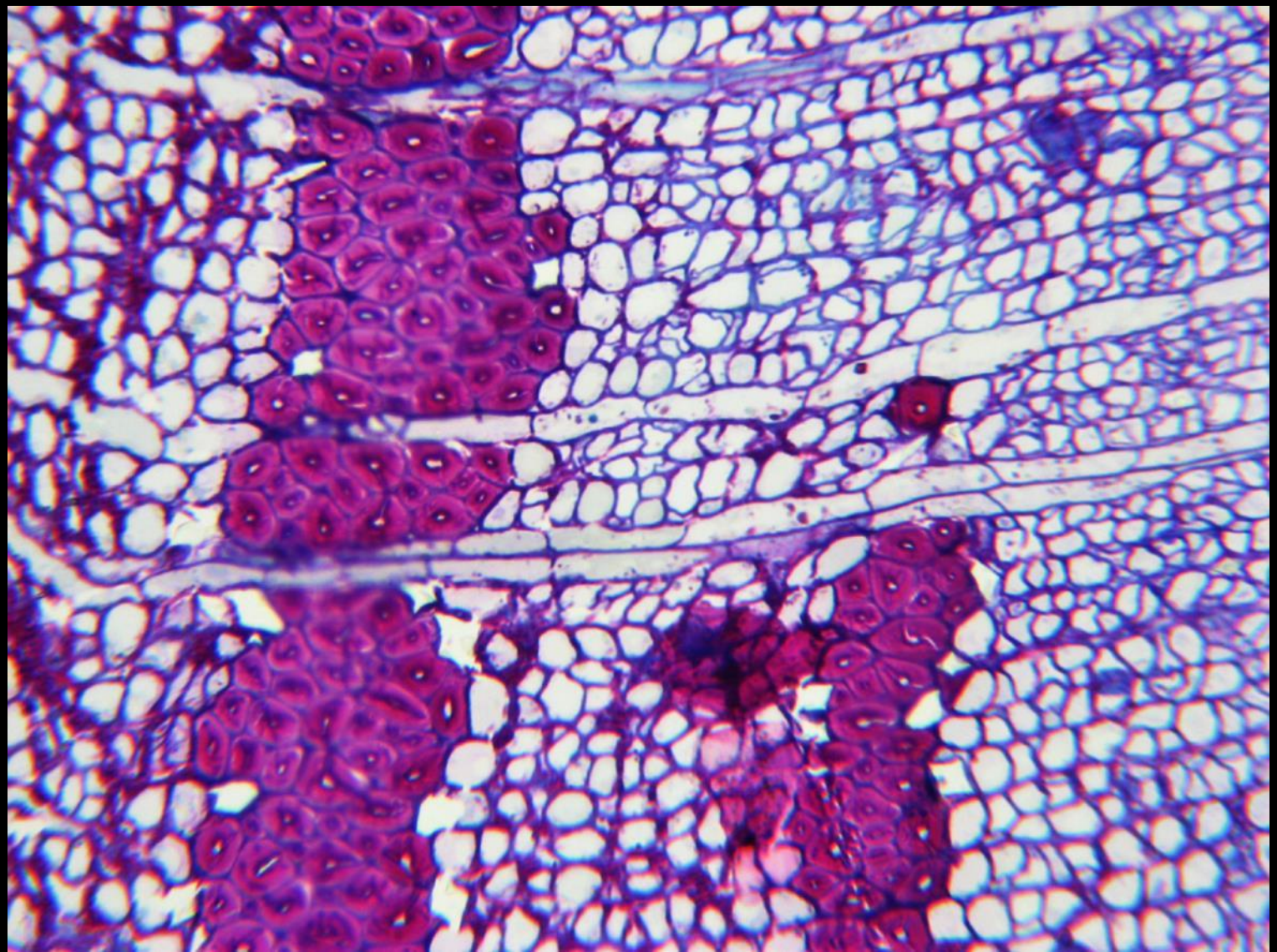
HLB Stems Untreated B.R.-2010



HLB Petioles Treated B.R.-2010



HLB Stems Treated B.R.-2010



Is the Phloem Functional?

- Methods have been used with other plants studying phloem uptake or translocation (TL)
- In collaborative work with Fan Jing we began using a fluorescent dye for phloem studies in HLB affected and healthy citrus in greenhouse trees
- We have since used it to see what is happening with nutrient treatments at these locations listed previously

Source-Sink

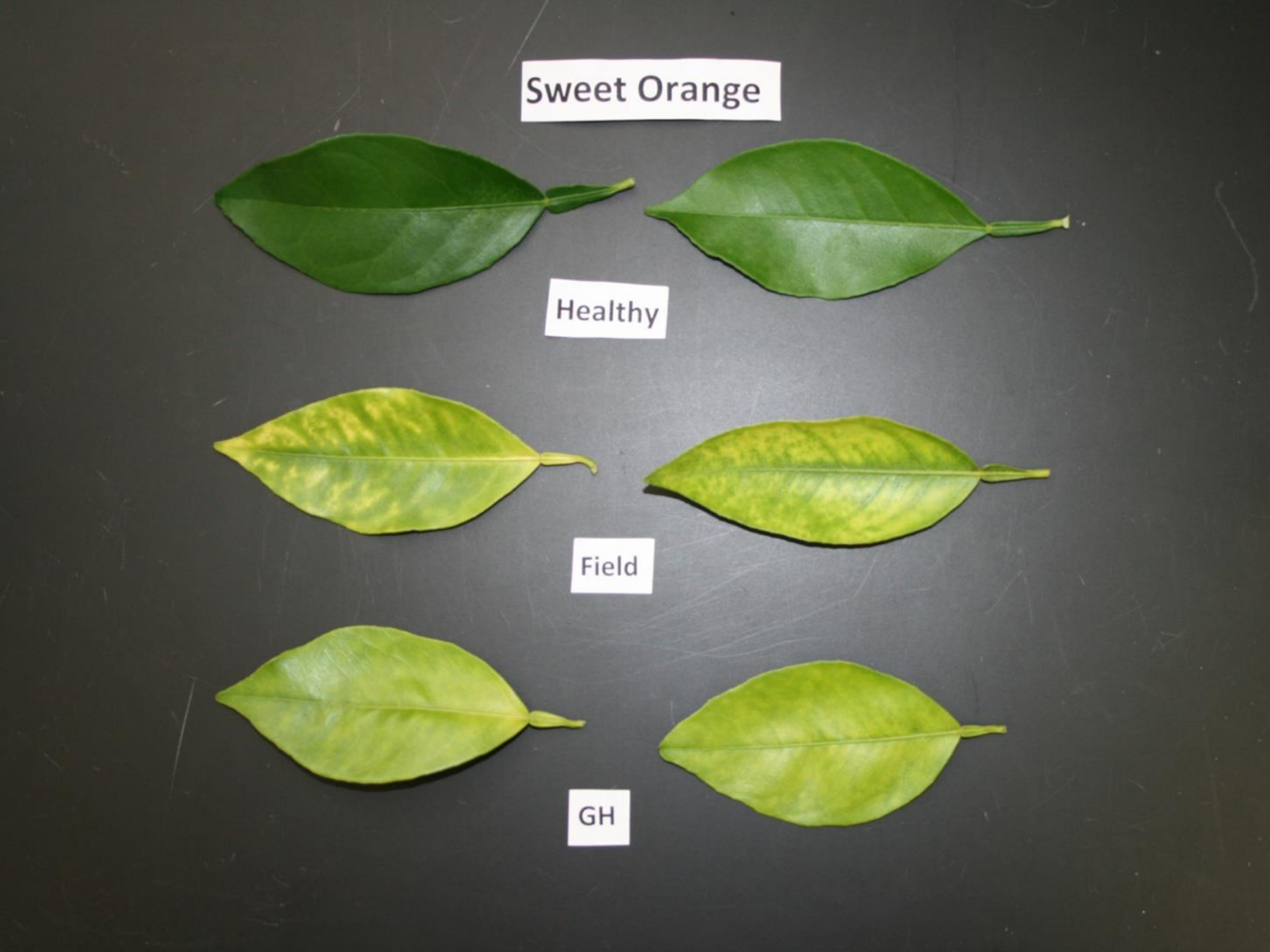
- Functionally a plant can be divided into source and sink
- Sources are parts where net fixation of carbon dioxide occurs
- Sinks are sites where assimilates are stored or used
- Allocation of assimilates between plant parts occurs via transport in the phloem.

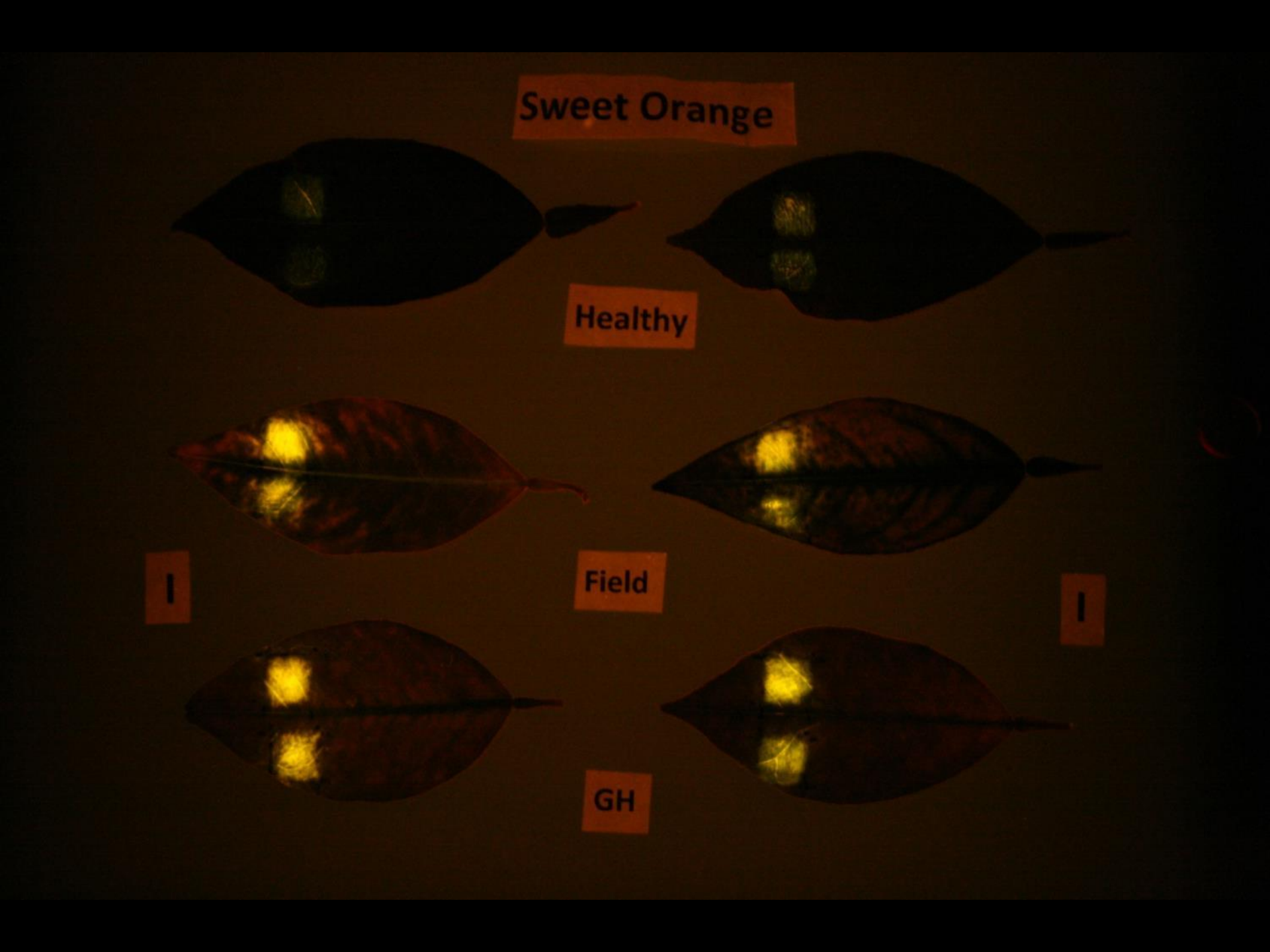
Source-Sink of Citrus

- Herbaceous crop plants accumulate these materials in source leaves during the photoperiod and evacuate them at night
- Citrus leaves have a system that is different
- Annual changes occur in carbohydrate levels & represent a combination of developmental and seasonal trends with the demands exerted by developing leaves and shoots and reproductive (flowers and fruits) sinks

Failure of Source/Sink

- Plants may have adverse environmental conditions (stress) and diseases that inhibit this source to sink process
- HLB is a phloem disease with infection by Ca. Liberibacter asiaticus and the production of collasped necrotic phloem that is not functional

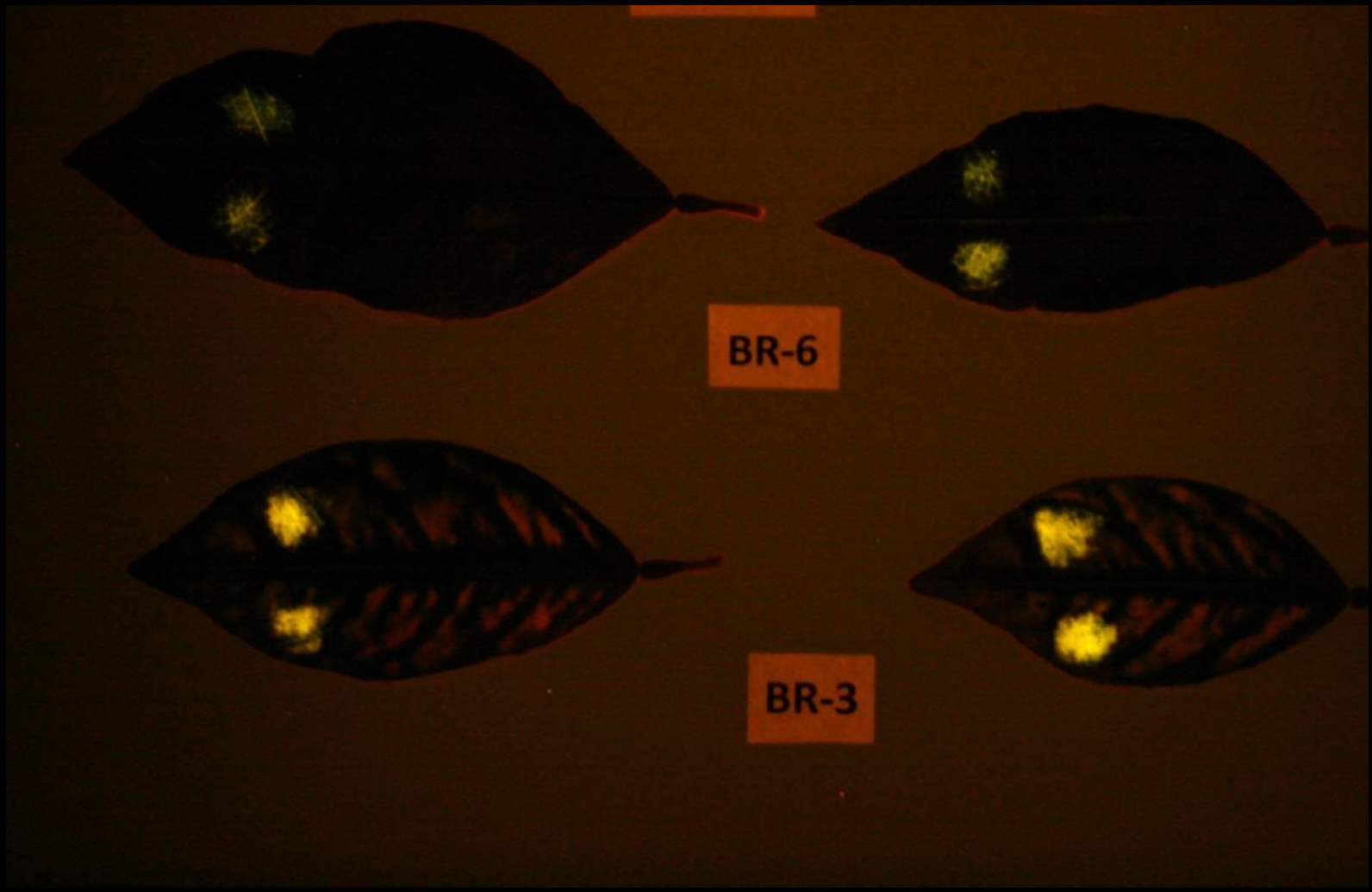

Sweet Orange
Healthy
Field
GH

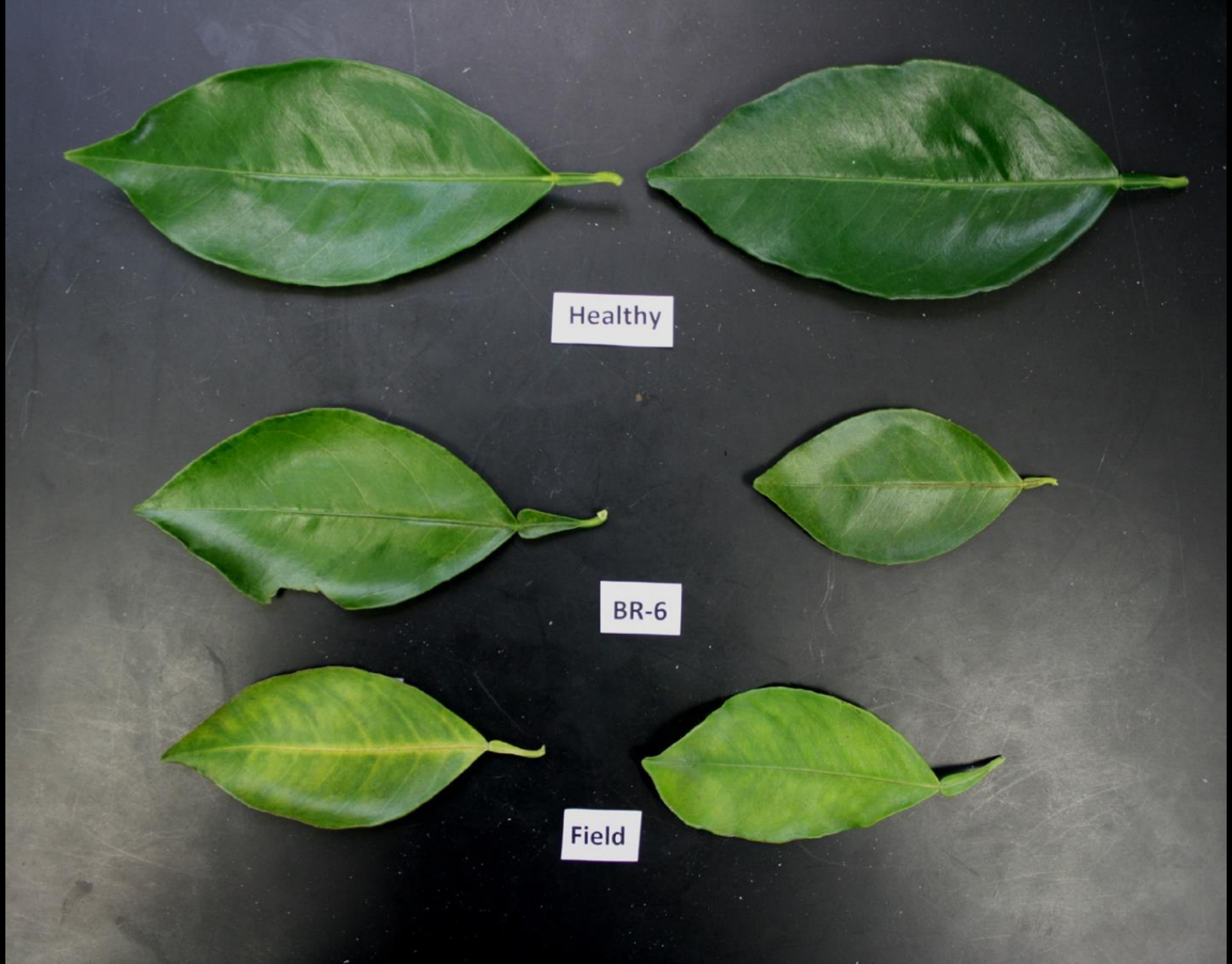

Sweet Orange
Healthy
I
Field
I
GH

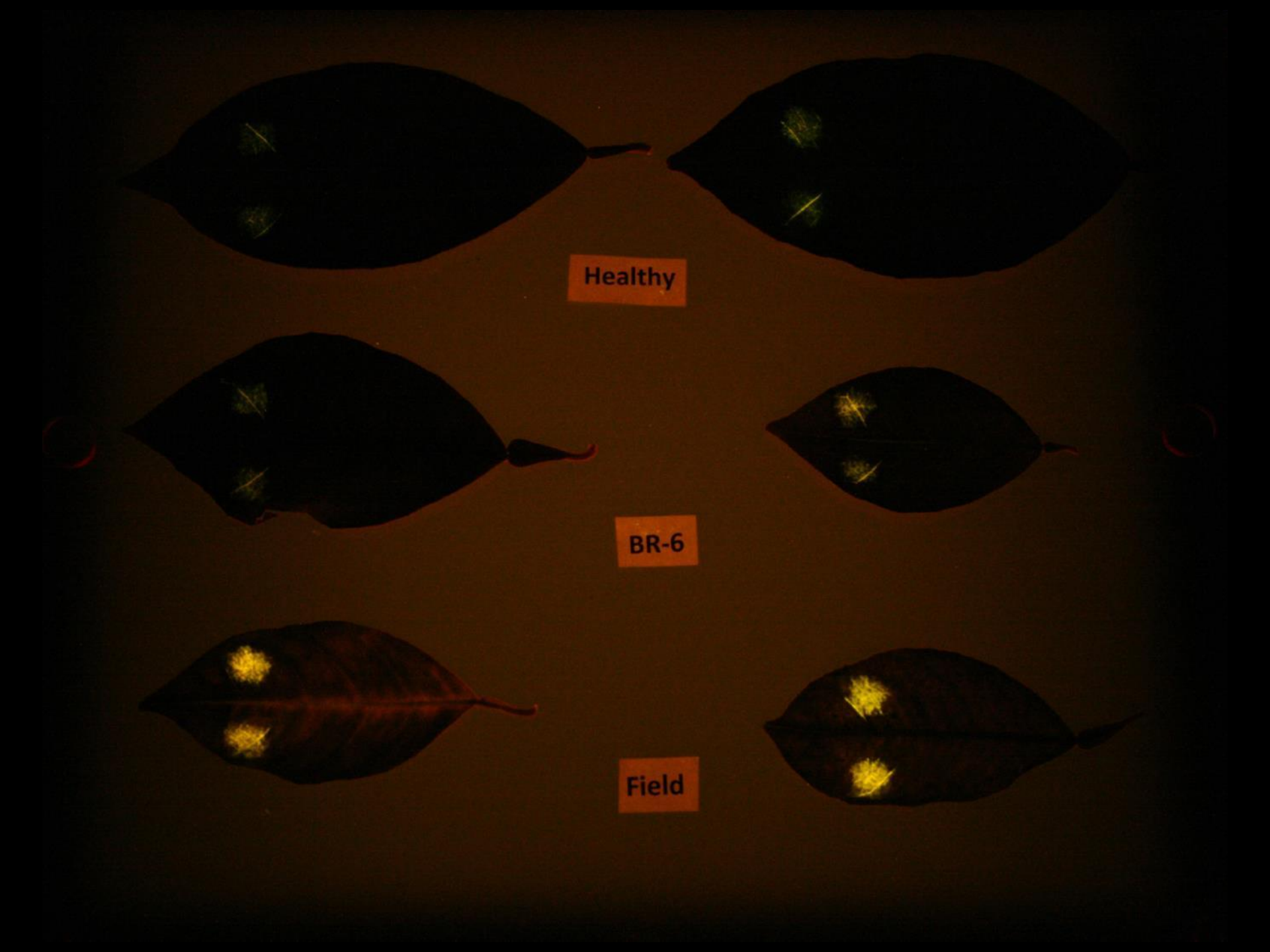
BR6: Row 4 Tree 15 (treated)

BR3: Row 6, Tree 18 (untreated)

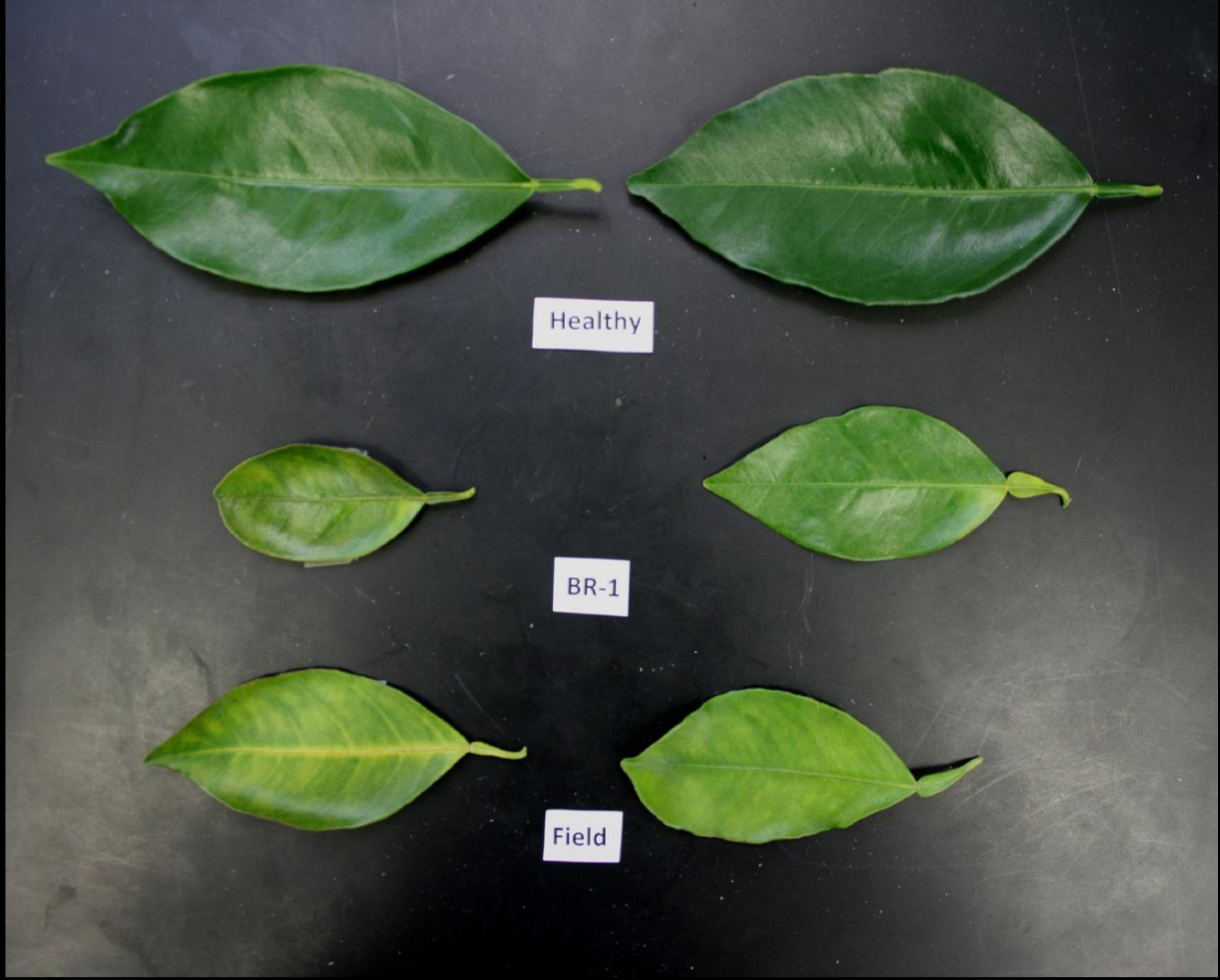

Healthy
BR-6
BR-3

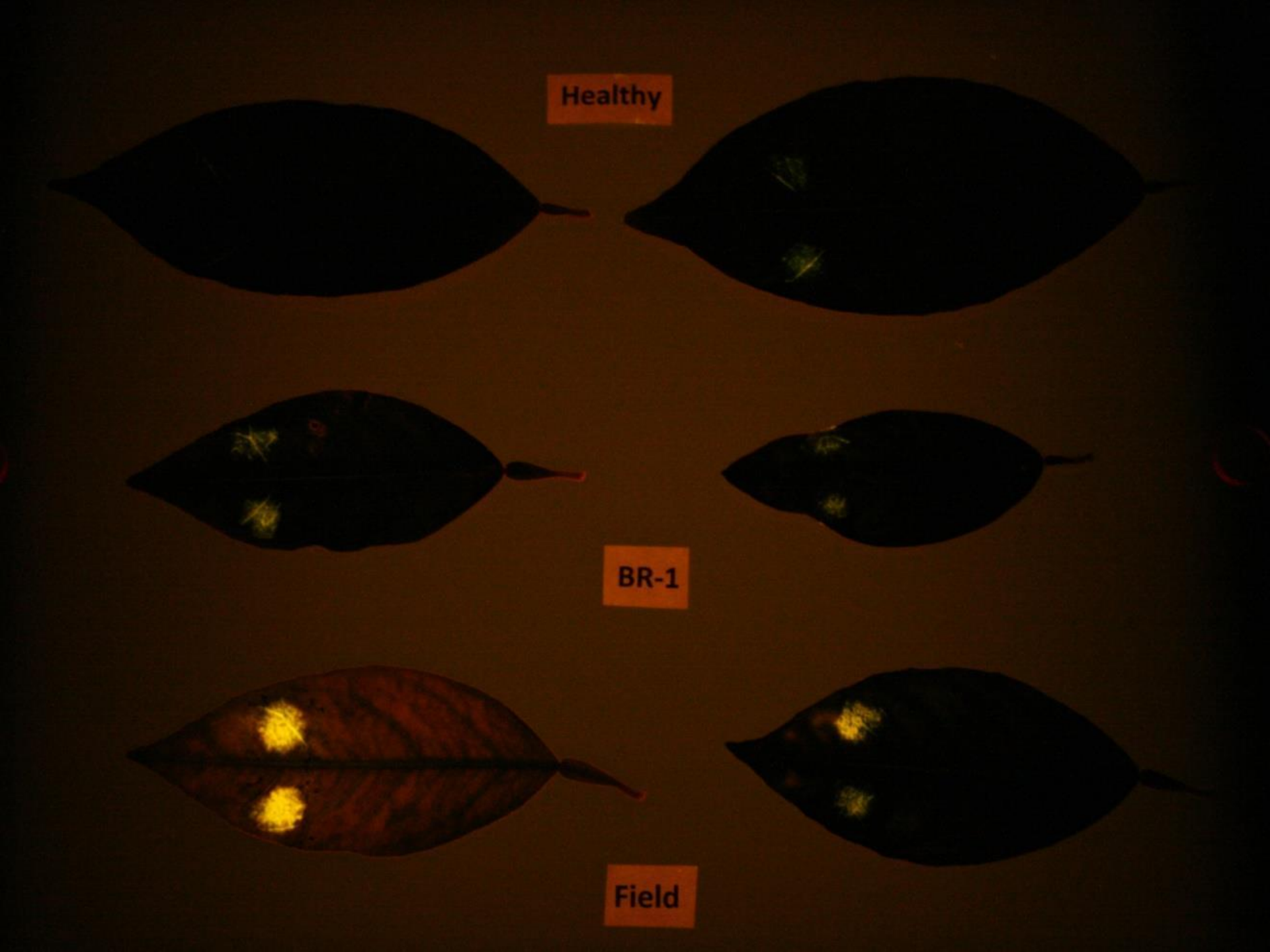

BR-6
BR-3


Healthy
BR-6
Field

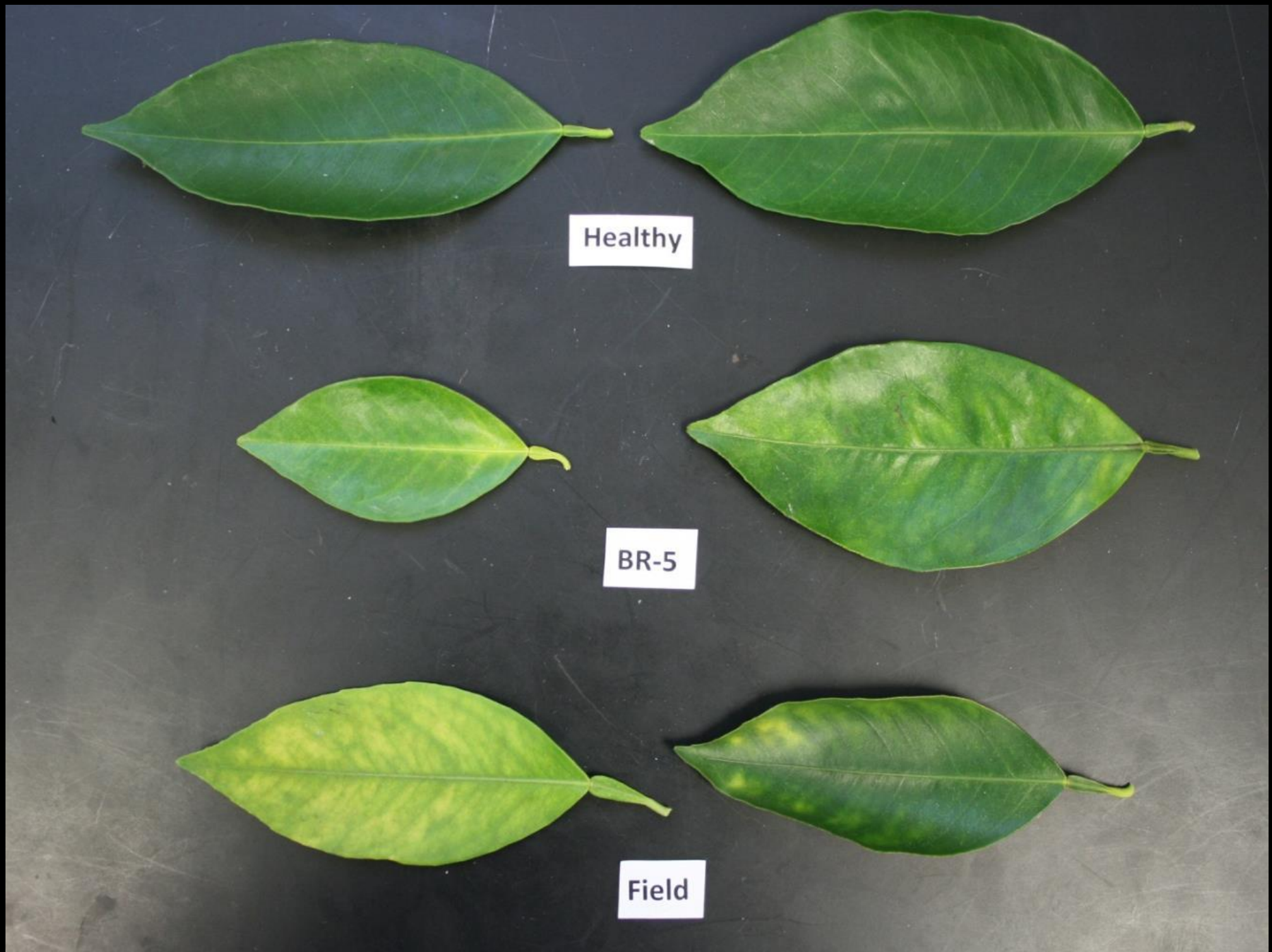

Healthy
BR-6
Field

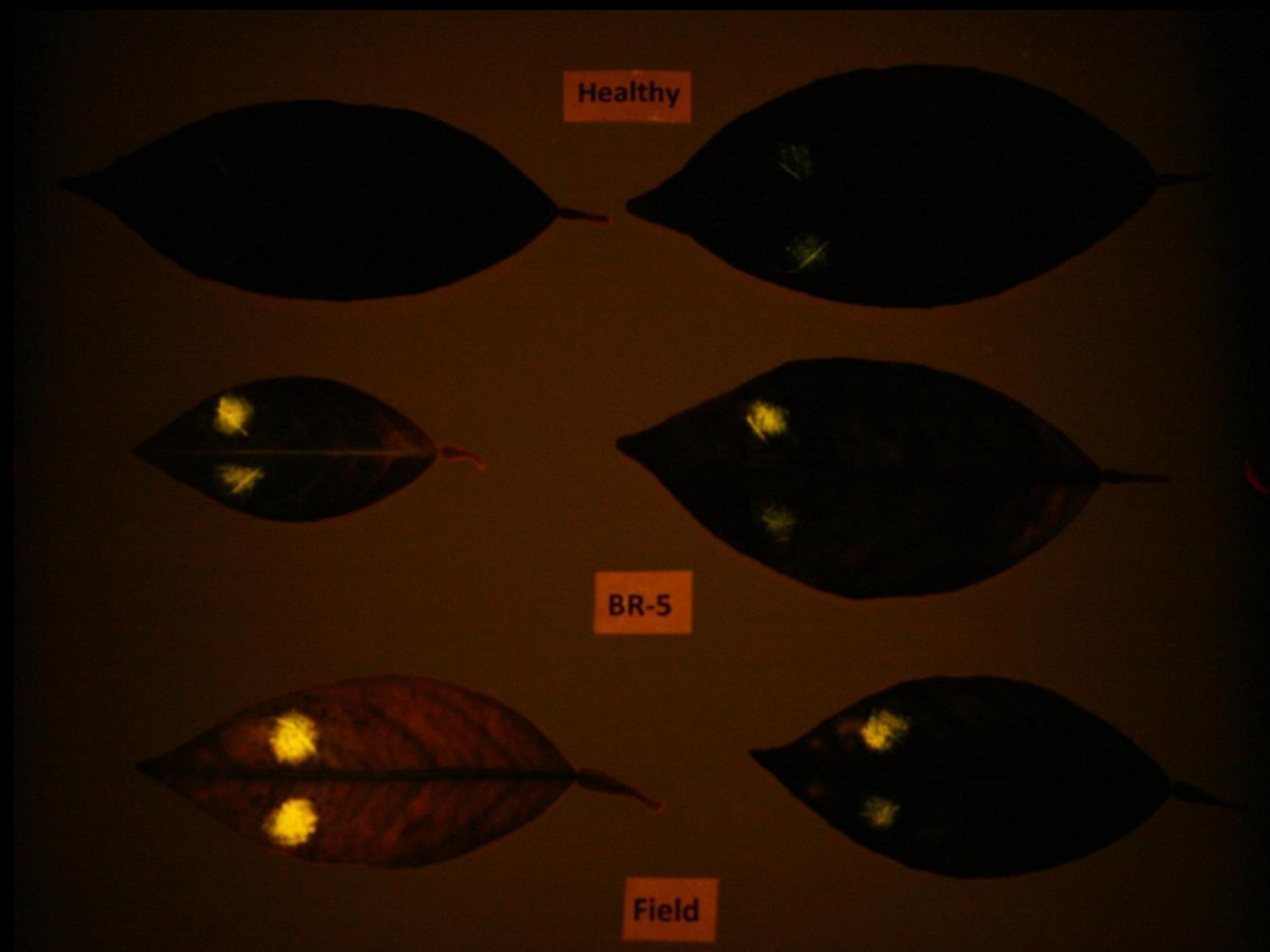
BR1:Row 3, Tree 18


Healthy
BR-1
Field

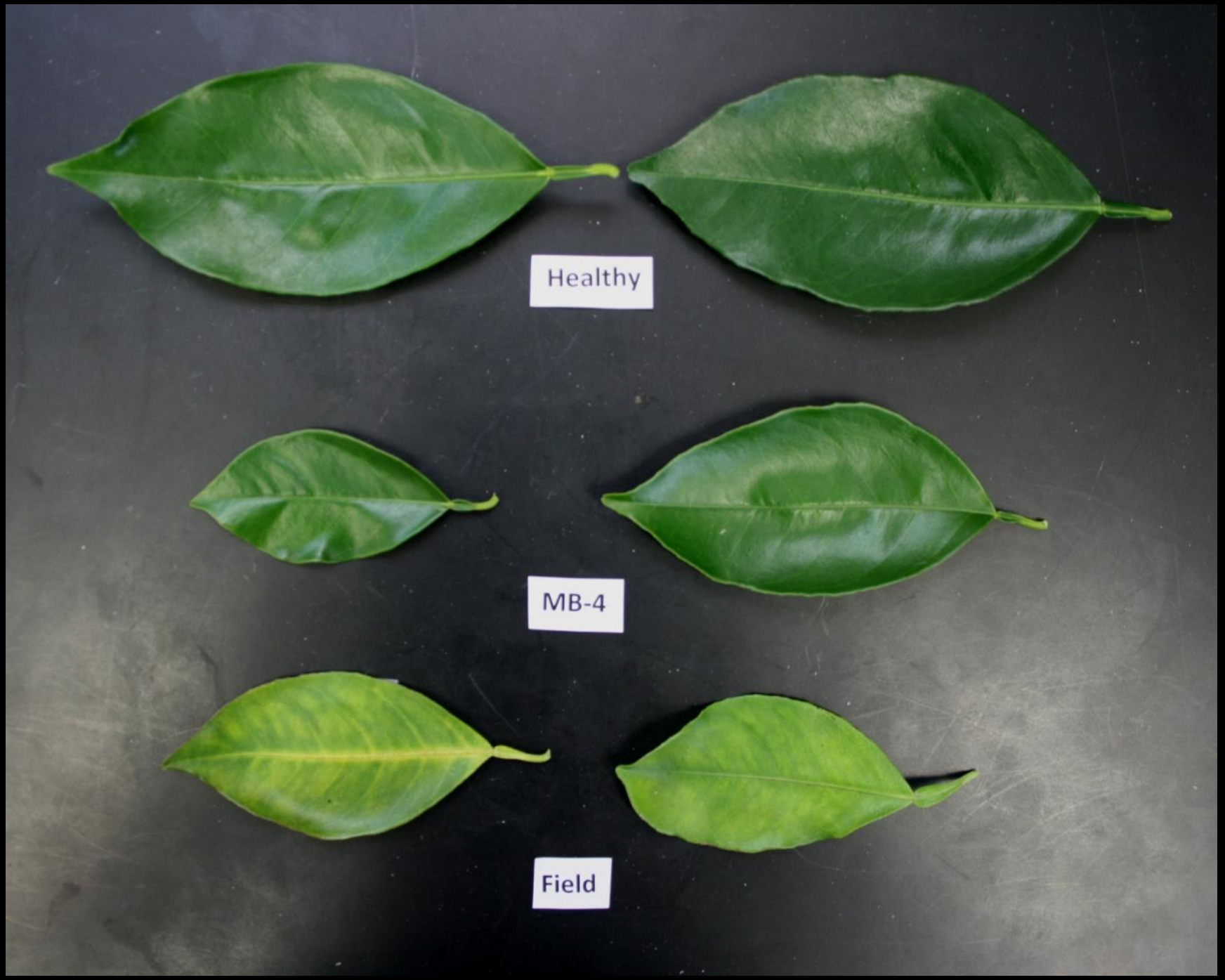

Healthy
BR-1
Field

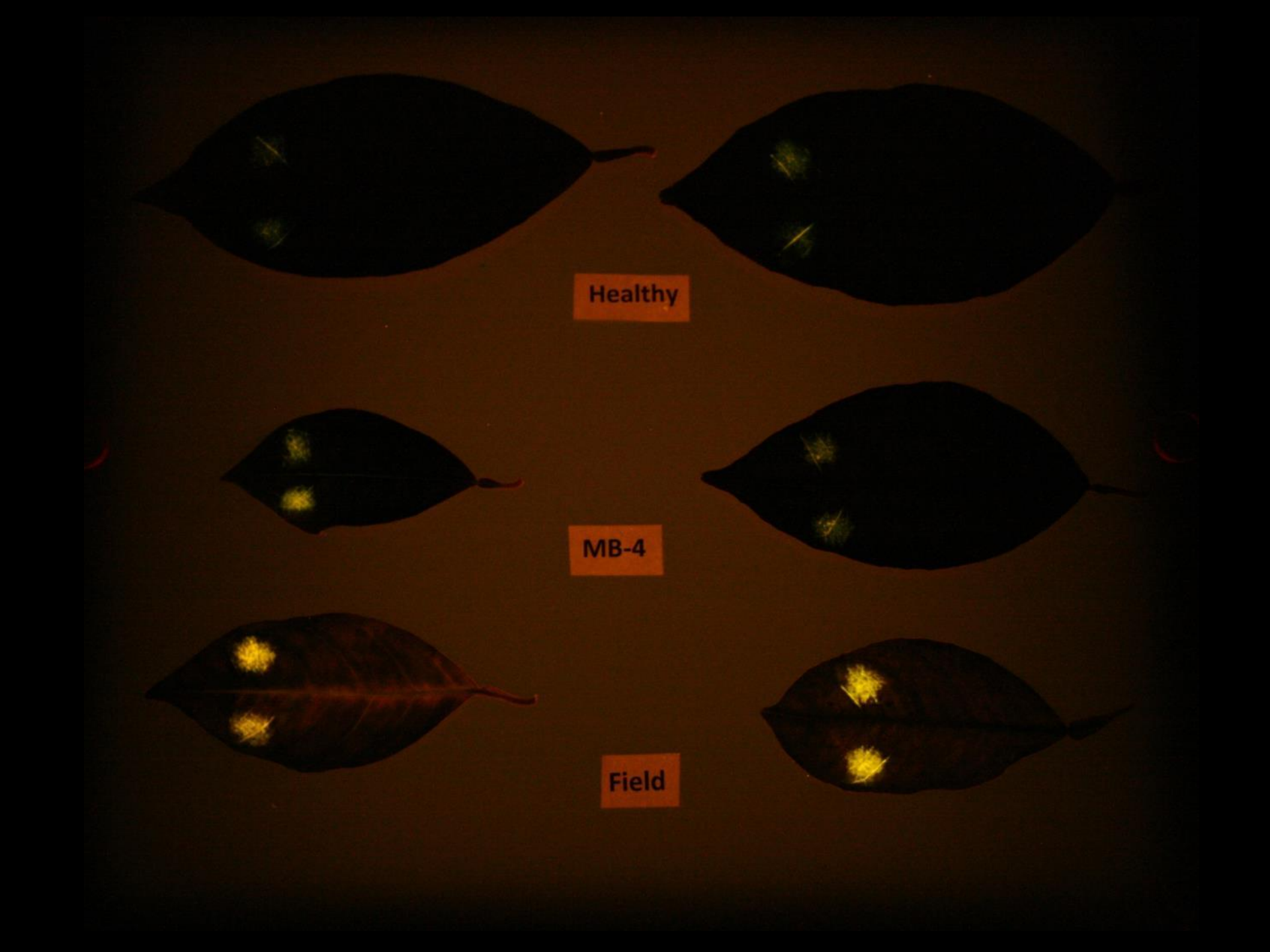
BR5: Row 3, Tree 21


Healthy
BR-5
Field


Healthy
BR-5
Field

Orange Hammock


Healthy
MB-4
Field


Healthy
MB-4
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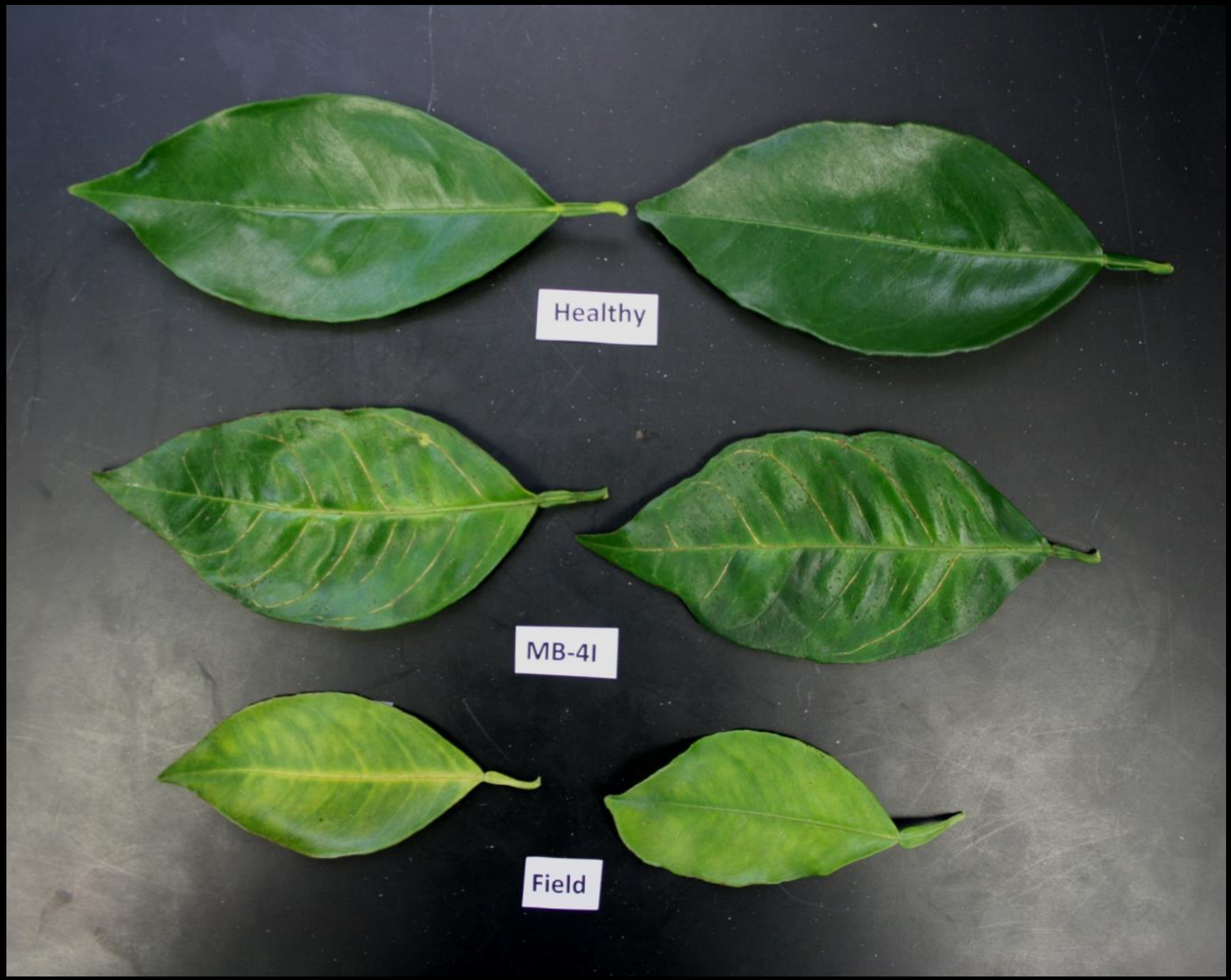
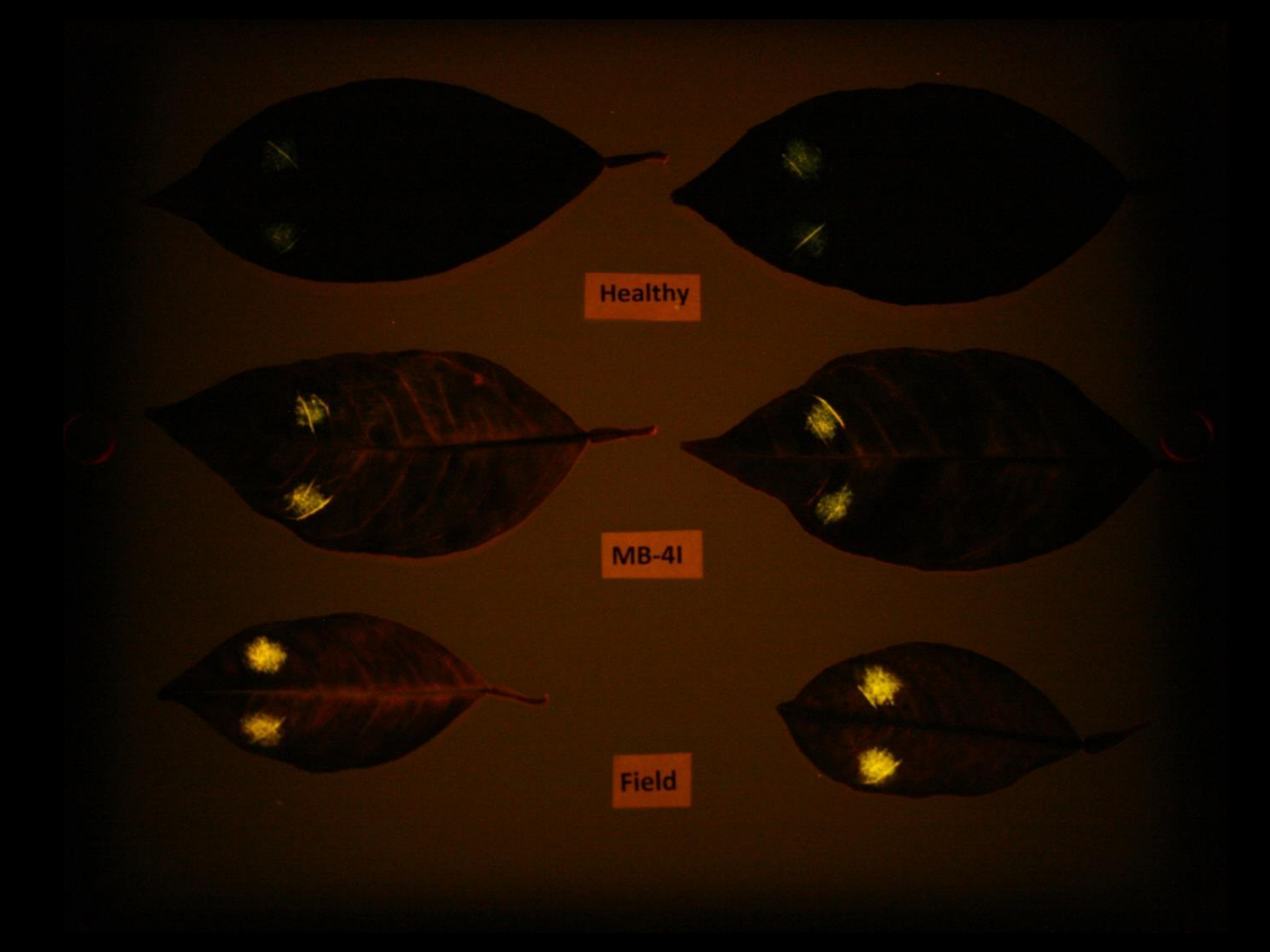
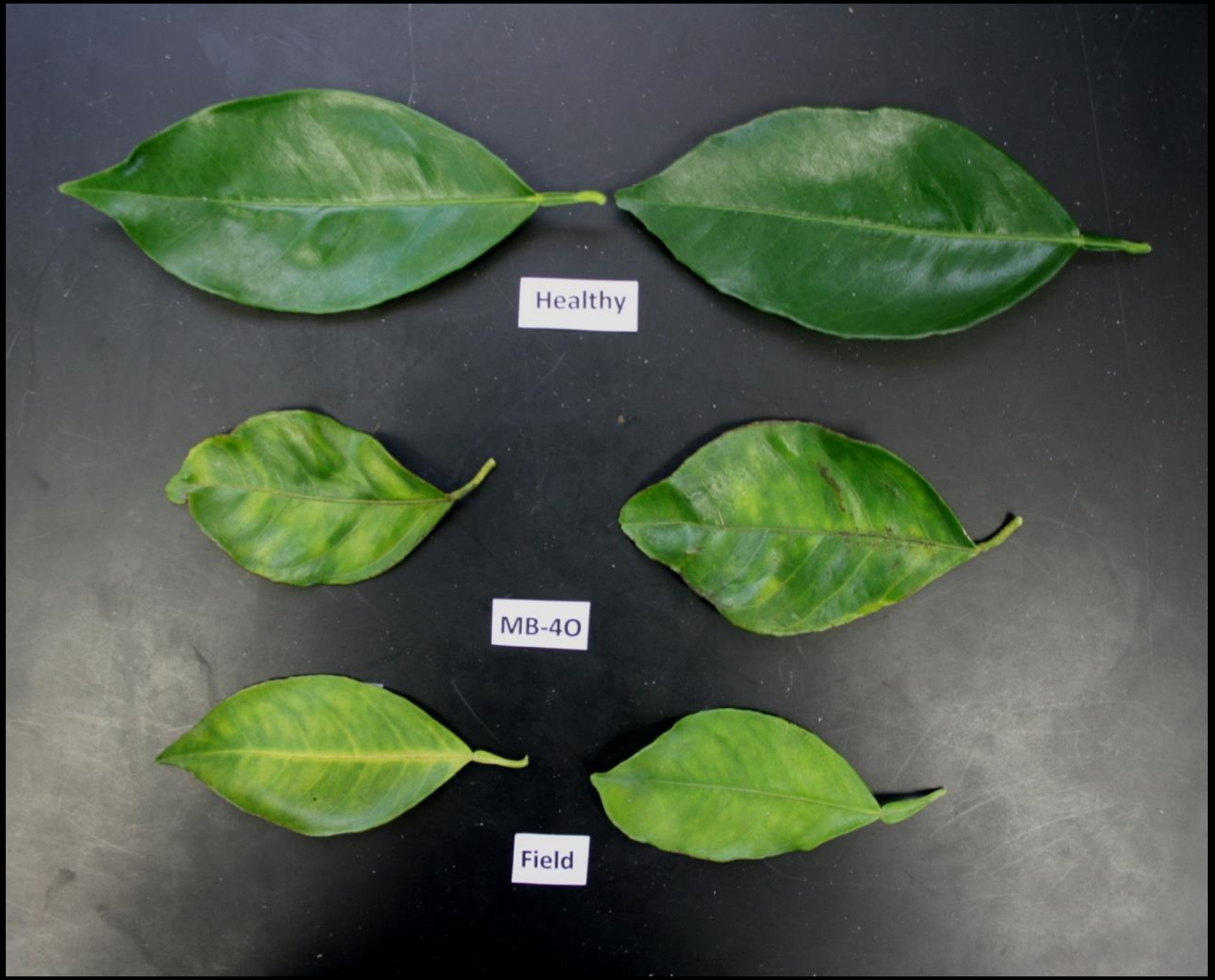
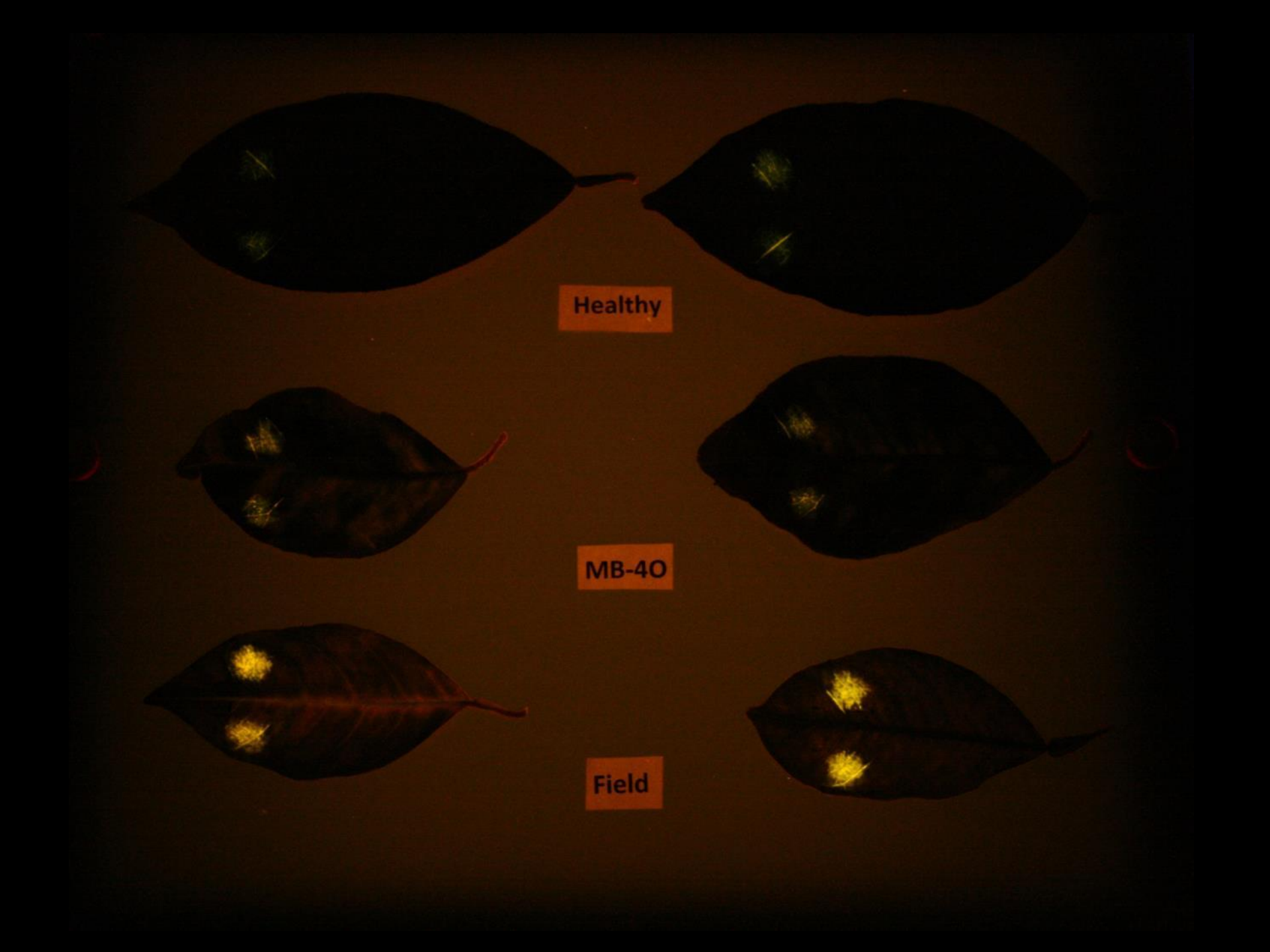

Healthy
MB-4I
Field


Healthy
MB-4I
Field


Healthy
MB-40
Field


Healthy
MB-4O
Field

Can We Measure the Amount of Dye Taken Up?

- Yes with microtiter plate assays that measure the amount of dye in the spots and just subtract that amount from the amount of dye applied to get the amount of dye translocated


B-35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Comparison of Healthy and HLB Affected Samples Averages (collected 12-14-11)	
Healthy Leaves	247 ug/ml
HLB Leaves	83 ug/ml

Comparison of Nutrient Treated and Untreated Healthy and HLB Affected Samples Averages (collected 01-03-12)	
Healthy Water Sprayed Leaves	240 ug/ml
Healthy Nutrient Sprayed Leaves	332 ug/ml
HLB Water Sprayed Leaves	53 ug/ml
HLB Nutrient Sprayed Leaves	90 ug/ml

Comparison of Nutrient Treated and Untreated Healthy and HLB Affected Samples Averages (collected 01-06-12)	
Healthy Water Sprayed Leaves	238 ug/ml
Healthy Nutrient Sprayed Leaves	276 ug/ml
HLB Water Sprayed Leaves	74 ug/ml
HLB Nutrient Sprayed Leaves	80 ug/ml

Comparison of Nutrient Treated and Untreated Healthy and HLB Affected Samples Averages (collected 01-27-12)	
Healthy Water Sprayed Leaves	395 ug/ml
Healthy Nutrient Sprayed Leaves	477 ug/ml
HLB Water Sprayed Leaves	132 ug/ml
HLB Nutrient Sprayed Leaves	201 ug/ml

Comparison of Nutrient Treated and Untreated Healthy and HLB Affected Samples Averages (collected 01-31-12)	
Healthy Water Sprayed Leaves	279 ug/ml
Healthy Nutrient Sprayed Leaves	373 ug/ml
HLB Water Sprayed Leaves	113 ug/ml
HLB Nutrient Sprayed Leaves	198 ug/ml

Comparison of HLB Nutrient Treated with Untreated Healthy (Orange Hammock Jan 2013)	
Sample	Translocated Dye
Healthy Greenhouse Leaves	330 ug/ml
HLB Nutrient Sprayed Fruit Leaves	344 ug/ml
HLB Sprayed Mature Leaves	328 ug/ml
HLB Nutrient Sprayed Young Leaves	277 ug/ml
HLB average	316 ug/ml

So What's Happening?

- Cytological results of experiments conducted by Rouse at the SWREC show improvement of HLB trees treated with nutrients e.g. new phloem with more open elements as compared to untreated HLB affected trees
- Results from Orange Hammock grove show similar results (HLB PCR positive treated trees)
- Fluorescent uptake tests show functional phloem in treated trees vs untreated but not as good as healthy unaffected trees

- Fluorescent assay works well however there is some seasonal variability in how much dye moves
- Annual changes occur in carbohydrate levels & represent a combination of developmental and seasonal trends exerted by the tree
- Re-establishing the source to sink connection by producing new phloem for photosynthetic products to move to sinks (fruit and new flushes and maybe roots)
- Phloem may still degrade and be blocked since the tree is still diseased

- Currently working on a larger database for these results and an in-field on-tree assessment of the phloem translocation in treated trees

- Funding for the project came from the Citrus Research and Development Foundation
- Current funding for this project ends June 30


2011/ 7/19 13:15