Meeting the Challenge of the Asian Citrus Psyllid in California Nurseries

A two-day workshop in Riverside, California

June 11- 12, 2009


Florida-Foundation Block


Brazil-Citrus Nursery

Organizing Committee:

T. Delfino-California Citrus Nursery Society
A. Eskalen-Dept. of Plant Pathology & Microbiology, University of California Riverside
R. Lee-USDA- ARS, National Clonal Germplasm Repository for Citrus and Dates
G. Vidalakis-Citrus Clonal Protection Program, Dept. of Plant Pathology & Microbiology, University of California Riverside

Invited Speakers:

J. Ayres-Fundecitrus, Brazil
J. Bethke-UC, CA
G. Baze-Golden Pacific Structures, CA
T. Delfino-CCNS, CA
F. Dixon-Wells Fargo, CA
D. Elder-American Ag Credit, CA
T. Gast-Southern Gardens Citrus, FL
P. Gomes-CHRP, USDA -APHIS, NC
E. Grafton-Cardwell-UCR, CA
D. Howard-AgraTech, CA
N. Jameson-Brite Leaf Nursery, FL
R. Keijzer-KUBO, The Netherlands
P. Llatser-AVASA, Spain
S. McCarthy-CDFA, CA
G. Vidalakis-UCR-CCPP, CA

Registration: http://ccpp.ucr.edu & http://eskalenlab.ucr.edu

Location:
Sunkist Center
Citrus State Historical Park
9400 Dufferin Avenue
(Corner of Van Buren Blvd)
Riverside, California

Sponsored by:

California Citrus Nursery Board
Citrus Research Board
Stuewe & Sons, Inc. Tree Seedling Nursery Containers
Golden Pacific Structures, A Rough Brothers Company
AgraTech Greenhouse Manufacturers
Bayer CropScience

Information on line at: http://eskalenlab.ucr.edu

Georgios Vidalakis <vidalg@ucr.edu>

Seed Transmission of HLB and CTV ELISA Testing in Screenhouses

Workshop
Meeting the Challenge of the Asian Citrus Psyllid in California Nurseries

G. VIDALAKIS
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Dept. of Plant Pathology & Microbiology
University of California
Riverside

June 11-12, 2009

Seed Transmission of HLB

Early Reports

Tirtawidjaja 1981. Proc. Int. Soc. Citriculrure. 469 - 471

- No citrus vein phloem degeneration (CVPD) were observed on seedlings obtained from normal-size fruits, although they had been taken from CVPD-affected trees

- Seeds derived from the smaller fruits produced some stunted, chlorotic seedlings

- Three of these (out of ~100) had the same narrow, mottled appearance of insect inoculated seedlings

International Research Conference on Huanglongbing

http://www.doacs.state.fl.us

http://www.doacs.state.fl.us/pi/hlb_conference/Proceedings.pdf

Orlando, Florida • December 2008

Tomato, Pepper, & Tamarillo

Liefting, L.W. et al. 2008
Proc. Int. Res. Conf. on HLB. Orlando, FL, #5.17, pg184

Seed transmission studies failed to detect the Ca. Liberibacter in:

- 1,030 tomato seedlings
- 225 pepper seedlings
- 225 tamarillo seedlings

Grown from infected seed

Periwinkle , Dodder, and Citrus

L. Zhou et al. 2008 Phytopathology 98:S181
Proc. Int. Res. Conf. on HLB. Orlando, FL, #3.2, pg112

- Ca. Las was detected in up to 53% of all seeds tested both from HLB-infected periwinkle and dodder

- Germination rates were normal for the Ca. Las-positive seeds from both plant species

- The periwinkle and citrus progenies from HLB-affected plants did not show blotchy-mottling

- They exhibited up to 80% vein yellowing, leaf curling and yellowing only when stressed by nutrient deficiency

- Symptoms disappeared after stress removed

Citrus & Murraya

J. S. Hartung et al. 2008.
Proc. Int. Res. Conf. on HLB. Orlando, FL, #5.5, pg166

•319 seedlings of *Murraya paniculata*, rough and 'Meyer' lemon, sour orange, grapefruit and 'Valencia' sweet orange

•The large majority of seedlings did not show any symptoms

•None of the seedlings tested positive for Ca. Las by a real-time PCR with one set of primers

However

Sour Orange-Different set of primers

J. S. Hartung et al. 2008.
Proc. Int. Res. Conf. on HLB. Orlando, FL, #5.5, pg166

- 9 of 89 sour orange seedlings show abnormal growth patterns which include stunting, defoliation and chlorosis

- One of these sour orange seedlings in particular was severely stunted and shows symptoms similar to HLB

- This seedling was positive for the presence of Ca. Las when tested by a real-time PCR with another set of primers

Duncan & Ruby Red grapefruit & Hamlin sweet orange

Shatters, Jr., R. G. et al. 2008
Proc. Int. Res. Conf. on HLB. Orlando, FL, #5.11 pg 173

- HLB-like symptomatic seedlings tested with qPCR.
- Less than 10% of the seedlings tested positive for Ca. Las
- The detection of Ca. Las did not always correlate with symptoms
- Ca. Las was also detected by qPCR in seedlings grown from surface sterilized seed germinated in sterile environment
- Dissection of the sterile-grown seedlings showed that the highest detectable level of Las was in the seedling roots
- HLB-symptomatic plants developed more slowly than asymptomatic plants however, most lost HLB symptoms over time and tested negative in subsequent tests

Pineapple sweet orange-2006

Graham, J. H. et al. 2008
Proc. of the Int. Res. Conf. on HLB. Orlando, FL, #5.12 pg 174

- Based on qPCR testing it is known that seed coats from infected fruit contain high titers of Ca. Las

- From the 59 seedlings sampled (including 45 Ca. Las negative seed coats), 7 plants were either positive or questionable

- Upon re-assay, 3 of the 7 plants were positive for Ca. Las

- Of these 3 plants, only one tested positive in subsequent RT-PCR testing

- Additional 356 seedlings were tested and none found positive or questionable

Pineapple sweet orange-2007

Graham, J. H. et al. 2008
Proc. of the Int. Res. Conf. on HLB. Orlando, FL, #5.12 pg 174

- Fruit were collected from 8 HLB symptomatic trees

- Extracted seed classified as healthy (28%), off colored-gummy (29%) or aborted (43%)

- 723 seedlings germinated from the healthy (359) and off-colored (344) seed were assayed by RT-PCR a year later

- 6 out of 723 (6 out of 344 off-colored seeds) tested Ca. Las positive after two assays

Carrizo citrange

Graham, J. H. et al. 2008
Proc. of the Int. Res. Conf. on HLB. Orlando, FL, #5.12 pg 174

Fruit were collected from 2 HLB positive Carrizo citrange seed source trees

Source #1: 142 seedlings – 2 positive for Ca. Las by qPCR

Source #2: 148 seedlings – 5 positive for Ca. Las by qPCR

Final Thoughts

- Ca. Las appears to be seed transmitted in different citrus species at a low rate
- The pathogens exists in both internal and external seed tissues
- However, the seed transmitted factor does not cause severe HLB symptoms and death….
- …since, symptom development is erratic and disappear after time or when stress factors are removed
- There is also the possibility that another unknown component of the HLB syndrome is not seed transmitted and hence the above observations
- Results vary with test methodologies used
- Not graft transmission experiments have been performed for a true rootstock-scion system

CTV ELISA Testing in Screenhouses

The Experiment

Objective #1: Monitor the temperature fluctuations inside and outside the screen house over time

LREC Protected Foundation Block:

- Monitor temperature every hour in- and out- doors

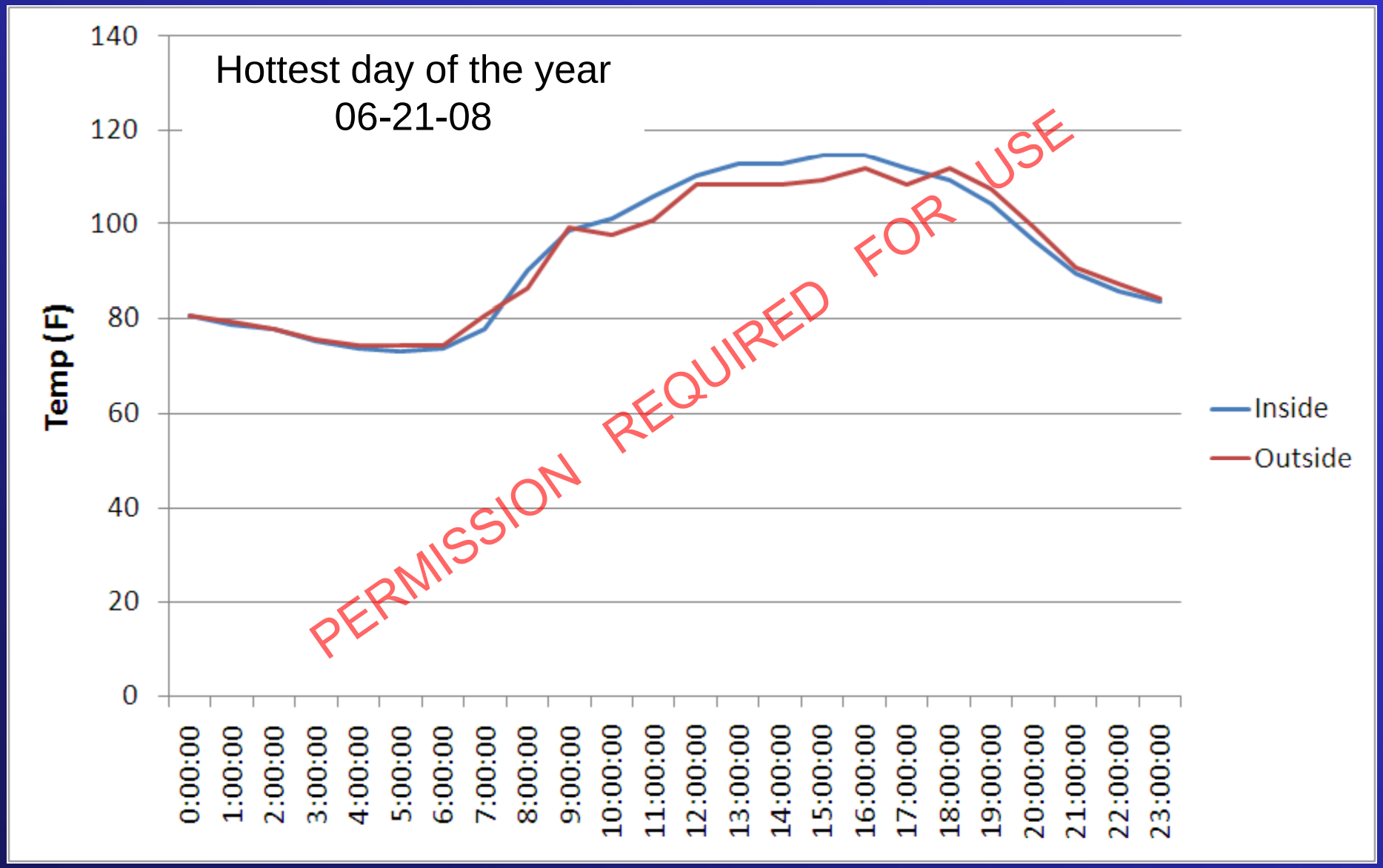
Objective #2: Perform CTV ELISA testing on different citrus varieties infected with different CTV isolates under screen over time correlate with temperature

Riverside Quarantine Screenhouse:

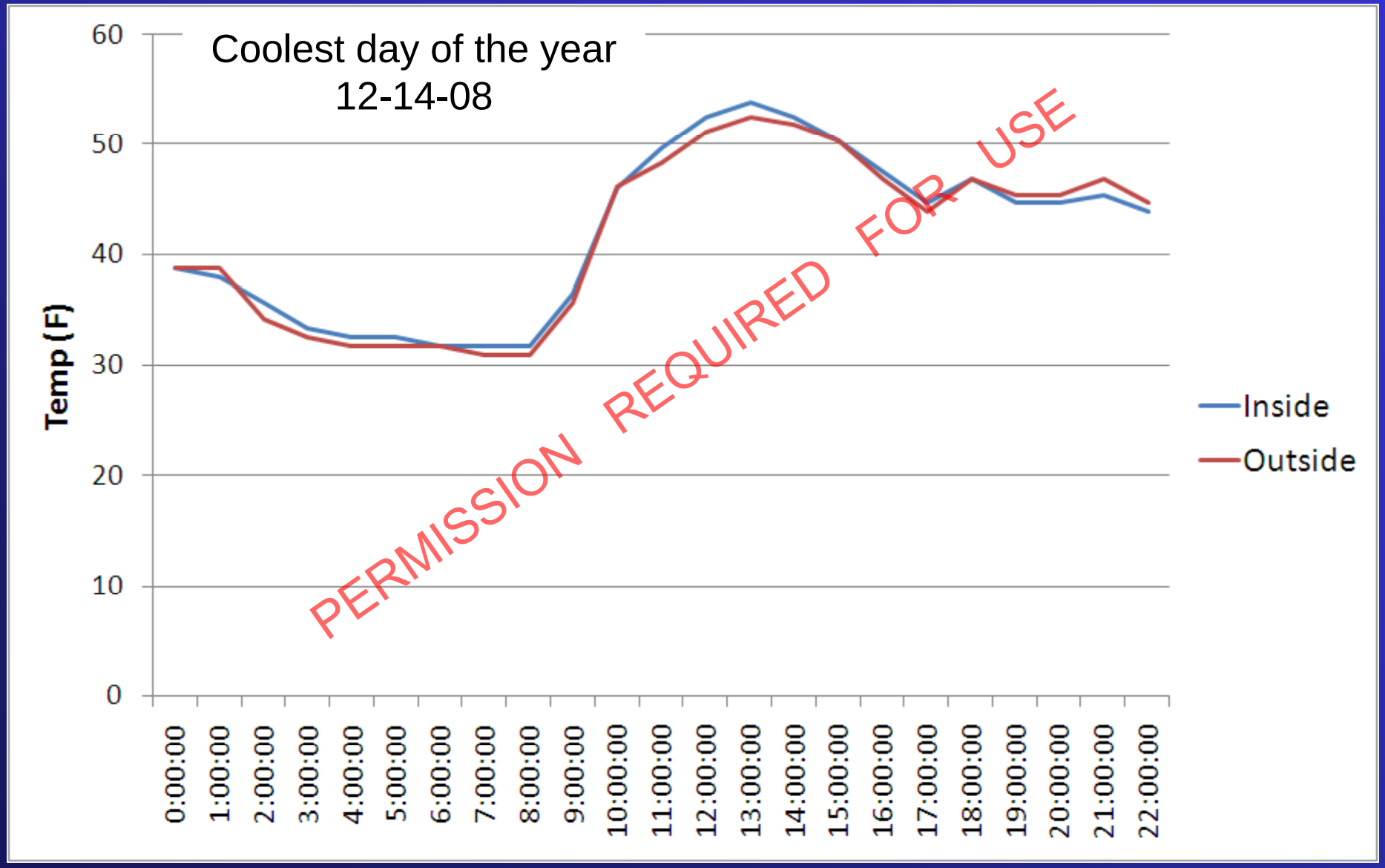
- 6 Sweet orange and 6 Satsuma mandarins
- 3 different CTV isolates (Mild-T 519, Moderate, & FB-07)
- 2 ELISA tests
- Twice every month
- High and Low temperatures monitored

Objective #1:
Monitor the temperature fluctuations inside and outside the screen house over time

LREC Protected Foundation Block:
Monitor temperature every hour in- and out- doors



LREC Protected Foundation Block:
Monitor temperature every hour in- and out- doors



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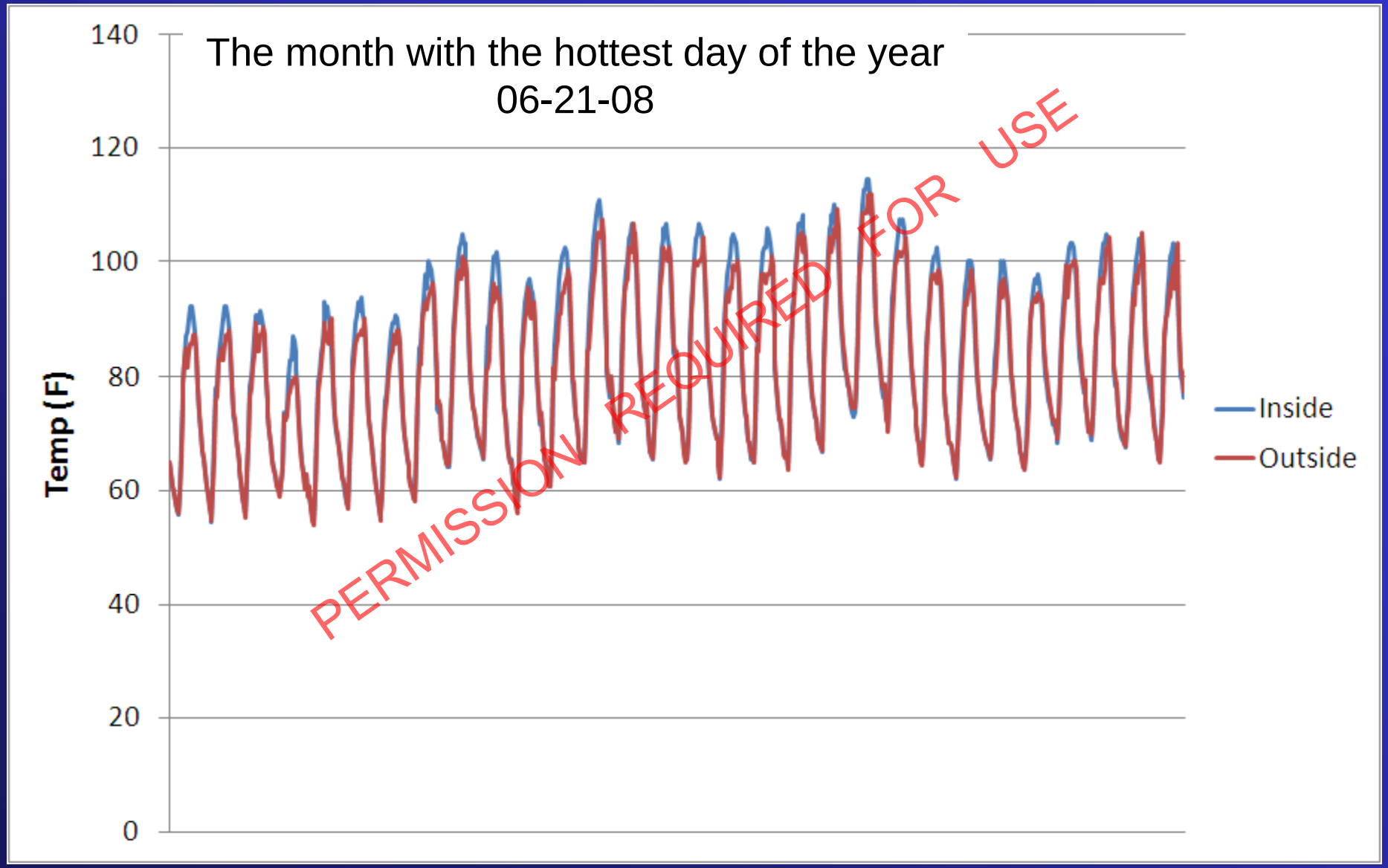

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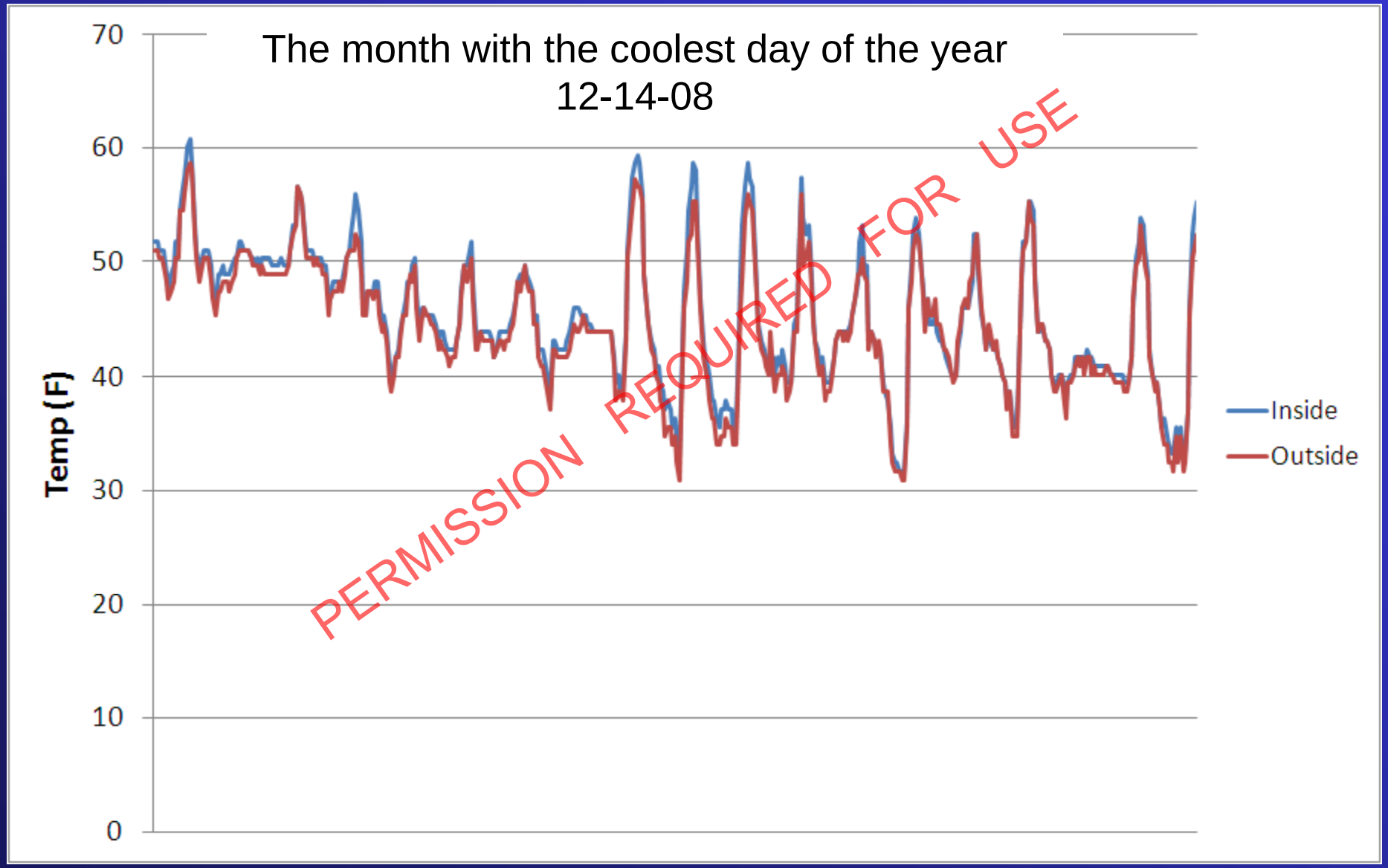
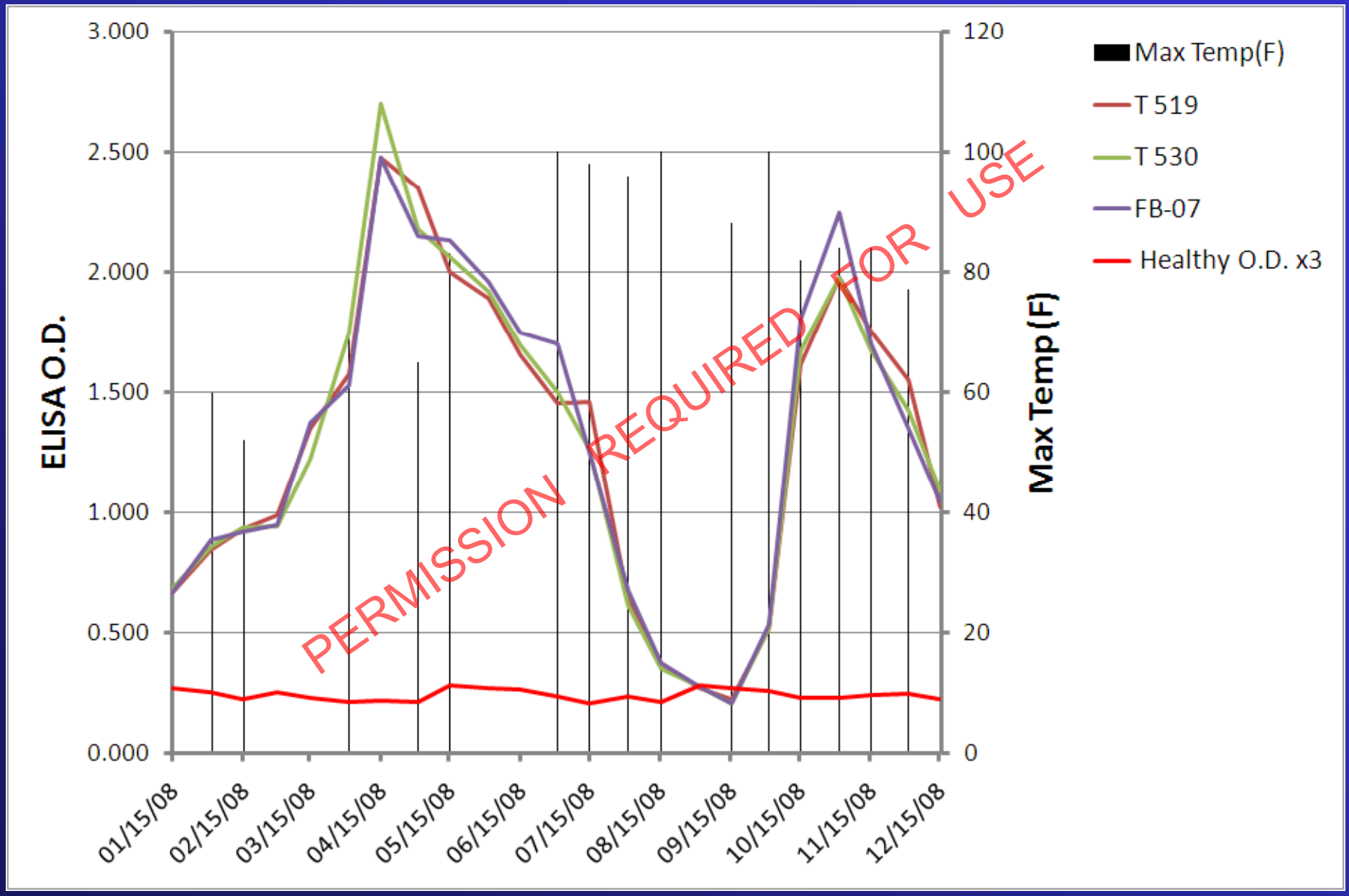


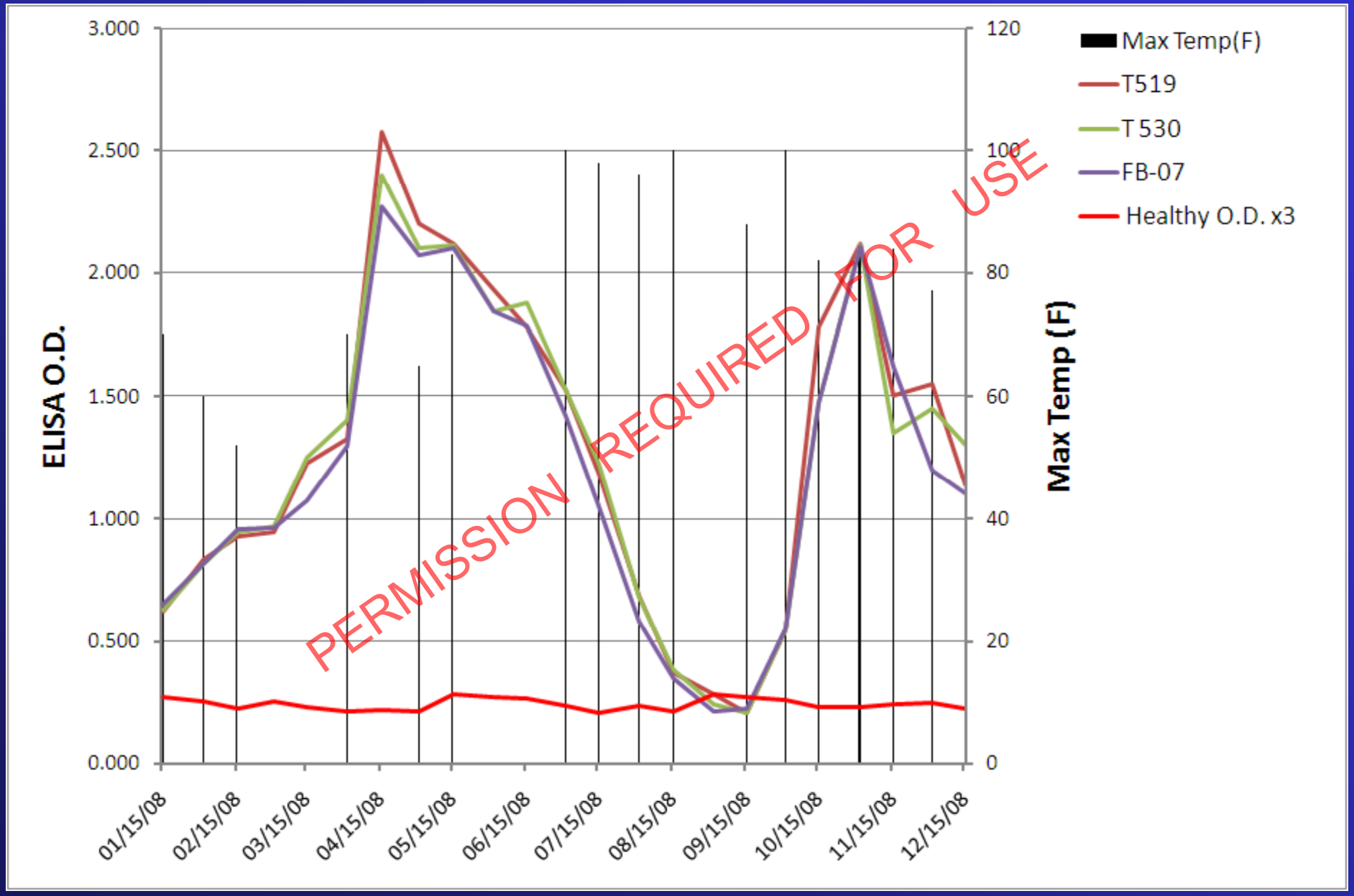
Objective #2

CTV ELISA testing on different citrus varieties infected with different CTV isolates under screen over time

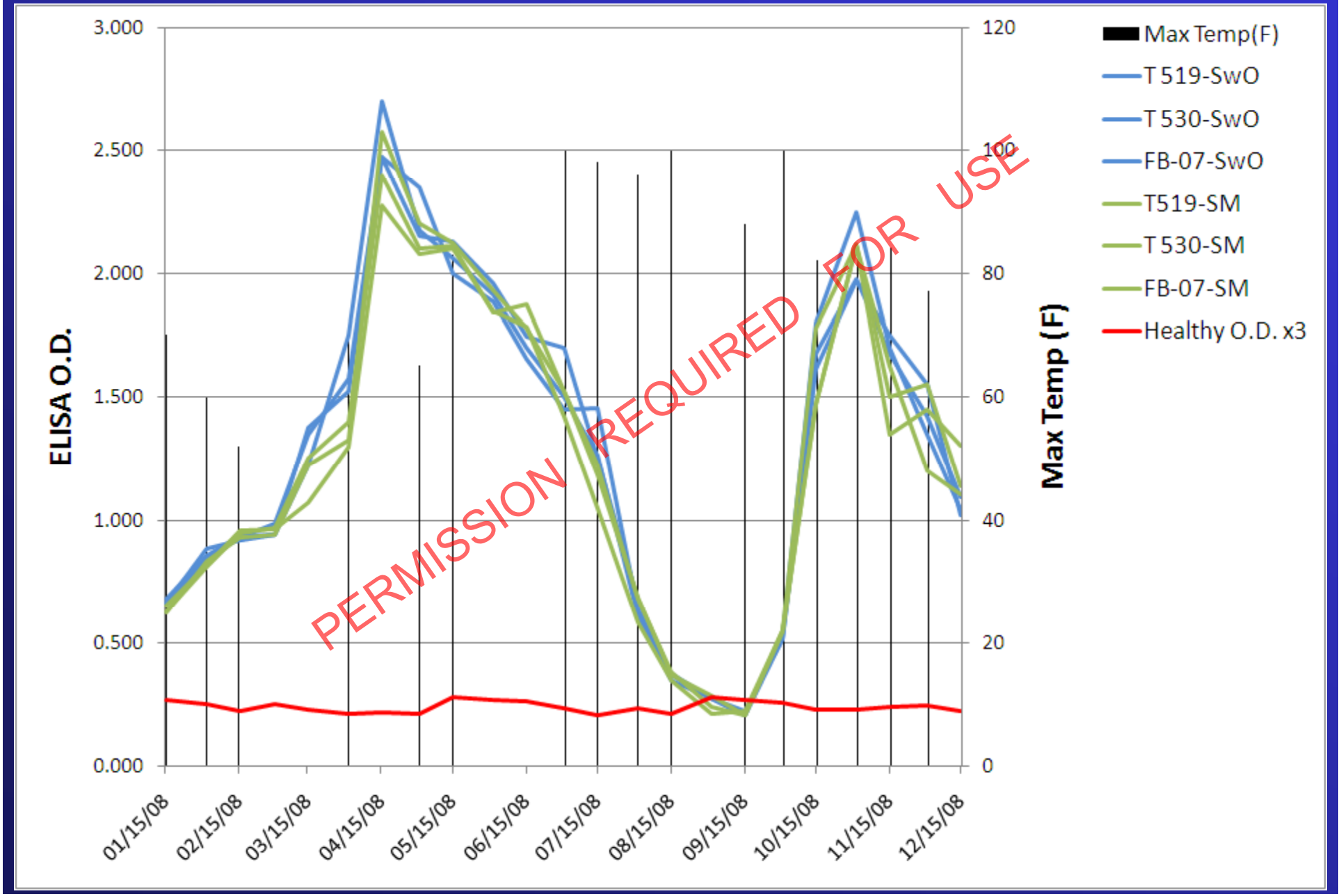
CTV + Sweet Orange



CTV + Satsuma Mandarin



Sweet Orange & Satsuma Mandarin

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