# Carrot cavity spot diagnostics serving CA carrot growers

**Isolation and characterization of carrot cavity spot pathogens at CSU Bakersfield**

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March 2021

# Carrot Cavity Spot

important disease of carrots worldwide

small brown sunken circular or elliptical lesions on the tubers
(cellulolytic activity leading to necrosis)

several *Pythium* species can cause this disease
- *P. violae*
- *P. sulcatum*
- *P. ultimum*

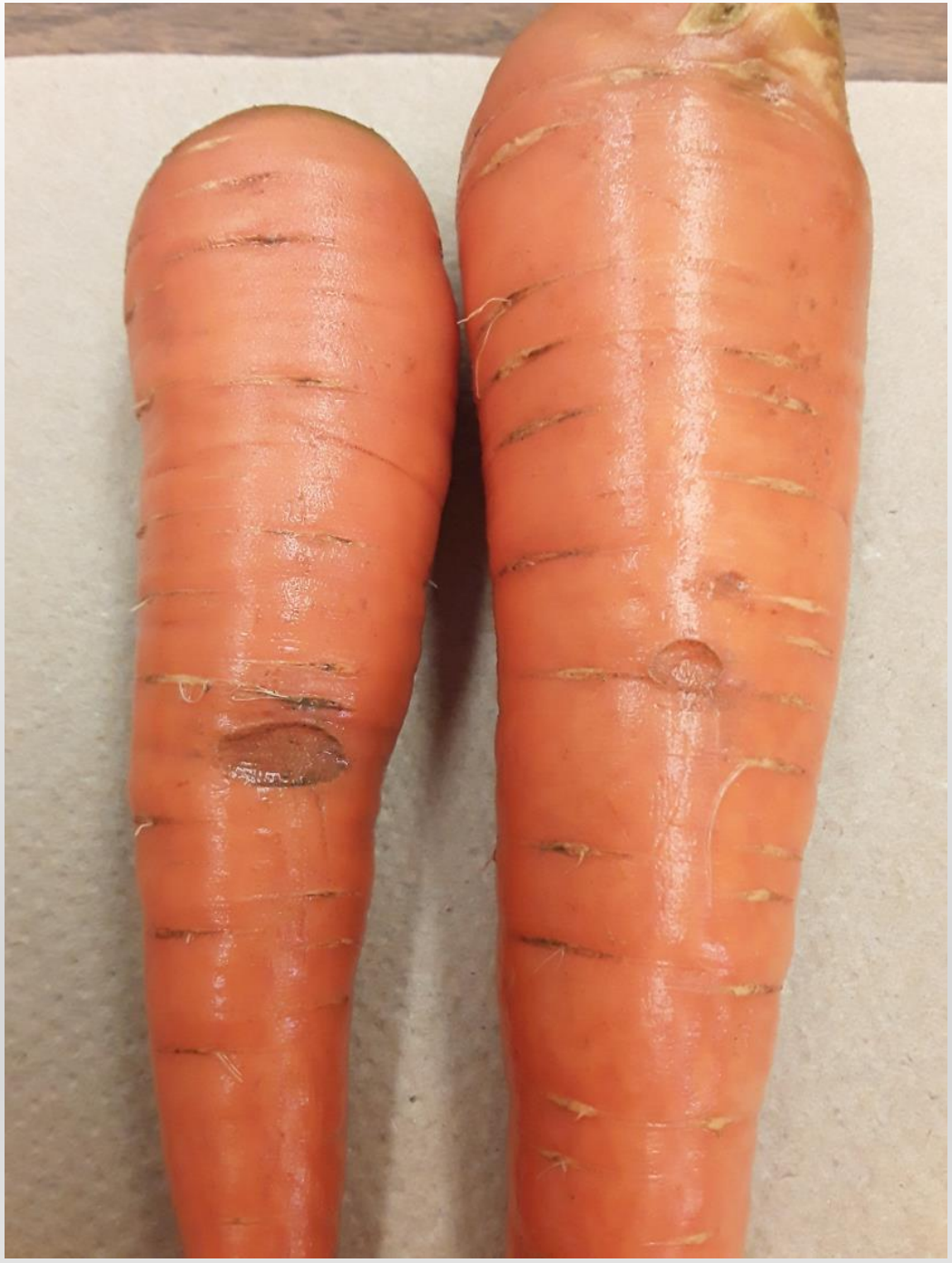
belonging to the oomycetes or water molds
- fungal-like organisms
- produce spores that can swim towards their host

affected tubers are rejected for the fresh as well as processing market

often overlooked/unnoticed

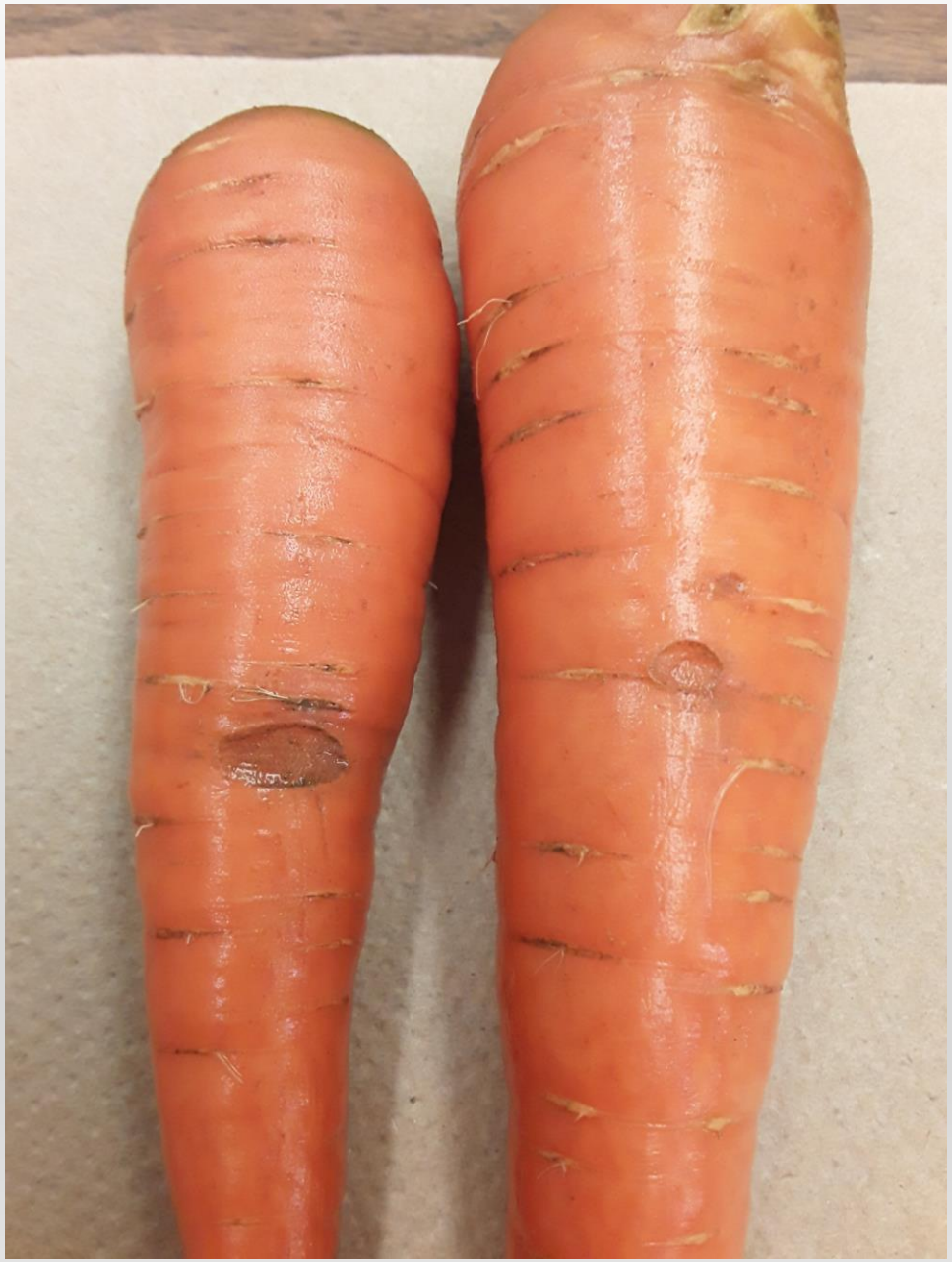
managed through the use of metalaxyl/mefenoxam
- resistance becomes a problem
- increased degradation in the soil

not used in organic farming



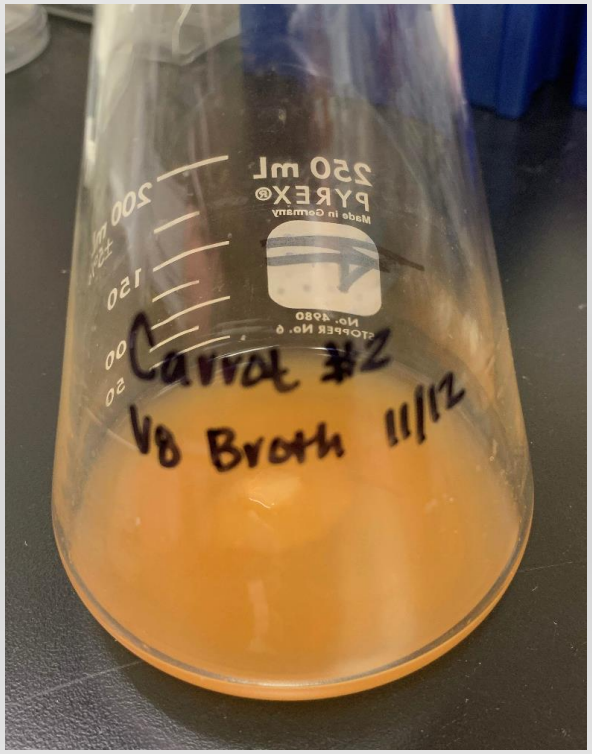
# Isolation of oomycete pathogens from cavity spot lesions

- carrots were washed well in tap water
- lesions were aseptically removed and cut into 2-4 pieces
- lesion tissue was pressed into PARP agar
- incubation in the dark at room temperature (±23$^{o}$C)
- part of the hyphae (outer edge) was transferred to fresh PARP and later to CMA



# Growing isolates for genomic DNA extraction

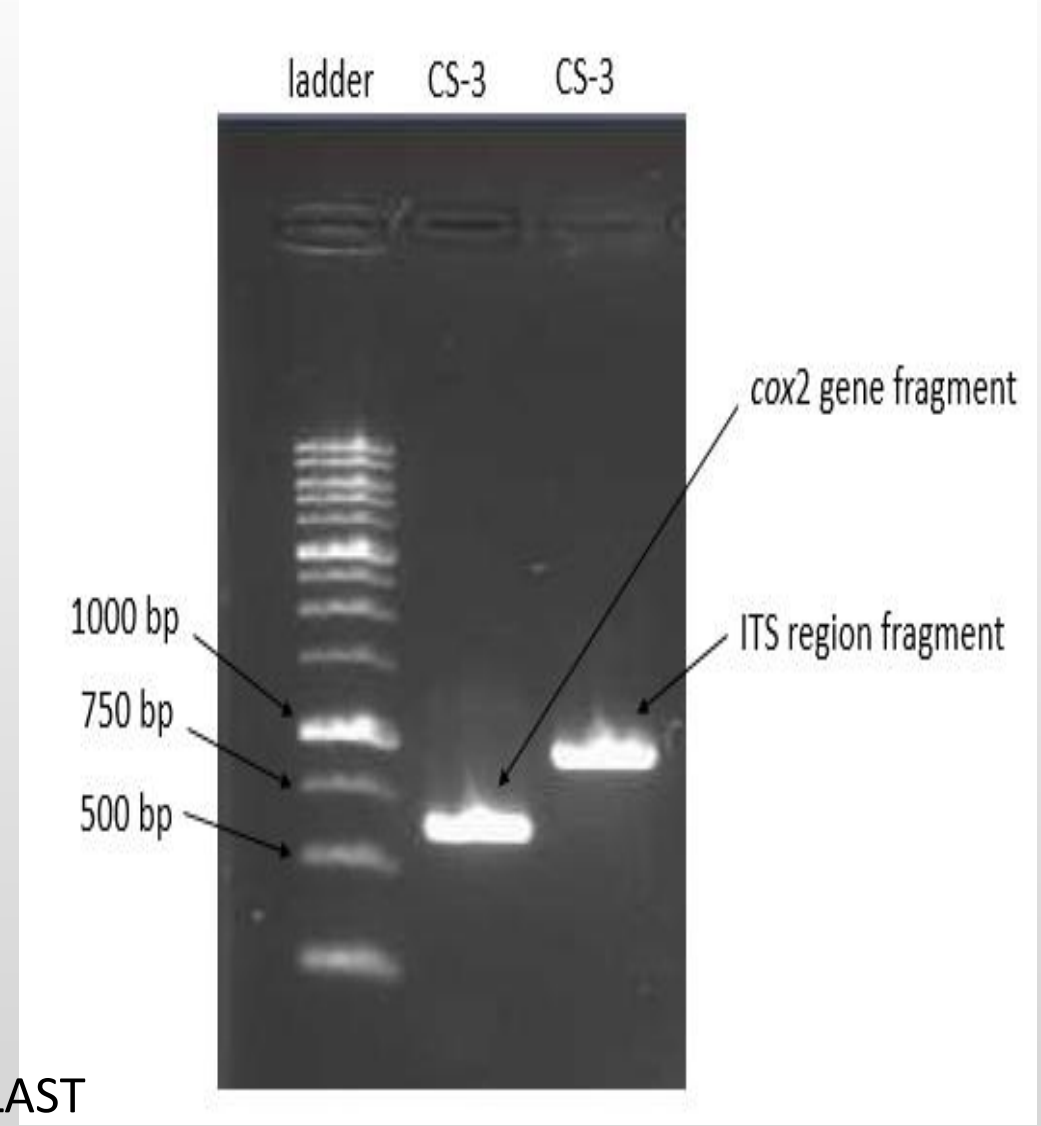
- agar plug with active mycelium was transferred to 15 ml V8 broth
- incubation for 4 days in the dark at room temperature (±23$^{o}$C)
- genomic DNA extraction with the DNeasy  Plant Mini Kit (Qiagen)
- measurement of DNA concentration on Nanodrop





# Molecular identification of oomycete strains

- amplification of two genetic fingerprint regions
  - *cox2* gene (Choi *et al*., 2015)
    → fragment of 628 bp
  - ITS region (Schroeder *et al.*, 2006)
    → fragment of ± 1000 bp
- verification on agarose gel
- purification
- send for sequencing (Laragen, Inc., Culver City, CA)
- sequence analysis and identification through online database BLAST

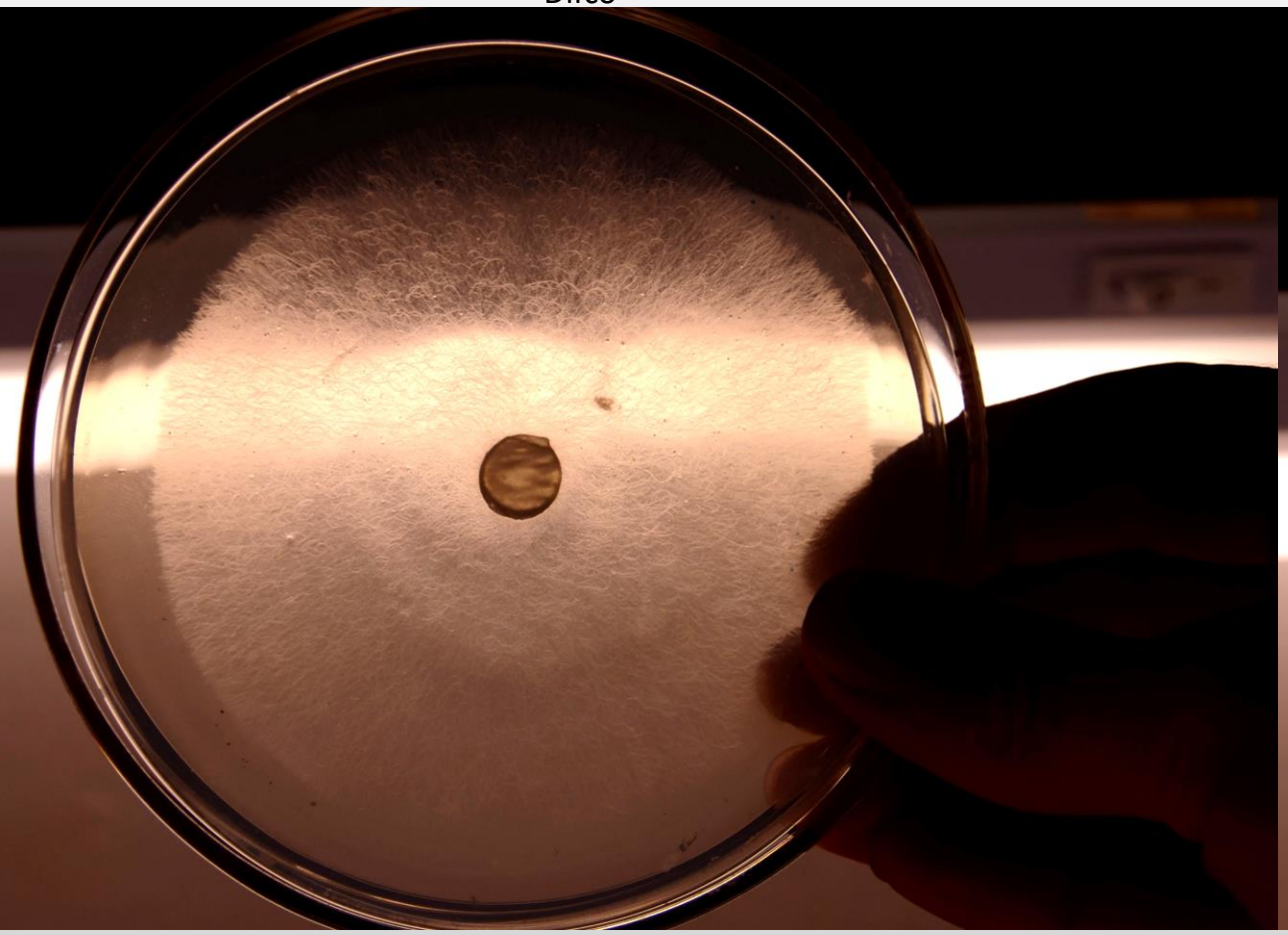



Choi, Y., Beakers, G., Glocking, S., Kruse, J., Nam, B., Nigrelli, L., Ploch, H., Shivas, R.G., Telle, S., Voglmayr, H., and Thines, M. (2015). Towards a universal barcode of oomycetes - a comparison of the *cox*1 and *cox*2 loci. *Molecular Ecology Resources* 15 (6): 1275-1288.

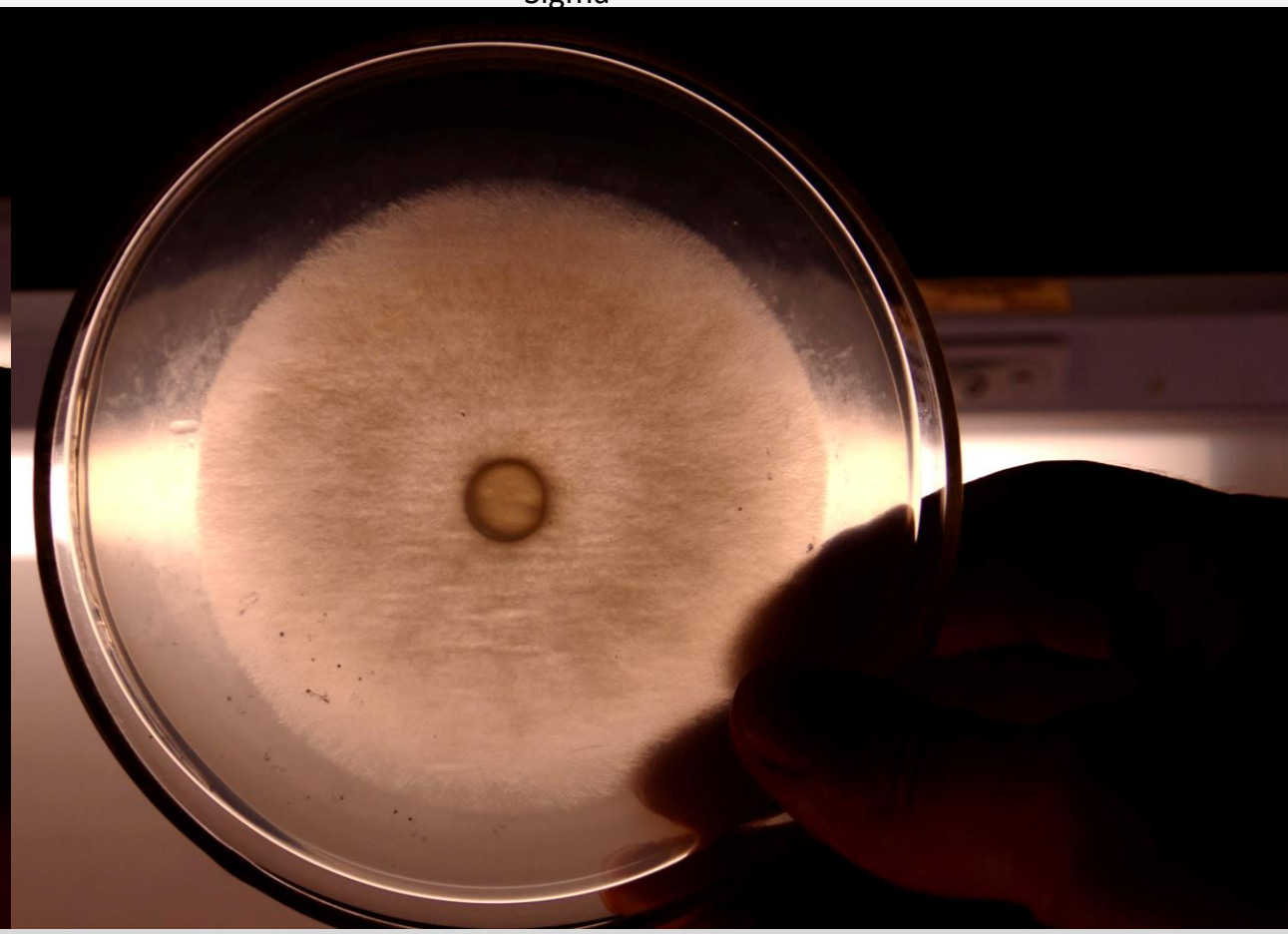
Schroeder, K.L., Okubara, P.A., Tambong, J.T., Lévesque, C.A., and Paulitz, T.C. (2006). Identification and quantification of pathogenic *Pythium* spp. from soils of eastern Washington using real-time polymerase chain reaction. *Phytopathology* 96:637-647.

# Amplification of genetic fingerprint regions directly on the hyphae

$CMA_{Difco}$



$CMA_{Sigma}$



$CMA_{Difco}$ is best used for hyphal tip transfer because individual hyphae are better visible

$CMA_{Sigma}$ enables more lush growth preferred for direct amplification

# Molecular identification of the isolated and received strains

| Original name | Isolated from | Identified as | Working name |
|---|---|---|---|
| Cavity spot isolate 1 (CS-1) | Conventional field | *Pythium violae* | *Pv*-2 |
| Cavity spot isolate 2 (CS-2) | Organic field | *Pythium spinosum* | *Ps* |
| Cavity spot isolate 3 (CS-3) | Conventional | *Pythium violae* | *Pv*-1 |
| *Pythium violae* WSU | received from Dr. L. du Toit, originally isolated from CA | *Pythium violae* | *Pv*-C |

used as a control for our diagnostics

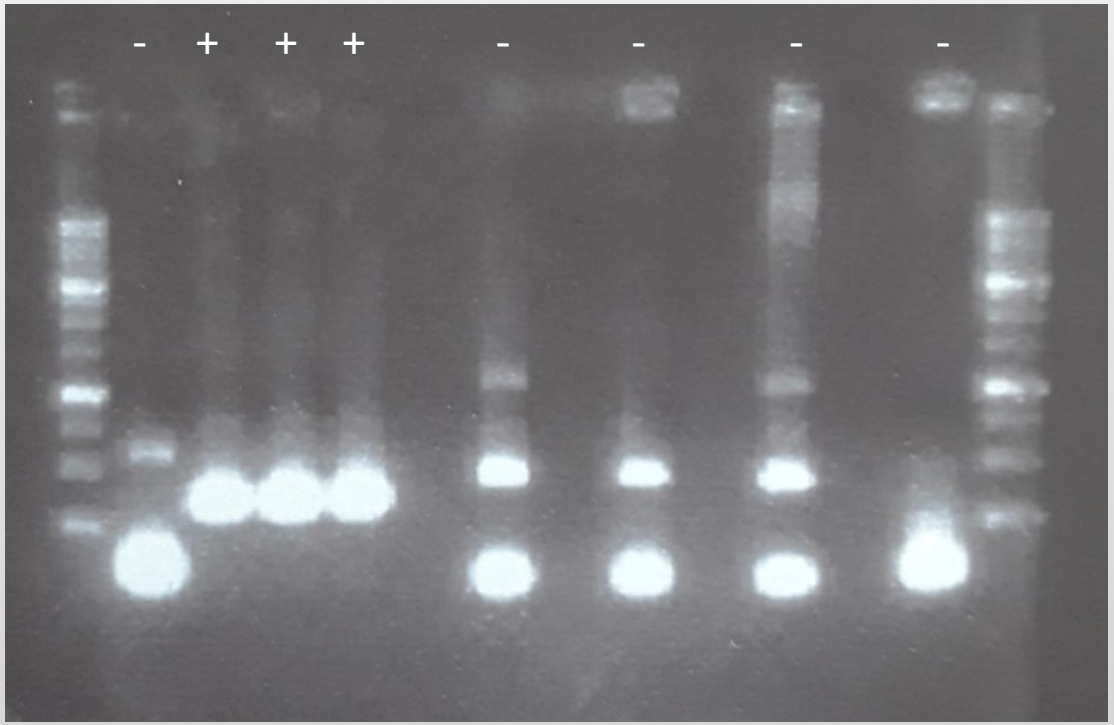
**ready to accept up to 100 samples for local growers for identification (funded by CFCAB)**

contact me at ifrancis@csub.edu

**Amplification with *P. violae* specific primers**

primers designed within the ITS region that should be specific to *P. violae* (Klemsdal *et al.*, 2008)

water *P. violae* non-*P. violae* oomycetes or fungi




the primer positions in the ITS region (underlined)

```
CS-3-Pv-1       TGTGTGTGCACAGCAATG-----------------TGTGTG----TGCGGGACTGGCTGA
CS-1-Pv-2       TGTGTGTGCACAGCAATG-----------------TGTGTG----TGCGGGACTGGCTGA
CS-PW-Pv-C      TGTGCGTGCACAGCAATG-----------------TGTGTG----TGCGGGACTGGCTGA
CS-2-Ps         TATGTGCCTACTGCACTGCTGACTTTGCATTCATTTGTATGGTCTTGGCGGAGTGGCGGG
Ps-SL           TATGTGCCTACTGCACTGCTGACTTTGCATTCATTTGTATGGTCTTGGCGGAGTGGCGGG
                * ** *   ** *** **                 *** **    **  *** **** *

CS-3-Pv-1       GTGCAGATGTGAAGTGTCTCGCTGGCCTACTTCTCTCTTTGGGGAGTGGACAGGTATCGA
CS-1-Pv-2       GTGCAGATGTGAAGTGTCTCGCTGGCCTACTTCTCTCTTTGGGGAGTGGACAGGTATCGA
CS-PW-Pv-C      GTGCAGATGTGAAGTGTCTCGCTGGCCTGCTTCTCTCTTTGGGGAGTGGACAGGTATCGA
CS-2-Ps         TTGCAGATGTGAAGTGTCTCGCTA--TGGTTGGCATTTGTAATGAATGCACAGCTTGCGA
Ps-SL           TTGCAGATGTGAAGTGTCTCGCTA--TGGTTGGCATTTGTAATGAATGCACAGCTTGCGA
                 **********************      *    * * *   ** ** **** *  ***
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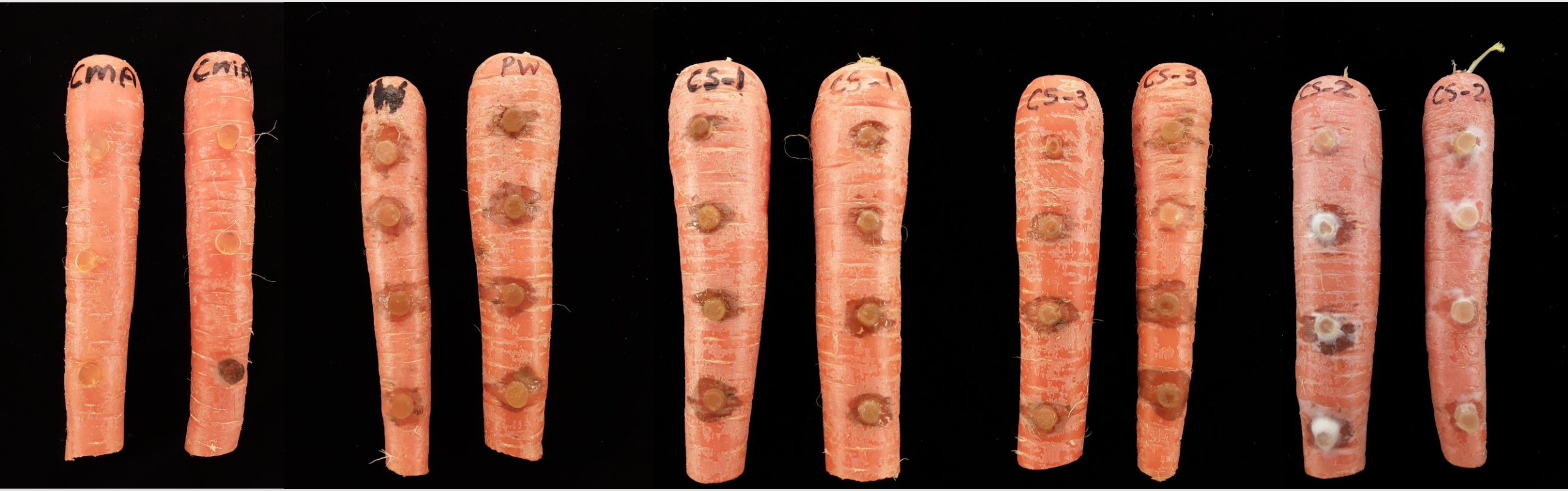
Klemsdal, S.S., Herrero, M.L., Wanner, L.A., Lund, G., and Hermansen, A. (2008). PCR based identification of *Pythium* spp. causing cavity spot in carrots and sensitive detection in soil samples. *Plant Pathology* 57:877-886.

# Carrot disk assay

CMA disks with active growth of *Pythium* were placed on mature freshly harvested (48h) carrots and incubated in a moist environment at 24°C in the dark
pictures taken at 5 dpi

Non-inoculated | *Pv*-C | *Pv*-2 | *Pv*-1 | *Ps*



**Repeat carrot disk assay**

with *P. spinosum* strain received from Dr. Cassandra Swett (UC Davis) and isolated from a cavity spot lesion from organically grown carrots in the Riverside area

/ *Pv*-C *Ps* *Ps*-SL

# Soil assay

*Pythium* grown in V8 broth for 4 days

mixed with hand mixer

added to sand : peat moss mixture (50:50, autoclaved twice for 30 min)

transferred to tree seedling pots (cleaned with ethanol and dried)

4 carrot seeds per pot (thinned to 1 seedling per pot)

under light  (16h photoperiod) at 23$^{o}$C

# Soil assay

reinoculated at 5.5 weeks

reinoculated at 12.5 weeks

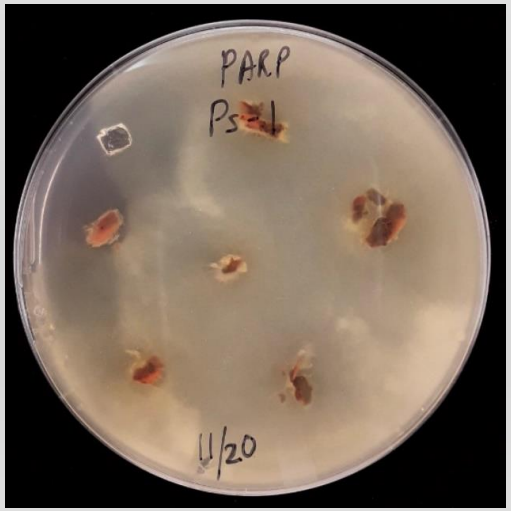
harvested at 16 weeks

# Soil assay

contamination with *Fusarium*

but the different *P. violae* strains were reisolated

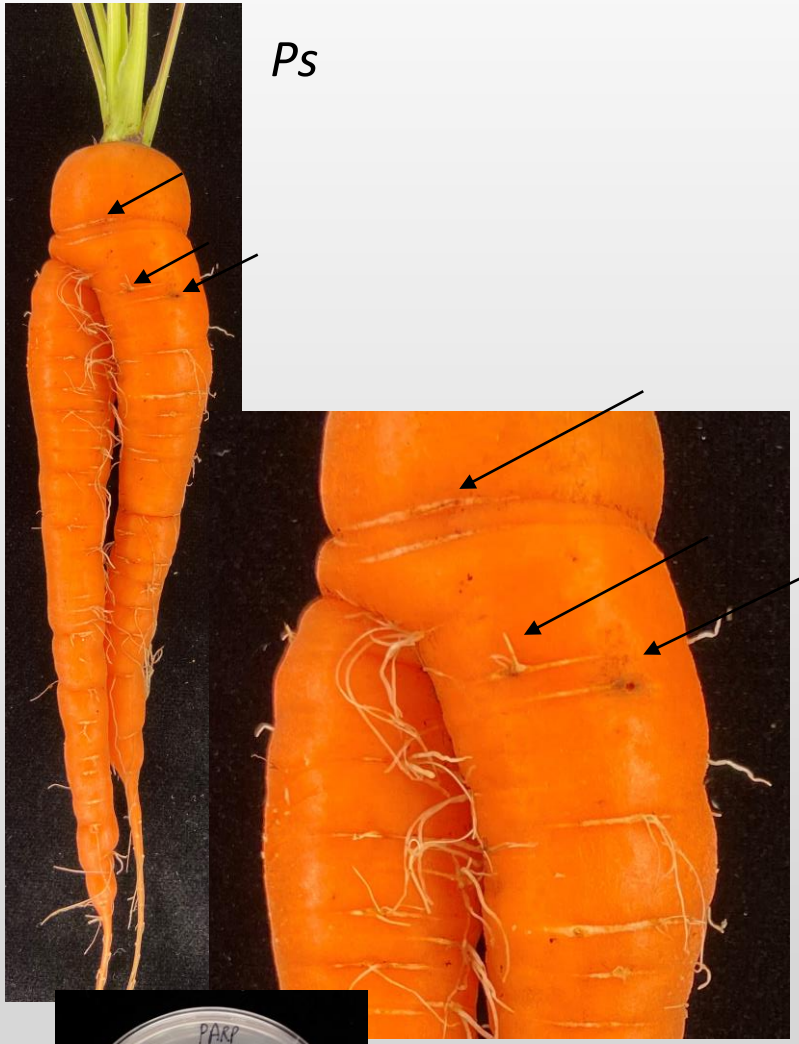
from the lesions as well

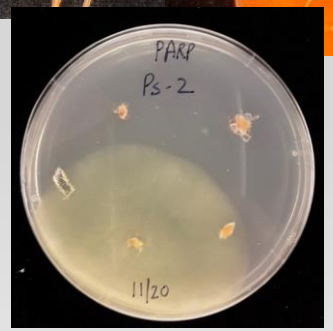



*P. violae* (*Pv*-C, *Pv*-1, *Pv*-2)

# Soil assay

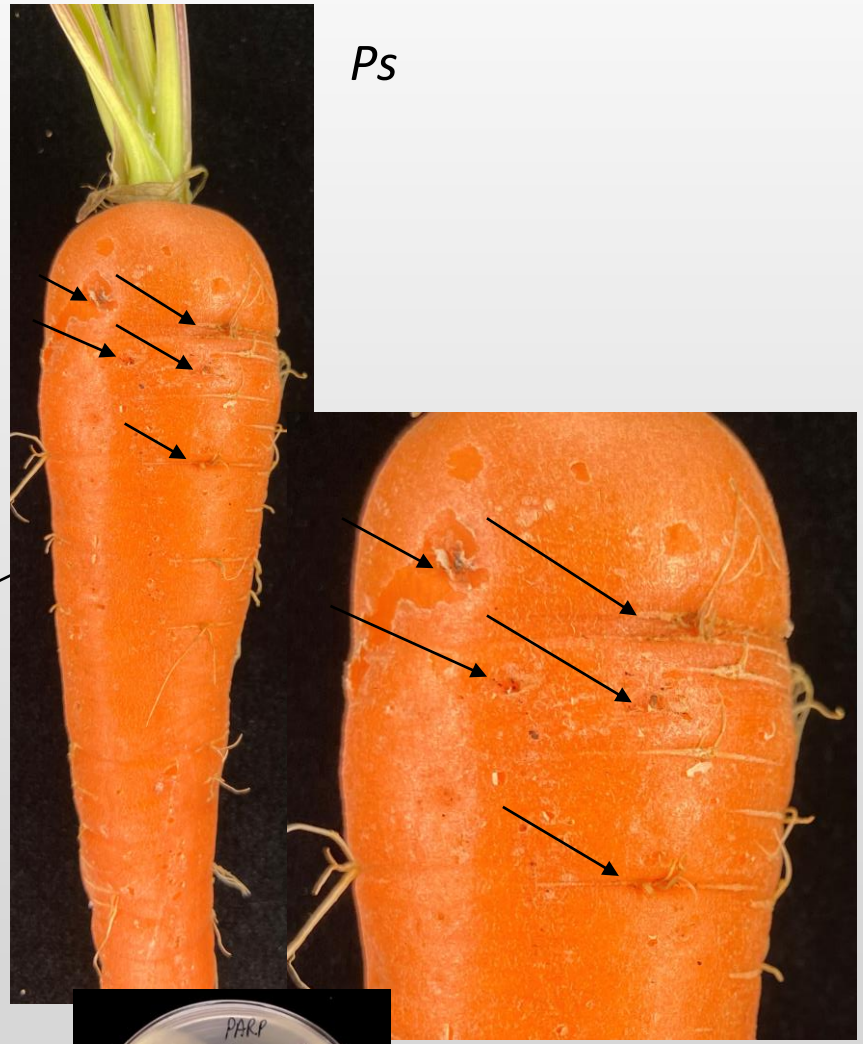
*Ps*





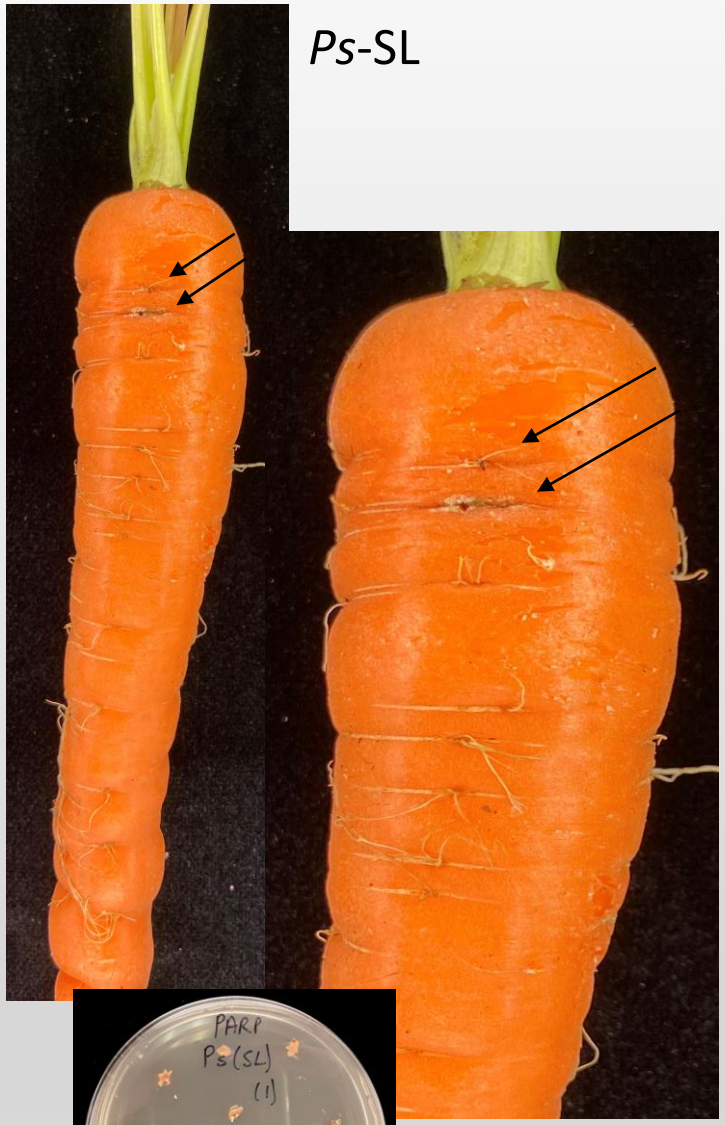

reisolated and identified as *Ps*

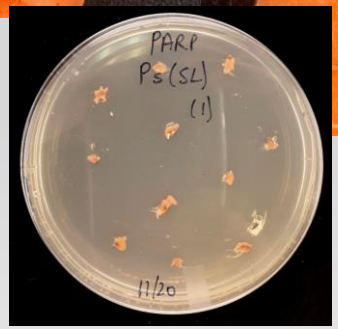
*Ps*




reisolated and identified as *Ps*

*Ps*-SL






reisolated and identified as *Ps*-SL

# Future directions for our research

The bacterial genus *Streptomyces* is renowned for the production of antimicrobial compounds

153 *Streptomyces* isolates
were isolates from diverse soils in the Bakersfield area

four local *Streptomyces* isolates strongly inhibited of *P. violae* and other oomycetes
some of them also inhibited *Fusarium*, *Sclerotium rolfsii*, and/or *Sclerotinia sclerotiorum*

# Acknowledgements


**Misbah Chaudhry**
Graduate student (M.Sc.) at CSUB

**Dr. Jaspreet Sidhu**
**Joe Nunez**
UCCE Kern County

**Dr. Lindsey du Toit** (Washington State University)

**Dr. Cassandra Swett** (University of California, Davis)

**Funding:**

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