



Screening for Resistance to Curtoviruses in Chile Pepper

Rebecca Creamer, Melina Sedano, Nhan Lam,
Ismael Escobar, Teresa Cross
Entomology, Plant Pathology, and Weed Science
New Mexico State University

Biological properties of curly top virus



- Infects a wide host range of dicot crops and weeds
- Crops: peppers, tomatoes, sugarbeets, spinach, melons, beans
- Found in vascular system, not seed transmitted
- Young plants most susceptible to infection
- Transmitted by the beet leafhopper

Symptoms of curly top in chile

- Severely stunted plants
- Some plants have chlorotic rolled leaves
- Small rounded fruit
- Stiff plants with brittle leaves
- Symptoms appear 1-2 weeks after infection



Management Options

- Heavy seeding
- Delay thinning
- Weed removal
- Insecticides - systemics to decrease leafhopper numbers
- Predictive model
- Plant resistance in bean, sugarbeet, tomatoes
- Test for resistance in chile



Plant Resistance

- Bean - single gene resistance to virus
- Sugarbeet - multigene tolerance to virus
- Tomato – field resistance/tolerance in Saladmaster, Roza, Rowpak, Columbian
- Chile - field resistance/tolerance in NuMex Las Cruces Cayenne, Tabasco



Methods of Resistance

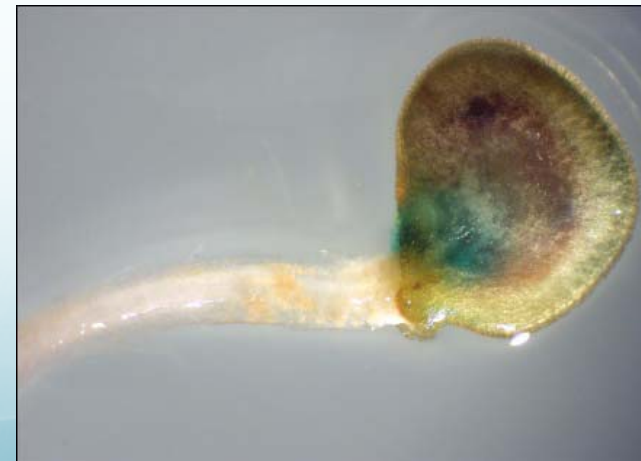
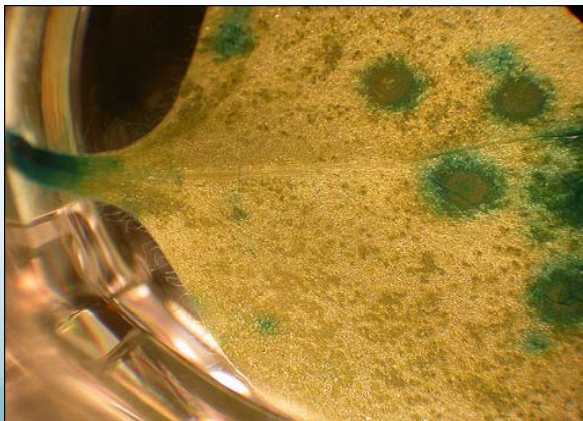
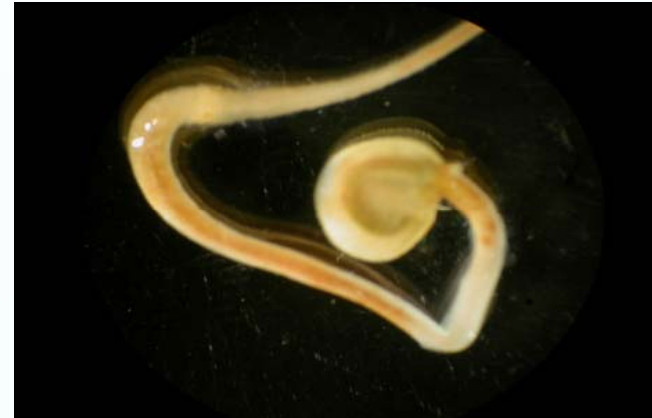
- Resistance prevents virus replication
 - Single gene, no infection
- Resistance allows replication, but prevents virus movement
 - Possible single gene, infection only in inoculated leaf
- Resistance to insect transmission
 - Multigene, tolerance
 - Insect won't land on plant, feed on plants
 - Insect doesn't prefer feeding on the plant

Laboratory screening

- Agroinoculation with BSCTV/BMCTV recombinant infectious clone
 - Tomato seedlings-apical meristem removed, add bacteria with syringe
 - Peppers-germinated seeds inoculated with bacteria using minuten pins
- Leafhopper transmissions using BSCTV
- Plants screened for virus using PCR and ELISA

pGUS Inoculations

Vascular puncture inoculation of chile with *Agrobacterium* containing pCAMBIA1390-GUS. Chile seedlings were punctured with minuten pins, and GUS activity was visualized using X-gluc as a substrate to provide a blue color.



Screening for BCTV resistant plants by using *Agrobacterium*

- Inoculated plants with BCTV clone containing tandem of replication region in *Agrobacterium*

➤ Method

- Prick small holes in the meristem of young plants
- Drop 2-3 μl of *Agrobacterium* solution into hole
- Incubate for 3 days
- Transfer plants from culture plate to pots
- Test for BCTV by PCR, 4 wks after inoculation.

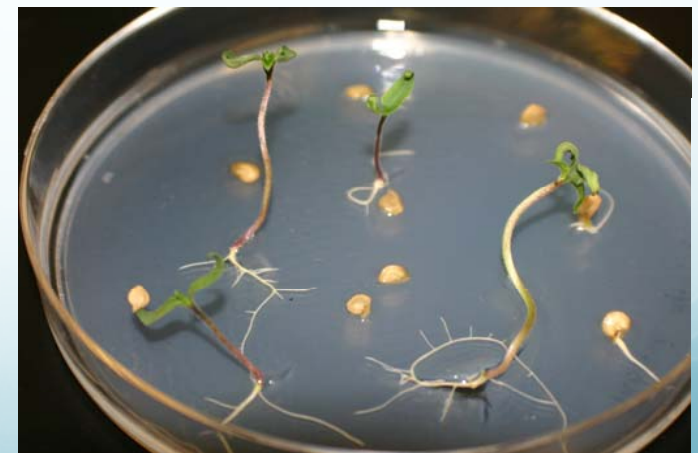
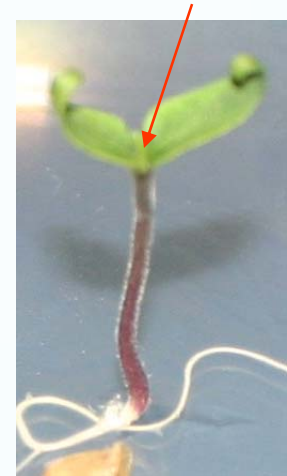


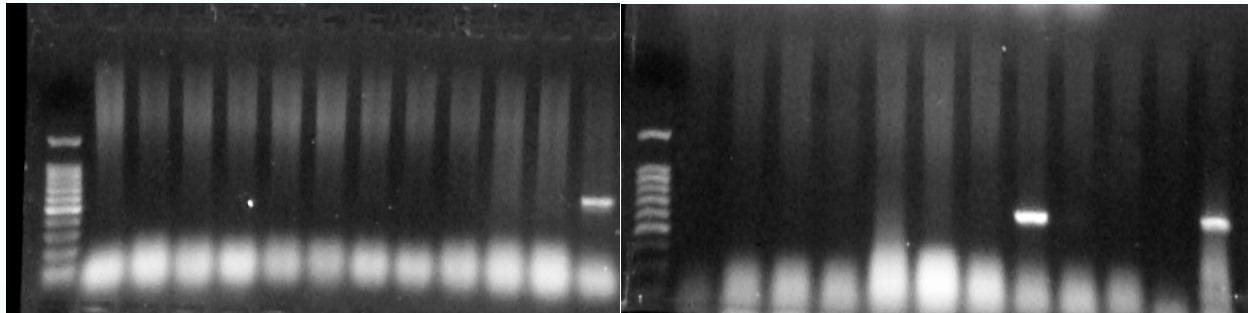
Table 2. Inoculation of tomato varieties using vascular puncture after meristem removal.

Variety	No. plants survived/ no. inoculated with pGD	No. plants infected/no. surviving with pGD (%)	No. plants survived/ no. inoculated with pCAMBIA1390-GUS	No. GUS plants/no. surviving (%)
Lauro 12	21/45	9/21 (42.9%)	7/15	4/7 (57.1%)
CTR 05-01	30/45	18/30 (60.0%)	12/15	7/12 (58.3%)
CTR 05-03	43/45	33/43 (76.7%)	13/15	9/13 (69.2%)
CVF-11	41/45	22/41 (53.7%)	14/15	7/14 (50.0%)
Roza	28/45	23/28 (82.1%)	11/15	3/11 (27.3%)
Columbian	24/45	22/24 (91.7%)	7/15	3/7 (42.9%)
Saladmaster	39/45	22/39 (56.4%)	10/15	6/10 (60.0%)
Rutgers	29/45	20/29 (69.0%)	9/15	6/9 (66.7%)

Results are totals from at least three trials.

Results of screening for BCTV resistant plants

M 1 2 3 4 5 6 7 8 9 10 11 12 M 13 14 15 16 17 18 19 20 21 22 23 24



1-20: virus inoculated
Tabasco plants

21-22: uninoculated tabasco
plant

23: PCR negative control

24: PCR positive control



Pepper-Vascular Puncture

Pepper Variety	Infected/Total Tested	% Infection
NM 6-4	19/28	67.9%
Tabasco	4/24	16.7%
NuMex Las Cruces cayenne	8/28	28.6%

Testing for Leafhopper Resistance

- Beet leafhopper
 - Reared on BCTV-infected sugarbeets
 - 1-10 leafhoppers/plant for 18 hrs
 - Stained leaves for salivary sheaths



Leafhopper Transmission

Pepper Variety	1 LH/plant # infected/plants tested		3 LH/plant # infected / tested	
NM 6-4	4/6			
Tabasco	0/4	0/20	6/36	
PI 205167	0/6			
PI 205174	0/4			
PI 533 10383	0/5	6/9		
PI 312 10335	4/4			
Grif 9303	0/3			
NuMex Bailey Piquin	1/6	4/4		

Leaf Staining

- Stain used is acid fuschin
- Stylet tracks and pu Puncture
 - ◆ Puncture = limited feeding
 - ◆ stylet track = extensive feeding

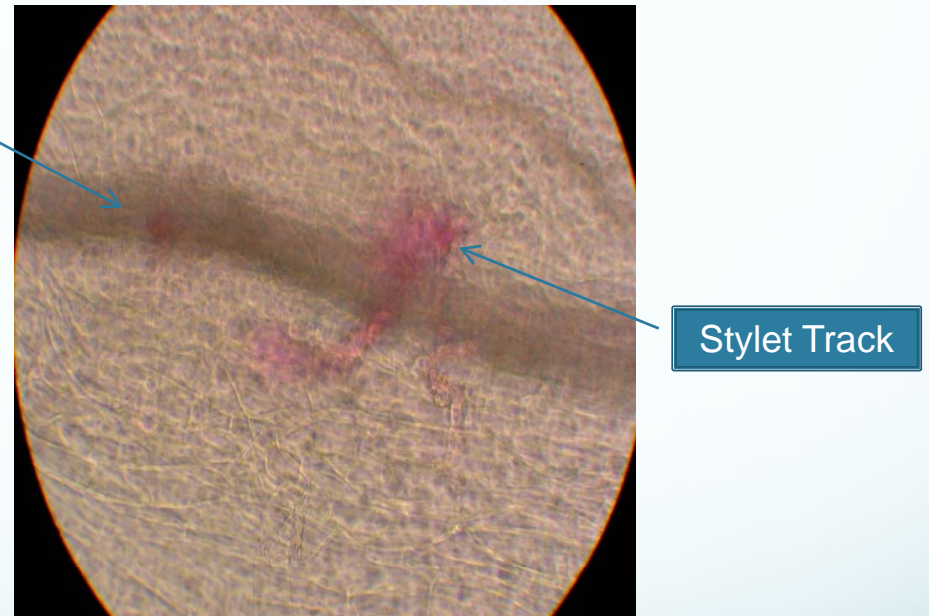


Table 4. Stylet sheath staining of beet leafhopper feeding on peppers.

Pepper Variety	No. plants tested	Punctures		Stylet Sheaths	
		Total	Average/plant	Total	Average/plant
NuMex Las	30	106	3.5	0	0
Cruces Cayenne					
Tabasco	20	21	1.0	0	0
New Mexico 6-4	30	1246	41.5	2	0.06

Results are total from two trials, each using 5 leafhoppers/plant.

Conclusions

- Vascular puncture effective inoculation method for rapid screening for R gene resistance
- Leafhopper transmission/stylet sheath staining can be used to screen for other types of resistance
- Tabasco and NuMex Las Cruces cayenne are field resistant/tolerant to curly top virus infection
- Field resistance/tolerance in several tomato varieties
- Mechanism of resistance has not been established, but is effective in both field and greenhouse and includes leafhopper non-preference for feeding