

Cucumber green mottle mosaic virus in California

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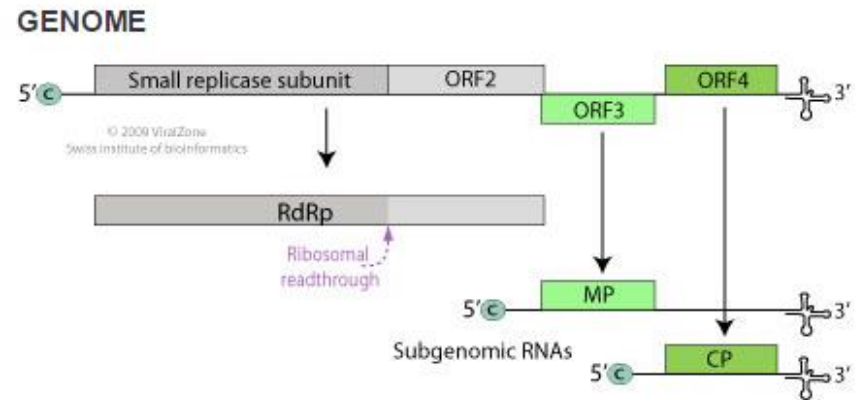
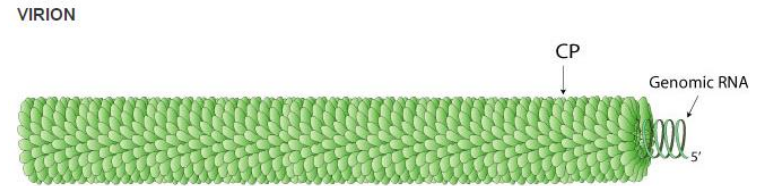
Overview

- CGMMV biology
- CGMMV in California
- Isolate sequencing
- Diagnostics research
- Watermelon green mottle mosaic virus



The virus

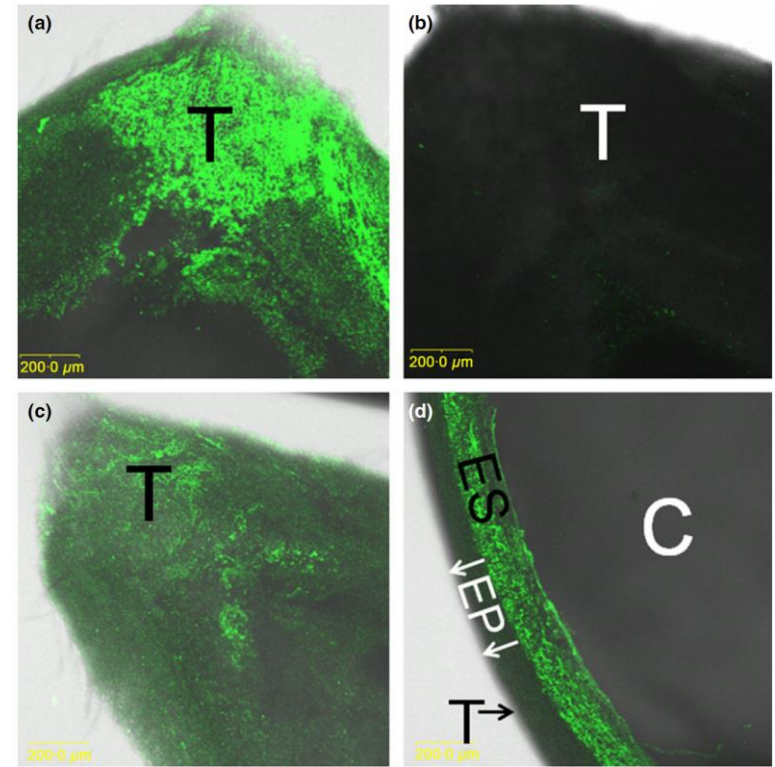
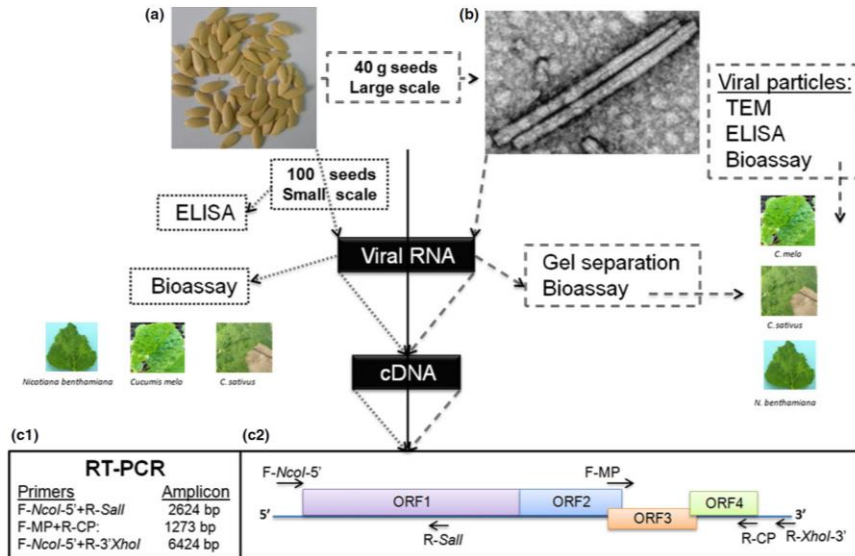
- Family *Virgaviridae*, genus *Tobamovirus*
- 6,421-6,425 nucleotide +ssRNA genome
- Four protein open reading frames
- Mechanically transmitted
- Seed transmitted



Seed disinfection treatments do not sufficiently eliminate the infectivity of *Cucumber green mottle mosaic virus (CGMMV)* on cucurbit seeds

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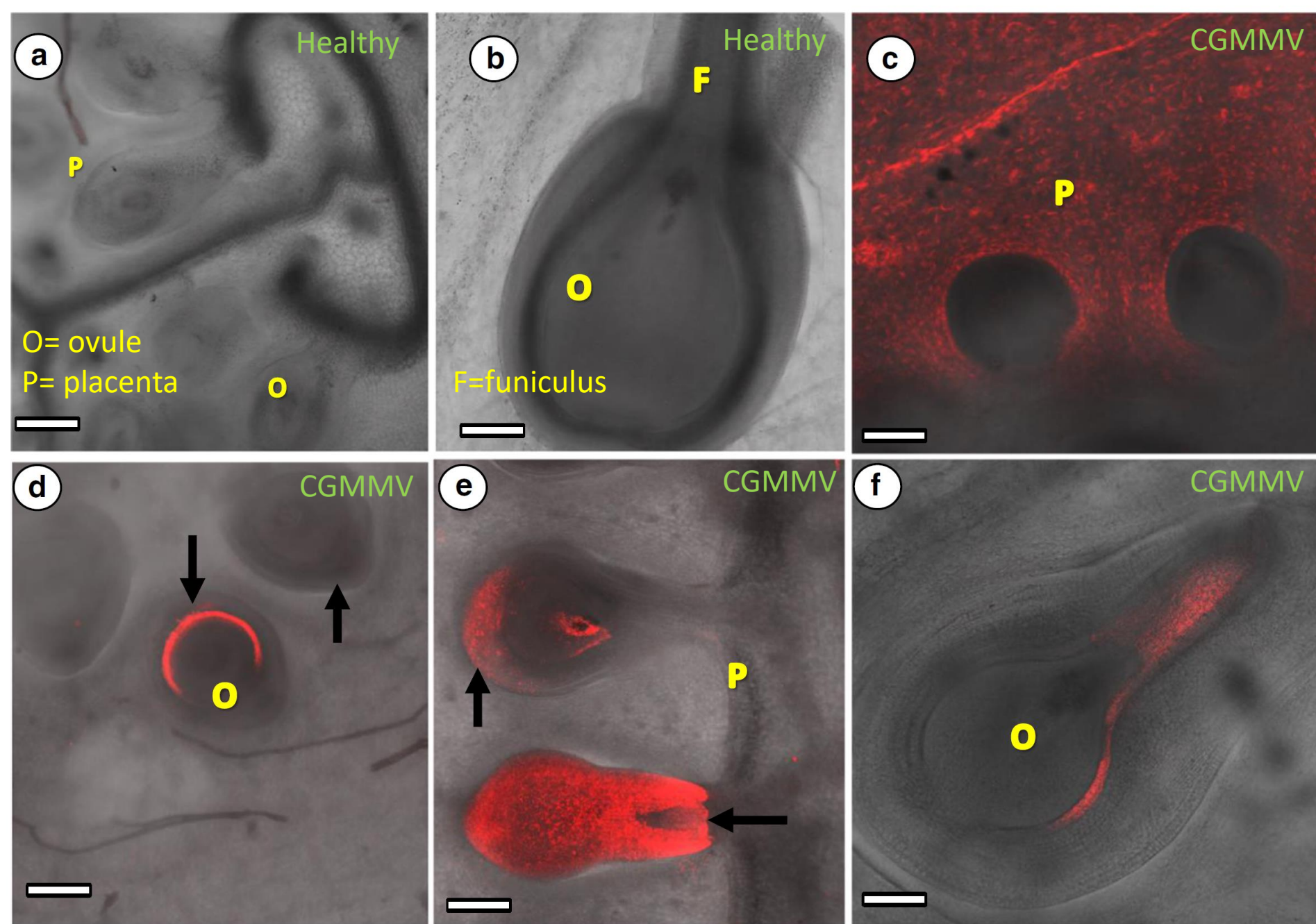
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Fluorescent *in-situ* hybridization of: A. CGMMV infected testa B. CGMMV free seed testa C. Heat+TSP treated CGMMV testa D. Inner tissue of heat+TSP treated seed. T=testa EP=endopleura

Analysis	Amount of seeds	Seed treatment			
		Control (untreated)	Chemical (10% TSP)	Heat (72°C, 72 h)	Combined treatment (10% TSP+heat 72°C, 72 h)
ELISA	40 g	+	+	+	+
	100 seeds	+	+	+	+
Fluorescence labelling	10 seeds	+	+	+	+
TEM	40 g	+	+	+	+
RNA	40 g	+	+	+	-
PCR for CP-MP	40 g	+	+	+	-
	100 seeds	+	+	+	+
LD-PCR	40 g	+	+	+	-
	100 seeds	+	+	-	-
Biological test	40 g	+	+	+	+
	100 seeds	+	+	+	+

+, presence of CGMMV; -, absence of CGMMV.



Bar = 100 μ m.

Insights into the maternal pathway for *Cucumber green mottle mosaic virus* infection of cucurbit seeds

Protoplasma (2019) 256:1109–1118
<https://doi.org/10.1007/s00709-019-01370-6>

How many plants develop systemic infections of CGMMV from contaminated seeds?

Watermelon planting density: 5,000 plants per acre

CGMMV watermelon seed transmission rate: 1-5%

Seed contamination rate: 1 in 2,000 or 1/500

$1,000 \text{ acres} * 5,000 \text{ plants/acre} * 1/2,000 \text{ seeds} * 1-5\% = 25 \text{ to } 125 \text{ CGMMV infected plants}$

$1,000 \text{ acres} * 5,000 \text{ plants/acre} * 1/5,000 \text{ seeds} * 1-5\% = 10 \text{ to } 50 \text{ CGMMV infected plants}$

2018 US watermelon production 113,000 acres (USDA-Economic research Service)

Triploid watermelon field
San Joaquin County, CA

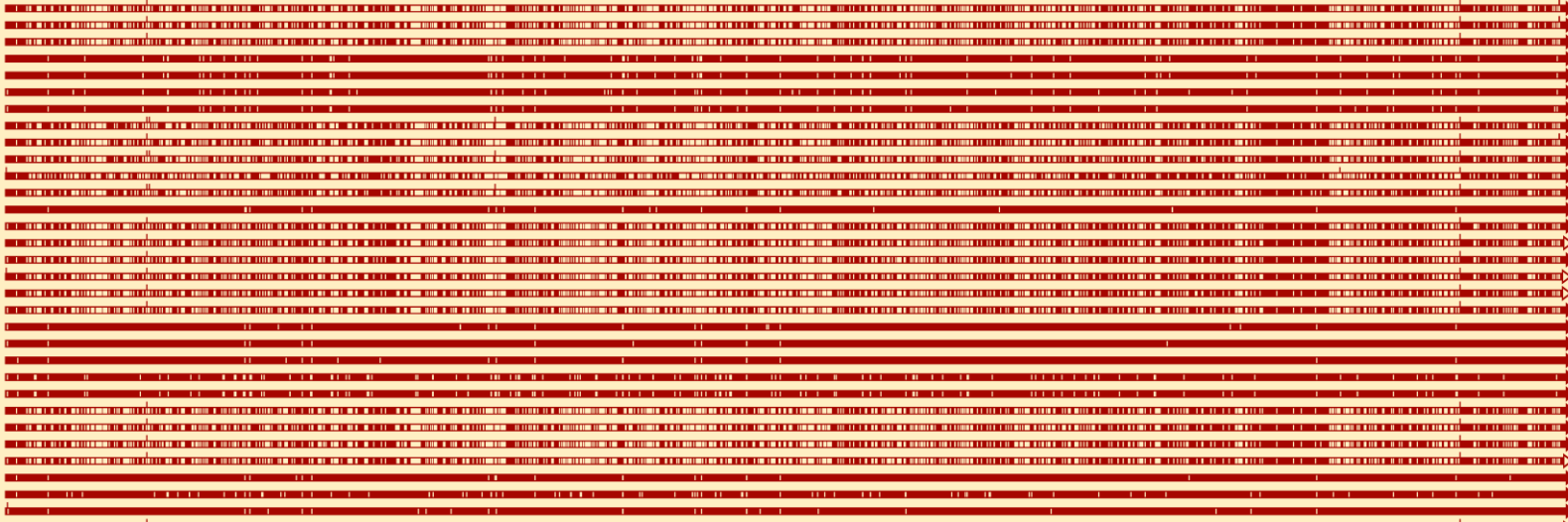
August 2014

100% CGMMV infection rate in field due to
mechanical transmission

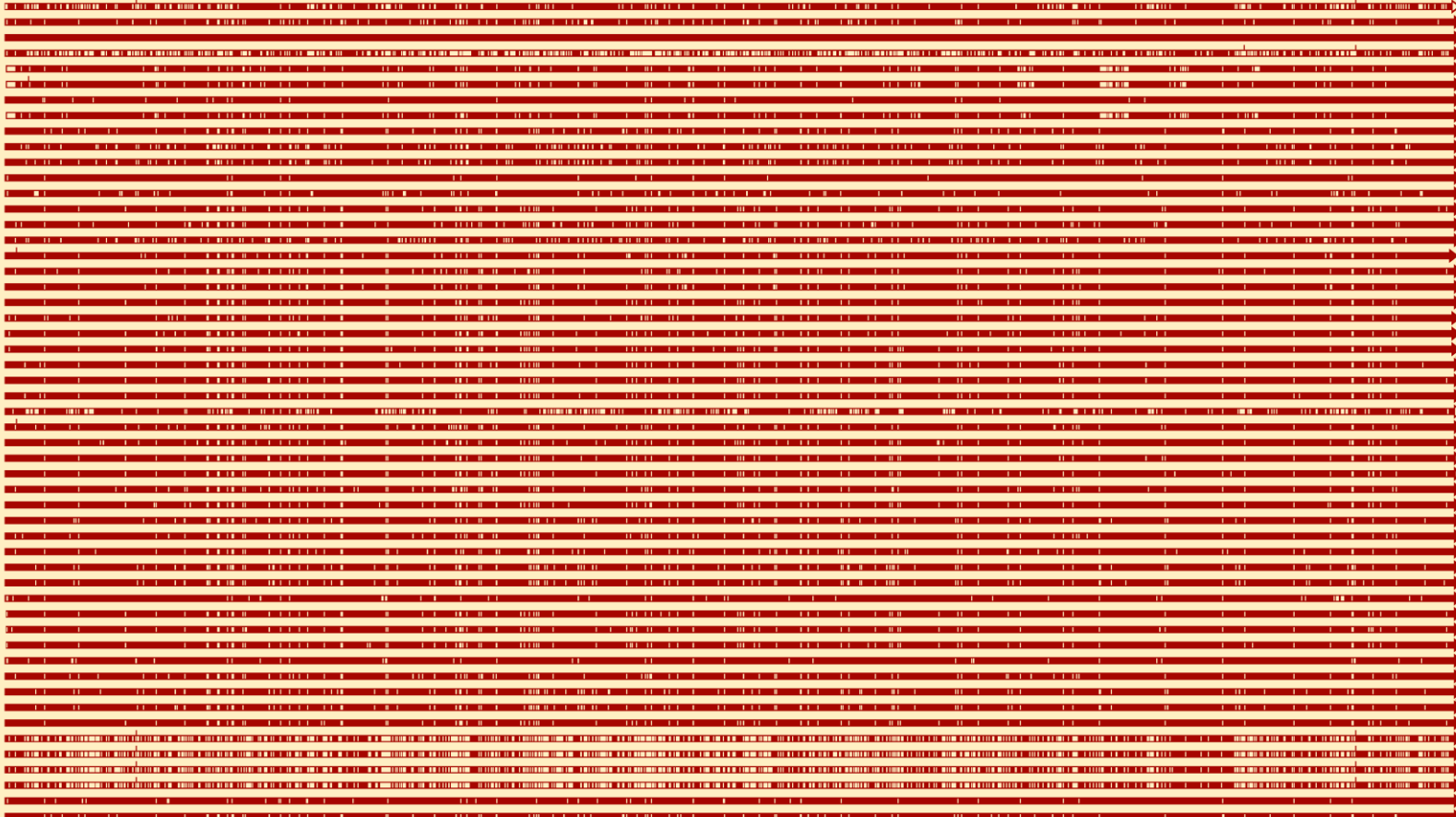


California timeline

- 2013: One county, 5 acres of seed production
- 2014: Four counties, all in triploid watermelon, three isolates of CGMMV
- 2015: no reported CGMMV
- 2016: ?
- 2017: three seed production fields (post-harvest seed detection), one small farm in Fresno county
- 2018: seven seed production fields (one field detection, six post-harvest seed detections); one small farm in Fresno county



UC Davis
isolates

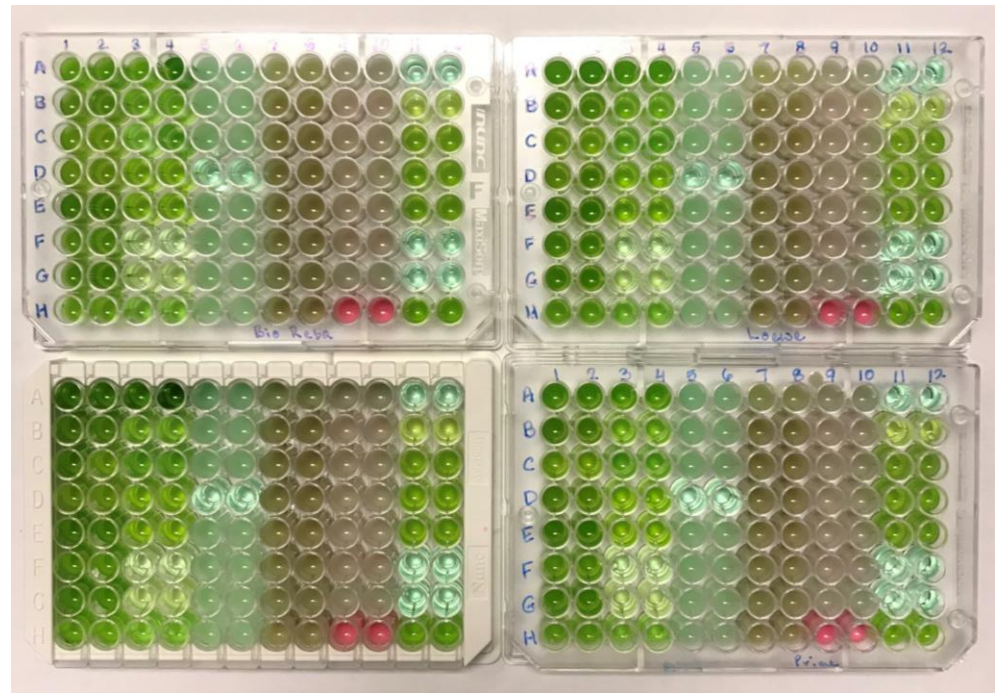


CA isolates

GenBank
full length
genome
sequences

ELISA kit comparison

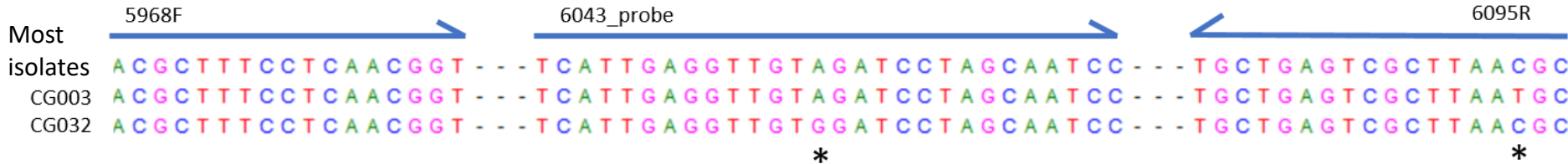
- Compare sensitivity and specificity
 - CGMMV
 - ZGMMV
 - WGMMV
- Consistent samples across reagent sets
- All UC Davis isolates were tested
- Seed samples tested from 1 infected seed to 1:5,000



Most sensitive
and specific:
Agdia and PRI

GenBank	Collection ID	Prime 1.5 hours		BioReba 1.5 hours		Loewe 1.5 hours		Agdia 1.5 hours	
MH271408	CG002	3.5	3.5	0.54	0.554	1.2	1.172	0.989	0.969
MH271409	CG003	3.5	3.5	0.341	0.344	0.889	0.932	1.037	1.016
MH271410	CG004	3.5	3.5	0.412	0.401	1.051	1.132	1.045	1.01
MH271412	CG006	3.5	3.5	0.64	0.648	1.51	1.552	1.032	1.026
MH271413	CG007	2.035	2.197	0.269	0.271	0.531	0.554	0.998	1.03
MH271414	CG008	3.5	3.5	0.884	0.897	1.517	1.462	1.022	1.059
MH271415	CG009	3.5	3.5	0.789	0.789	1.324	1.347	0.995	1.01
MH271416	CG010	3.5	3.5	1.064	1.089	1.556	1.636	0.814	0.807
MH271417	CG011	3.5	3.5	1.374	1.413	2.001	2.039	0.92	0.92
MH271418	CG012	3.5	3.5	1.179	1.257	1.862	1.955	0.996	1.019
MH271419	CG013	3.5	3.5	0.405	0.44	1.081	1.106	1.108	1.05
MH271420	CG014	3.5	3.5	0.406	0.383	1.088	1.116	1.01	1.007
MH271421	CG015	3.5	3.5	0.393	0.39	1.091	1.109	1.042	1.01
MH271424	CG019	3.5	3.5	0.305	0.302	1.034	1.034	1.062	1.038
MH271425	CG020	3.5	3.5	1.311	1.319	2.083	2.078	1.015	1.007
MH271427	CG022	1.408	1.401	0.206	0.2	0.365	0.364	0.983	1.009
MH271430	CG025	3.5	3.5	0.57	0.665	0.896	0.943	0.929	0.899
MH271431	CG026	0.517	0.639	0.114	0.11	0.272	0.26	0.929	0.91
MH271432	CG027	3.5	3.5	0.769	0.792	1.248	1.25	0.902	0.858
MH271433	CG028	3.5	3.5	0.293	0.293	0.963	0.973	0.913	0.893
MH271434	CG029	3.5	3.5	0.401	0.405	1.212	1.202	0.909	0.886
MH271435	CG030	3.5	3.5	0.417	0.398	1.031	1.016	0.999	0.886
MH271436	CG031	2.666	2.744	0.363	0.361	0.595	0.563	0.931	0.916
MH271437	CG032	3.5	3.5	0.298	0.302	0.781	0.781	0.922	0.937
MH271438	CG033	2.281	2.367	0.298	0.302	0.547	0.521	0.944	0.969
MH271439	CG034	0.619	0.744	0.112	0.116	0.217	0.214	0.91	0.906
MH271440	CG035	2.622	1.984	0.222	0.213	0.432	0.433	0.883	0.907
MH271441	CG036	3.5	3.5	0.383	0.382	1.135	1.145	0.951	0.969
MH271443	CG038	3.5	3.5	0.478	0.485	1.116	1.109	0.891	0.898
MK070867	WGMMV	3.5	3.5	0.548	0.541	1.196	1.236	0.101	0.102
	CDFA wm	3.5	3.5	0.504	0.509	1.172	1.187	3.5	3.5
	ZGMMV	2.62	2.766	0.155	0.17	0.435	0.437	0.091	0.09
	Agdia +	3.5	3.5	0.579	0.584	1.149	1.171	3.5	3.5
	Seed neg	0.183	0.156	0.086	0.086	0.088	0.098	0.091	0.091
	seed 1	3.5	3.5	0.576	0.564	1.324	1.364	3.5	3.239
	seed 1:100	2.328	2.314	0.197	0.19	0.344	0.36	3.131	3.5
	seed 1:500	2.135	2.19	0.199	0.206	0.332	0.342	3.024	2.848
	seed 1:1,000	1.786	2.739	0.172	0.176	0.287	0.312	1.298	1.343
	seed 1:2,000	0.454	0.415	0.107	0.096	0.089	0.144	0.346	0.347
	seed 1:5,000	0.152	0.149	0.086	0.093	0.144	0.089	0.145	0.147
	Positive	3.5	3.5	1.636	1.575	2.746	2.675	0.108	0.11
	Negative	0.141	0.134	0.094	0.102	0.093	0.089	0.088	0.089
	OD threshold	0.413		0.294		0.273		0.265	

RT-qPCR design and validation



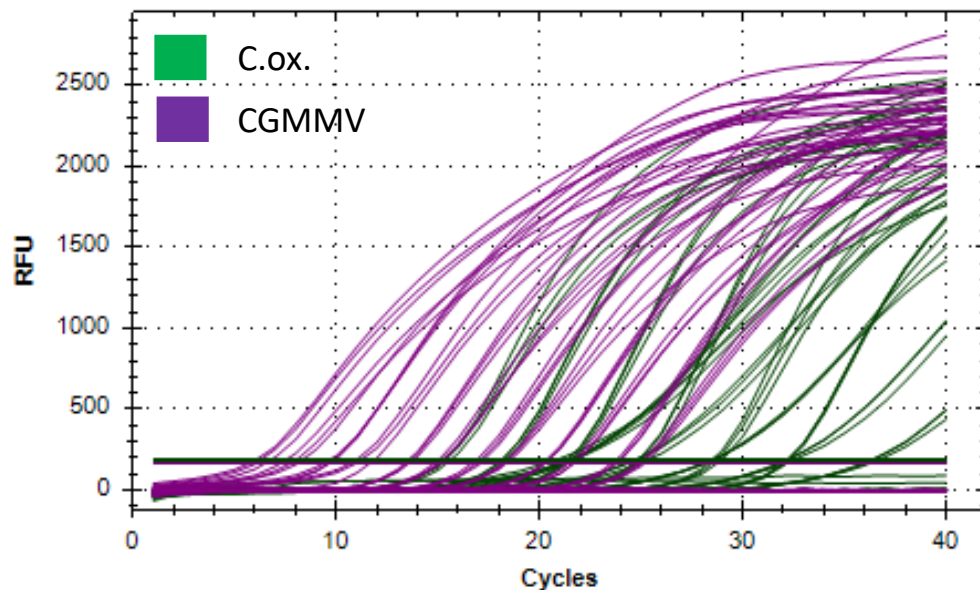
- Designed from alignment containing 100 genome sequences of CGMMV (38 UC Davis, 62 GenBank)
- Amplicon length limited to 200 nucleotides
- Most highly conserved domains exist within coat protein sequence

Cytochrome oxidase internal control assay from Weller *et al.* (2000) Appl. And Env. Micro. 66(7) 2853-2858

Species/Abbrv	CGTTCGCATTCCAGATTATCCA	TGCTTACGCTGGATGGAA TGCCCT	GTTYGGYYCCTTATATAATCCGTAGTTG
1. COX Fwd	CGTTCGCATTCCAGATTATCCA	TGCTTACGCTGGATGGAA TGCCCT	GTTYGGYYCCTTATATAATCCGTAGTTG
2. COX probe	CGTTCGCATTCCAGATTATCCA	TGCTTACGCTGGATGGAA TGCCCT	GTTYGGYYCCTTATATAATCCGTAGTTG
3. COX Rvrs	CGTTCGCATTCCAGATTATCCA	TGCTTACGCTGGATGGAA TGCCCT	GTTYGGYYCCTTATATAATCCGTAGTTG
4. MG947207.1:Cucumis melo var. momordica mitochondrion complete sequence	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
5. JF412792.1:Cucumis melo subsp. melo mitochondrial sequence	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
6. HQ860792.1:Cucumis sativus cultivar Calypso complete sequence	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
7. EU069548.1:Cucumis metuliferus cytochrome oxidase subunit 1 (cox1)	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
8. EU069547.1:Cucumis melo cytochrome oxidase subunit 1 (cox1)	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
9. AJ223416.1:Cucumis sativus mitochondrial coxi gene	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
10. CP026480.1:Citrullus lanatus cultivar Charleston Gray	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
11. GQ856147.1:Citrullus lanatus mitochondrion complete genome	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
12. EU156499.1:Luffa acutangula cytochrome oxidase subunit 1	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
13. EU069549.1:Neoachmandra indica cytochrome oxidase subunit 1	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
14. EU069546.1:Citrullus lanatus subsp. vulgaris cytochrome oxidase subunit 1	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
15. GU321912.1:Cucurbita pepo cytochrome oxidase subunit 1	G T A T G C C A C G G C G C A T T C C A G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
16. GQ856148.1:Cucurbita pepo mitochondrion complete genome	G T A T G C C A C G G C G C A T T C C A G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
17. EU281033.1:Echinocystis lobata cytochrome oxidase subunit I	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C A G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C A G G A T G G A A T G C C C T T A G C A G T T T T G G C T C T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T
18. CP026481.1:Citrullus lanatus cultivar Charleston Gray	G T A T G C C A C G T C G C A T T C C T G A T T A T C C A G A T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T T T T A T A T A T C C G T A G T T G G G A T T	T G C T T A C G C T G G A T G G A A T G C C C T T A G C A G T T T T G G C T T T T A T A T A T C C G T A G T T G G G A T T	G T T Y Y G G Y Y C C T T A T A T A T C C G T A G T T G G G A T T

Sample	Fluoro.	Technician 1									Technician 2								
		CGMMV only			COX only			Duplex CGMMV/COX			CGMMV only			COX only			Duplex CGMMV/COX		
0.006 ng/ μ L	Cy5	24.21	24.24	24.14	-	-	-	24.92	25.07	25.03	24.02	24.13	23.99	-	-	-	24.02	24.04	24.14
	HEX	-	-	-	32.76	32.33	32.38	36.04	36.35	26.18	-	-	-	31.74	32.01	31.92	36.32	36.6	37.7
0.06 ng/ μ L	Cy5	20.39	20.43	20.31	-	-	-	20.95	21.14	21.37	20.29	20.25	20.27	-	-	-	20.29	20.39	20.44
	HEX	-	-	-	28.6	28.35	28.47	31.36	31.66	31.58	-	-	-	28.05	28.04	27.95	32.71	32.56	33.42
0.6 ng/ μ L	Cy5	16.88	16.89	16.75	-	-	-	25.54	17.42	17.63	16.8	16.82	17.03	-	-	-	16.91	16.87	17.02
	HEX	-	-	-	24.84	24.7	24.82	27.54	28.71	27.82	-	-	-	24.81	24.91	24.18	29.12	29.09	29.03
6 ng/ μ L	Cy5	13.52	13.42	13.34	-	-	-	14.09	14.22	14.22	13.37	13.47	13.52	-	-	-	13.44	13.67	13.63
	HEX	-	-	-	21.4	21.33	21.31	23.75	24.36	24.14	-	-	-	20.83	20.82	20.79	24.87	25.51	25.39
60 ng/ μ L	Cy5	9.88	9.78	9.73	-	-	-	10.68	10.56	11.12	9.67	9.94	10.1	-	-	-	10.05	10.05	10.54
	HEX	-	-	-	18.07	17.88	17.87	20.77	21.02	20.88	-	-	-	18	17.45	17.57	22.25	21.72	22.69
600 ng/ μ L	Cy5	7.53	7.4	7.17	-	-	-	8.17	7.83	7.58	6.81	7.04	7.04	-	-	-	7.27	7.37	7.11
	HEX	-	-	-	15.33	15.21	15.23	18.73	19.34	19.78	-	-	-	15.1	15.15	14.5	23.73	25.17	23.92
Healthy NB 600ng/ μ L	Cy5	N/A	N/A	N/A	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A	-	-	-	N/A	N/A	N/A
	HEX	-	-	-	22.1	22.83	22.46	22.57	22.16	22.36	-	-	-	22.74	22.85	22.79	22.54	22.35	22.87
NTC 1	Cy5	N/A	N/A	N/A	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A	-	-	-	N/A	N/A	N/A
	HEX	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
NTC 2	Cy5	N/A	N/A	N/A	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A	-	-	-	N/A	35.52	N/A
	HEX	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A	-	-	-	N/A	N/A	N/A	N/A	39.43	N/A

RT-qPCR amplification curves



Weed sampling and GH screening

- GH: common weed species in CA
 - Field seed collections
 - Greenhouse grown
 - Mechanical inoculation
- Fields associated w/ CGMMV
 - 2018 collection from 2013/2014 sites: 1,960 samples, 23 species, no CGMMV found
 - 2019 collection from 2018 sites: 2,000 samples, 24 species, no CGMMV found (so far)



Sugar baby' watermelon	<i>Citrullus lanatus</i>	systemic
Straight 8' cucumber	<i>Cucumis sativus</i>	systemic
Sugar pumpkin	<i>Cucurbita maxima</i>	systemic
Crookneck squash	<i>Cucurbita pepo</i>	systemic
Opo	<i>Lagenaria siceraria</i>	systemic
Bittermelon	<i>Mormodica charantia</i>	systemic
Nicotiana benthamiana	<i>Nicotiana benthamiana</i>	systemic
Quinoa	<i>Chenopodium quinoa</i>	systemic
Chenopodium captitatum	<i>Chenopodium captitatum</i>	systemic
Chenopodium amaranticolor	<i>Chenopodium amaranticolor</i>	local lesion
Datura, Locoweed	<i>Datura stramonium</i>	local lesion
Bells of Ireland	<i>Molucella laevis</i>	systemic
Common purslane	<i>Portulaca oleracea</i>	nonhost
Green amaranth	<i>Amaranthus viridis</i>	nonhost
Chenopodium amanita	<i>Chenopodium amanita</i>	nonhost
Giant parsnip	<i>Heracleum maxima</i>	nonhost
Morning glory	<i>Convolvulus arvensis</i>	nonhost
Khella	<i>Ammi visnaga</i>	nonhost
Conyza, Horseweed	<i>Erigeron canadensis</i>	nonhost
Common amarath	<i>Amaranthus retroflexus</i>	nonhost
Black nightshade	<i>Solanum nigrum</i>	nonhost
Cow parsnip	<i>Heracleum lanatum</i>	nonhost
Little mallow	<i>Malva parviflora</i>	nonhost
Castor bean	<i>Ricinus communis</i>	nonhost
Bull thistle	<i>Cirsium vulgare</i>	nonhost
Cocklebur	<i>Xanthium stumerium</i>	nonhost
Sharpleaf cancerwort	<i>Kickxia elantine</i>	nonhost
Hoary cress	<i>Lepidium draba</i>	nonhost
Knotweed	<i>Polygonum ssp.</i>	nonhost
Wild mustard	<i>Brassica ssp.</i>	nonhost

Watermelon green mottle mosaic virus in California

- Accidental identification in diagnostic samples
- Discovered in Fresno County
- Very small acreage farms for intrastate markets
- Mixed infections with CGMMV
- 3 distinct isolates so far

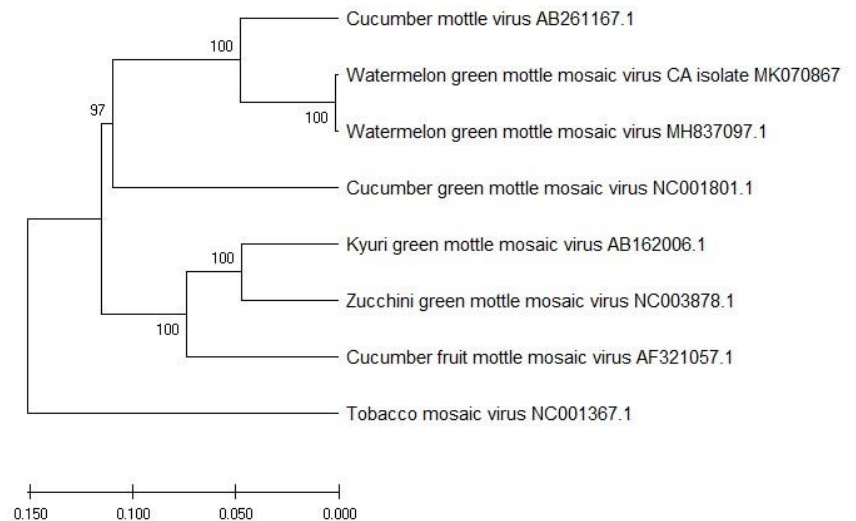


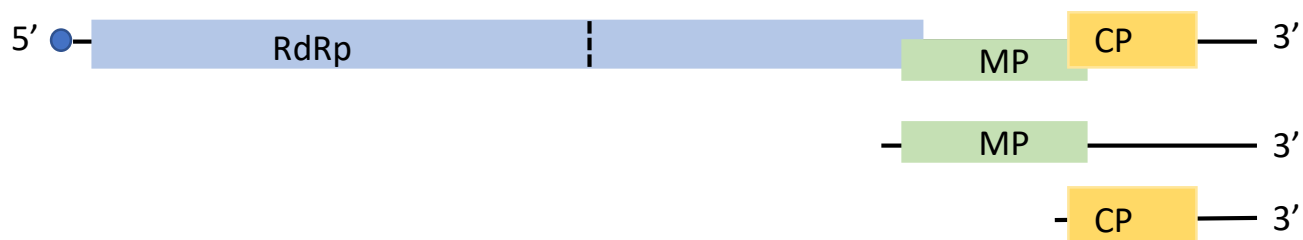
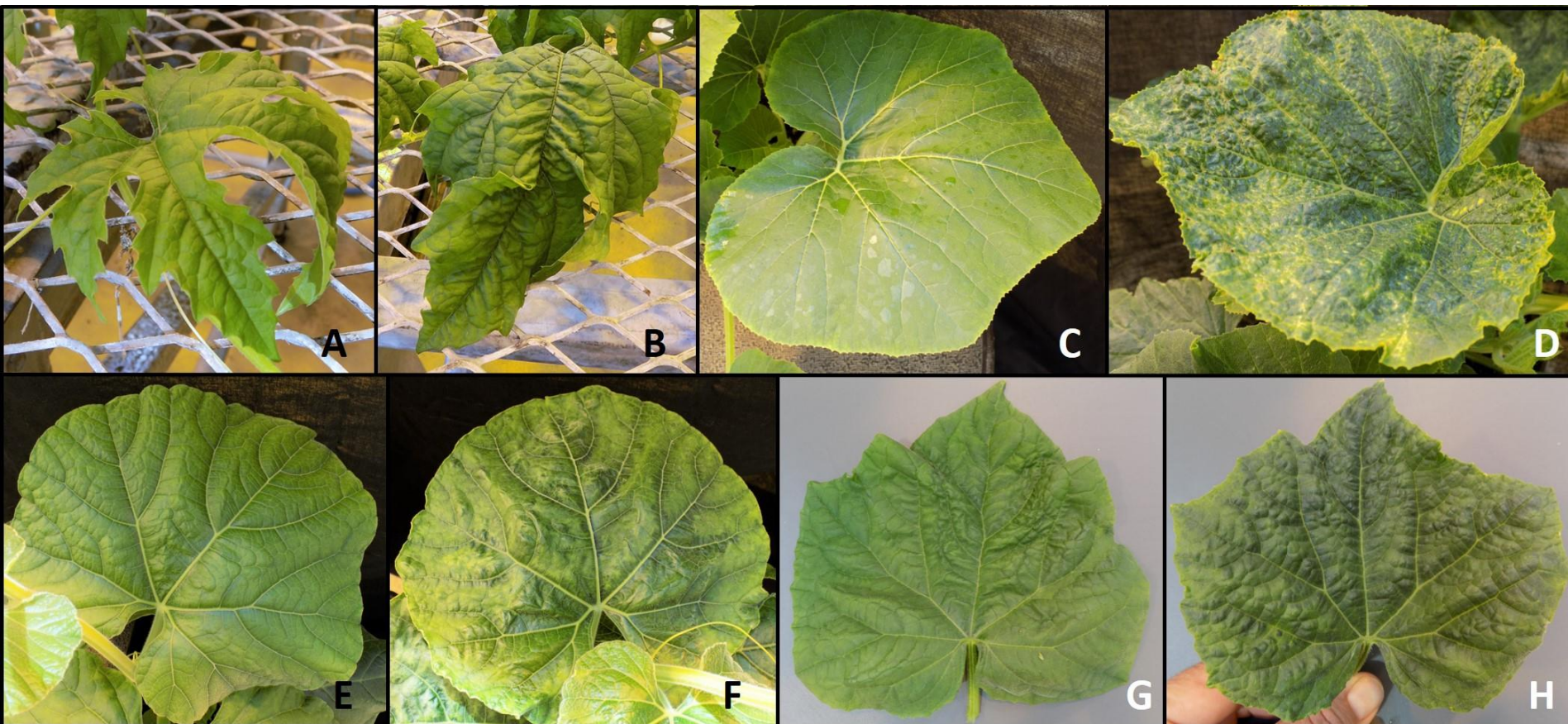
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First report of watermelon green mottle mosaic virus in North America

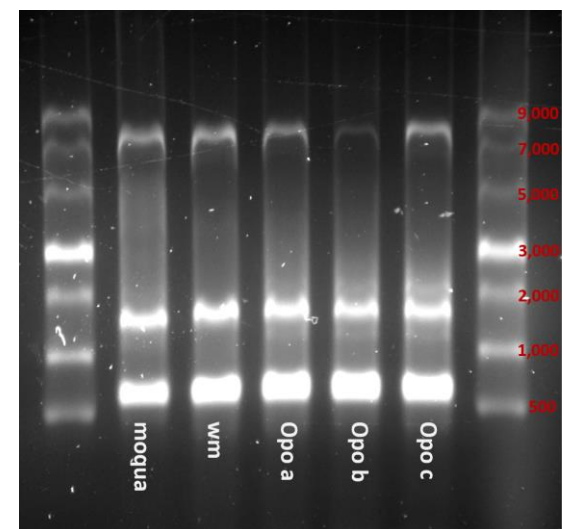
Tera Pitman, Katie Posis, Tongyan Tian, Charles Belanger, Avijit Roy, and Bryce Falk

Published Online: 17 Jul 2019 | <https://doi.org/10.1094/PDIS-02-19-0308-PDN>





WGMMV genome 6,482 nucleotides



A photograph of a watermelon plant with several large, lobed green leaves and two bright yellow flowers. The plant is set against a light blue background with soft shadows.

Thank you

Research supported by:

CDFA Specialty Crop Block Grant

USDA-APHIS-PPQ

American Seed Trade Association

Special thanks to: Bryce Falk (for running an awesome lab)
Tongyan Tian and Katie Posis (for sharing interesting samples)
Suraj Gurung (for having interesting hobbies)