USDA APHIS-funded studies to assess and identify potential reservoir/weed host plants for CGMMV in California cucurbit seed production areas.

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Introduction

Cucumber green mottle mosaic virus (CGMMV) has been an ongoing issue in California since it was first discovered in 2013 (Tian et al. 2014). The following year, 2014, saw the largest outbreak of the disease, which caused economic losses to seedless triploid watermelon production in four counties. The California cucurbit and seed industries were alarmed at the scope of the outbreak. Prior to 2013 CGMMV, a seed transmitted virus, was not tested for in seed lots. After 2014 testing was required and voluntarily implemented by the majority of cucurbit seed producers working in California. Subsequent years, 2015-2018, saw sporadic detections of CGMMV. All but one detection occurred in seeds coming from fields in northern California, or plants in seed increase fields. To try to determine if weed species are hosts for CGMMV and have the potential for carrying over CGMMV between years, we started surveying fields with past detections. In 2018 the fields surveyed were associated with detections from 2013-2016, and no positive weed samples were found. This year we surveyed weeds and volunteer cucurbits from fields associated with CGMMV detections in 2018. Fields were located in Yolo, Sutter, Solano, and Colusa counties. This report describes the field conditions during sampling and the testing results. In summary, four sites were visited for a total of 10 visits in which samples were collected resulting in approximately 2,400 individual plants tested by RT-qPCR for CGMMV. There were no positive detections.

Research Plan

The research objective was to sample plants during the 2019 growing season from field locations associated with 2018 CGMMV detections in attempts to identify potential reservoir/weed host plants for CGMMV that might occur in California cucurbit seed production areas. Weed species and volunteer cucurbits were of primary interest. The excluded weeds were: all monocots, bindweed (*Convovulus avensis*), little mallow (*Malva parviflora*), amaranth (*Amaranthus viridis*), wild mustard (*Brassica* spp.), and jimsonweed (*Datura stramonium*). All excluded species were shown to not host CGMMV under greenhouse conditions after mechanical inoculation. Horseweed (*Erigeron canadensis*) was also tested under greenhouse conditions and found not to be a systemic host for CGMMV, but due to anecdotal observations in fields we kept the species on the collection list. The species collected were: cucumber, squash, watermelon, sunflower, nightshade (*Solanum* spp.), cocklebur (*Xanthium strumerium*), fleabane (*Erigeron* ssp.), hairy mallow (*Albutilon* ssp.), hemlock (*Conium maculatum*), lambs' quarter (*Chenopodium album)*, red sorrel (*Rumex acetosella*), henbit (*Lamium amplexicaule*), horseweed (*Erigeron canadensis*), pineapple weed (*Matricaria discoidea*), common groundsel (*Senecio vulgaris*), prickly lettuce (*Lactuca serriola*), willowherb (*Epilobium* ssp.), and common knotweed (*Polygonum aviculare*). Each sample was comprised of 5 individual plants of one species collected within a 1-meter radius of the initial selection and placed in a plastic bag. GPS location information was recorded for each sample to allow revisiting sites in the event of a positive detection. Location information has been redacted from this report. Samples were kept in an insulated cooler for transport to the lab, and then kept at 4^{o}C until processed. Sample processing occurred within 5 days of collection. Each sample was

subsampled for RNA extraction and backup tissue in case of RNA sample loss. For RNA extraction, 200mg of leaf tissue representing all plants in the sample were placed in 2mL microcentrifuge tubes. Tissue backup material representing all plants of an individual sample were put into 15mL centrifuge tubes, labeled with sample information, and stored at -80^{o}C.

RNA extraction was performed using Trizol® Reagent (ThermoFisher Scientific, Waltham, MA) following the manufacturers' instructions. The resulting RNA was then analyzed for concentration and purity by Nanodrop, and concentrations adjusted to 100-400ng/µL. Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) was carried out as a two-step process. First, total RNA was reverse-transcribed into cDNA using the iScript Reverse Transcription Supermix kit (catalog #1708840, Bio Rad, Hercules, CA) with 2uL RNA sample volume. Following cDNA synthesis, qPCR was performend with CGMMV detection duplexed with cytochrome oxidase (COX) internal control using iQ Multiplex Powermix (catalog #1725849, Bio Rad). CGMMV primers and probe amplify a 127 nucleotide amplicon in the coat protein gene and are CGMMV:5968_Fwd 5'-ACGCTTTCCTCAACGGT-3', CGMMV_6043_probe 5'-5Cy5/TCATTGAGGTTGTAGATCCTAGCAATCC/3IAbRQSp-3', and CGMMV_6095_Rvrs 5'-GCGTTAAGCGACTCAGCA. The COX primers and probes are as described by Weller et al. (2001). Two replicates of qPCR were analyzed for each sample. Samples with COX CQ values greater than 25 or with Cq values not in agreement, values different by 1 Cq, were re-tested. If the initial sample did not have adequate RNA quality or quantity then a new sample was pulled from the freezer backup, RNA extracted, and tested by RT-qPCR.

Field Descriptions

Each field site was visited three times during the growing season, from April to July. There was a fourth collection period planned, but the late rains that occurred in northern California in later April to early May caused very muddy field conditions, and site visits had to be postponed.

Site 1 was fallow at the first collection on April 16th and had been actively managed for weeds. There were volunteer watermelon and cucumber from the previous cropping cycle and we collected 24 samples, 6 of which were weed species. The second collection occurred on May 28th. The field had been planted to tomato, and more weeds and volunteers were present, but still not many. We collected 75 samples. The third site visit occurred on July 1st, and there were no observable weeds within the tomato canopy. We collected 52 samples of various species from the field margins. Overall, this site was very actively managed for weeds and the species composition, once present, did not change through the season.

Site 2 was producing winter wheat, and the grower requested that we not impact the crop stand if possible. We focused on the field margins, within the first meter of wheat to the edge of the road bed. This site was also actively managed for weeds and had volunteer cucumber and squash from the previous cropping cycle. The collection dates were April 18th, May 22nd, and June 25th. We visited for a fourth collect July 6th. Unfortunately, the field had been harvested, ripped, and leveled prior to that date, and there were no living plants anywhere in the field.

Site 3 was also planted to winter wheat and we were again asked not to disturb the stand. There was active weed management, and few weeds and volunteers were observed. Collection dates were April 23rd and May 28th. We sought permission for entering the field in June but couldn't establish communication. We visited the site on July 1st for a final collection but the field had been harvested, ripped, and leveled. There were no living plants in the field.

Site 4 was fallow at the first site visit and had almost no weeds present. It was planted to sunflowers and very actively managed for weeds. We had difficulties establishing communication with the site contact early in the season, so the collection dates were May 30th and June 27th. We revisited the site on July 7th to attempt a third collection but the field had been trenched for irrigation, destroying all the areas we had previously found to collect weed samples from. The sunflowers were also large enough at that point that in-field collection wasn't feasible. There were no plants other than sunflowers we could get to, so no samples were collected.

Results

From the 10 field collection events at four field sites we collected approximately 4,300 individual plants, resulting in 485 tested samples. No sample tested positive for CGMMV.

Images

Site 1 collection on April 16th, 2019. The field was previously sprayed with herbicide, and plants were sparse.

Site 1 collection on May 28th. Tomato transplants are growing and volunteer cucurbits were growing in a few places within the field. Weed species were very sparse.

Site 1 collection July 1st. Weeds and volunteers within the field were difficult to identify under the tomato canopy.

Site 2 collection April 23rd, showing field margins previously treated for weed control.

Site 2 visit July 1st, 2019. No plants were present to collect from.

Site 3 collection April 18th showing field border weed density.

Site 3 collection on May 22nd, showing weeds and cucurbit volunteers along the field border.

Site 3 collection on June 25th. It appeared that broadleaf chemical control had been applied between site visits, and the field margins had been cultivated.

Site 4 collection May 30th, showing fairly clean field borders. Some samples were collected from unter the high tension power poles in the field.

Site 4 collection June 27th, 2019.

References

Tian, T., K. Posis, C.J. Maroon-Lango, V. Mavrodieva, S. Haymes, T.L. Pitman, and B.W. Falk. 2014. First Report of Cucumber Green Mottle Mosaic Virus on Melon in the United States. Plant Disease. 98(8): 1163.

Weller, S.A., J.G. Elphinstone, N.C. Smith, N. Boonham, and D.E. Stead. 2001. Detections of *Ralstonia solanacerarun* Strains with a Quantitative, Multiplex, Real-Time, Fluorogenic PCR (TaqMan) Assay. Applied and Environmental Microbiology. 66(7):2853-2858.

County	Date	# samples	# plants	# species
Sutter	4/16/2019	24	132	6
Solano	4/18/2019	74	347	11
Colusa	4/23/2019	40	112	9
Solano	5/22/2019	95	401	10
Sutter	5/28/2019	75	196	5
Colusa	5/28/2019	21	79	6
Yolo	5/30/2019	11	43	3
Yolo	6/27/2019	24	94	5
Solano	6/25/2019	65	325	2
Sutter	7/1/2019	53	213	8

Common name	Species
cucumber	*Cucumis sativus*
squash	*Cucurbita pepo*
watermelon	*Citrullus lanatus*
sunflower	*Helianthus annuus*
nightshade	*Solanum* spp.
cocklebur	*Xanthium strumerium*
fleabane	*Erigeron* spp.
Mallow	*Albutilon* spp.
hemlock	*Conium maculatum*
Lamb's quarter	*Chenopodium album*
Red sorrel	*Rumex acetosella*
Henbit	*Lamium amplexicaule*
Pigweed	*Amaranth* spp.
Horseweed, Conyza	*Erigeron canadensis*
Pineappleweed	*Matricaria discoidea*
Common groundsel	*Senecio vulgaris*
Prickly lettuce	*Lactuca serriola*
Willowherb	*Epilobium* spp.
Common knotweed	*Polygonum aviculare*

			CGMMV RT-qPCR		COX RT-qPCR	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
1	cucumber	5	N/A	N/A	15.07	14.81
2	cucumber	5	N/A	N/A	18.01	22.02
3	cucumber	5	N/A	36.37	18.18	18.93
4	nightshade	5	N/A	N/A	20.1	19.24
5.1	cucumber	5	36.13	N/A	18.57	18.04
5.2	cucumber	5	N/A	N/A	18.39	18.74
5.3	cucumber	5	N/A	N/A	24.07	24.18
5.4	cucumber	5	N/A	N/A	21.24	21.23
6	watermelon	5	N/A	N/A	20.8	25.61
7	watermelon	5	N/A	N/A	19.93	19.98
8	cocklebur	5	N/A	N/A	16.8	17.51
9	watermelon	5	N/A	N/A	19.78	19.82
10	watermelon	5	N/A	N/A	17.01	16.88
11	watermelon	5	N/A	N/A	21.01	21.59
12	fleabane	5	38.02	N/A	19.8	19.85
13	watermelon	5	N/A	N/A	18.45	18.56
14	cucumber	5	N/A	37.97	18.09	18.16
15	cucumber	5	N/A	N/A	18.18	18.53
16	cucumber	5	N/A	N/A	21.03	19.12
17	cucumber	5	N/A	N/A	26.32	26.27
18	cucumber	5	N/A	N/A	17.53	17.45
19	nightshade	5	N/A	N/A	18.95	19.33
20	watermelon	3	N/A	N/A	18.06	17.33
21	watermelon	4	N/A	N/A	17.13	16.9
22	fleabane	5	N/A	N/A	20.04	34.7
23	watermelon	5	N/A	N/A	18.84	18.79
24	Albution spp.	5	36.34	N/A	16.59	15.91

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
25	Chenopodium album	5	N/A	N/A	24.87	24.01
26	Conyza canadensis	5	N/A	N/A	28.52	28.87
27	cucumber	5	N/A	N/A	15.39	15.14
28	Chenopodium album	5	N/A	N/A	18.42	18.54
29	cucumber	5	N/A	N/A	15.39	14.6
30	cucumber	5	N/A	N/A	12.99	12.94
31	hemlock	5	39.55	N/A	21.66	22.08
32	nightshade	1	36.18	N/A	17.12	16.72
33	cucumber	5	N/A	N/A	13.86	13.65
34	cucumber	5	N/A	N/A	16.79	17.45
35	cucumber	4	N/A	N/A	16.69	15.86
36	Amaranth	1	N/A	38.54	20.96	23.39
37	cucumber	5	N/A	N/A	25.37	24.53
38	Portulaca oleracea	5	N/A	N/A	17.31	20.43
39	cucumber	5	N/A	N/A	13.21	12.84
40	nightshade	5	N/A	36.37	22.68	22.4
41	Red sorrel	5	N/A	N/A	29.39	28.3
42	Lamium amplexicaule	1	N/A	35.4	21.81	20.75
43	cucumber	5	N/A	N/A	18.99	16.99
44	cucumber	5	N/A	38.64	13.25	13.06
45	Chenopodium album	3	N/A	N/A	22.37	22.62
46	cucumber	5	37.18	N/A	13.4	13.75
47	cucumber	5	N/A	N/A	15.71	15.02
48	cucumber	5	N/A	37.33	14.02	14.04
49	Amaranth	5	N/A	N/A	16.04	17.13
50	cucumber	5	N/A	N/A	13.92	13.6
51	cucumber	5	35.44	N/A	13.76	14.01
52	cucumber	5	37.6	N/A	12.09	11.86
53	Chenopodium album	5	N/A	N/A	22.67	22.6
54	cucumber	5	N/A	N/A	12.9	13.58
55	cucumber	5	N/A	N/A	15.02	15.08
56	Chenopodium album	5	38.11	N/A	22.81	22.92
57	cucumber	5	N/A	N/A	15.6	15.75
58	cucumber	5	35.11	N/A	13.92	14.12
59	Chenopodium album	5	38.45	N/A	23.08	23.06
60	Chenopodium album	5	N/A	N/A	22.42	21.95
61	Chenopodium album	5	N/A	N/A	23.11	22.89
62	cucumber	5	35.98	N/A	15.88	15.84
63	Chenopodium album	5	N/A	N/A	26.73	26.87
64	Chenopodium album	5	N/A	N/A	17.18	16.25
65	cucumber	5	N/A	N/A	14.09	14.06
66	cucumber	5	N/A	N/A	23.79	26.01
67	Chenopodium album	3	N/A	N/A	16.5	16.32
68	cucumber	5	N/A	N/A	23.4	23.96
69	Chenopodium album	5	N/A	N/A	24.88	23.93

70	Chenopodium album	5	N/A	N/A	24.14	25.41
71	Chenopodium album	5	N/A	N/A	22.85	22.58
72	Nightshade	5	N/A	N/A	20.2	19.45
73	Chenopodium album	1	N/A	N/A	21.01	20.06
74	squash	5	N/A	36.45	18.33	18.19
75	squash	5	N/A	36.9	17.83	16.38
76	squash	5	N/A	36.36	20.56	19.84
77	Chenopodium album	5	N/A	N/A	23.27	22.96
78	squash	5	N/A	N/A	21.69	21.74
79	? Amaranth spp?	5	N/A	N/A	27.62	28.47
80	squash	5	N/A	N/A	18.04	19.09
81	Conyza canadensis	5	N/A	N/A	24.12	23.81
82	squash	5	N/A	N/A	21.24	21.21
83	squash	5	N/A	N/A	25.53	25.04
84	squash	5	N/A	N/A	23.02	23.21
85	squash	5	N/A	N/A	23.5	24.21
86	squash	5	N/A	N/A	22.03	21.16
87	squash	5	N/A	N/A	21.04	19.45
88	squash	5	N/A	N/A	17.34	16.58
89	squash	5	N/A	36.88	20	21.11
90	squash	5	N/A	N/A	17.5	16.64
91	Chenopodium album	2	N/A	38.4	21.22	21.01
92	squash	5	N/A	N/A	21.36	21.43
93	squash	5	N/A	N/A	23.4	23.34
94	squash	5	N/A	N/A	24.32	28.1
95	squash	11	N/A	36.43	20.49	18.2
96	squash	5	N/A	37.45	20.81	19.73
97	squash	2	N/A	N/A	21.32	20.93
98	squash	3	N/A	N/A	15.89	15.39

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
99	amaranth	1	N/A	38.99	26.83	22.96
100	chenopodium album	2	38.89	N/A	22.36	22.13
101	amaranth	5	36.59	38.89	23.01	24.87
102	conyza	5	39.49	N/A	27.63	27.59
103	amaranth	3	N/A	N/A	24.51	24.54
104	conyza	5	N/A	N/A	29.42	29.85
105	conyza	3	N/A	N/A	26.82	26.89
106	thistle	1	N/A	N/A	30.96	31.33
107	conyza	5	N/A	N/A	22.14	22.38
108	conyza	3	N/A	N/A	30.74	30.37
109	thistle	1	N/A	N/A	24.77	24.38
110	Lamium amplexicaule	1	36.51	N/A	23.2	21.55
111	Amaranthus retroflexus	1	36.55	37.1	30.84	30.63
112	Common groundsel	4	N/A	N/A	23.84	27.92
113	Senecio vulgaris	3	N/A	N/A	25.57	25.64
114	conyza	5	N/A	N/A	26.03	25.32
115	conyza	1	N/A	N/A	32.49	34.33
116	Senecio vulgaris	1	N/A	N/A	24.01	24.31
117	Senecio vulgaris	3	N/A	N/A	24.61	23.03
118	conyza	5	N/A	N/A	20.5	20.7
119	chenopodium album	1	N/A	N/A	25.66	24.84
120	amaranth	1	N/A	N/A	27.61	27.68
121	amaranth	1	N/A	N/A	26.15	26.65
122	conyza	1	N/A	N/A	22.75	21.56
123	Matricaria discoidea	2	N/A	N/A	25.29	24.34
124	chenopodium album	5	N/A	36.18	21.92	21.92
125	amaranth	5	N/A	38.14	27.85	27.07
126	Senecio vulgaris	2	N/A	N/A	21.73	21.73
127	amaranth	1	N/A	N/A	21.42	20.74
128	sunflower	1	N/A	N/A	25.48	25.99
129	Senecio vulgaris	1	N/A	N/A	23.34	21.93
130	conyza	5	38.07	N/A	24.66	24.96
131	amaranth	5	N/A	N/A	27.39	26.87
132	conyza	5	N/A	N/A	31.01	30.74
133	conyza	5	N/A	N/A	20.32	20.93
134	chenopodium album	1	N/A	N/A	23.96	23.81
135	Senecio vulgaris	3	N/A	N/A	22.16	22.76
136	chenopodium album	1	N/A	N/A	21.88	21.81
137	conyza	5	N/A	N/A	23	22.76
138	Senecio vulgaris	3	N/A	N/A	15.19	9.49

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
139	watermelon	1	N/A	N/A	26.13	25.32
140	cucumber	5	N/A	N/A	26.43	26.46
141	cucumber	5	N/A	N/A	26.18	26.37
142	cucumber	5	N/A	N/A	22.09	22.36
143	cucumber	5	N/A	N/A	25.23	25.47
144	cucumber	5	N/A	N/A	26.02	26.17
145	cucumber	5	N/A	N/A	26.9	27.01
146	cucumber	5	N/A	N/A	22.88	22.83
147	cucumber	5	N/A	N/A	28.85	28.32
148	cucumber	5	N/A	N/A	26.87	26.88
149	cucumber	5	N/A	N/A	22.87	22.45
150	Lactuca serriola	1	N/A	N/A	20.68	20.82
151	Nightshade spp.	5	N/A	N/A	24.57	24.58
152	cucumber	5	N/A	N/A	22.02	22.07
153	cucumber	5	N/A	N/A	25.21	25.13
154	Nightshade spp.	5	N/A	N/A	22.64	22.35
155	cucumber	5	N/A	N/A	27.18	26.11
156	cucumber	5	N/A	N/A	22.61	22.36
157	cucumber	5	N/A	N/A	24.93	25.69
158	cucumber	5	N/A	N/A	29.08	29.11
159	cucumber	5	N/A	N/A	24.72	24.62
160	cucumber	5	N/A	N/A	23.44	23.36
161	cucumber	5	N/A	N/A	22.76	22.57
162	cucumber	5	N/A	N/A	28.94	28.58
163	cucumber	5	N/A	N/A	22.69	24.35
164	Nightshade spp.	5	N/A	N/A	23.67	23.68
165	Nightshade spp.	5	N/A	N/A	24.11	24.52
166	Nightshade spp.	5	N/A	N/A	29.43	39.12
167	cucumber	1	31.7	N/A	28.14	28.24
168	Nightshade spp.	1	N/A	N/A	27.55	27.98
169	squash	1	N/A	N/A	22.47	22.64
170	squash	1	N/A	N/A	27.58	27.93
171	Nightshade spp.	5	N/A	N/A	27.56	27.79
172	squash	2	N/A	N/A	24.23	23.97
173	Nightshade spp.	5	N/A	N/A	25.75	26.03
174	Nightshade spp.	2	N/A	N/A	24.49	24
175	Chenopodium spp.	1	N/A	N/A	25.45	25.77
176	Amaranth spp.	5	N/A	N/A	24.33	24.21
177	Nightshade spp.	5	N/A	N/A	24.9	24.56
178	Nightshade spp.	5	N/A	N/A	27.01	26.68
179	Nightshade spp.	1	N/A	N/A	26.93	25.01
180	squash	1	N/A	N/A	23.86	23.59
181	Nightshade spp.	5	N/A	N/A	25.74	25.18
182	Chenopodium spp.	5	N/A	N/A	25.51	25.54
183	Amaranth spp.	5	N/A	N/A	27.36	27.29

	CGMMV		COX	
	Cq 1	Cq 2	Cq 1	Cq 2
CGMMV+	12.08	12.12	24.3	24.47
water	N/A	N/A	N/A	N/A

184	Amaranth spp.	5	N/A	N/A	27.95	27.36
185	Epilobium spp.	3	N/A	N/A	33.52	33.93
186	Chenopodium spp.	5	N/A	N/A	25.82	26.07
187	Chenopodium spp.	5	N/A	N/A	25.74	25.95
188	Amaranth spp.	2	N/A	N/A	26.14	26.01
189	Conyza spp.	5	N/A	N/A	31.42	31.31
190	squash	1	N/A	N/A	21.83	21.49
191	Chenopodium spp.	5	N/A	N/A	24.79	24.96
192	Conyza spp.	5	N/A	N/A	34.37	35.37
193	squash	5	N/A	N/A	24.39	23
194	Sunflower	1	N/A	N/A	29.68	29.15
195	squash	5	N/A	N/A	21.67	22.07
196	Amaranth spp.	5	N/A	N/A	25.85	26.03
197	Conyza spp.	5	N/A	N/A	26.74	26.57
198	squash	5	N/A	N/A	23.36	23.35
199	Chenopodium spp.	5	N/A	N/A	24.06	24.29
200	Lactuca serriola	1	N/A	N/A	26.31	25.89
201	Conyza spp.	5	N/A	N/A	22.49	22.18
202	squash	5	N/A	N/A	27.63	27.02
203	Epilobium spp.	5	N/A	N/A	22.82	22.84
204	squash	5	N/A	N/A	21.6	21.67
205	squash	5	N/A	N/A	24.31	24.41
206	squash	5	N/A	N/A	23.58	23.73
207	Epilobium spp.	5	N/A	N/A	33.37	33.73
208	squash	5	N/A	N/A	26.57	26.26
209	Lactuca serriola	1	N/A	N/A	26.76	26.71
210	squash	5	N/A	N/A	24.64	24.66
211	Conyza spp.	5	N/A	N/A	25.74	25.72
212	squash	5	N/A	N/A	24.48	23.68
213	Epilobium spp.	5	N/A	N/A	24.68	24.36
214	squash	5	N/A	N/A	23.67	23.71
215	Epilobium spp.	5	N/A	N/A	24.74	25
216	Chenopodium spp.	5	36.55	N/A	24.22	24.41
217	squash	5	N/A	N/A	24.72	24.02
218	Anthemis cotula	2	N/A	N/A	22.66	32.84
219	Epilobium spp.	5	N/A	N/A	24.36	24.38
220	squash	5	N/A	N/A	26.38	26.2
221	squash	5	37.09	N/A	23.35	23.14
222	Chenopodium spp.	5	N/A	N/A	27.61	27.27
223	Conyza spp.	5	N/A	N/A	28.16	28.44
224	squash	5	N/A	N/A	26.45	26.79
225	squash	5	N/A	N/A	25.3	25.1
226	squash	5	N/A	N/A	23.61	23.6
227	Chenopodium spp.	5	N/A	N/A	27.04	26.89
228	Sunflower	1	N/A	N/A	26.31	27.43
229	Sunflower	1	N/A	N/A	28.06	28.24
230	Chenopodium spp.	5	N/A	N/A	28.86	28.54

231	Chenopodium spp.	5	N/A	N/A	27.01	26.57
232	Sunflower	5	N/A	N/A	28.96	28.8
233	squash	5	N/A	N/A	27.22	26.87

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
234	watermelon	2	N/A	N/A	20.98	20.62
235	cucumber	5	N/A	N/A	21.95	21.65
236	watermelon	2	N/A	N/A	19.52	19.6
237	Amaranth spp.	5	N/A	N/A	26.39	26.13
238	watermelon	1	N/A	N/A	20.26	20.32
239	watermelon	1	N/A	N/A	23.23	23.31
240	Chenopodium album	5	N/A	N/A	27.08	26.92
241	watermelon	5	N/A	N/A	19.41	19.82
242	watermelon	5	N/A	N/A	20.16	20.54
243	Amaranth spp.	5	N/A	N/A	24.69	24.92
244	watermelon	5	38.99	38.41	21.13	21.47
245	Abutilon theofrasti	5	N/A	N/A	20.74	21.44
246	Amaranth spp.	5	N/A	37.81	18.15	18.11
247	Conyza canadensis	1	N/A	N/A	27.38	27.96
248	Conyza canadensis	2	N/A	N/A	26.44	26.39
249	Conyza canadensis	5	N/A	N/A	23.69	23.75
250	Amaranth spp.	5	N/A	N/A	26.31	26.13
251	Conyza canadensis	2	N/A	N/A	23.61	23.94
252	Conyza canadensis	5	N/A	N/A	24.27	23.82
253	Conyza canadensis	2	N/A	N/A	26.87	29.75
254	Conyza canadensis	5	N/A	N/A	19.55	19.18
255	Chenopodium album	1	N/A	N/A	27.3	27.07

			CGMMV		COX			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2		Cq 1	Cq 2	Cq 1	Cq 2
256	Conyza canadensis	1	N/A	N/A	23.85	23.59	CGMMV+	12.45	12.28	23.79	23.53
257	watermelon	1	N/A	N/A	22.72	22.78	water	N/A	N/A	N/A	N/A
258	cucumber	5	N/A	N/A	21.04	21.06					
259	watermelon	1	N/A	N/A	20.33	20.27					
260	watermelon	1	N/A	N/A	21.21	21.23					
261	Velvetleaf (Abutilon)	5	N/A	N/A	20.37	20.37					
262	watermelon	1	N/A	N/A	20.49	20.47					
263	cucumber	5	N/A	N/A	21.16	21.13					
264	cucumber	5	N/A	N/A	21.39	21.6					
265	watermelon	1	N/A	N/A	20.4	20.65					
266	watermelon	1	N/A	N/A	22.66	22.66					
267	watermelon	1	N/A	N/A	23.1	23.15					
268	watermelon	1	N/A	N/A	26.93	26.83					
269	watermelon	1	N/A	N/A	25.4	25.28					
270	watermelon	1	N/A	N/A	21.1	23.53					
271	watermelon	1	N/A	N/A	23.28	23.36					
272	Velvetleaf (Abutilon)	1	N/A	N/A	21.91	22.42					
273	Velvetleaf (Abutilon)	2	N/A	N/A	22.14	22.56					
274	cucumber	5	N/A	N/A	20.86	20.77					
275	watermelon	5	N/A	N/A	21.69	21.7					
276	cucumber	5	38.36	N/A	22.04	22.14					
277	Velvetleaf (Abutilon)	1	N/A	N/A	20.13	20					
278	Conyza canadensis	1	N/A	N/A	30.35	30.38					
279	cucumber	1	N/A	N/A	20.03	20.04					
280	watermelon	5	N/A	N/A	21.43	21.49					
281	watermelon	1	N/A	N/A	22.23	22.31					
282	watermelon	5	N/A	N/A	25.87	26.01					
283	cucumber	5	N/A	N/A	22.05	22.22					
284	Conyza canadensis	1	N/A	N/A	20.23	20.17					
285	watermelon	1	N/A	N/A	20.64	20.51					
286	watermelon	2	N/A	N/A	26.16	26.55					
287	watermelon	1	N/A	N/A	20.18	20.36					
288	watermelon	1	N/A	N/A	22.3	22.37					
289	Conyza canadensis	5	N/A	N/A	29.4	29.43					
290	watermelon	5	N/A	N/A	21.65	22.08					
291	cucumber	5	N/A	N/A	20.38	20.52					
292	Velvetleaf (Abutilon)	3	39.62	N/A	22.98	24.02					
293	watermelon	3	N/A	N/A	27.92	28.01					
294	watermelon	1	N/A	N/A	21.3	22.36					
295	watermelon	2	N/A	N/A	23.64	24.05					
296	watermelon	2	N/A	N/A	25.96	26.23					
297	watermelon	1	N/A	N/A	23.3	23.52					
298	Conyza canadensis	1	N/A	N/A	30.43	30.76					
299	watermelon	2	N/A	N/A	26.86	27.06					
300	watermelon	2	N/A	N/A	23.13	27.88					

301	Amaranth spp.	3	N/A	N/A	26.76	27.1
302	cucumber	1	N/A	N/A	21.24	22.87
303	watermelon	1	N/A	N/A	23.98	24.52
304	watermelon	5	N/A	N/A	21.12	21.31
305	watermelon	1	N/A	N/A	24.53	24.64
306	cucumber	2	N/A	N/A	21.56	21.56
307	watermelon	1	N/A	N/A	24.29	24.22
308	cucumber	5	N/A	N/A	23.55	25.82
309	watermelon	5	N/A	N/A	22.25	22.66
310	Velvetleaf (Abutilon)	5	N/A	N/A	22.56	23.25
311	watermelon	2	38.73	N/A	22.03	22.19
312	watermelon	1	N/A	N/A	20.39	21.21
313	watermelon	1	N/A	N/A	21.69	21.94
314	Velvetleaf (Abutilon)	3	N/A	N/A	24.7	24.81
315	watermelon	1	N/A	N/A	22.13	22.19
316	watermelon	5	N/A	N/A	24.12	25.46
317	watermelon	1	N/A	N/A	21.12	22.71
318	watermelon	3	N/A	N/A	25.25	26.19
319	watermelon	2	N/A	N/A	22.75	22.83
320	watermelon	2	N/A	N/A	22.46	22.54
321	cucumber	5	N/A	N/A	20.55	21.06
322	cucumber	5	N/A	N/A	20.43	21.03
323	Conyza canadensis	1	N/A	N/A	24.73	24.66
324	cucumber	5	N/A	N/A	20.53	21.22
325	watermelon	1	N/A	N/A	21.47	21.59
326	cucumber	5	N/A	N/A	21.33	21.5
327	cucumber	5	N/A	N/A	21.19	21.12
328	watermelon	1	N/A	N/A	27.71	27.71
329	Conyza canadensis	5	N/A	N/A	22.71	23.08
330	cucumber	5	N/A	N/A	27.69	28.21
331	Velvetleaf (Abutilon)	1	N/A	N/A	22.28	22.44

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
332	Polygonum aviculare (common knotweed)	5	N/A	N/A	29.27	29.84
333	Polygonum aviculare (common knotweed)	5	N/A	39.14	22.32	22.19
334	Polygonum aviculare (common knotweed)	5	N/A	N/A	27.74	27.46
335	Polygonum aviculare (common knotweed)	3	N/A	N/A	27.9	27.88
336	Polygonum aviculare (common knotweed)	2	N/A	N/A	22.36	22.34
337	Polygonum aviculare (common knotweed)	5	N/A	N/A	23.44	23.24
338	Polygonum aviculare (common knotweed)	3	N/A	N/A	21.19	21.21
339	Abultilon threophrasti (velvetleaf)	1	N/A	N/A	20.41	20.44
340	Polygonum aviculare (common knotweed)	5	N/A	N/A	28.68	29.01
341	Polygonum aviculare (common knotweed)	5	N/A	N/A	29.08	29.22
342	Polygonum aviculare (common knotweed)	2	N/A	N/A	30.72	30.79
343	Amaranth spp.	2	N/A	N/A	26.51	26.86

	CGMMV		COX	
	Cq 1	Cq 2	Cq 1	Cq 2
CGMMV+	12.45	12	23.79	23.53
water	N/A	N/A	N/A	N/A

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
344	Cucumber	5	N/A	N/A	28.37	28.62
345	Squash	5	N/A	N/A	25.93	25.88
346	Squash	5	N/A	N/A	23.61	23.71
347	Cucumber	5	N/A	N/A	23.22	23.32
348	Squash	5	N/A	N/A	23.01	23.34
349	Squash	5	N/A	N/A	24.2	24.23
350	Cucumber	5	N/A	N/A	23.64	23.5
351	Squash	5	N/A	N/A	22.79	22.61
352	Squash	5	N/A	N/A	23.29	23.38
353	Squash	5	N/A	N/A	28.49	28.56
354	Squash	5	N/A	N/A	22.4	22.64
355	Cucumber	5	N/A	N/A	22.8	22.97
356	Cucumber	5	N/A	N/A	23.19	23.44
357	Cucumber	5	N/A	N/A	22.76	22.83
358	Cucumber	5	N/A	N/A	22.85	22.86
359	Squash	5	N/A	N/A	23.13	23.09
360	Cucumber	5	N/A	N/A	24.25	23.48
361	Cucumber	5	N/A	N/A	22.86	22.74
362	Cucumber	5	N/A	N/A	23.15	23.29
363	Cucumber	5	N/A	N/A	23.26	23.25
364	Cucumber	5	N/A	N/A	23.1	22.88
365	Squash	5	N/A	N/A	23.7	23.51
366	Cucumber	5	N/A	39.49	23.64	23.46
367	Cucumber	5	N/A	N/A	21.83	21.78
368	Squash	5	N/A	N/A	22.83	23.14
369	Cucumber	5	N/A	N/A	21.19	21.26
370	Cucumber	5	N/A	N/A	21.39	21.73
371	Squash	5	N/A	N/A	21.18	21.68
372	Cucumber	5	N/A	N/A	20.58	20.85
373	Cucumber	5	N/A	N/A	22.96	23.09
374	Squash	5	N/A	N/A	21.89	21.88
375	Amaranth	5	N/A	N/A	26.53	26.56
376	Squash	5	N/A	N/A	22.85	22.89
377	Squash	5	N/A	N/A	23	23.16
378	Squash	5	N/A	N/A	21.45	21.49
379	Squash	5	N/A	N/A	22.14	22.09
380	Squash	5	N/A	N/A	22.4	22.5
381	Squash	5	39.01	N/A	22.38	22.58
382	Squash	5	N/A	N/A	22.97	23.06
383	Squash	5	N/A	N/A	22.6	22.63
384	Squash	5	N/A	N/A	23.03	23.22
385	Squash	5	N/A	N/A	22.28	22.13
386	Squash	5	N/A	N/A	22.25	22.99
387	Cucumber	5	N/A	N/A	21.32	21.6
388	Squash	5	N/A	N/A	21.84	22.24

	CGMMV		COX	
	Cq 1	Cq 2	Cq 1	Cq 2
CGMMV+	12.45	12.28	23.79	23.53
water	N/A	N/A	N/A	N/A
CGMMV+	12.76	12.71	24.19	24.43
water	N/A	N/A	N/A	N/A

389	Squash	5	N/A	N/A	22.02	22.23
390	Squash	5	N/A	N/A	22.83	23.1
391	Squash	5	N/A	N/A	22.47	22.82
392	Cucumber	5	N/A	N/A	21.99	22.19
393	Squash	5	N/A	N/A	23.13	23.08
394	Squash	5	N/A	N/A	23.38	23.19
395	Cucumber	5	N/A	N/A	23.84	23.26
396	Squash	5	N/A	N/A	22.72	22.89
397	Cucumber	5	N/A	N/A	23.22	23.24
398	Squash	5	N/A	N/A	23.12	23.39
399	Cucumber	5	N/A	N/A	28.72	29.07
400	Squash	5	N/A	N/A	24.09	24.14
401	Squash	5	37.86	N/A	24.25	24.45
402	Cucumber	5	38.39	N/A	23.8	23.8
403	Cucumber	5	N/A	N/A	23.15	23.43
404	Cucumber	5	N/A	N/A	22.09	22.19
405	Amaranth	5	N/A	N/A	24.99	25.06
406	Squash	5	37.46	37.38	22.45	22.44
407	Squash	5	N/A	N/A	25.01	25.15
408	Squash	5	N/A	N/A	22.85	22.92

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
409	Abitulon threophrasti	5	N/A	N/A	22.8	23
410	Asclepias spp.	5	N/A	N/A	21.65	21.57
411	Abitulon threophrasti	4	N/A	N/A	23.68	23.84
412	Abitulon threophrasti	2	N/A	N/A	26.31	28.18
413	Amaranth	5	38.34	N/A	22.9	23.13
414	Abitulon threophrasti	5	N/A	N/A	26.88	26.68
415	Amaranth	2	38.56	N/A	23.45	23.52
416	Abitulon threophrasti	5	N/A	38.34	26.35	26.41
417	Amaranth	5	N/A	N/A	21.81	21.71
418	Abitulon threophrasti	5	N/A	N/A	25.77	25.92
419	Amaranth	1	N/A	N/A	27.12	27.05
420	Amaranth	5	N/A	N/A	25.71	25.6
421	Amaranth	5	N/A	N/A	26.41	26.34
422	Amaranth	1	N/A	N/A	25.74	25.59
423	Amaranth	1	N/A	N/A	24.01	23.64
424	Abitulon threophrasti	5	N/A	N/A	24.01	23.64
425	Amaranth	1	N/A	N/A	26.79	26.95
426	Amaranth	2	37.95	N/A	28.26	28.41
427	Abitulon threophrasti	5	N/A	N/A	23.32	23.71
428	Alfalfa	5	N/A	N/A	22.47	22.15
429	Xanthium spinosum	5	N/A	N/A	28.85	29.05
430	Xanthium spinosum	5	36.81	37.96	26.01	25.34
431	Xanthium spinosum	5	N/A	N/A	29.3	31.48
432	Xanthium spinosum	5	N/A	N/A	22.81	22.28

	CGMMV		COX	
	Cq 1	Cq 2	Cq 1	Cq 2
CGMMV+	12.76	12.71	24.19	24.43
water	N/A	N/A	N/A	N/A

			CGMMV		COX	
Sample #	Species	# plants	Cq 1	Cq 2	Cq 1	Cq 2
433	Asclepias fascicularis	5	N/A	N/A	27.32	27.56
434	Asclepias fascicularis	5	38.3	36.37	23.58	23.66
435	Asclepias fascicularis	5	N/A	N/A	26.52	26.71
436	Asclepias spp.	5	N/A	N/A	34.86	35.11
437	Amaranth	5	38.68	38.96	26.03	25.86
438	Amaranth	5	N/A	N/A	28.47	28.26
439	Amaranth	5	N/A	N/A	26.05	26.28
440	Abitulon threophrasti	5	N/A	N/A	21.69	21.72
441	Amaranth	5	38.84	N/A	28.22	28.13
442	Amaranth	1	N/A	N/A	31.31	32.17
443	Proboscidea louisianica	4	N/A	N/A	23.97	23.71
444	tomatillo	2	N/A	N/A	24.98	26.02
445	thistle	1	N/A	N/A	35.05	35.98
446	Asclepias fascicularis	5	N/A	N/A	38.25	39.77
447	Asclepias fascicularis	5	N/A	N/A	25.31	25.15
448	Amaranth	1	38.25	N/A	26.41	26.64
449	Abitulon threophrasti	3	N/A	N/A	22.53	22.64
450	Amaranth	5	38.94	36.26	25.77	25.89
451	Asclepias fascicularis	1	N/A	N/A	38.77	34.75
452	Amaranth	5	N/A	N/A	27.91	29.5
453	Asclepias fascicularis	5	N/A	N/A	27.22	26.66
454	Amaranth	5	N/A	N/A	29.17	28.61
455	Asclepias fascicularis	5	N/A	N/A	26.39	26.48
456	Amaranth	1	36.96	N/A	27.33	27.47
457	Abitulon threophrasti	5	N/A	37.91	28.22	28.61
458	Abitulon threophrasti	1	N/A	N/A	24.15	24.32
459	Abitulon threophrasti	4	36.84	N/A	21.75	22.03
460	Abitulon threophrasti	3	37.04	N/A	21.75	21.7
461	Amaranth	5	38.18	N/A	26.81	27
462	tomatillo	5	N/A	38.03	27.22	27.28
463	Horseweed	5	N/A	N/A	29.37	30.2
464	Amaranth	5	N/A	35.43	25.61	25.61
465	Abitulon threophrasti	5	37.2	37.85	21.83	21.74
466	Amaranth	5	N/A	37.65	28.63	28.77
467	Amaranth	5	N/A	38.11	27.04	27.22
468	Nightshade	3	38.03	37.93	27.26	27.13
469	Abitulon threophrasti	1	36.43	37.02	23.53	23.41
470	Asclepias fascicularis	5	N/A	N/A	N/A	N/A
471	Abitulon threophrasti	5	37.19	36.91	25.68	25.88
472	Amaranth	5	N/A	N/A	26.75	26.97
473	Abitulon threophrasti	1	N/A	37.04	26.03	26.08
474	Amaranth	5	37.6	N/A	25.26	25.18
475	Amaranth	5	37.38	38.42	22.75	24.32
476	Amaranth	5	36.81	37.57	24.64	24.74
477	Amaranth	3	38.48	37.63	26.12	26.05

478	Abitulon threophrasti	5	N/A	N/A	24.45	24.6
479	Amaranth	5	N/A	N/A	29.36	29.86
480	Abitulon threophrasti	5	37.77	N/A	20.47	20.34
481	Amaranth	5	38.48	36.92	22.86	23.1
482	Asclepias fascicularis	5	N/A	N/A	N/A	N/A
483	Horseweed	1	N/A	N/A	29.55	29.35
484	Amaranth	5	N/A	39.06	23.99	23.95
485	Amaranth	2	39.21	38.87	29.01	29.13

	CGMMV		COX	
	Cq 1	Cq 2	Cq 1	Cq 2
CGMMV+	12.41	12.52	25.23	25.21
water	36.51	36.59	N/A	N/A