Ecological and Epidemiological Factors Affecting Carrot Motley Dwarf Development in Carrots Grown in the Salinas Valley of California

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ABSTRACT

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The geographic and temporal incidence of carrot motley dwarf (CMD) and the partial host ranges of the CMD viruses and their aphid vector, *Cavariella aegopodii*, were investigated. The CMD viruses—carrot redleaf luteovirus and carrot mottle virus—and *C. aegopodii* were found to have limited host ranges that overlap in carrot but in no other plant species growing in the Salinas Valley. Field studies assessing the incidence of CMD in spring carrots revealed that CMD development was closely associated with overwintered carrot fields. Little to no CMD developed in spring fields that were distant from overwintered carrot fields or when no overwintered carrot fields were present. Susceptibility of carrot cultivars to CMD ranged from good resistance to extreme susceptibility. These data suggest that time of planting, location in relation to overwintered carrot fields, and carrot cultivar are all important factors in disease development.

Carrot motley dwarf (CMD) is a viral disease complex comprising carrot redleaf luteovirus (CRLV) and carrot mottle virus (CMoV) (13). CMD affects carrots (*Daucus carota* L.) and other members of the Umbelliferae family. The disease has been reported to occur worldwide, wherever carrots are grown in cool conditions (10). CRLV is a putative member of the luteovirus group and is transmissible by the willow-carrot aphid (*Cavariella aegopodii* Scop.) in a circulative, nonpropagative manner (5). CMoV is the type member of the umbravirus group and is mechanically transmissible (13). However, when plants are coinfected with CRLV and CMoV, as in the CMD complex, *C. aegopodii* can transmit both viruses (5,14).

CMD was first described in the Salinas Valley region of California in 1956 and has caused severe crop losses (8,10,11). Reddening, yellowing, and stunting (Fig. 1) make infected plants, especially those infected at a young age, unmarketable. Carrots are planted from December through July; those planted in late winter to early spring are the most severely affected, and those planted later are not seriously affected. Currently, no practices successfully control CMD in early carrots.

The epidemiology of CMD in California carrots has not been previously studied. We used serological (enzyme-linked immunosorbent assay [ELISA]) and biological (aphid transmission) assays, along with mapping studies, to follow and characterize CMD development in carrot crops. In addition, we evaluated weed and crop plants as possible hosts for the CMD viruses.

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MATERIALS AND METHODS

Insect vector maintenance and virus transmission. Carrot plants naturally infected with the CMD viruses were obtained from fields in the Salinas Valley. Nonviruliferous *C. aegopodii* were reared on healthy carrots (cv. Six Pak II) in a climate-controlled room at 24 C and with 16 hr of light. The aphids were allowed to feed on virus-infected leaves for 24 hr, then were transferred to caged seedlings for a 48- to 72-hr inoculation access period. At the completion of this period, the cages were removed and the plants were sprayed with the insecticide permethrin (Pounce). The plants were then transferred to a shaded, insect-proof greenhouse with ambient temperature.

Virus host range. Carrots inoculated by viruliferous aphids were maintained under the same conditions as those used for virus transmission studies. The host range study included, in addition to carrots, fennel (*Foeniculum vulgare* Mill.), celery (*Apium graveolens* L.), chervil (*Anthriscus cereifolium* (L.) Hoffm.), cilantro (*Coriandrum sativum* L.), *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Nicotiana benthamiana* Domin., *N. clevelandii* Gray, *N. edwardsonii* Christie & Hall, parsley (*Petroselinum crispum* (Mill.) Nym. ex A.W. Hill), and poison hemlock (*Conium maculatum* L.). For CMoV, both aphid transmission (with CRLV) and mechanical (sap) inoculation methods were used. The CMoV partial host range was determined by grinding leaves from infected plants in 2× GKP buffer (0.1 M K_2HPO_4, 2.5% Celite, 0.5% bentonite, 2.5% pyrophosphate, pH 8.5) (3) and by mechanically inoculating leaves of healthy young test plants. Fourteen days post inoculation, test plants were assessed for symptoms and assayed by back-inoculation to indicator plants (cilantro).

The CRLV and CMoV partial host ranges were also determined by aphid

transmission. Viruliferous aphids were used to inoculate plants, which were kept in the same greenhouse growing conditions as those used for the virus transmission studies. The plants were examined visually for symptom development and by ELISA.

Insect vector host range. To determine the partial host range of *C. aegopodii*, 10 aphids were placed on three plants each of fennel, carrot, celery, chervil, cilantro, parsley, and poison hemlock and allowed to multiply for 2 wk under the same conditions as those used to rear aphids. The number of aphids was then determined and the average number per plant calculated. This experiment was repeated twice.

Field sample collection and CMD incidence. Carrot fields surveyed in the Salinas Valley included any overwintered fields from the previous growing season, newly planted fields adjacent to overwintered fields, and fields at various distances and directions from the overwintered fields. In addition, other potential crop (celery, cilantro, parsley) and weed (fennel, poison hemlock) hosts were assayed. For the 1991 growing season, 500 leaves were collected at random from each of the fields and the percentage of symptomatic leaves was determined. Leaves were also chosen at random for use in bioassay studies (aphid transmission) to verify results obtained by visual assessment of symptoms. For the 1992 growing season, 50–60 leaves were collected at random from each of the fields surveyed and samples were examined by bioassay and ELISA. Plants from two overwintered fields from the 1991 growing season were also subjected to ELISA and bioassay. The fields from which the samples were collected for both years were depicted on a map of the entire growing region, along with the planting date, date of assay(s), proximity of overwintered fields, and percentage of visual symptoms. Overwintered fields from the 1992 growing season were examined by ELISA in January 1993; 50 leaves were collected and assayed.

Virion purification. Cilantro plants infected with both CRLV and CMoV were harvested 2–3 wk post inoculation and used for virion purification. Virions were extracted from 300 g of fresh tissue by a modified method for luteovirus virion purification (2,9,12).

Antisera production. Antisera to the CRLV capsid protein were produced by administering virions purified from plants infected with the CMD viruses in three intramuscular injections of 100 μg each to a New Zealand white rabbit. The first injection was of virions emulsified with an equal volume of Freund's complete adjuvant, and the second and third injections, given at 2-wk intervals after the first, were of virions emulsified with an equal volume of Freund's incomplete adjuvant. The immunized rabbit was bled 10 days after each injection.

Serological assays. Serological assays were conducted using double antibody sandwich ELISA as described by Clark and Adams (1). Antisera were cross-absorbed using a 1:50 dilution of healthy sap, and IgGs were purified using protein A-Sepharose columns. IgGs and alkaline phosphatase-conjugated IgGs were prepared from CRLV antisera and used at 1 μg/ml and 1/250 dilution, respectively. Plant extracts were prepared by grinding leaf tissue in a leaf press using a PBST-PVP-OVA (1× phosphate buffered saline, 0.05% Tween 20 [v/v], 2% polyvinylpyrrolidone 40 [w/v], 0.2% ovalbumin [w/v], pH 7.4) grinding buffer at a 1:5 (w/v) ratio. Healthy plant tissue was used as a control, and the ELISA threshold was determined by calculating the mean plus three times the standard deviation of the absorbance (A_{405}) value of the healthy samples (eight per plate).

Cultivar screening. Forty-four carrot cultivars, including nine commonly grown in the Salinas Valley, were screened for CMD susceptibility or resistance by inoculating 10 3-wk-old plants of each cultivar via aphid transmission. The plants were placed in a constant-temperature growth chamber at 16 C for 21 days, at which time the plants were rated visually on a scale of 0 = no symptoms to 10 = most severe symptoms (reddening, yellowing, and stunting). For plants showing few or no symptoms, aphid transmissions were done back to indicator plants (cilantro) to confirm virus infection.

Fig. 1. Carrot plants in the Salinas Valley with reddening, yellowing, and decreased size, typical symptoms of carrot motley dwarf.

Table 1. Partial comparative host range for carrot redleaf luteovirus (CRLV) and carrot mottle umbravirus (CMoV)

Host	Aphid transmission[a]		Mechanical inoculation[b]
	CRLV	CMoV	CMoV
Fennel (*Foeniculum vulgare*)	–[c]	–	–
Carrot (*Daucus carota*)	+	+	–
Celery (*Apium graveolens*)	–	–	–
Chervil (*Anthriscus cereifolium*)	+	+	–
Cilantro (*Coriandrum sativum*)	+	+	+
Chenopodium amaranticolor	–	–	–
C. quinoa	–	–	–
Nicotiana benthamiana	–	–	–
N. clevelandii	–	+	+
N. edwardsonii	–	–	–
Parsley (*Petroselinum crispum*)	–	–	–
Poison hemlock (*Conium maculatum*)	–	–	–

[a] Willow-carrot aphids (*Cavariella aegopodii*) were allowed 24-hr acquisition access periods on plants infected with both CRLV and CMoV, then 72-hr inoculation access periods.
[b] Plants were sap-inoculated with plant tissue infected with CRLV and CMoV and ground in 2× GKP buffer.
[c] + = Host and – = nonhost for virus.

RESULTS

Host range. To compare the abilities of CRLV and CMoV to cause disease symptoms and to identify plant species that could serve as reservoirs of these viruses, a partial host range study was done (Table 1). Generally, 100% of the test plants of a susceptible host species

were infected in aphid transmission studies. Only *N. clevelandii* and cilantro could be infected by mechanical inoculation and only by CMoV; also, only 10–15% of the test plants were infected per experiment.

Aphid transmission of the CMD viruses showed that carrot, chervil, and cilantro were host plants for CMD. Interestingly, we were not able to infect parsley plants under greenhouse conditions, although one field sample yielded the CMD viruses. Plants infected with both viruses showed severe systemic discoloration (reddening and yellowing), mottling, and stunting (Fig. 1). Symptoms induced in plants infected only by CMoV were less severe than those induced by both viruses. CMoV alone induced systemic yellowing and mottling 12–16 days post inoculation in *N. clevelandii* and cilantro plants. No plants were infected only by CRLV.

Insect vector host range. To determine which plants might serve as reservoirs for the aphid vector in and around carrot fields in the Salinas Valley, a partial host range of *C. aegopodii* was identified. After the 2-wk test period, carrots were found to be the best host for *C. aegopodii* (Fig. 2); celery, parsley, and fennel were also good aphid hosts. Chervil was a good host for the aphids as well as the viruses but is not grown commercially in the Salinas Valley.

Cultivar screening. The effects of CMD varied among carrot cultivars both in natural field conditions and under controlled growth chamber conditions. The difference often was dramatic (Table 2), ranging from no symptoms in cv. Boston to reddening, yellowing, and stunting in cv. Danvers. Of the nine cultivars commonly grown in the Salinas Valley, Danvers was the most susceptible and Imperator 58 and CVC-14, an Imperator type, were the most resistant.

Field sample collection and CMD incidence. Of the 40 spring carrot fields surveyed for CMD incidence during the 1991 growing season, 23 are shown in Figure 3A. Ten fields had high levels (>20%) of infected plants; 100% of the plants were symptomatic in six of these fields and 50% were symptomatic in two. Two of the fields with 100% symptomatic plants were plowed and replanted in April 1991; when plants from these fields were visually assessed on 12 June 1991, none was infected. Plants from all 40 fields were assessed visually, and those from 10 fields were also examined by bioassay (aphid transmission) to confirm data obtained by visual assessment. Bioassay data corresponded perfectly with data from the visual assays.

Analysis of the field data indicated that the fields with CMD-affected carrots were all planted between December 1990 and February 1991; in other fields planted during this same time period, however, the incidence of CMD was low (Fig. 3A). Further surveys showed that all the CMD-affected fields were either adjacent to or directly south (downwind) of the overwintered fields. Plants in the five overwintered fields showed typical CMD symptoms and 100% were infected with the CMD viruses, as confirmed by bioassay. Carrots in these overwintered fields were present in January 1991 when the new crops began to emerge. Carrots planted in the same area as the severely affected fields but later in the growing season (i.e., April) showed much lower levels (<10%) of infection.

During the 1992 growing season, very little CMD was observed in winter/spring carrots (Fig. 3B). However, there were four overwintered carrot fields from the 1991 growing season. One of these overwintered fields was approximately 40 miles north of the main carrot production area, and there were no other carrot fields within 10 miles. Plants in this field were not affected by the CMD viruses, whereas plants in the other three

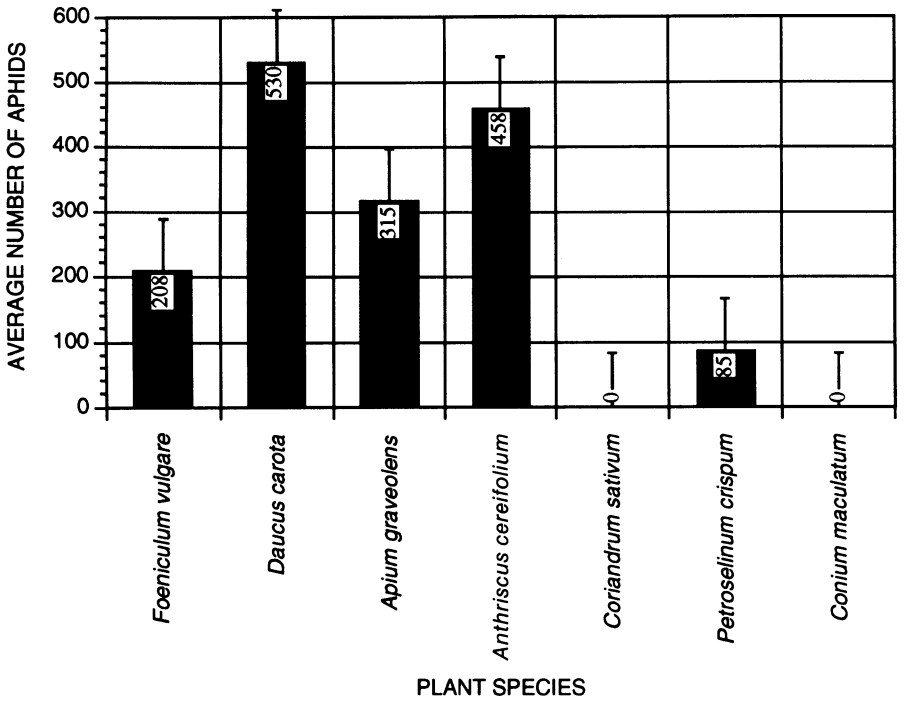

Fig. 2. Partial host range of the willow-carrot aphid (*Cavariella aegopodii*). Ten aphids were placed on three plants of each species, and the plants were kept caged at 20 C and 16 hr of light for 2 wk, at which time the number of aphids was counted. The average number of aphids per plant after two repetitions of the experiment is shown for each species.

Table 2. Results of carrot cultivar screening for resistance or susceptibility to carrot motley dwarf[a]

Cultivar[b]	Disease severity rating[c]
Boston	0
Nansen	0
Apache	2
Bolero	2
Caro Best	2
Condor	2
Cosmos	2
CVC-14*	2
Dominator	2
FMX 350	2
Imperator 58*	2
Navajo	2
Niz	2
Plato	2
Tino	2
Vita Sweet	2
Berdie	4
Bertan	4
Caro Pack	4
Cello King	4
Daybreak	4
FMX 268	4
FMX 291	4
Goldmine	4
HMX 5280	4
Nantes*	4
Narmen	4
Nice	4
Orlando Gold	4
Primo	4
Top Pak	4
Vilm	4
Avenger	6
Carrot Seed Pak*	6
Chantenay	6
Estelle	6
Huron	6
Bang*	8
Beta III*	8
Gold King*	8
Flame	8
Sierra*	8
Six Pak II	8
Danvers*	10

[a]Ten plants of each cultivar were aphid-inoculated with the CMD viruses, kept in a constant-temperature growth chamber (16 C) for 21 days, and then evaluated.
[b]* = Commonly grown in the Salinas Valley.
[c]On a scale of 0 (no symptoms) to 10 (reddening, yellowing, and stunting).

overwintered fields showed high levels (88, 92, and 100%) of infection (Fig. 3B). Visual inspection also showed that plants in the field with 100% infection were colonized by *C. aegopodii*. The only significant CMD incidence occurred in the new fields planted adjacent to this field. Because our previous data suggested a relationship between overwintered carrot fields and CMD incidence in spring carrots, growers sprayed plants in the other two fields with an insecticide in early January to reduce or eliminate the potential aphid vectors. No CMD occurred in adjacent spring-planted carrots. As a result, the only fields significantly affected by CMD in the 1992 growing season were the three planted immediately adjacent to the 100%-infected untreated field (Fig. 3B).

For both growing seasons, potential weed and alternate crop hosts were also tested for presence of the viruses. The only other species infected by the CMD viruses were cilantro plants grown in the vicinity of carrot fields. None of the other plants found in the vicinity of carrot fields, including fennel, poison hemlock, parsley, and celery, contained the viruses.

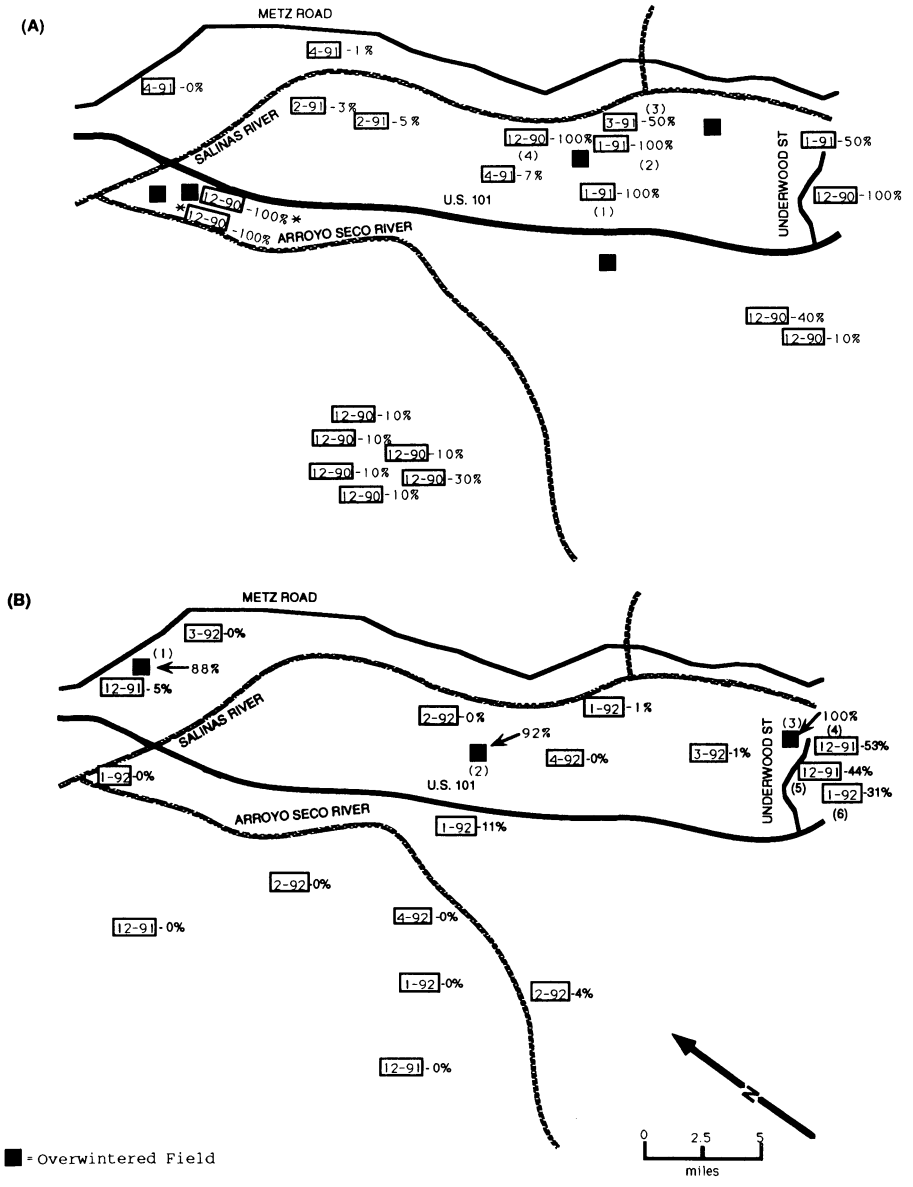

Fig. 3. (A) Salinas Valley carrot fields for the 1991 growing season. Five hundred leaves were collected from each field and examined visually for symptoms. The location, the month of planting (in box), and the percentage of symptomatic leaves are indicated for each field. Two fields that were plowed and replanted and subsequently had 0% symptomatic plants are indicated by an asterisk. Numbered fields (in parentheses) are examples of those that were planted either adjacent to or south of overwintered fields and had disease incidences of >50%. **(B)** Salinas Valley carrot fields for the 1992 growing season. Fifty to 60 leaves were collected from each field and examined by ELISA and bioassay. The location, the month of planting (in box), and the percentage of infected leaves are indicated for each field. Overwintered fields 1 and 2 were sprayed with an insecticide to control aphid populations, and overwintered field 3 was not sprayed. Fields 4–6 were the only ones with disease incidences of >11% for the growing season.

DISCUSSION

In California, CMD is limited to the Salinas Valley and the cool, coastal carrot-growing regions. Because the disease is most destructive to young plants grown during cool, low-light intensity conditions, the late winter and early spring conditions are ideal for CMD development (5).

The data given here suggest that a primary factor affecting CMD development and spread is the number and proximity of overwintered carrot fields to newly planted early spring fields. Weeds or other crops do not seem to be significant sources for CMD spread. Host range studies on both the viruses and the aphid vector indicated that carrots were the best host for both. Other plant hosts for the viruses either were not found in the Salinas Valley (e.g., chervil) or were not good hosts for the aphid vector (e.g., cilantro). Although cilantro was a good host for both CMD viruses, aphid transmission of the viruses from infected plants was inefficient because most aphids died during the acquisition access period (*data not shown*). Plant species that were found to be good hosts for the aphid vector either were not found in the Salinas Valley (e.g., chervil) or were not hosts for the viruses (e.g., celery, parsley, and fennel). Thus, of the species tested and observed to occur in the carrot-growing region, carrots were the best host for both the viruses and the aphid vectors. Carrot crops were assayed each month during the growing season and showed increasing percentages of infection in late fall to early winter (*data not shown*). During late spring to summer, carrot plants showed a low level of infection. Overwintered carrot crops located in the carrot-growing region always showed a high percentage of plants infected by the CMD viruses, although they did not show effects of the disease.

When data from the 1991, 1992, and early 1993 growing seasons were compared, a decrease in the number and severity of CMD-affected fields was seen during the later two seasons. The major differences between the growing seasons was the number of overwintered fields and the location of these fields in relation to the newly planted fields, along with the levels of *C. aegopodii* in the overwintered fields. Each of the five overwintered fields in 1991 contained high levels of aphids and CMD. In 1992, however, all but one of the overwintered fields were sprayed with insecticide, and high levels of CMD developed only in new carrots adjacent to the unsprayed field. Each of the five overwintered fields from the 1992 growing season, present as new plants began to emerge for the

1993 growing season, was located in the southern end of the valley. Samples from two of these overwintered fields assayed in January 1993 indicated a 98% infection rate, and each field was treated with insecticide to eliminate the aphid population. Three carrot fields were newly planted adjacent to the overwintered carrot fields, and these showed a CMD incidence of <0.5% when assayed in March 1993.

All these data suggest that carrots are a major factor in CMD disease development in the Salinas Valley. Similar results were found for CMD in central Washington, where volunteer carrots and carrots grown for seed provided a year-round cycle of hosts for the CMD viruses and thus perpetuated the disease cycle (7). This situation is also similar to that found with celery mosaic potyvirus (CeMV) in California celery and beet mosaic potyvirus (BtMV) in California sugar beets (4,6). For both of these viruses, the major sources of primary inoculum were the overwintering crops or crop residues left in the fields (6). Similar to CMD, CeMV has a host range limited to the Umbelliferae (6). Also similar to CMD, the aphid vector of CeMV is closely linked to celery in the disease cycle (6). A celery-free period was developed in California to break the disease cycle and therefore control the disease (6). With BtMV, distance of newly planted fields from old fields was found to play an important role in disease development, and planting new fields at a greater distance from overwintered fields gave good control (4). However, a period during which no overwintered crops were left in the ground (i.e., a beet-free period) provided a more practical approach for disease management (6).

Our data collected over 3 yr show that the carrot plants present in fields from November through January are nearly 100% infected and therefore pose a significant risk to newly planted fields. Ideally, the number of overwintered fields could be limited and the newly planted fields could be grown a substantial distance from the overwintered fields. If planting at a certain time of year and close to overwintered fields is necessary, more resistant cultivars could be used to reduce the risk of a CMD epidemic developing. This possible control strategy was evident in field situations. Entire fields of the cultivar Danvers that were planted early in the growing season and close to overwintered carrot fields were severely affected by CMD. When one of the more resistant cultivars, such as CVC-14, was planted adjacent to Danvers, the differences in CMD effects were sometimes dramatic. Although the CVC-14 plants were infected and showed symptoms, they were not devastated, as were the Danvers plants. This strategy is not always practical, however, because most growers are contracted to grow a specific amount of each cultivar each year, which could prevent planting large amounts of certain cultivars. If necessary, control of the aphid population in the overwintered carrots could serve to reduce or eliminate the potential for widespread development of CMD.

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