Identification, Seed Transmission, and Host Range Pathogenicity of a California Isolate of Melon Necrotic Spot Virus

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ABSTRACT

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A 30-nm diameter isometric virus with host range restricted to the Cucurbitaceae was isolated from declining muskmelons. Systemic infections were produced only in *Cucumis melo* and *C. anguria* var. *longipes*. The virus had a dilution end point of 10^{-4}, a thermal inactivation point of 60 C and its longevity in vitro was 32 days. The virus was seed transmitted in commercial melon seeds and in an aphid-resistant melon inbred line. No symptoms were produced and virus could not be recovered from the top leaves in 8.8% of 78 melon lines, and hypersensitive reaction without systemic spread of virus was observed on 53.2% of those lines. The remaining 38% were systemically infected by the virus. Studies of host range, symptomatology, physical properties, transmission, and serology indicated that the virus is a strain of muskmelon necrotic spot virus.

During an extensive breeding program designed to incorporate aphid resistance from a naturally occurring resistant muskmelon *Cucumis melo* L. into cultivars, a mechanically transmissible virus causing decline and premature death of the aphid-resistant parent and hybrid plants was isolated. Sanitation measures and careful cultivation practices had little effect on the incidence of the disease. Natural infections with typical systemic symptoms were observed only in older plants and only in one or two cultivars in mixed plantings grown in isolation in insect-free greenhouses; those observations suggested that the causal agent was seedborne with no symptom expression in young plants. The virus also caused a shock reaction and death in plants of Golden Beauty casaba melon (GBC).

Complications caused by a seed-transmitted virus in the breeding program, as well as the potential for dissemination of an apparently new virus disease of melons in California, motivated this study. This paper describes the identification of the virus as a strain of muskmelon necrotic spot virus (MNSV) (8), the seed transmission of this virus, and sources of resistance to the virus in *Cucumis melo* cultivars, breeding lines, and plant introductions.

MATERIALS AND METHODS

Virus sources and inoculation procedures. The California strain of muskmelon necrotic spot virus (MNSV-C) was isolated from melon breeding lines grown in greenhouses in the USDA FR/SEA Boyden Entomological Lab., Riverside, CA. The virus was mechanically transmitted to GBC melon which served as the virus donor plant.

Virus inoculum was prepared by grinding inoculated cotyledons or systemically infected young top leaves of GBC melon with a mortar and pestle in 115 mM K_2HPO_4 (1 g of tissue per 2 ml of K_2HPO_4 solution), and filtering the brei through a double layer of cheesecloth. All infectivity assays were performed by rubbing corundum-dusted leaves of Crimson Sweet watermelon with cotton swabs wetted with inoculum.

Host range. Four of a group of six plants from each host species tested were sap inoculated. AT eight and 30 days after inoculation, pooled samples from inoculated leaves and separate pooled samples from uninoculated leaves of each test species were back-inoculated to watermelon.

Physical properties of MNSV-C in crude sap. Thermal inactivation point, dilution end point, and longevity in vitro of crude sap were determined according to the procedures of Ross (11).

Virus purification. Tissue from GBC melon plants inoculated at the first-leaf stage and harvested when they became systemically infected (8 to 15 days) was used for virus purification. Tissue was homogenized in a Waring Blendor in 68 mM K_2HPO_4, 5 mM ethylenediaminetetraacetate (EDTA), 13 mM mercaptoethanol, and 0.5% bentonite (2 ml of solution per gram of tissue). The liquid was expressed through a double layer of cheesecloth and centrifuged for 10 min at 10,000 rpm in a Sorvall SS-34 rotor. Further clarification was performed by lowering the pH of the supernatant fluid to 5.2 with concentrated HCl and letting it stand for 2 hr at 5 C, or by incubating the extract overnight with 8.5% butanol (v/v) followed again by centrifugation for 10 min at 10,000 rpm in the same rotor.

The resulting low speed supernatant fluid was centrifuged for 2 hr at 30,000 rpm in a Beckman No. 30 rotor. Pellets were resuspended overnight in 25 mM potassium phosphate buffer containing 2 mM EDTA, pH 7.2. One volume of fractionated bentonite (10 mg/ml) prepared according to Fraenkel-Conrat et al

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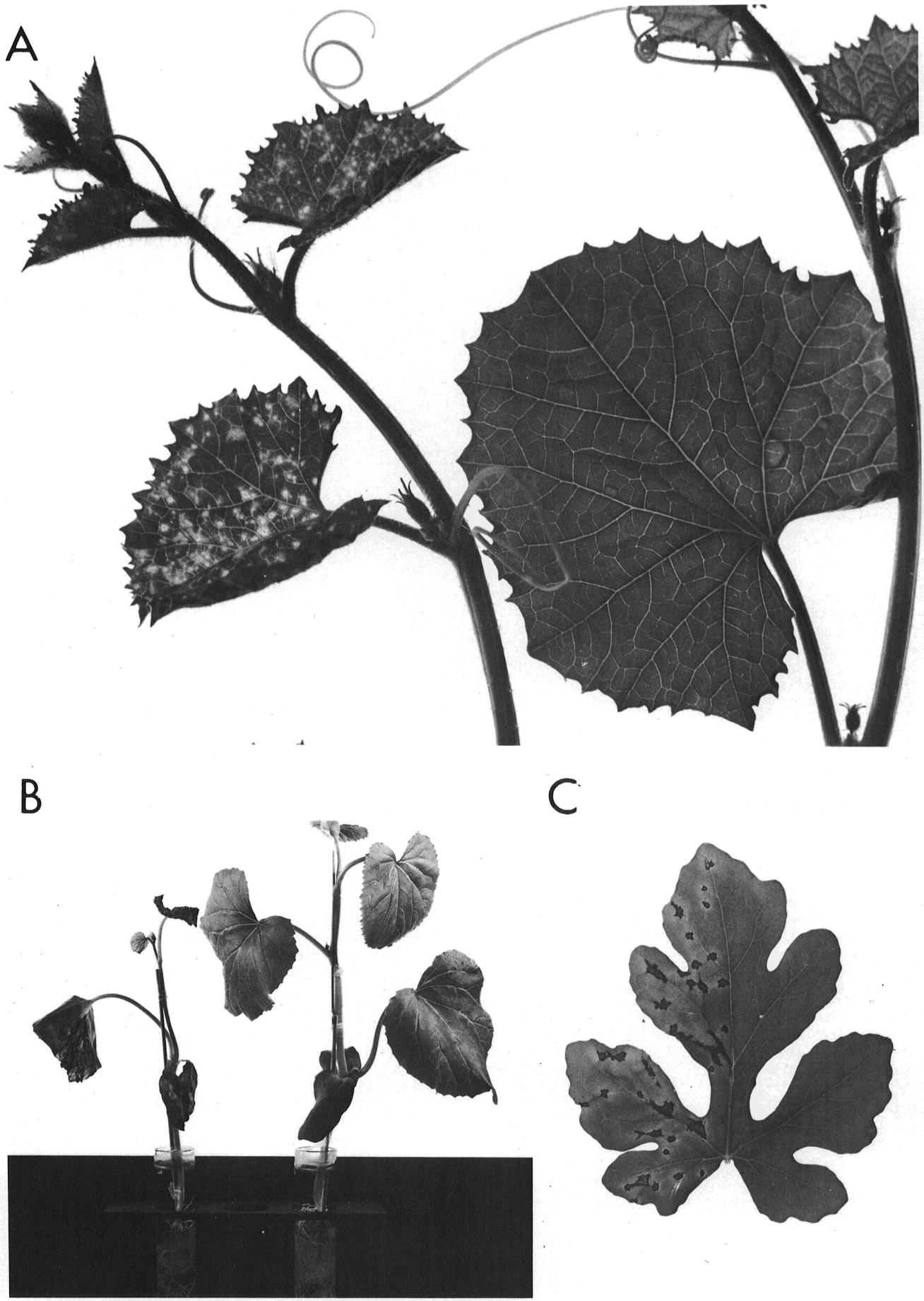

Fig. 1. Symptoms induced by muskmelon necrotic spot virus, California isolate (MNSV-C) in melons. **A)** (left) Systemic symptoms in MNSV-C inoculated Golden Beauty casaba (GBC); (right) uninoculated GBC. **B)** (left) Shock reaction of GBC melon seedling inoculated with MNSV-C at cotyledon stage; (right) uninoculated GBC melon. **C)** (left side) Local lesions induced by MNSV-C in watermelon leaf; (right side) uninoculated.

(6) was mixed with two volumes of the resuspended pellet solution, shaken, and centrifuged 10 min at 10,000 rpm in a Sorvall SS-34 rotor. High speed centrifugation followed by bentonite treatment was repeated and the final low speed supernatant fluid was layered on 20 to 50% (w/v) linear sucrose gradients and centrifuged at 27,000 rpm for 5 hr at 25 C in a Beckman SW 27 rotor. Virus-containing zones were collected with an ISCO density gradient fractionator, diluted with an equal volume of double distilled water, and concentrated by centrifugation at 30,000 rpm in a Beckman No. 30 rotor for 2.5 hr. The pellets were resuspended in double distilled water, mixed with one-half volume of fractionated bentonite, and centrifuged at low speed as before. The resulting supernatant fluid was purified further by repeating the sucrose gradient centrifugation and ultracentrifugation as previously described. The final virus pellets were resuspended in double distilled water and stored at 5 C if not used immediately.

Electron microscopy. Electron micrographs of purified MNSV-C preparations stained either with 2% phosphotungstic acid or 2% aqueous uranyl acetate were made with a Hitachi Model HU-12 electron microscope operating at 75 kV.

Serology. Antiserum to MNSV-C was produced in New Zealand white rabbits. Two intramuscular injections, each containing 0.7 mg in 1 ml of double distilled water emulsified with 1 ml of Freund's complete adjuvant, were given 6 days apart followed by one intravenous injection containing 0.25 mg of virus 6 days after the second intramuscular injection. Serum was collected 6 and 10 days after the intravenous injection and the titer was determined by the ring interphase precipitation test (2). Antisera against cucumber mosaic virus, squash mosaic virus, and wild cucumber mosaic virus was supplied by R. N. Campbell, turnip yellow mosaic virus antiserum by P. R. Desjardins, and muskmelon necrotic spot virus antiserum by Y. Saito. Serological relationships were observed in Ouchterlony agar double diffusion tests using 0.6% agarose dissolved in phosphate (10 mM)-buffered saline and containing 0.1% sodium azide.

Seed transmission tests. Seed transmission tests were performed in steam-sterilized, recycled, peat-vermiculite potting mix as described by Boodley and Sheldrake (5). One hundred plants each from a commercial GBC melon seed stock and from the aphid-resistant inbred line 91213, were used for each test. Plants were isolated from each other and protected from insects by covering with air-inflated and ventilated plastic bags as described by Bartlett and Katz (3). Plants were watered with Ward's solution, micronutrient concentration at 0.1 M (13), as needed and leached

TABLE 1. Reaction of plants to inoculation with a California isolate of muskmelon necrotic spot virus

Systemic infection
- Cucurbitaceae:
  - *Cucumis anguria* var. *longipes* (Hook, F.) Meeuse (PI 282442)
  - *C. melo* L. 'Honeydew'
  - *C. melo* 'Golden Beauty' casaba
  - *C. melo* var. *makuwa* 'Nanbu-kin' (91492)
  - *C. melo* var. *makuwa* 'Large Green', 'ao-a' (91270)[y]
  - *C. melo* var. *makuwa* 'Ginsen', 'Silver Spring'
  - *C. melo* var. *makuwa* 'White Skin Pear', 'Shirokawa Nashi'

Localized infection
- Cucurbitaceae:
  - *Benincasa hispida*[z]
  - *Citrullus fistulosus* (PI 164460)
  - *C. fistulosus* Stocks (PI 374216)
  - *C. lanatus* (Thunb.) Matsum. & Nakai 'Crimson Sweet'
  - *C. lanatus* 'Klondike'
  - *Cucumis africanus* L. f. (PI 374151)
  - *C. dinteri* Cogn. (PI 374209)
  - *C. dipsaceus* Ehrenb. ex Spach (PI 193498)
  - *C. hardwickii* Royle (PI 374209)
  - *C. heptadactylus* Naud. (PI 282446)
  - *C. leptodermis* Schweick. (PI 282448)
  - *C. melo* 'Crenshaw'
  - *C. melo* 'Honeydew Green Flesh'
  - *C. melo* var. *conomon* 'Enzie 1'
  - *C. melo* var. *makuwa* 'Silver' (91272)
  - *C. metuliferus* E. Mey. ex Naud. (PI 202681)
  - *C. myriocarpus* Naud. (PI 420149)
  - *C. meeusii* C. Jeffrey (PI 376068)
  - *C. sativus* L. 'Ashby'
  - *C. sativus* 'Improved Long Green'
  - *C. sativus* 'National Pickling'
  - *C. sativus* 'Palace Pride'[z] ('Oriental Pickling'-type)
  - *C. zeyheri* Sond. (PI 282450)
  - *Momordica balsamina* L.

Immune
- Amaranthaceae:
  - *Gomphrena globosa* L.
- Balsaminaceae:
  - *Impatiens balsamina* L.
- Chenopodiaceae:
  - *Chenopodium amaranticolor* Coste & Reyn.
  - *C. quinoa* Willd.
- Compositae:
  - *Ageratum* sp.
  - *Calendula officinalis* L.
  - *Zinnia elegans Jacq.*
- Cruciferae:
  - *Alyssum murale* Waldst. & Kir.
  - *Brassica nigra* L.
  - *B. oleracea* L. 'Green Ball'
- Cucurbitaceae:
  - *Cucumis* sp. (PI 273650)
  - *C. anguria* L. (PI 196477)
  - *C. prophetarum* subsp. *dissectus* (PI 193967)
  - *Cucurbita maxima* Duch. 'Buttercup'
  - *C. maxima* 'True Hubbard'
  - *C. pepo* L. 'Cocozelle Bush'
  - *C. pepo* 'Golden Summer Crookneck'
  - *C. pepo* 'Golden Zucchini'
  - *C. pepo* 'Hybrid Zucchini'
  - *C. pepo* 'Small Sugar'
  - *Lagenaria siceraria* (from Peru)
  - *L. siceraria* (from Mexico)
  - *Luffa aegyptiaca* Mill.
- Leguminosae:
  - *Glycine max* (L.) Merr.
  - *Phaseolus vulgaris* L. 'Pinto'
  - *P. vulgaris* 'Speckled Butter'
  - *Pisum sativum* L. 'Alderman'
  - *P. sativum* 'Bijou' sweet pea
  - *P. sativum* 'Royal' sweet pea
  - *Vicia faba* L.
  - *Vigna unguiculata* (L.) Walp. 'Black Eye'
- Scrophulariaceae:
  - *Antirrhinum majus* L.
- Solanaceae:
  - *Capsicum annuum* L. 'Mexican chili'
  - *Datura stramonium* L.
  - *Lycopersicon esculentum* Mill. 'Rutgers'
  - *Nicotiana glutinosa* L.
  - *N. tabacum* L. 'Turkey'
  - *N. tabacum* 'Xanthi'
  - *Physalis pubescens* L.
- Umbelliferae:
  - *Coriandrum sativum* L.

[y] Progeny number assigned by G. W. Bohn.
[z] Inoculated leaves showed no symptom expression, but virus was recovered by assay on watermelon.

with tap water once a week.

Response of *Cucumis melo* cultivars, breeding lines and plant introductions to inoculation with MNSV-C. Seventy-eight entries, including the most popular cultivars, breeding lines, and plant introductions of *Cucumis melo* were inoculated. Plants were grown in silica sand, five plants per 10 cm diameter paper pot with five replicates. Plants were fertilized daily with Ward's solution and leached once a week with tap water.

The first inoculation was made when plants were at the one- or two-leaf stage. Plants were thinned to one per pot 20 days later. Inoculations were repeated at intervals of 30 and 45 days. Pooled samples from each genotype were assayed on watermelon 30, 60, and 90 days after the first inoculation. Some healthy-appearing plants were maintained in screened cages for seed production and were assayed 5 mo after the first inoculation. The experiment was repeated and test plants were assayed once for systemic infection.

RESULTS

Symptomatology and host range. Local lesions on the inoculated leaves, followed by systemic chlorotic spotting (Fig. 1-A) and severe stunting, were the initial symptoms of the disease. The chlorotic leaf pattern became necrotic with age, and finally veins, petioles, and stems developed necrotic streaks after which the shoot or the entire plant ultimately died. Two additional unique symptoms occurred that could be used for diagnostic purposes.

TABLE 2. Response of *Cucumis melo* muskmelon cultivars, accessions, and plant introductions to inoculation with a California isolate of muskmelon necrotic spot virus

Systemic infection-38%
- Netted-type melons:
  - Delicious 51 (Dessert 145-20)[a,b]
  - Dulce
  - Glabrous 45
  - Hale's Best Jumbo (Burpee 6827)[c]
  - Imperial 45 (Niagara 4945)[d]
  - Imperial 45 (Northrup-King W41129-R435)[e]
  - PMR 45 (Dessert 159-11R)
  - PMR 45 (Ferry-Morse 15120-11856)
  - Spartan Rock (Dessert)[a]
  - SR 91 (Ferry-Morse 11875)[f]
  - Top Mark (Dessert)
  - WMR Ms (male-sterile)[a]
  - WMR 29 (17013-M_1)
- Honeydew-type melons:
  - Gold Rind (Robinson 60202)[a,g]
- Persian-type melons:
  - Persian (70122 BSR S_3)
- Casaba-type melons:
  - Golden Beauty (Burpee 5247)
  - Golden Beauty (Burpee)
  - Golden Beauty (Dessert 142-18)
  - Golden Beauty (Dessert)
  - Golden Beauty (Hollar 7300)[h]
  - Golden Beauty (Northrup-King 41029-1-1)
  - Golden Beauty (Northrup-King 41029-R514)
  - Golden Beauty (Northrup-King 41032-60300)
  - Leopard (Dessert)
  - Santa Claus (Northrup-King 41029-60200)
- Exotic melons:
  - *Cucumis melo* var. *mormordica* (91164)
  - Deserta Naja (from Russia)
  - Earl's Favorite (from Japan)
  - Little's Chinese Cucumber (90637), PI 371795
  - PI 255478

Localized infection-53.2%
- Netted-type melons:
  - Ambrosia (Burpee 5016)
  - Armstrong (Dessert 3341)
  - Banana (Dessert 140-9)
  - Dessert Sun (Dessert 161-2)
  - Harvest Queen (Dessert 174-8)
  - Heart of Gold (Dessert 14922)
  - Honey Mist (Burpee 5128)
  - Honey Rock (Burpee 6063)
  - Iroquois (Northrup-King)
  - PMR 88 (Davis)
  - Top Mark (Northrup-King 42435-1215)
  - WMR Ms2 (male-sterile)
  - Zink's Bush
- Honeydew-type melons:
  - Baby Slip (Hollar 4695)
  - Green Flesh (Dessert 144-19)
  - Orange Flesh (Northrup-King 4117-20400)
- Casaba-type melons:
  - Armenian
  - Black Spanish (70102)
  - Crenshaw (Dessert 3819-17)
  - Crenshaw (Ferry-Morse 1539)
  - Crenshaw (Ferry-Morse 3909-11804)
  - Crenshaw (Ferry-Morse 7012-11804)
  - Crenshaw (Northrup-King 41041-R399)
  - Crenshaw (70111 BSR S_3)
  - Crenshaw (70112 BSR S_3)
  - Crenshaw (70113 BSR S_3)
  - Crenshaw (70114 BSR S_3)
  - Crenshaw (70116 BSR S_3)
  - Crenshaw (70117 BSR S_3)
  - Crenshaw (70118 BSR S_3)
  - Crenshaw (70119 BSR S_3)
  - Crenshaw (70120 BSR S_3)
  - Crenshaw (70121 BSR S_3)
  - Golden Canary (Dessert)
- Exotic melons:
  - Perfect Flower
  - Ojen (from Israel)
  - Earl's Favorite (from Japan)
  - PI 124111 (from India)
  - *Cucumis melo* var. *utilissimus* (from India)
  - Oriental cucumber
  - PI 145594 (from India)

Immune-8.8%
- Netted-type melons:
  - Improved Gulfstream
  - Perlita
  - Planters Jumbo
  - PMR 5
  - WMR 29 (16995-M_1)
- Honeydew-type melons:
  - PMR 61090

[a] Virus recovered from fruit flesh and/or top leaves 6 mo after inoculation.
[b] Dessert Seed Company.
[c] Burpee Seed Company.
[d] Niagara Seed Company.
[e] Northrup-King Seed Company.
[f] Ferry-Morse Seed Company.
[g] Robinson Seed Company.
[h] Hollar Seed Company.

Casaba type melons inoculated with MNSV-C at the cotyledon or one-true-leaf stage collapsed when the virus became systemic (Fig. 1-B). Crenshaw melons inoculated at the same stage showed mosaic symptoms on uninoculated top leaves. Virus could not be recovered from these symptomatic leaves either by direct assay on index plants or by extracting them for purification. However, virus could be recovered readily from inoculated leaves. Plants also showed a severe hypocotyl necrosis just below the inoculated leaves, which could cause death of the plant without upward systemic spread of the virus.

The host range of MNSV-C was restricted to the Cucurbitaceae. Twenty-seven species from eight other plant families were immune to the virus (Table 1). Systemic symptoms of MNSV-C were produced only on *Cucumis melo* (several cultivars) and *Cucumis anguria* var. *longipes*. Localized infections were produced on some *Cucumis*, *Citrullus* (Fig. 1-C), and *Luffa* spp.; on *Lagenaria siceraria* (collected from California [*L. siceraria* collected from Peru and Mexico were immune]); and on *Momordica balsamina*.

Physical properties of MNSV-C in sap. Sap extracted from infected GBC remained infective when heated at 60 C for 10 min but not at 65 C for the same time. Sap diluted to 10^{-4} remained infectious but not that diluted to 10^{-5}. Crude sap held for 32 days at room temperature also was infectious.

Purification of MNSV-C. Clarification by lowering the sap pH to 5.2 with concentrated HCl or extraction with 8.5% butanol, followed by two cycles of differential centrifugation and two cycles of sucrose density gradient centrifugation yielded 4 to 6 mg of virus per 100 g of infected GBC tissue. The higher yields were obtained from winter-grown plants. The virus sedimented as a single component in sucrose density gradient columns. Material collected from the UV-absorbing band produced typical systemic symptoms on GBC melon and local lesions on watermelon. Electron microscopic examination of virus preparations on carbon-backed grids stained with 2% aqueous phosphotungstic acid or uranyl acetate revealed the presence of isometric particles 30 nm in diameter (Fig. 2).

Serology. Antiserum with a titer of 1:1,024, as determined by the ring interphase precipitin test with a constant antigen concentration of 50 μg/ml, was obtained against MNSV-C. Immunodiffusion patterns of purified virus and extracts from healthy and infected GBC melon tissue tested against MNSV-C antiserum are shown in Fig. 3-A. MNSV-C antiserum also was tested in agar double diffusion against cucumber mosaic virus, squash mosaic virus, wild cucumber mosaic virus, turnip yellow mosaic virus, and tomato bushy stunt virus without any reaction. Likewise, antisera to cucumber mosaic virus, squash mosaic virus, wild cucumber mosaic virus, and turnip yellow mosaic virus failed to react with purified MNSV-C. Muskmelon necrotic spot virus (MNSV) antiserum, however, reacted with purified virus and virus-infected GBC melon tissue (Fig. 3-B). Serological relationships between MNSV-C and MNSV could not be determined because MNSV was not available.

Seed transmission studies. No diseased plants were observed until 64 days after planting in all three tests. Four of 98 plants of line 91213 and one of the 100 GBC melon plants showed definite systemic chlorotic-necrotic symptoms in the first test 68 to 75 days after planting. Inoculation of watermelon leaves with sap from all systemically infected plants induced typical black, spreading, necrotic local lesions (Fig. 1-C). In the second test, three of 100 GBC and none of 100 of the 91213 melon plants showed systemic symptoms. In the third test, plants of line 91213 expressed no symptoms, and the virus was not detected by infectivity assay 30 and 50 days after planting. However, six plants of 100 developed symptoms within 64 to 74 days after planting.

Response of *Cucumis melo* to inoculation with MNSV-C. Movement of the virus appeared to be erratic in the plants of many melon lines. Systemic symptoms appeared as early as 6 to 10 days or as late as 2 or 3 mo after first inoculation. In a few tests, virus was recovered from fruit flesh and/or the top leaves 6 mo after inoculation although the plants (Delicious #51 [Dessert Seed Co., 145-20], Spartan Rock [Dessert Seed Co.], and Honeydew Gold Rind [Robinson Seed Co., 60202]) showed no systemic symptoms. In other tests, systemic symptoms disappeared and virus no longer could be recovered from the plants of PI 255478 (91138) a bulk self of 90254.

Melons showing no symptoms when inoculated with MNSV-C were: cantaloupe cultivars Improved Gulf Stream, Perlita, Planters Jumbo, PMR 5, and breeding line WMR 29 (16995-M) and PMR honeydew line 61090-M (Table 2). All other lines or cultivars showed local lesions or systemic infection by the virus.

The data from both experiments indicate that 13 seed stocks of 32 netted melons; one of five honeydews; one of one Persian; 10 of 12 casabas; three of nine exotic melons, and two of four plant introductions were susceptible to systemic infection to MNSV-C (Table 2). The remaining stocks (45 of 79), reacted to infection by producing local lesions on inoculated leaves, but the virus did not spread systemically.

DISCUSSION

The host range of MNSV-C was restricted to the Cucurbitaceae. Several other viruses: Cucumber virus 3 (CV 3) and cucumber virus 4 (CV 4) (1) (both related serologically to tobacco mosaic virus [4]); bottle gourd mosaic virus (12); wild cucumber mosaic (9);

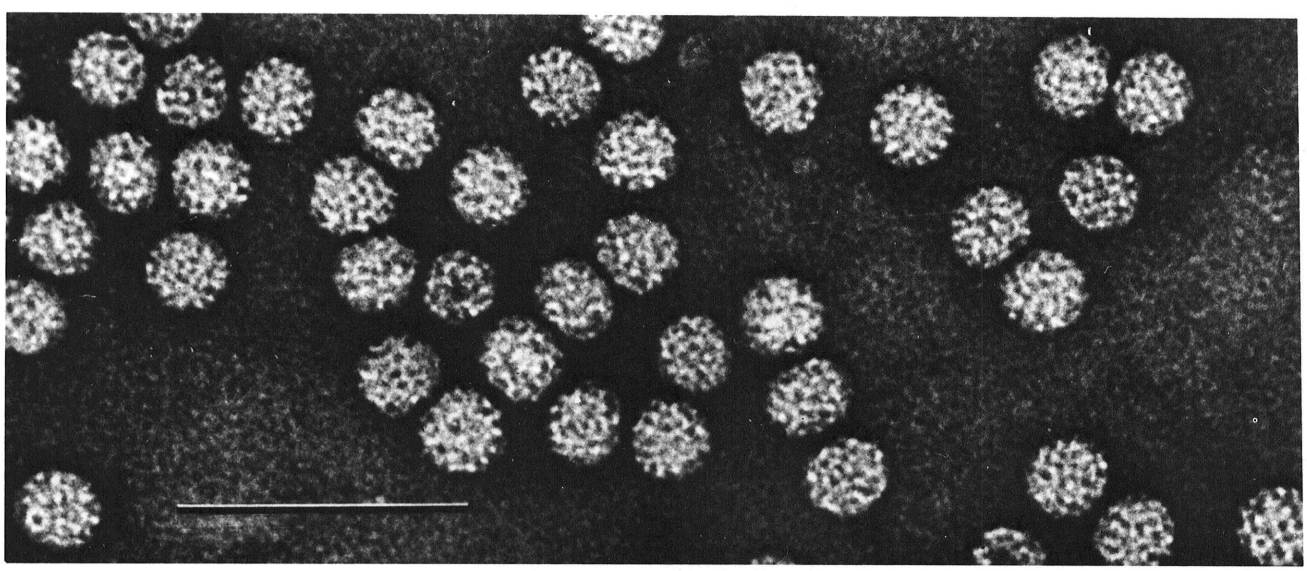

Fig. 2. Electron micrograph of muskmelon necrotic spot virus, California isolate (MNSV-C), particles purified from infected Golden Beauty casaba melon, stained with 2% phosphotungstic acid on carbon-backed grids. Bar represents 100 nm.

watermelon mosaic virus 1 (10,14); and muskmelon necrotic spot virus (MNSV) also are restricted to that family. Of the viruses that have been mentioned, only CV 3, CV 4, MNSV, and MNSV-C failed to infect *Cucurbita pepo* (summer squash). Cucumber viruses 3 and 4, however, are both rod-shaped viruses which systemically infect *Cucumis sativus* (cucumber) and *Citrullus lanatus* (watermelon), whereas MNSV-C and MNSV are isometric viruses that induced only local lesions in those two hosts. MNSV-C, like MNSV, produced local lesion without systemic symptoms in three of the cultivars of *Cucumis melo* var. *conomon* that were tested. Both MNSV-C and MNSV failed to produce symptoms on *Luffa aegypiaca* and *Cucumis sativus* (Palace Pride) from Japan, although MNSV-C could be recovered from the inoculated leaves of Palace Pride. At present, there are no known vectors for either virus.

Based on symptomatology and similarities in host range, physical properties, particle characteristics, and serological relationships, MNSV-C is considered to be identical to or a strain of MNSV. Cross-reaction between both viruses and their antisera needed for the exact determination of their relationship could not be made because MNSV was no longer available. Muskmelon necrotic spot virus was described by Kishi (8) as a new virus disease of muskmelons grown in greenhouses in Japan. Our isolate, MNSV-C, was likewise first observed in muskmelons grown in greenhouses. Field surveys of cucurbit viruses in California carried out by Lindberg et al (9), Grogan et al (7), and Milne et al (10) did not indicate the presence of MNSV; either it was not present or symptoms were not expressed under field conditions. Field surveys to detect MNSV-C were not a part of the present work, but the virus was found in seed transmission tests of commercial seed stocks. Since melons for commercial seed are grown in the field, the virus already has been introduced into the field but has escaped detection either because disease incidence is low or because symptom expression is very mild or absent.

It is important for a plant breeding program to begin with parent stock free of seed-transmitted virus to avoid the infection of progeny. Unfortunately, MNSV-C in young plants could not be detected until symptoms were expressed, usually 60 or more days after emergence. In spite of this shortcoming, available melon germplasm was screened for MNSV-C resistance. If lack of symptoms and a hypersensitive reaction are considered resistant reactions, 62% of the plant material tested was found to be resistant to MNSV-C. Lack of assayable virus 3 mo after inoculation was our criterion for resistance. However, it is possible, as it was in three of 14 plants saved for seed and originally designated as resistant, that virus may be recovered at some time later than 3 mo after inoculation (Table 2). The virus either must have been present in such low concentration or localized in such a way that detection was impossible by our methods.

Although MNSV-C does not appear to be prevalent in melon growing areas in California, there is some evidence that inoculum sources exist there. The virus was recovered from plants grown in isolation from these grown from seed produced in California. Under favorable environmental conditions, the disease could become limiting to melon production. The disease agent, however, has been identified and it can be controlled by the resistance to the disease found in some American muskmelon cultivars.

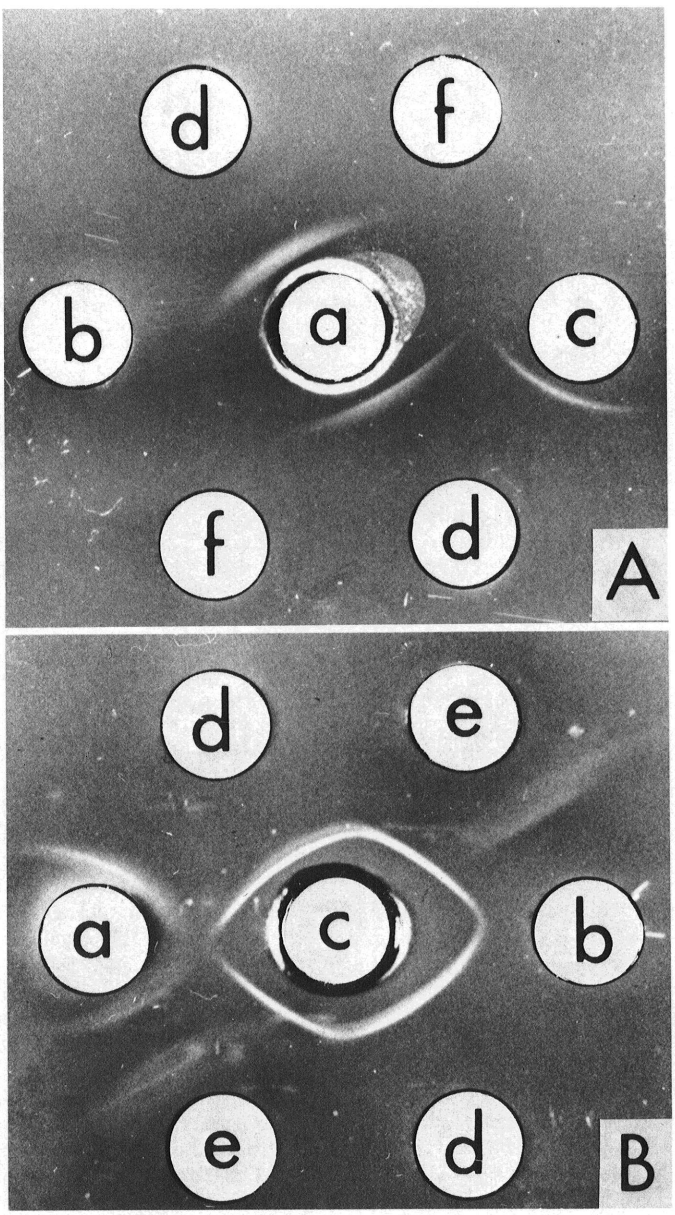

Fig. 3. Immunodiffusion analysis of intact particles of muskmelon necrotic spot virus, California isolate (MNSV-C) with anti-MNSV (type strain) and anti-MNSV-C sera. **A)** Sap from MNSV-C infected melon. **B)** Sap from healthy melon. **C)** Purified MNSV-C. **D)** MNSV-C antiserum. **E)** MNSV antiserum. **F)** Normal serum.

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