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ISOLATION AND CHARACTERIZATION OF TWO VIRUSES FROM CUCURBITA FOETIDISSIMA HBK, BUFFALO GOURD

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ISOLATION AND CHARACTERIZATION OF TWO VIRUSES FROM *CUCURBITA FOETIDISSIMA* HBK, BUFFALO GOURD

by

Martha Elizabeth Meyer Rosemeyer

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This thesis has been approved on the date shown below:

William P. Bemis — Oct. 7. 1982

WILLIAM P. BEMIS
Professor of Plant Sciences

Date

DEDICATION

This manuscript is dedicated to the universal principle of love, whose waves are beating our shores at all times, gently reminding us that it is to be taken into all our activities--especially work, for work is love made visible.

> In a natural ecosystem, biological balance results from a diverse network of interacting organisms in dynamic equilibrium. Each species is adapted to the prevailing environment and each is a source of food for others. However, each species also has one or more mechanisms to endure or escape its competitors and natural enemies, and each ecological niche is occupied both in space and time. Disease epidemics are rare in this situation.
>
> Agriculture usually goes counter to the mechanisms of biological balance. The diverse network of species is replaced with a single genotype (the crop). Often the crop is not well adapted to the environment provided, and is grown without proper benefit to the natural mechanisms of disease escape and endurance. Moreover, empty niches are created through tillage, pesticides, and other practices. The result is more disease which, itself, is a natural force in the maintenance of biological balance.
>
> R. J. Cook (1981)

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ABSTRACT

Striking viruslike disease symptoms on field-grown buffalo gourd, Cucurbita foetidissima HBK, have been observed in the Tucson and Phoenix, Arizona, areas. A partial cause of this disease syndrome has been isolated and characterized as squash mosaic virus (SMV) and cucumber mosaic virus (CMV). The CMV isolated from buffalo gourd appears to be an unusual strain: at least two other strains of CMV were not able to infect buffalo gourd. If buffalo gourd seedlings were inoculated with the SMV or CMV isolate, a systemic mosaic appeared, but after several weeks new leaves were symptomless.

Three types of disease syndromes, characterized by the presence or absence of mosaic and distortion, were observed. These could not be associated with SMV or CMV. The pathogen responsible for the distortion symptoms has not been identified but appeared to be non-mechanically transmissible. Buffalo gourd was found resistant to mechanically inoculated watermelon mosaic virus-2 and tobacco ringspot virus.

INTRODUCTION

The buffalo gourd, Cucurbita foetidissima HBK (Figures 1 and 2), is a xerophytic cucurbit indigenous to western North America and has great potential for arid-land agriculture (Bemis, Curtis, et al., 1978). It is currently being domesticated by the University of Arizona Buffalo Gourd Research Team led by W. P. Bemis, J. W. Berry, and C. W. Weber. Curtis first described the native buffalo gourd as a potential crop plant in 1946. In 1974, Curtis and Rebeiz published an extensive report covering 6 years of field research on domestication of the buffalo gourd in Lebanon. Research was terminated due to the retirement of Curtis and the Lebanese civil war (Bemis, Berry, et al., 1978). Seed from remnants of the Curtis collection (originally from one location in Texas) was used to start the domestication program at The University of Arizona.

The buffalo gourd plant has numerous vines bearing entire heart-shaped leaves. One plant may cover an area as large as 12.2 m^2 (40 ft^2). Each season it can produce an abundant crop (up to 200) of gourds 5-7 cm in diameter. Seeds of the gourds are edible when processed and contain 33% crude protein and 33% crude fat. The oil is of high quality, containing 45–65% linoleic acid (Berry et al., 1976). Estimated yields are approximately 1000 kg/ha oil and 1000 kg/ha protein (Bemis, Berry, et al., 1978).

The buffalo gourd has a large perennial root capable of reaching 50 kg in three or four seasons (Bemis, Berry, et al., 1978).

Figure 1. Buffalo gourd in its native habitat. -- Photographed on the Navajo Indian Reservation (Arizona) by Gary Nabhan.

Figure 2. Buffalo gourd under domestication. -- The vines are pressed to the ground to stimulate adventitious root development. Photograph by Robert Azzi (from Fitchett, 1972).

Starch content of the root is about 14–18% by wet weight and 50-60% by dry weight. The starch is edible but must be processed due to the presence of bitter cucurbitacins (Berry, Schreerens, and Bemis, 1978).

Preliminary studies have tested the suitability of roots for gasohol production (Gathman et al., 1979). Buffalo gourd is able to produce approximately 2400 liters of ethanol per hectare (250 gal/acre), which compares very favorably with the production from corn and sorghum at 2100 L/ha (230 gal/acre) but is less than that of sugar beets at 2700 L/ha (290 gal/acre). However, these traditional crops are grown on fertile well-watered land.

Buffalo gourd (Figure 1) is ecologically adapted to the semiarid regions of western North America. It presently ranges from central Mexico north to Wyoming and east to Missouri (Bemis, Curtis et al., 1978). In Arizona it is feral from an altitude of 1000–2300 m (3000–7000 ft).

Buffalo gourd is well adapted to semiarid growing conditions. It can withstand a minimum rainfall of 27–30 cm (11–12 in.) (Bemis, 1979, personal communication). Thus its potential for arid-land agriculture. The National Academy of Sciences (1975) has recommended buffalo gourd as a candidate for crop domestication and development in arid lands.

In the wild, buffalo ground appears to be disease free, but under cultivation the crop is susceptible to numerous diseases, as is common when wild plants undergo cultivation. Agriculture, as traditionally practiced, disturbs the ecological system of an area. This causes a biological imbalance, which, in turn, creates a favorable

situation for disease epidemics (Cook, 1981). Horsfall and Cowling (1978, p. 3) wrote in Plant Disease: An Advanced Treatise "For convenience in planting, tending, and harvesting, man has clustered his plants together on the most fertile land. This is convenient not only for man but for the pathogens that cause epidemics."

In the summer of 1978, mosaic symptoms and later leaf size reduction, distortion, and shortened internodes were observed on 1- and 2-year-old buffalo ground plants at the University of Arizona Campbell Avenue Farm. The purpose of this thesis was to identify and characterize the viral pathogens infecting these buffalo ground plants.

As a result of research on melon virus diseases, a mosaic-inciting pathogen was isolated from Cucurbita foetidissima in California (Middleton, 1947); however, techniques were not sophisticated enough and virus taxonomy not clear enough to identify the pathogen.

Mosaic virus diseases have been a continuous threat to the spring production of cantaloupes (Cucumis melo L. cv. reticulatus) in southwestern and central Arizona (Nelson, 1964; Nelson, Allen, and Tuttle, 1968; Nelson and Tuttle, 1969). Cantaloupe acreage in central Arizona and southern California decreased 95% and 75%, respectively, in the years from 1952 to 1962 due to the crown blight disease complex of which mosaic viruses appear to be an important factor (Nelson, 1964).

Squash mosaic virus (SMV), cucumber mosaic virus (CMV), watermelon mosaic virus (WMV) strains 1 and 2 (WMV-1, WMV-2), and curly top virus (CTV) are known to be present in cucurbit-growing areas of Arizona and California (Grogan, Hall, and Kimble, 1959; Nelson and Tuttle, 1969). Of the three mosaic viruses, CMV, WMV, and SMV,

CMV and WMV-2 strain have been most consistently found in the Yuma Valley of southwestern Arizona (Nelson and Tuttle, 1969). Cucumber mosaic virus is an aphid-borne, 30-nm, isometric cucumovirus (Francki, Mossop, and Hatta, 1979), and WMV is an aphid-borne, 750-nm, flexuous filament of the potyvirus group (Van Regenmortel, 1971). Cucumber mosaic virus has been more prevalent than WMV in central and northern California (70% vs. 11% of samples). However, in the desert valleys of southern California, 93% of the samples showed WMV and 15% showed CMV in 1956–58 (Grogan et al., 1959). Watermelon mosaic virus-2 has been found in 100% of the samples in five areas of Yuma County from 1960–66. On the other hand, CMV was found in only three of five areas and when present its incidence ranged from 32% to 75%, with an average 16% (Nelson and Tuttle, 1969).

Although CMV is found less often than WMV in southeastern Arizona, its effects on individual plants are more severe. Plants infected with CMV at an early stage of growth may die, unlike those infected with WMV, or if they survive, are more stunted and yield less than do WMV-infected plants of the same age (Nelson, 1964).

The epidemiology of CMV and WMV in Yuma County involves both cultivated and uncultivated annual and perennial plants. The green peach aphid, Myzus persicae Sulzer, and the melon aphid, Aphis gossypii Glover, present all year round (Coudriet and Tuttle, 1963), are the vectors of CMV and WMV (Coudriet, 1962). Cucumber mosaic virus is transferred from the perennial periwinkle, Vinca rosea L., which is commonly grown as an ornamental around homes, to nearby cantaloupe plants. Watermelon mosaic virus is transferred from

overwintering hosts such as mallow (Malva pariflora L.), sour clover (Melitotus indicas L.), and sweet pea (Lathyrus odoratus L.) to spring cantaloupe. Some mallow are able to survive the summer in sheltered locations and support aphid populations. Seedlings of winter mallow and sour clover appear again in fall and are infected from the oversummering sour clover and mallow, and the cycle begins anew (Nelson and Tuttle, 1969).

Squash mosaic virus, a cucumber beetle-transmitted, 30-nm, isometric cucumovirus (Campbell, 1971), has been found in Arizona and less often than WMV and CMV. In Yuma County in 1961, it was found in 0.45% of the cantaloupe sampled (Nelson et al., 1962). In southern California and Yuma County in 1956–58, it was present in 0.01% of the plants sampled in north-central California in 0.26% (Grogan et al., 1959). However, Flock and Mayhew (1981) found 5–10% of the cucurbits in southern California to be infected with SMV in 1977–78. The virus is more commonly found in autumn due to the large populations of cucumber beetles present at this time (Nelson and Tuttle, 1969).

One distinguishing characteristic of SMV, besides its narrow host range (mainly limited to Cucurbitaceae) is its seed transmissibility. Seed transmission as high at 94% has been reported (Rader, Fitzpatrick, and Hildebrand, 1947) in some hosts, although it may not be seed transmitted in others. Two strains of SMV can be distinguished on the basis of symptoms and seed transmissibility (Nelson and Knuhtsen, 1973a, 1973b).

Curly top virus (CTV), a leafhopper-transmitted, 20-nm, isometric geminivirus (Thomas and Mink, 1979), has caused serious damage

in diverse crops, including cucurbits, west of the Rocky Mountains (Hills and Taylor, 1954). In 1958 curly top virus was associated with major losses of cantaloupe in the Southwest, except Yuma County (Flock, Laird, and Dickson, 1960). In Yuma County, losses of cantaloupe due to CTV were light from 1960–68 (Nelson and Tuttle, 1969). Field and greenhouse studies have shown that the earlier the cantaloupe seedlings are infected, the more severe the damage (Hills and Taylor, 1954; Flock et al., 1960). Differences in symptom severity have also been noted with different strains (Duffus and Gold, 1973).

Squash leaf curl (SLC) is a recently described disease of squash in central and southern California and western Arizona (Flock and Mayhew, 1981). The causal agent has recently been identified as a geminivirus by Dodds et al., 1982. It is not mechanically transmitted but is vectored by the sweet potato whitefly, Bemisia tabaci. The symptoms on banana squash, Cucurbita maxima, include interveinal chlorosis or mottling associated with vein clearing or green vein banding, stunt and upright bending of new shoots, leaf margin curl, and vein thickening. Enations were frequently found on the undersides of the leaves. These symptoms were also found in field-grown 'Yellow Crookneck,' 'Zucchini,' and scallop-type squash (all Cucurbita pepo) and were induced in greenhouse tests of other types of squash and pumpkins. However, the symptoms on watermelon, cucumber, and cantaloupe (including 'Casaba' and 'Crenshaw' melon) were mild chlorosis and stunting with some vein clearing (Flock and Mayhew, 1981).

The symptoms of SLC on cotton distinguish it from cotton leaf curl. Flock and Mayhew (1981) stated that because cotton leaf crumple has been eradicated in California since 1967 by plow-up regulation preventing stub cotton, the pathogens causing SLC and cotton leaf crumple could not be the same. However, cotton leaf crumple was present in California from 1978–81 due to the presence of stub cotton (Nelson, 1981a, personal communication).

Tobacco ringspot virus (TRSV), a nematode-transmitted, 28-nm, isometric nepovirus (Stace-Smith, 1970a), has been found as far west as Texas, where it causes the pimple's disease of watermelon (Rosberg, 1953; McLean, 1960). Though particularly searched for in California (Grogan et al., 1959) and Arizona (Nelson, 1981b, personal communication), it has not been found. The TRSV is rarely seed transmitted in cucurbits (Stace-Smith, 1970a).

Tomato ringspot virus (TmRSV) is nematode-transmitted, 28-nm, isometric nepovirus (Stace-Smith, 1970b). Though present on cucurbits in the eastern United States (Provvidenti, Robinson, and Munger, 1978), it has not been found on cucurbits in the western United States (Grogan et al., 1959).

Bean yellow mosaic virus–severe strain (BYMV-S), an aphid-borne 750-nm, flexuous filament of the potyvirus group (Bos, 1970), has been reported in the northeastern United States on squash (Provvidenti and Uyemoto, 1973) but has not been report in the West (Grogan et al., 1959; Nelson and Tuttle, 1969).

MATERIALS AND METHODS

Plant viruses are characterized by accumulating positive information on virus properties. The shape of the particle, either rod, filamentous, isometric, or bacilliform, is observed by electron microscopy. The host range, determined by inoculating diagnostic plant species with the virus, eliminates certain virus groups. Finally, serological tests, which are more specific, usually identify the virus by the unique antibody-antigen reaction involved.

In conjunction with the characterization of the causal agent(s), pathogenicity must be proved by Koch's postulates, establishing the causal relationship between pathogen and disease. As for nematode-caused diseases (Mountain, 1960), Koch's postulates for bacterial or fungal pathogen-caused diseases must be modified for a virus-caused disease because viruses cannot be separated from the host plant and cultured on artificial media. Therefore, the virus can only be isolated from other pathogens by incoculation onto and maintenance in another host plant. Extracts from this plant, either crude or purified, are then transferred to a healthy plant of the original host species. Expression of the original symptomology and subsequent reidentification of the pathogen completes Koch's postulates.

Vegetative Propagation of Buffalo Gourd

Cuttings were taken October 1978 from five buffalo gourd plants expressing symptoms from the University of Arizona Campbell Avenue

Farm (CAF) Plot 1. The cut was made below the second or third node with a razor blade dipped in 95% ethanol. The cuttings were temporarily stored in an ice chest until placed for rooting in a vermiculite–perlite mix under mist.

Virus Detection by Electron Microscopy

An extract from each of the five cuttings was examined in the transmission electron microscope (Hitachi-500) for presence of virus particles. One gram of tissue was chopped with a razor blade into a vial containing 5 ml of 0.01 M phosphate buffer pH 7. After a few minutes, the extract was shaken and one drop placed on a glass slide. One drop of 4% phosphotungstic acid (PTA) pH 7 was added and the two drops mixed with a pasteur pipette. A carbon-coated, 300-mesh, copper grid was then touched to the drop and the excess preparation drained off one side by a small piece of filter paper. A minimum of 10 apertures were viewed in search of viruslike particles. Extracts of the five isolates were also examined in this manner at other steps in the characterization.

Inoculation Methods and Virus Isolation

Leaves of the rooted buffalo gourd cutting were ground in a mortar and pestle with 0.1 M phosphate buffer pH 7 at a proportion of approximately 1 g of tissue to 10 ml of buffer. Carborundum (360 grit) was placed in the mortar and pestle while grinding. The resulting inoculum was then rubbed with a plumber's acid brush on the cotyledons and two to four true leaves of seedling cucurbits (12 to 16 days old). Before inoculation, the true leaves were observed for

symptoms of seed-borne SMV. The exact cucurbits used (Table 1) varied with the assay but always included three of the following species: Cucurbita pepo cv. Summer Yellow Crookneck squash and Sugar Pumpkin, Cucurbita maxima cv. Big Max pumpkin, Cucumis melo cv. Hale's Best Jumbo cantaloupe, and Citrullis lanatus cv. Florida Giant watermelon.

The seedlings were inoculated four times over a period of 3 to 4 weeks and were observed for 3 to 4 weeks after the last inoculation. Extracts of inoculated plants exhibiting symptoms were checked by electron microscopy for the presence of virus particles. This entire procedure was repeated three times. All plants were maintained in greenhouses at temperatures of 21–27°C day/16–21°C night in winter and 32–38°C day/21–27°C night in summer.

Host Range

Indicator Plants

Diagnostic plants of the appropriate age (Table 1) were inoculated according to the procedure described above with cucurbit tissue exhibiting symptoms. The plants were routinely observed for symptom development over a period of at least 30 days.

Test of Pathogenicity of Isolates by Koch's Postulates

After buffalo gourd seedlings had been inoculated and scored for symptoms, 10 seedlings of 'Big Max' pumpkin, 8 of 'Caserta' squash, and 6 of 'Summer Crookneck' squash were inoculated with extracts from the buffalo gourd seedlings that showed symptoms.

Table 1. Species, cultivar, age in days, and preinoculation size of indicator plants for host range studies

Species	Cultivar	Age in Days	Size
Cucurbita pepo	'Sugar Pumpkin'	14–20	2-3 true leaves
	'Summer Crookneck' squash	14–20	2-3 true leaves
	'Caserta' squash	14–20	2-3 true leaves
Cucurbita maxima	'Big Max' pumpkin	14–20	2-3 true leaves
Cucumis melo	'Hale's Best Jumbo' cantaloupe	18–20	2-3 true leaves
Citrullus lanatus	'Florida Giant' watermelon	20–30	2-3 true leaves
Cucumis sativus	'Straight Eight' cucumber	20–30	2-3 true leaves
	'National Pickling' cucumber	20–30	2-3 true leaves
Chenopodium amaranticolor		30–45	4-8 true leaves
Nicotiana tabacum	'Xanthi' tobacco	30–45	2-6 true leaves
Lycopersicon esculentum	'Bonny Best' tomato	30–40	4-6 true leaves
Phaseolus vulgaris	'Kentucky Wonder' bean	12–14	Cotyledon or first true leaf
Vigna unquiculata	'Blackeye' cowpea	8–10	Cotyledons only
Pisum sativum	'Progress #9' pea	21	10-15 cm high
Gomphrena globosa		30–40	15-20 cm high
Cucurbita foetidissima	AZ #1 reciprocal (142 x 158) 1978, W. P. Bemis, University of Arizona, Tucson	9–11	Cotyledons or first true leaves

These cucurbit seedlings had been previously tested for seed-borne SMV by inoculating groups of 12 of their half-cotyledons to two 'Caserta' seedlings, who themselves had been tested for seed-borne SMV by immune electron microscopy (IEM). (See section entitled "Immune Electron Microscopy" for procedure.) After symptoms appeared on the pumpkin and squash, extracts from these plants were tested for either SMV by host-range and IEM tests or for CMV by host-range tests.

Serology

Antiserum Acquisition and Preparation

The source and preparation of the antisera used in this study are summarized in Table 2.

Serological Tests

Double-diffusion Agar Gel Tests. Agar gel tests for SMV were performed according to the method of Ball (1974). Twenty-five milliliters 0.75% agar (Appendix) were poured into 100 × 15-mm plastic petri dishes. A Shandon 7-hole borer with 1-cm center well, 0.7-cm side wells, and 2-cm center-to-center radius was used to wells in the agar. The gel was removed from the holes using a pasteur pipette attached to a small vacuum pump. The borer was sterilized with ethanol after every five plates. Crude viral sap was prepared by grinding leaf tissue in a mortar and pestle with 0.1 M phosphate buffer pH 7 at a ratio of 1 gm tissue to 5 ml buffer. After the antiserum and crude sap were placed in the wells, the plates were left at room temperature for

Table 2. Antiserum preparation and acquisition

Virus	Source	Inoculum	Purification Host	Titer
SMV-1H	Nelson[a]	Colorado seed-borne	Cucumis melo	1/700
CMV-242	ATCC[b]	CMV-S	Datura stramonium	1/256
CMV-242a	ATCC	CMV-S	Cucurbita pepo	1/128
CMV-260	ATCC	CMV-D	Nicotiana tabacum cv. Xanthi	1/512
CMV-30	ATCC	CMV-Com	Nicotiana tabacum cv. Xanthi	1/256
TRSV-157	ATCC	TRSV, from grape	Nicotiana tabacum	unknown
TmRSV-174	ATCC	TmRSV-174, from grape	Cucumis sativus	1/1024
AMV	Nelson	unknown	unknown	1/256
PVY NC-57	Gooding[c]	PVY NC-57	unknown	unknown

a. M. R. Nelson, University of Arizona, Department of Plant Pathology, Tucson.

b. American Type Culture Collection, Rockville, Maryland.

c. G. V. Gooding, North Carolina State University, Department of Plant Pathology, Raleigh.

48 hours and then examined for precipitate bands with a fluorescent light box.

Agar gel plates for CMV serology were made according to the method of Scott (1968), using American Type Culture Collection (ATCC) CMV PV-242 antiserum. Two known strains of CMV, ATCC CMV PV-242 (CMV-S from South Africa) and ATCC CMV PV-59 (isolated by E. G. Ruppel from sugar beet at Mesa, Arizona), were used as controls. Both the controls and the unknowns were propagated in 'Xanthi' tobacco and 'Sugar Pumpkin.'

Immune Electron Microscopy. Standard preparations for immune electron microscopy were performed as outlined in the section entitled "Virus Detection." To test the antigenicity of the virus, one drop of antiserum diluted 1:9 with distilled water was combined with one drop of the crude plant sap on a glass slide and mixed well. Then two drops of the 4% phosphotungstic acid stain were added and the preparation again mixed. The grid was loaded and drained as above. Preparations of buffer, nonhomologous antiserum, and healthy tissue served as controls.

The grids were observed in the electron microscope and scored for presence or absence of a precipitation reaction or particle decoration. If the antiserum and antigen (virus) are homologous and in proper concentrations, the virus particles appear clumped and are covered with a mantle of antibody.

Though unnecessary for identification of SMV, more elaborate variations of the basic IEM technique described above were attempted

with the CMV (known and buffalo gourd isolate (29V)). Many combinations of buffers, fixatives, and stains were used (Table 3). In addition, the formaldehyde treatment was varied in concentration and time. One gram of tissue was chopped in 5 ml of buffer, with and without fixative, and stained according to the particular test used.

Derrick Technique. The Derrick technique of immune electron microscopy (Derrick and Brlansky, 1976) was also employed with the the CMV isolated from buffalo gourd (29V). Milne and Luisoni (1975) have successfully used this technique with CMV, and their modification of the basic Derrick technique is described here. The antiserum was diluted 1:300, 1:500, and 1:1000. The grids were floated carbon-side down on the antiserum for 5–7 min, washed with 20 drops of 0.01 M pH 7 phosphate buffer, and excess drained without blotting. Then the grids were floated on crude plant sap, as prepared for the previous IEM tests, for 15–25 min, depending on the experiment. Finally, 40–50 drops of distilled water, followed by 5 drops of 2% uranyl acetate stain, were dropped singly on the grid and then the grid was drained. Virus particles in randomly chosen fields were counted at 17K in the electron microscope, a technique used by Roberts (1980). The number of fields viewed depended on the titer of the particle, but Roberts (1980) used 15 fields as a minimum. Five to 20 fields were viewed in four trials, and the range or average in number of particles per field recorded.

Table 3. Buffers, fixatives, and stains used with CMV in immune electron microsopy

Buffers	Fixatives	Stains
0.01 M phosphate pH 7	formaldehyde 2% - 30,60 min 5% - 30,60,90 min 10% - 30 min	4% phosphotungstic acid pH 7
		2% uranyl acetate in water pH 4
0.2 M borate pH 9	4% glutaraldehyde	2% uranyl acetate in acetate buffer pH 3
0.2 M acetate pH 5	1% osmium tetroxide	2% uranyl formate pH 3
0.2 M acetate pH 6.8		4% phosphomolybdic acid pH 7
distilled water pH 6.8		4% cadmium iodide pH 5
		4% ammonium molybdate pH 7
		4% silver nitrate pH 6.2

Field Observations

Symptoms

Disease symptoms and general physiological characteristics of 41 buffalo gourd plants at the University of Arizona Campbell Avenue Farm (CAF) were observed bimonthly during the growing season from September 1978 through 1979 and again in June 1980. The presence and extent of the following characteristics were recorded: mosaic, including size of spots; distortion; rolling of leaf margins; wavy or irregular leaf margins; puckered or irregular veins; and rugosity of the leaf. Maximum leaf size, number of leaves per node, differences in symptoms between old and new leaves, and overall plant size or vigor were also recorded. The plants were staked and flagged for permanence.

Twenty-four of the plants were located in the Campbell Avenue Farm Plot 6 germplasm nursery, which contain the original and adventitious plants from seed collected in the western United States and Mexico in 1975. Nine of the observed plants were located in the Campbell Avenue Farm Plot 1 and were from either accession 142 or 158 selections 1 and 2. Eight of the observed plants were from roots collected from the University of Arizona Mesa Experiment Station and replanted at the Campbell Avenue Farm. (See "Search for Disease Resistance.")

Four tomato plants were placed in the germplasm nursery to check for presence of curly top virus. Three cantaloupe plants and one watermelon plant grown nearby on the farm were tested for presence of SMV, CMV, and WMV.

Environmental Conditions and Symptom Expression

Observations of symptoms and physiological characteristics of the 24 plants from the Campbell Avenue Farm Plot 6 were compared with the farm's temperature and precipitation data. The plants in this field were not irrigated in 1979.

Field Assay

Besides virus characterization, it was important to determine the following: extent of virus infection in the field, relative proportions of the CMV and SMV viruses, and whether virus could be isolated from apparently symptomless plants.

Two assays were performed: one at CAF Plot 6 (Assay 1) and one at CAF Plot 1 (Assay 2). For Assay 1 six buffalo gourd plants exhibiting typical viruslike symptoms and five symptomless plants were chosen. For Assay 2, five plants exhibiting symptoms and five randomly chosen plants, symptomless or not, were used. The buffalo gourd tissue was collected directly from the field, and the extracts were examined by electron microscopy and inoculated onto 'Big Max' pumpkin, 'Sugar Pumpkin,' and 'Florida Giant' watermelon. Buffalo gourd extracts from Assay 1 were inoculated onto the cucurbits three times and extracts from Assay 2 only once. The cucurbits were observed for 3 to 4 weeks. Finally, extracts of selected plants from both assays were inoculated onto diagnostic species, which were checked for SMV by IEM.

Seed Transmission

Greenhouse Tests

Seed of Arizona #1 reciprocal (142 x 158) buffalo gourd harvested from CAF Plot 1, known to contain virus-infected plants, was tested for the presence of seed-borne virus. One thousand seedlings were grown for 3 weeks in a plywood box (30 cm × 60 cm × 120 cm) containing sterilized soil. The plants were scored for symptoms and a pooled preparation of tissue from 15 abnormal seedlings was examined in the electron microscope. A separate preparation of tissue from 15 normal seedlings served as the control. At this time, extracts of abnormal plants were inoculated onto 20 'Sugar Pumpkin,' 3 'Big Max' pumpkin, 3 'Xanthi' tobacco, and 3 Chenopodium amaranticolor plants, which were observed for 1 month.

Seeds from gourds from four virus-infected plants were sown and observed. Because virus infection drastically reduces flowering and subsequently gourd production, only a few seeds could be tested. Two gourds of plant 30 (30-1 and 30-2) and one gourd each of plants 36, 22, and 200 provided seeds. Fifty seeds of 30-1, 22, and 200 were germinated in petri dishes in a growth chamber 14 h at 32°C (90°F), 10 h at 21°C (70°F) and then after 1 week planted in soil. The remainder of the seeds were planted directly in soil.

Field Tests

When vines were 15–90 cm long, 3500 plants of Arizona #1 reciprocal (142 x 158) were observed at the University of Arizona Marana Experimental Farm for symptoms due to seed-borne virus.

Abnormal plants were flagged and observed once a month throughout the 1979 growing season.

Crop-loss Measurement

Seed Yield

The gourds from CAF Plot 1 (planted in 1977) were harvested and threshed and the weight of seeds recorded. The weights of seed from 1978 and 1979 were compared with subjective observations concerning the extent of the virus disease in the field.

Root Starch Yield

In October 1979 prior to dormancy, six apparently healthy and nine diseased 18-month-old Arizona #1 reciprocal buffalo gourd plants were selected from CAF Plot J. The primary roots of these plants were dug, refrigerated for 3 months, lyophilized, and analyzed for the dry-weight percentage of starch using the anthrone reagent (Clegg, 1956). The mean and standard deviation of percent starch were calculated for both healthy and diseased root groups. Sample sizes were small because healthy plants were difficult to recognize. Adventitious roots of the disease-free selections were transferred to the greenhouse to screen for disease resistance and to assay for starch content under greenhouse conditions.

Infectivity Assays

Ten viruses and strains, representing six groups of viruses, were inoculated onto Arizona #1 reciprocal buffalo gourd seedlings that

had cotyledons or only one true leaf. Virus, source of virus, host, and indicator plants were recorded.

The buffalo gourd seedlings were inoculated three times at approximately weekly intervals. Appropriate indicator plants were also inoculated to verify the virulence of the inoculum. The buffalo gourd seedlings were observed at weekly intervals until 1 month after the last inoculation. At this time they were checked by electron microscopy for the appropriate virus particle and the extract inoculated onto indicator plants. After the appropriate interval of time for symptom development on the indicator plants, for example, 7 days for local lesions on Chenopodium amaranticolor to 21 days for systemic necrosis in pea, the indicator plants were scored for symptoms.

Search for Disease Resistance

Twelve buffalo gourd plants with virus symptoms and 10 without symptoms were selected from a severely infected Mesa Agricultural Experiment Station field. The 1-year-old roots were dug in October 1978 and replanted at the Campbell Avenue Farm in April 1979. Cuttings from a nearby infected pumpkin plant were taken to identify viruses present in the area. The plants were observed throughout 1979 and once in 1980.

RESULTS AND DISCUSSION

Vegetative Propagation of Buffalo Gourd

In general, buffalo gourd did not root well in the mist chamber; however, cuttings 11, 29, 33, 39, and 44 did root and these plants will be discussed here. A description of the field symptoms exhibited by these plants can be found in the section entitled "Symptoms."

Virus Detection by Electron Microscopy

Neither rod-shaped nor filamentous virus particles were observed in extracts of buffalo gourd by electron microscopy. Spherical virus particles in high titer were not detected; spherical virus particles in low titer may be confused with spheres present in healthy plant preparations.

Inoculation and Virus Isolation

The results of inoculating three cucurbits with extracts of buffalo gourd cuttings are shown in Table 4. In the first experiment the cucurbits were placed in the greenhouse and a growth chamber. Two repetitions of this experiment with only greenhouse-grown plants yielded similar results.

The 'Big Max' pumpkin is the most susceptible cucurbit of the three inoculated. None of the isolates infected watermelon. There appears to be range of titer (or virulence) from high for plant 11 to low for plant 33. The pumpkin plants in the growth chamber did not appear more susceptible than plants in the greenhouse. Electron

Table 4. Percentage of indicator plants exhibiting systemic mosaic following inoculation with extracts of cuttings from five different buffalo plants

Cuttings from Buffalo Gourd Plant Number	Indicator Plants[a]							
	Pumpkin				Cantaloupe, Greenhouse		Watermelon, Greenhouse	
	Greenhouse		Growth Chamber[b]					
	n[c]	%	n[c]	%	n[c]	%	n[c]	%
11	9	100	--	--	6	83	6	0
29	13	54	4	75	9	0	8	0
30	13	38	3	33	9	0	6	0
33	11	18	5	0	7	0	7	0
44	10	100	5	60	9	0	6	0
SMV-2[d]	7	100	2	100	4	50	3	0
Buffer inoculated	7	0	2	0	9	0	3	0

a. Indicator plants: 'Big Max' pumpkin, 'Hale's Best' cantaloupe, 'Florida Giant' watermelon.

b. Growth chamber maintained at 35°C for 16 h with incandescent and fluorescent light and 24°C for 8 h in the dark.

c. Total number of plants.

d. SMV-2 from 'Sugar Pumpkin.'

microscopic examination of cucrbitaceous hosts revealed neither rod-shaped nor filamentous virus particles.

Host Range

Indicator Plants

Results of inoculating the five unknown viruses, 11V, 29V, 30V, 33V, and 44V, isolated from buffalo gourd cuttings 11, 29, 30, 33, and 34, respectively, and five known viruses, SMV, CMV PV-242, CMV PV-59, TRSV, and TmRSV, on appropriate indicators plants (Table 1) are shown in Table 5. Because the indicator plants Chenopodium amaranticolor and Nicotiana tabacum cv. Xanthi were symptomless and uninfected following inoculation the viruses isolated from plants 11, 30, 33, and 44 most closely resemble SMV (Frietag, 1956). Watermelon mosaic virus (and BYMV-S, though Arizona is not near its geographic range) was eliminated because the unknown viruses are not filamentous.

Buffalo gourd isolate 29V appears to closely resemble CMV. Chenopodium amaranticolor and Vigna unquiculata (cowpea) reacted with local lesion, and 'Xanthi' tobacco showed mosaic symptoms, which does not occur if these plants are inoculated with SMV. Neither ringspots on 'Xanthi' or systemic necrosis on cowpea were present; therefore, TRSV and TmRSV seem less likely. Although neither of these viruses is known to be present in Arizona, the possibility of seed transmission, discovery, or introduction always exist.

Table 5. Host range of five viruses isolated in this study and five known viruses

Indicator Host	Symptoms[a] in Indicator Hosts									
	Unknown Virus					Known Virus				
	11V	29V	30V	33V	44V	SMV-2	CMV PV-242	CMV PV-59	TRSV PV-57	TmRSV
Cucurbita pepo 'Sugar Pumpkin'	le,m	sm	le,m	le,m	m	le,m	mm	sm	m	--
C. maxima 'Big Max' pumpkin	m	m	m	m	m	m	m	m	--	--
Cucumis melo 'Hale's Best Jumbo'	m	m	m	m	m	m	m	m	--	--
Citrullus lanatus 'Florida Giant'	ns	m,s	ns	ns	ns	ns	ns	m	--	--
Cucumis sativus 'Straight Eight'	cf	cf,m	cf	cf	nf	cf	m	m	LL,m[b]	LL,m[c]
Chenopodium amaranticolor	ns	LL	ns	ns	ns	ns	ns	LL	LL[b]	LL[c]
Nicotiana tabacum 'Xanthi'	ns	m	ns	ns	ns	ns	mm	m	rs,m	rs,m[c]
Lycopersicon esculentum 'Bonny Best'	ns	fl,m	ns	ns	ns	ns	mm	s,m	--	--

Table 5. Host range--Continued

Indicator Host	Symptoms[a] in Indicator Hosts: Unknown Virus 11V	29V	30V	33V	44V	Known Virus SMV-2	CMV PV-242	CMV PV-59	TRSV PV-57	TmRSV
Phaseolus vulgaris 'Kentucky Wonder'	ns	ns	ns	ns	ns	ns	ns	ns	LL,sn[b]	LL,sn[c]
Vigna unquiculata 'Blackeye'	ns	LL	ns	ns	ns	ns	LL	LL	LL,sn[b]	LL,sn[c]
Gomphrena globosa	ns	LL	ns	ns	ns	ns	ns	LL	--	--
Cucurbita foetidissima	--	--	mm	--	mm	mm	ns,(ns[d])	ns,(ns[d])	ns,(LL[d])	m[d]

a. cf = chlorotic fleck; nf = necrotic fleck; mm = mild mosaic (systemic); m = moderate mosaic (systemic); sm = severe mosaic (systemic); LL - local lesions; rs = ringspot; sn = systemic necrosis; le = lamina extension; s = stunting; fl = fern leaf; ns = no symptoms; -- = not tested.

b. From Stace-Smith (1970a).

c. From Stace-Smith (1970b).

d. From Provvidenti et al. (1978).

Test of Pathogenicity of Isolates by Koch's Postulates

Approximately one out of the six buffalo gourd seedlings inoculated with isolates 30V, 44V, and SMV-2 showed a mild systemic mosaic and vein clearing (Figure 3), which became symptomless in 1 to 2 weeks (Table 5), but when extracts of these infected plants were inoculated onto 'Big Max' pumpkin and 'Caserta' and 'Summer Yellow Crookneck' squash, mosaic symptoms developed. When Chenopodium amaranticolor and 'Xanthi' tobacco were inoculated with these extracts, the plants remained symptomless. Immune electron microscopy confirmed the presence of SMV.

Fifty percent (n = 18) of the buffalo gourd plants inoculated with isolate 29V (Table 4) developed a spotted systemic mosaic on recurving leaves (Figure 3). In three weeks, new leaves appeared symptomless. Local lesions developed on Chenopodium amaranticolor inoculated with extracts of these plants, and systemic mosaic developed on inoculated 'Xanthi' tobacco. Electron microscopy revealed neither rod-shaped or filamentous particles.

The mosaic symptoms due to SMV and CMV buffalo gourd isolates are much milder and of shorter duration on buffalo gourd than the symptoms exhibited by the original field plants. It is postulated that the relative severity of the symptoms in the field is either due to the field environment or to the presence of more than one causal agent for the described disease syndrome and that this part of the syndrome is not mechanically transferable. For further discussion, see section entitled "Symptoms."

Figure 3. Mosaic symptoms resulting from inoculation of buffalo gourd with SMV and CMV buffalo gourd isolates. -- Two weeks after inoculation, SMV incites a mild systemic mosaic and vein clearing (left) and CMV incites a severe systemic mosaic (middle). Leaf from healthy buffalo gourd is shown on right.

Serology

Double-diffusion Agar Gel Tests

The agar gel tests showed that isolates 11V, 30V, 33V, and 44V are SMV and that 29V is neither SMV or alfalfa mosaic virus (AMV) (Table 6). The titer of virus in buffalo gourd is very low, which confirmed the results of the electron microscopic examination of preparations of buffalo gourd. This experiment was repeated four times with similar results.

The results of CMV agar gel serology were inconclusive. In three separate tests, the suspected CMV strain (29V) and two known strains of CMV (PV-242 and PV-59) were challenged with CMV PVAS-242, but only one band appeared: PV-59 in 'Xanthi' tobacco.

Immune Electron Microscopy

The results of IEM with SMV-1H antiserum (Table 6) supported the results of the double-diffusion agar gel tests. The decorated SMV-2 and SMV(30) particle precipitated if combined with homologous antisera (Figures 4 and 5) and remained separate and undecorated with nonhomologous antiserum or buffer (Figures 6 and 7). Immune electron microscopic examination of CMV PV-242, CMV PV-59, and isolate 29V with CMV antisera were inconclusive. However, CMV and isolate 29V appeared to be similar in structure: both had an unusual hollow core (Figure 8). They are also quite similar in their reactions to PTA: CMV and 29V dissociated, unlike SMV and TRSV. Uranyl acetate stained CMV and 29V well; however, care must be exercised when using this stain because it causes the buffer salts to precipitate.

Table 6. Serological reactions of five isolates from buffalo gourd to SMV and AMV antisera

Isolate	Host	Reaction to Antiserum[a]		
		SMV		AMV
		Agar Gels	IEM	Agar Gels
11V	'Big Max' pumpkin	+		-
	'Sugar Pumpkin'	+		
	Buffalo gourd	-		
29V	'Big Max' pumpkin	-		-
	'Sugar Pumpkin'	-	-	
	Buffalo gourd	-		
30V	'Big Max' pumpkin	+		-
	'Sugar Pumpkin'	+	+	
	Buffalo gourd	-		
33V	'Big Max' pumpkin	+		-
	'Sugar Pumpkin'	+	+	
	Buffalo gourd	-		
44V	'Big Max' pumpkin	+		-
	'Sugar Pumpkin'	+	+	
	Buffalo gourd	-		
SMV	'Big Max' pumpkin	+		-
	'Sugar Pumpkin'	+	+	
	Buffalo gourd			
AMV	Medicago sativa cv. Hayden alfalfa	-		+

a. + = banding present or precipitation reaction in IEM test; - = banding absent or no precipitation reaction.

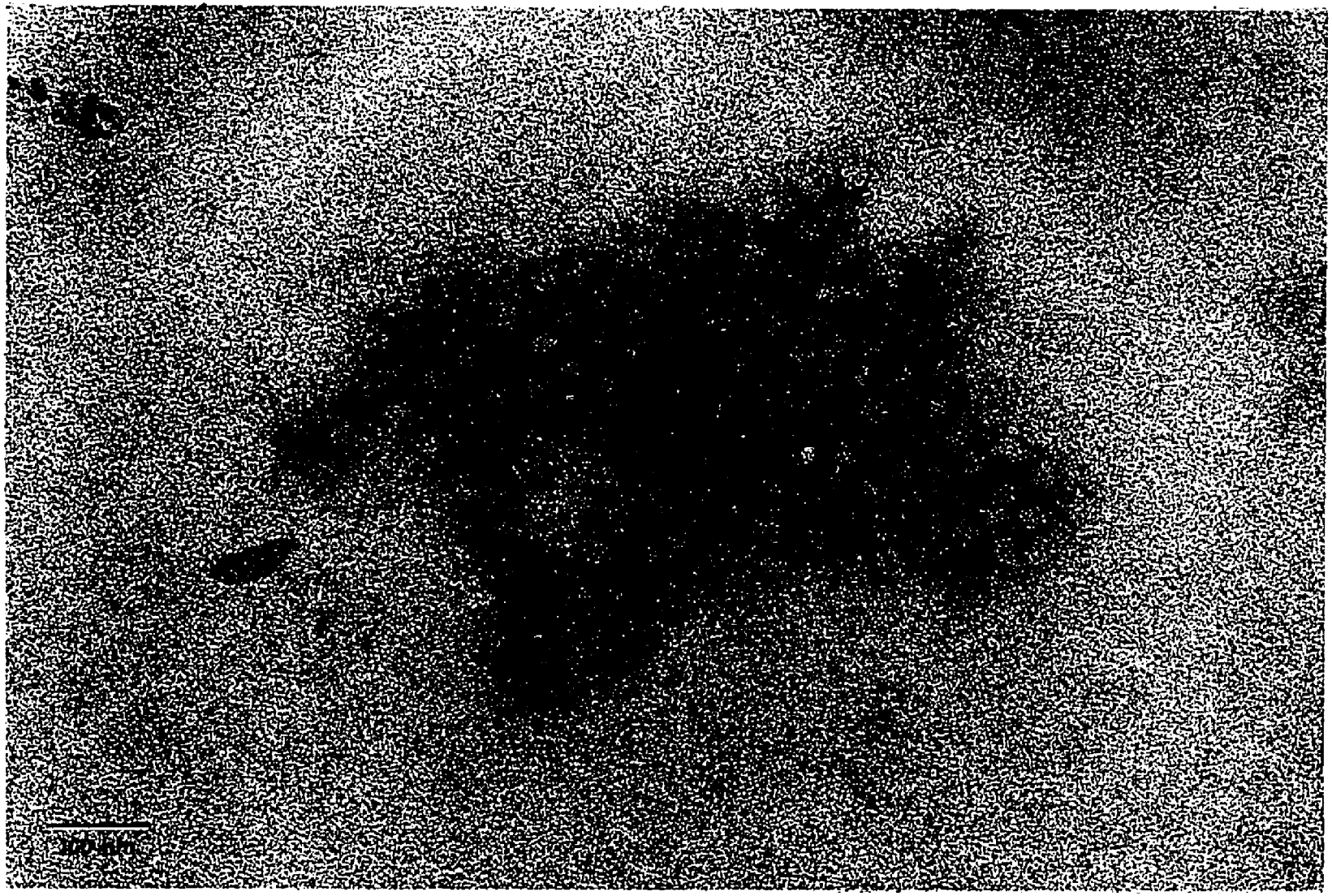

Figure 4. Precipitation of SMV-2 with SMV-1H antiserum. -- Buffered preparation of Cucurbita pepo cv. Sugar Pumpkin in crude sap stained with phosphotungstic acid (×158,000).

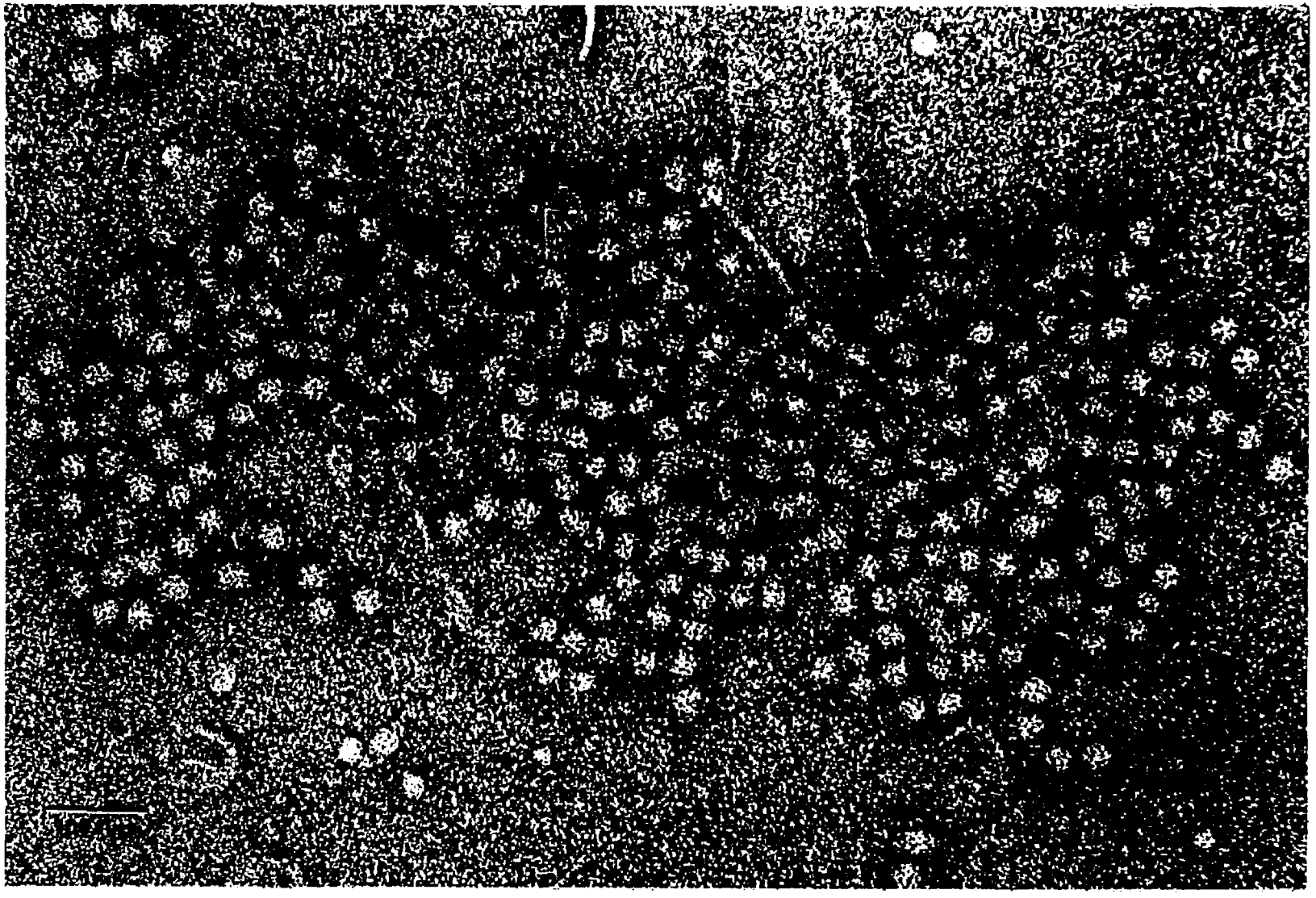

Figure 5. Precipitation of SMV isolated from buffalo gourd with SMV-1H antiserum. -- Note heavy antibody decoration of virus particles. This crude-sap preparation from Cucurbita pepo cv. Sugar Pumpkin was stained with phosphotungstic acid (×158,000).

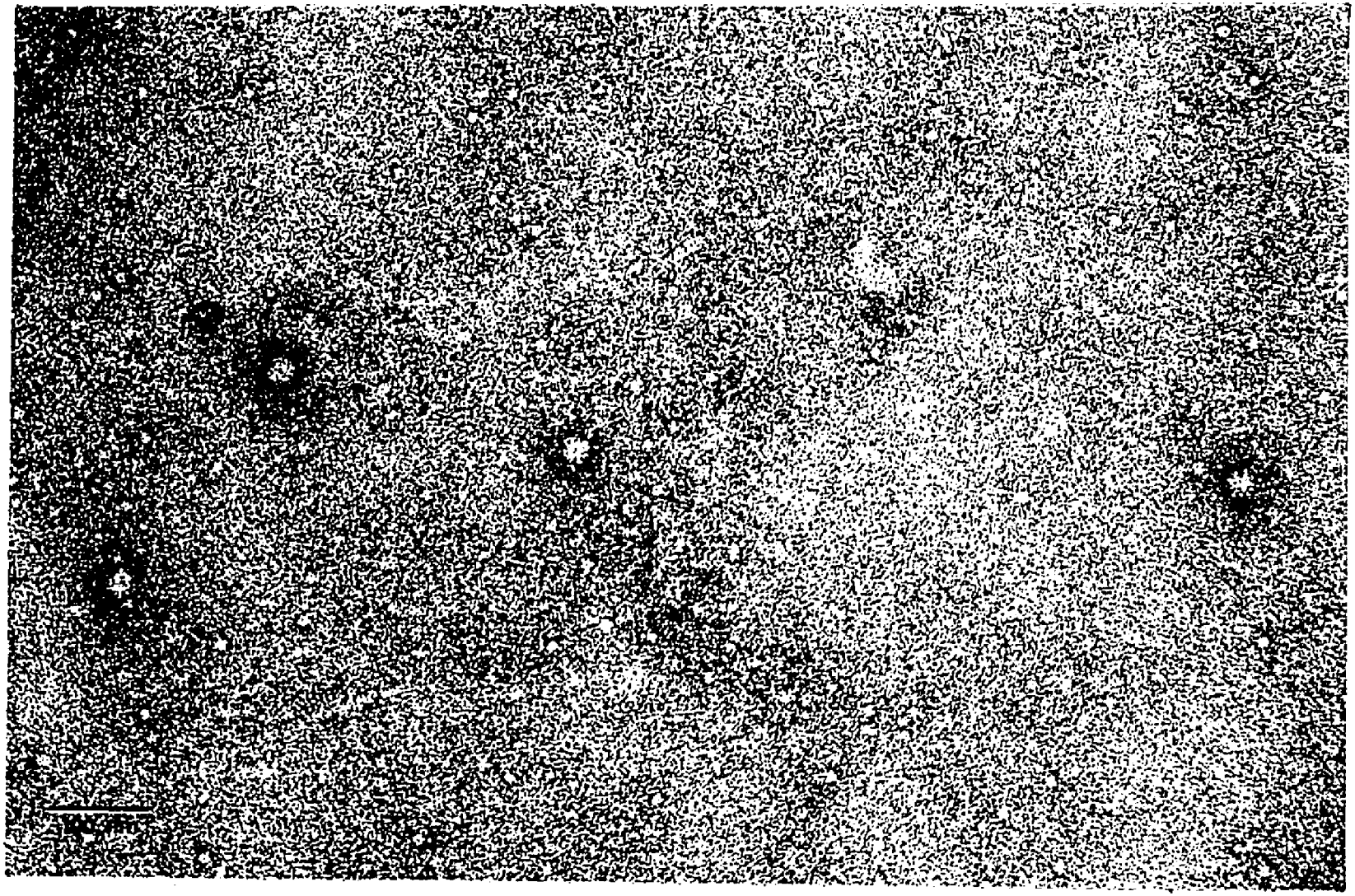

Figure 6. Squash mosaic virus isolated from buffalo gourd in phosphate buffer without antiserum. -- Both SMV isolated from buffalo gourd and SMV-2 showed no precipitation under these conditions. Crude-sap preparation from Cucurbita pepo cv. Sugar Pumpkin stained with phosphotungstic acid (×158,000).

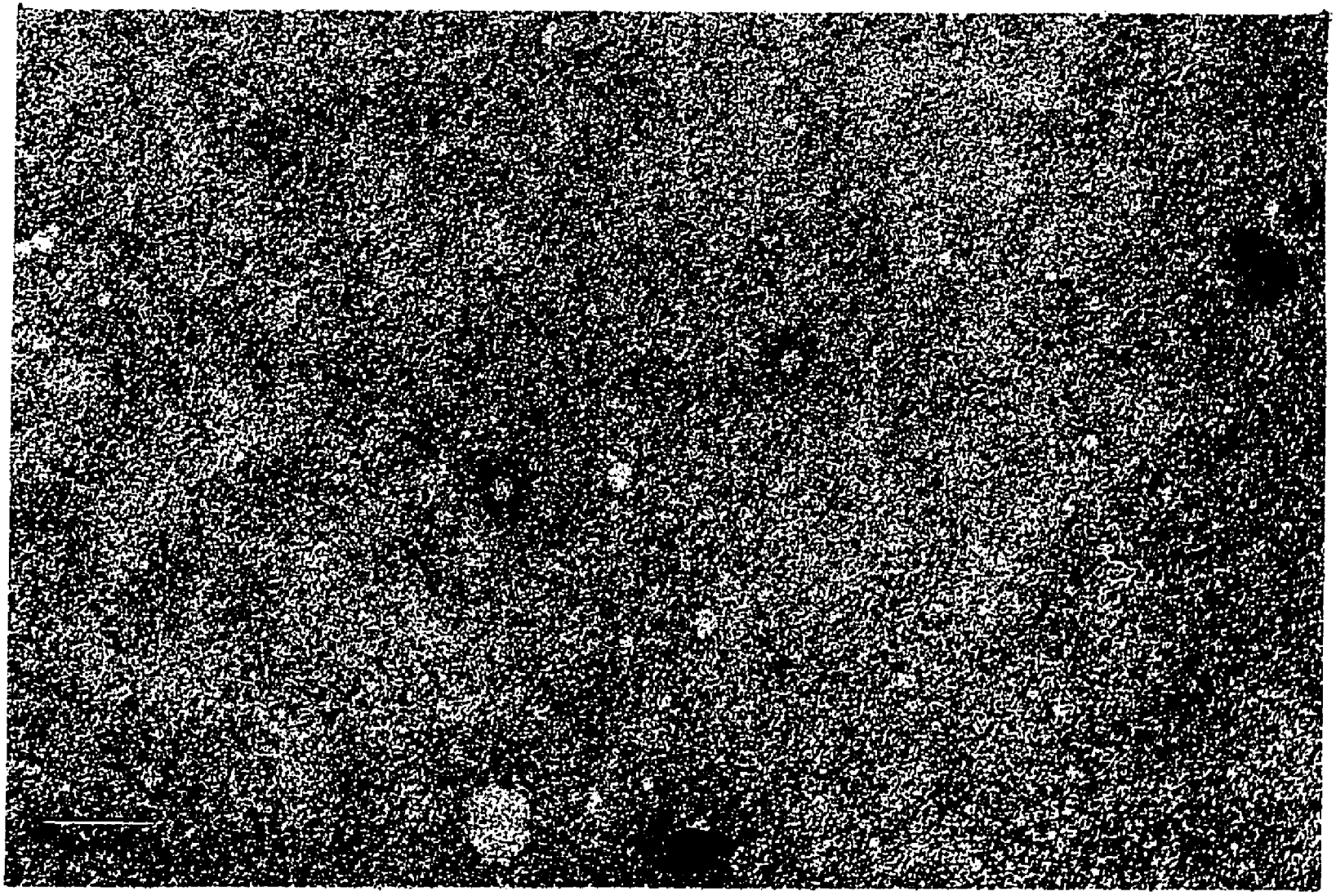

Figure 7. Squash mosaic virus isolated from buffalo gourd with CMV PVAS-242 antiserum. -- Both SMV and SMV-2 behaved identically under these conditions. Crude-sap preparation from Cucurbita pepo cv. Sugar Pumpkin, stained with phosphotungstic acid (×158,000).

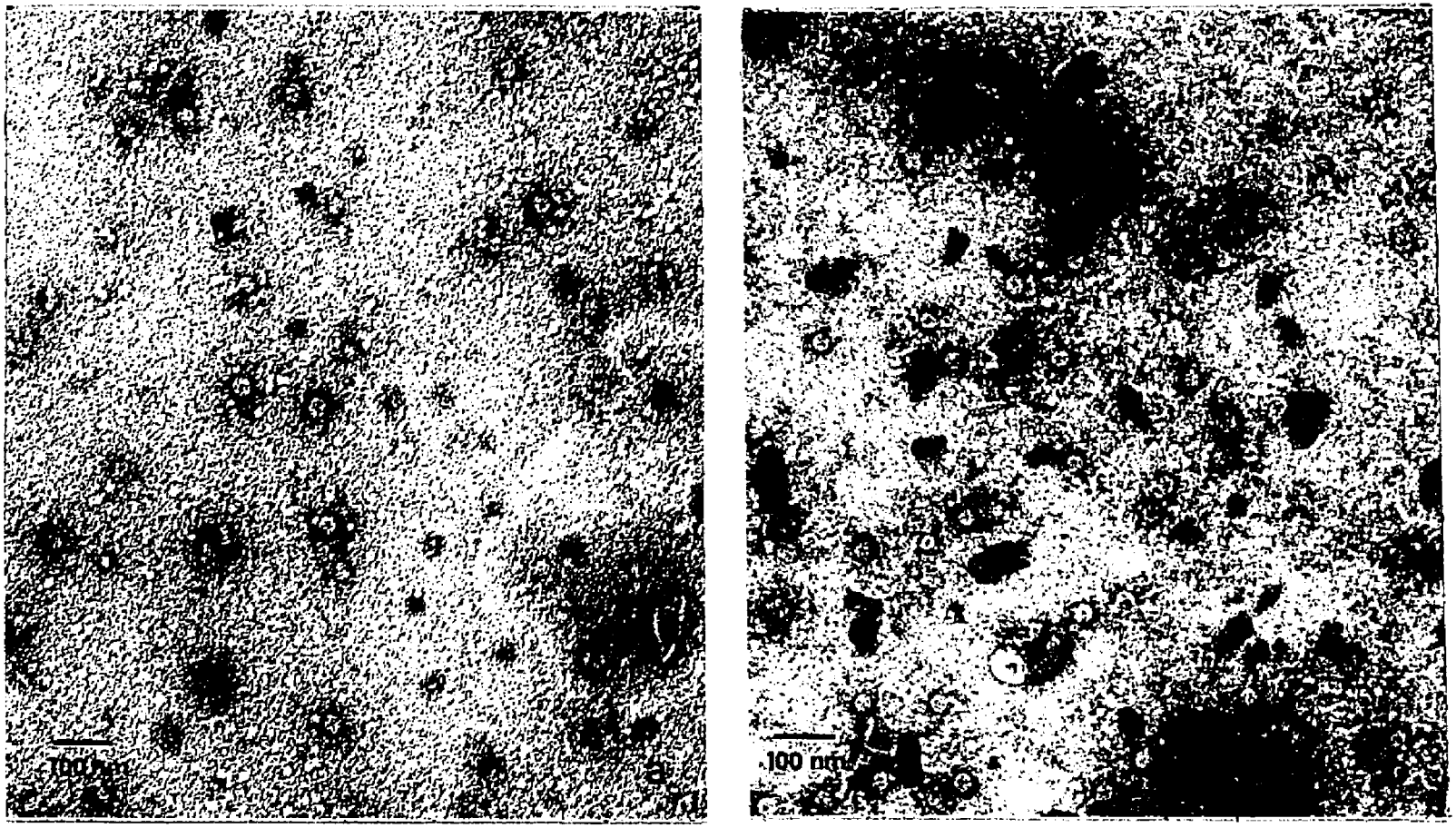

Figure 8. Cucumber mosaic virus ATCC PV-242 and buffalo gourd isolate 29V. -- (a) CMV (ATCC PV-242) in crude-sap preparation from 'Xanthi' tobacco, in distilled water, fixed with oxmium tetroxide, and stained with uranyl acetate; (b) CMV 29V isolated from buffalo gourd in crude-sap preparation from 'Xanthi' tobacco, in phosphate buffer and stained with uranyl acetate (×81,600).

Consequently, the grid must be washed with distilled water before staining. Other stains (Table 3) caused precipitation of the buffer and/or dissociation of the particle.

Both CMV and 29V tended to aggregate naturally in preparations without antiserum. Therefore, some precipitation occurred in the standard preparations; although from subjective observations there was much less precipitation than in preparations containing antisera. As a result, the Derrick technique, which avoids precipitation, was adopted.

Derrick Technique

In the Derrick technique, homology between virus and antiserum is determined by the number of virus particles present within a unit area (a field at a specified magnification) of a antiserum-treated grid as compared to buffer and heterologous antiserum-treated areas. At least a threefold increase in particle number per unit area is necessary to count the reaction as positive (Derrick and Brlansky, 1974; Milne and Luisoni, 1975; Lesemann, Bozarth, and Koenig, 1980). A threefold increase was not consistently observed, even with known CMV (Table 7); however, the small number of fields counted may have contributed to the inconsistency.

Strains of known CMV appeared to differ markedly in their overall immunogenicity (Table 7); for example, many more particles of CMV PV-242 (a strain from South Africa) adhered to the grid than particles of CMV PV-59 (a sugar beet isolate from Mesa, Arizona) with any CMV antiserum. The CMV PV-59 appears to be only slightly immunogenic to any CMV antisera, as is buffalo gourd isolate 29V.

Table 7. Range or mean of number of virus particles adhered per field at 17K magnification in four trials with the Derrick technique using five isolates and six antisera

Virus	Antiserum Preparation	Antiserum Dilution	Range or Mean of n Fields for Trial			
			I n ≥ 5	II n ≥ 10	III n ≥ 5	IV n ≥ 20
CMV PV-242	+ buffer		15	0–1	10	0–2
	+ 260	1:300	22–35	---	---	10–20
	+ 260	1:500	45–78	25–50	45	---
	+ 242a	1:500	11–39	50–80	95	---
	+ 30	1:500	---	---	140–160	40–60
	+ PVY	1:500	---	0–1	0–2	0–1
	+ TRSV	1:500	---	0–1	---	---
	+ TmRSV	1:500	---	0–1	---	---
CMV PV-59	+ buffer		0–4	0–1		0–1
	+ 260	1:300	0–4	---		---
	+ 260	1:500	25–30	3		0–4
	+ 242a	1:500	9–29	0–1		---
	+ 30	1:500	---	---		0–4
	+ PVY	1:500	0-1	0–1		0
	+ TRSV	1:500	---	0–1		---
	+ TmRSV	1:500	---	0–1		---

Table 7. Range or mean of number of virus particles--Continued

Virus	Antiserum Preparation	Antiserum Dilution	Range or Mean of n Fields for Trial			
			I n ≥ 5	II n ≥ 10	III n ≥ 5	IV n ≥ 20
CMV 29V	+ buffer		1–4	17		0–2
	+ 260	1:300	1–4	---		2–6
	+ 260	1:500	7–9	13		---
	+ 242a	1:500	0–1	17		---
	+ 30	1:500	---	---		0–1
	+ PVY	1:500	0–1	13		0–2
	+ TRSV	1:500	---	6		---
	+ TmRSV	1:500	---	4		---
PVY	+ buffer		45–70	2		1–2
	+ 260	1:300	30–40	---		1–2
	+ 260	1:500	29–37	1		---
	+ 242a	1:500	43–57	2		---
	+ 30	1:500	---	---		1–2
	+ PVY	1:500	75–100	8		3–4

Table 7. Range or mean of number of virus particles--Continued

Virus[a]	Antiserum Preparation	Antiserum Dilution	Range or Mean of n Fields for Trial			
			I n ≥ 5	II n ≥ 10	III n ≥ 5	IV n ≥ 20
TRSV	+ buffer			0–1		
	+ 260	1:500		0–1		
	+ 242_a	1:500		0–1		
	+ TRSV	1:500		17		
	+ TmRSV	1:500		0–1		

a. The virus host was Nicotiana tabacum cv. Xanthi except for PVY for which the host was Capsicum annuum cv. Tobasco. In Trial I the 'Xanthi' tobacco tissue was harvested 18 days after inoculation, in Trial II 15 days, and in Trials III and IV 12 days.

Although the serology was not conclusive, the preponderance of the evidence, host range, structure, and reaction to stains, leads to the conclusion that 29V is in fact a strain of CMV.

The titer of the antisera does not always seem as important as the strain similarity in the amount of virus adhered; for example, PV-242 (titer 1:128) in Trials II and III with PVAS-242 showed nearly twice the number of adhering particles as compared with PVAS-260 (titer 1:512).

Field Observations

Symptoms

Three major disease syndromes were found in the buffalo gourd fields at the Campbell Avenue Farm in 1979. Plants exhibiting a Type I syndrome had the following symptoms: mosaic (large or small spots) (Figure 9a); distortion of leaves, often including undulations or rolling of the leaf margin (Figure 9b); puckered veins (unequal growth of leaf laminae and veins) (Figure 9a); enations (29% of Type 1) (Figure 10a); reduced leaf size (as small as 2 cm); shortened internodes; and upright habit. An view of a plant exhibiting Type I symptoms is seen in Figure 11a. Seventy-nine percent of the 41 plants observed in 1979 had these symptoms. Cucumber mosaic virus and SMV were isolated from plants of Type I, plants 28, 30, 33, and 44, as previously mentioned.

A Type I variant expressed symptoms as above, but particularly during the latter part of the growing season produced as many as 20 small (1-4 cm) leaves per node (Figure 10b). In one plant these

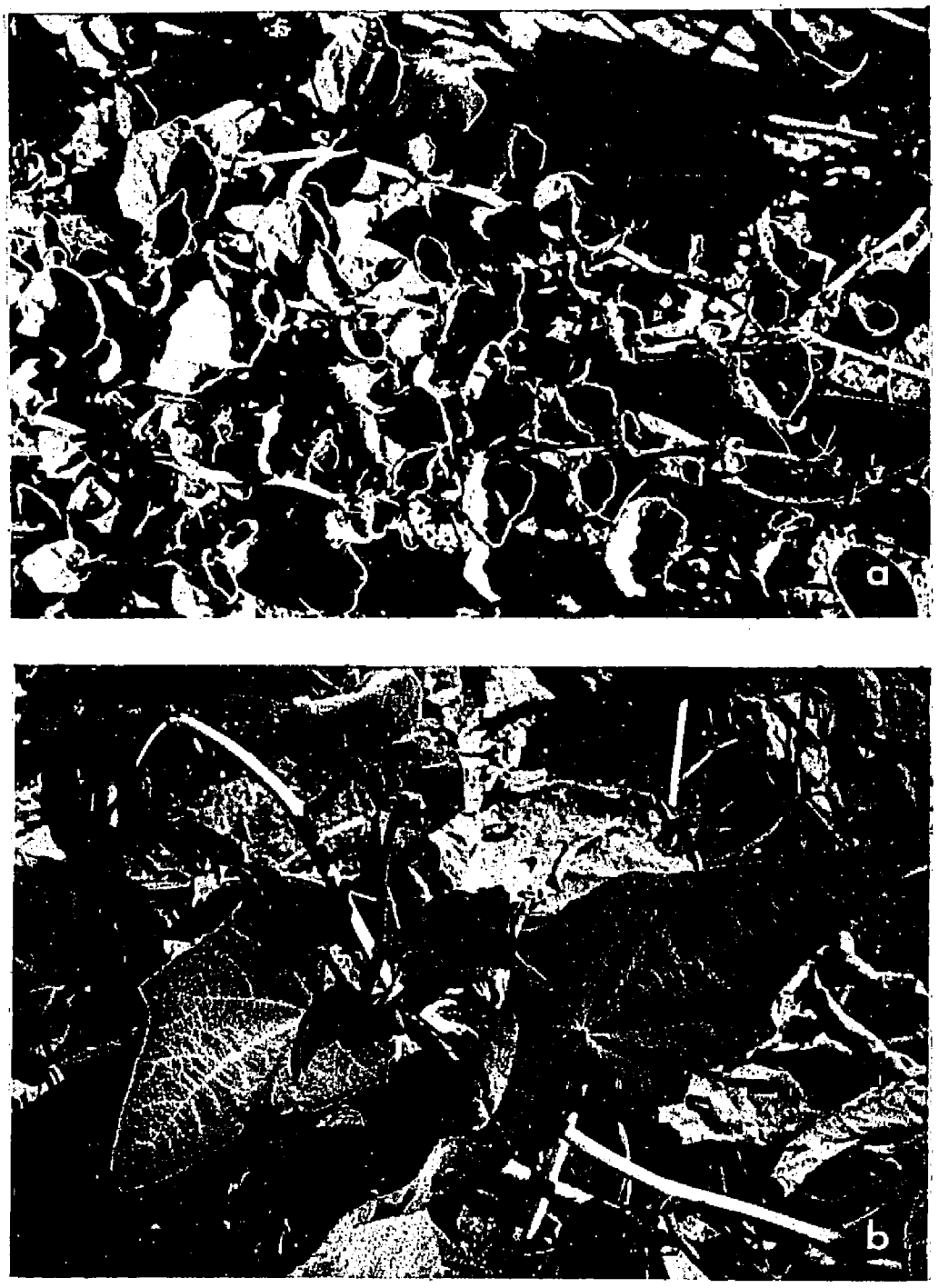

Figure 9. Mosaic and leaf distortion symptoms in field-grown buffalo gourd. -- (a) Mosaic and leaf distortion symptoms on leaves. Leaves are small, approximately 6 cm long when mature (25-30 cm normal). Note undulation of veins (white arrow). (b) Severe leaf distortion symptoms. Vein and blade growth appears asynchronous, resulting in leaf-blade puckering. Distorted leaves are approximately 10 cm long.

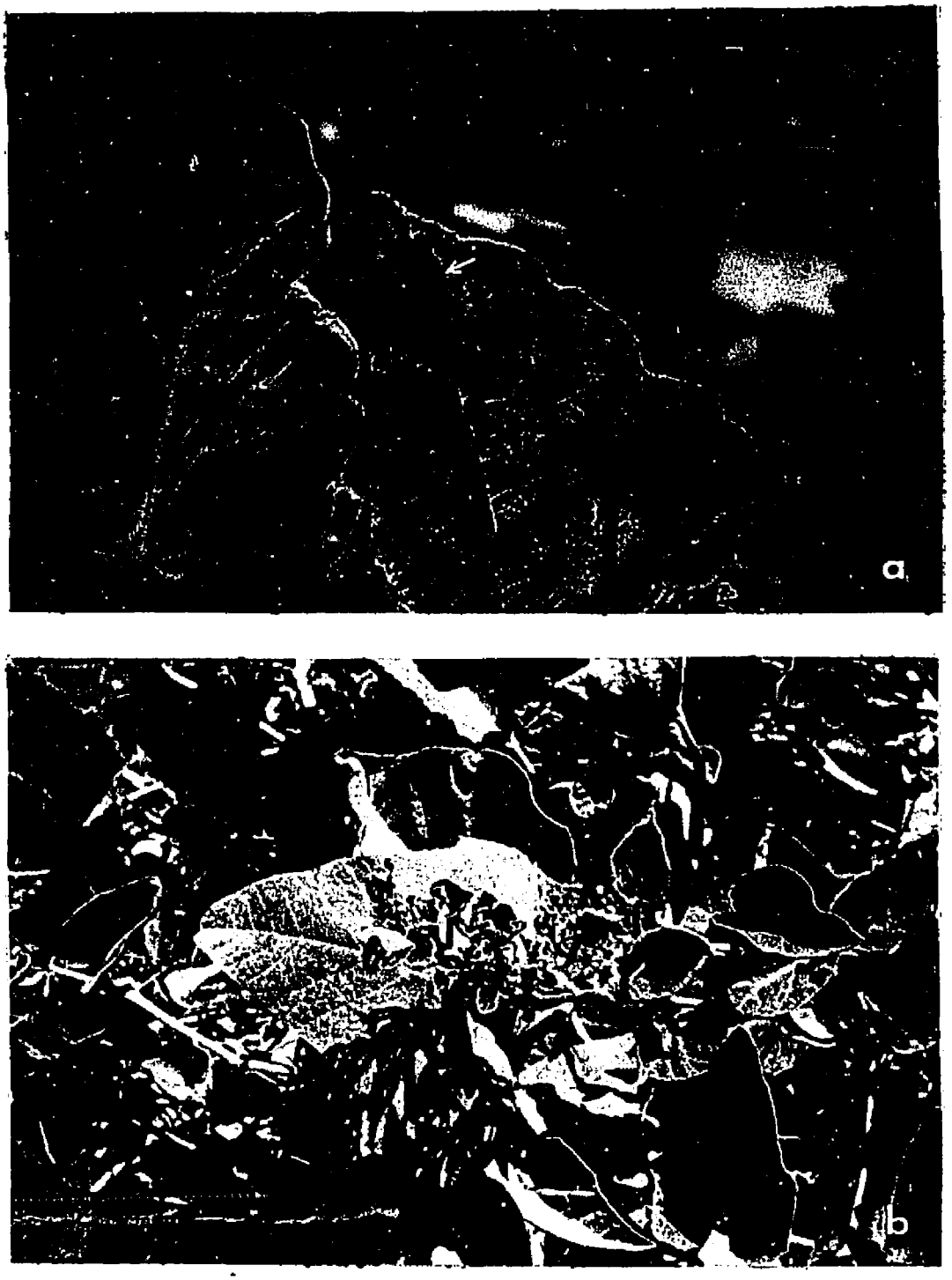

Figure 10. Enations and witches'-broom in field-grown buffalo gourd. -- (a) Enations on the underside of leaf (white arrow). Note leaf distortion and rolling caused by enations near the margins of the leaf on the left (black arrow). (b) Witches'-broom at terminus of runner. Leaves are 1-2 cm long and cupped.

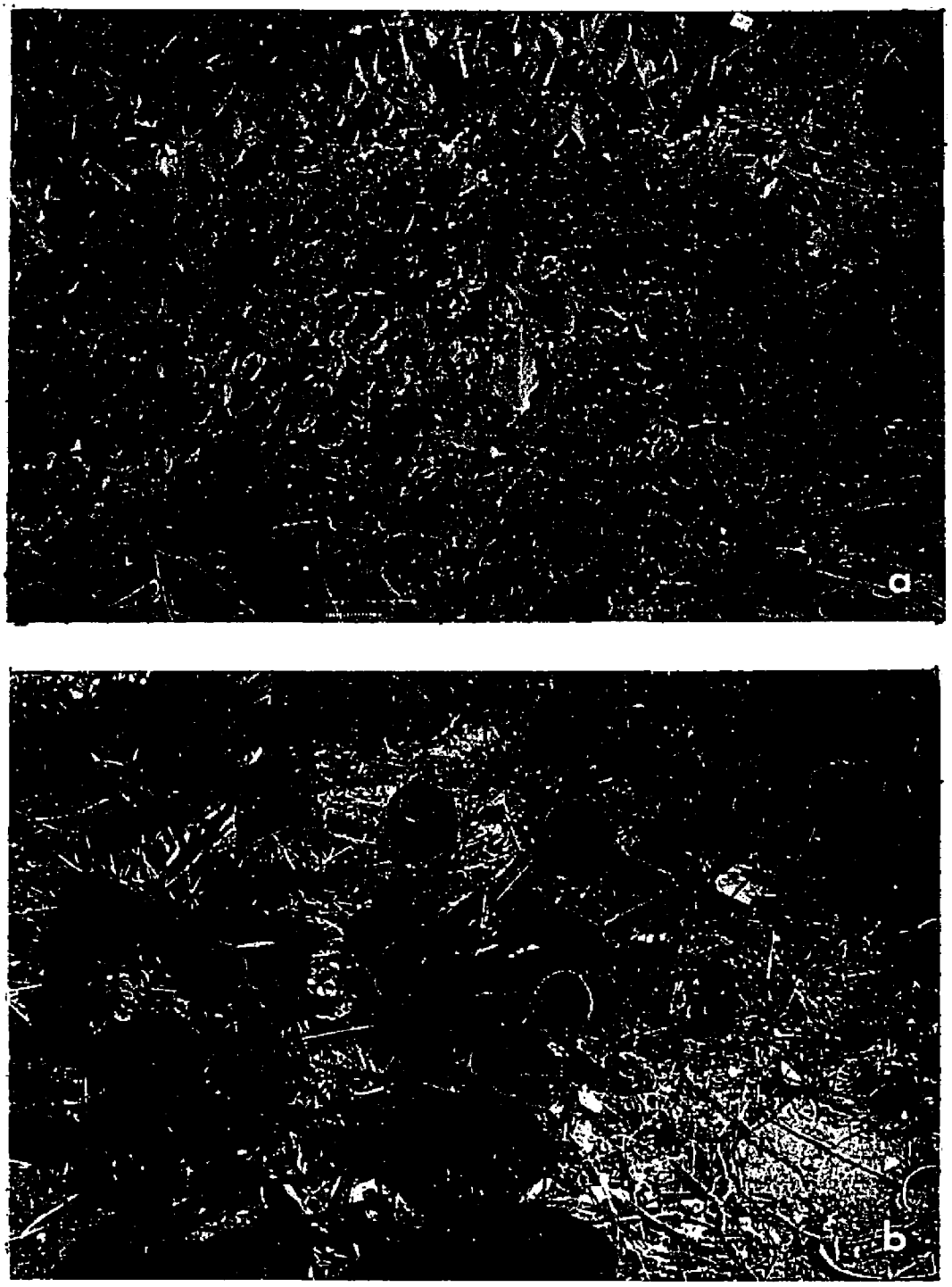

Figure 11. Type I and Type II symptom syndromes of field-grown buffalo gourd. -- (a) Plant in foreground exhibits Type I symptoms, mosaic and distortion. Healthy vine is in the background. Blue flag (upper center) is 3 cm × 4.5 cm. (b) Plant exhibits Type II symptoms, distortion without mosaic. Note curling of leaf margins.

witches'-broom symptoms were so acute that the plant had 1-cm leaves and 15-cm petioles, with many petioles emerging from one node. Ten percent of the plants showed these symptoms, among them plant 29 from which a strain of CMV has been isolated.

Plants with Type II symptoms (Figure 11b) had no mosaic but did have leaf distortion, including enations. Size of leaves was normal or only slightly reduced, and tips of vines were upright. Eight percent of the plants showed these symptoms.

Plants with Type III symptoms (Figure 12) had leaves with generalized chlorosis, seasonally showing mosaic. The maximum length of the leaves, which were cupped but not not distorted in any other way, was 3 cm; growing habit was upright. Three percent of the plants showed these symptoms, and although pathogen transfer was attempted at least four times, all results were negative.

Logically, the mosaic symptoms should be caused by SMV or CMV; however, mosaic symptoms on inoculated buffalo gourd plants in the greenhouse did not remain for more than a few weeks. Perhaps under field conditions the mosaic may be expressed more continually.

The distortion-type symptoms was not mechanically transferable and did not appear in buffalo gourd seedlings inoculated with CMV or SMV. It may be hypothesized that a nonmechanically transmittable organism such as curly top virus (CTV) or the newly described squash leaf curl (SLC) may be involved.

Curly top virus causes generalized chlorosis, leaf distortion with vein puckering, leaf margin roll, and shortened internodes on cucurbits. The vector is the sugar beet leaf hopper Circulifer tenellus

Figure 12. Type III symptom syndrome of field-grown buffalo gourd. -- Note chlorosis, reduced leaf size (1-2 cm long), leaf cupping, and reduced vigor.

(Keener, 1946; Giddings, 1948). Enations caused by CTV have only been seen on sugar beet (Nelson, 1981b, personal communication).

Squash leaf curl causes a chlorosis or mottle of interveinal tissue or an overall chlorosis; distortion, including margin roll and enations; and upright growth on cucurbits. Squash leaf curl has been found to be transmitted only by the whitefly Bemisia tabaci. The causal agent has been identified as a geminivirus (Dodds et al., 1982).

It seems likely that one or both of these organisms causes the leaf distortion, margin roll, puckering, enations, and upright growth present in Types I, II, and II. It may be hypothesized that Type I involves both mosaic organisms (SMV and CMV) as well as CTV or SLC; Type II is produced only by CTV or SLC; and Type II results from an acute infection by either CTV or SLC.

Symptoms of CTV were seen on four tomato plants transplanted to CAF Plot 6. Tomato is one of the crops most susceptible to CTV; in some parts of the western United States, almost a total loss of tomato plantings has been recorded (Keener, 1956). The symptoms of CTV in tomato, leaf rolling, generalized chlorosis, stunting, and reduced yields, were observed on these plants.

Curly top virus has been known to coexist naturally with a mosaic virus. Curly top virus and an unidentified mosaic virus were found in 1947 in guar planted at the Mesa Experiment Station (Keener, 1956).

Squash mosaic virus and WMV were identified in cultivated cucurbits from the Campbell Avenue Farm in 1979. Host range and serology tests revealed SMV in cantaloupe plantings and WMV in

watermelon. The vectors of SMV, CMV, and WMV were also observed in the fields in 1979. A paucity of aphids and a profusion of cucumber beetles, the spotted Acalymma trivittata Mann and western striped Diabrotica undecimpuctata tenella Le Conte, were observed (identification by the University of Arizona Department of Entomology).

Two plants, one from Type I, 30 (1978) and one from Type II, 60 (1979), showed fasciation in the fall (September, October). However, the symptom was not present in the succeeding year. The cause of fasciation is unknown.

Viruslike morphological abnormalities, similar to those described by Whitaker and Bemis (1964), were also observed on field-grown buffalo gourd plants. Certain lines of buffalo gourd tended to develop these genetically induced abnormalities, as one would expect.

Symptoms in each plant were similar through succeeding years. However, the sample size from 1979–1980 was small due to a 70% overwintering mortality rate (vs. 25% mortality in 1978–79). This may be have been due to the large outbreak of Erwinia-Fusarium-caused root rot during late 1979 (Butler, 1980) and/or the lethality of the virus complex.

Environmental Conditions and Symptom Expression

Mosaic and distortion have a tendency to either increase or stay the same but not to decrease during periods of growth, corresponding to periods of high precipitation. Both mosaic and distortion have a tendency to decrease or stay the same but not to increase during periods when growth occurred slowly or not at all, corresponding to

dry periods (Tables 8 and 9). Periods III and IV (Table 9) showed this relationship most markedly.

Temperature effects appeared to be less important; however, this is difficult to determine without control plants under identical field conditions but with lower temperatures. In Periods II and III, approximately June through September, 1979, the average of the daily maximum and minimum temperatures was over 26.5°C (80°F). Duration of mosaic through these periods was highly variable.

In general, there is a positive correlation between symptom expression, host plant growth, and virus concentration (Kasaanis, 1957). Cucumber mosaic virus concentration has been found to be correlated with symptom expression in 'Ashley' cucumber (Wood and Barbara, 1971) and tobacco (Cheo and Pound, 1952). The relative quantity of these parameters are in turn greatly dependent on the following: species of host plant; length of time infected; virus strain; and environmental conditions, including the availability of water, temperature, soil fertility, light intensity, duration, and photoperiod (Gibbs and Harrison, 1976). The correlation of symptom and virus concentration is valid only for any one combination of host species and virus; relative severity of symptoms caused by two different viruses in the same host or one virus in two different hosts is not correlated (Gibbs and Harrison, 1976).

Drought stress is believed to negatively affect virus concentration (Gibbs and Harrison, 1976) and would therefore decrease symptom severity, but this has not been well substantiated. In contrast, the effect of temperature on symptom expression, growth, and virus

Table 8. Temperature and precipitation at Campbell Avenue Farm in 1979

Month	Temperature			Precipitation	
	Maximum °C	Minimum °C	Monthly Average °C	Monthly Average cm	Daily Amount cm (date)
January	14.3	3.8	9.1	8.94	
February	19.4	4.8	12.1	2.11	
March	20.6	6.3	13.4	3.23	.25(2), .46(17), .30(20), 1.52(21), .25(28), .43(29)
April	27.7	9.6	18.6	0	
May	30.1	14.6	22.4	1.47	.51(9), .28(16), .41(20), .13(23), .15(26)
June	37.8	18.8	28.4	.43	.25(4), .18(8)
July	39.2	22.5	30.9	5.38	1.63(16), 1.02(18), 2.36(20), .38(30)
August	35.6	21.6	28.0	5.16	.9(4), .28(9), 2.79(12), .38(14), .79(16)
September	36.2	21.7	29.0	0	
October	30.9	14.6	22.8	.71	.71(21)
November	21.3	6.0	13.7	.89	.89(8)
December	20.3	5.3	12.8	.13	
Yearly Total				28.45	

Table 9. Number of plants at Campbell Avenue Farm Plot 6 exhibiting changes in mosaic and distortion symptoms during periods of growth and dormancy in 1979

Period	Date	Precip-itation (cm)	No. Plants Exhibiting Change[a]					
			Mosaic			Distortion		
			↑	=	↓	↑	=	↓
0[b]	Jan 1-Mar 2	11.3						
I	Mar 3-Jun 18	1.91	10	7	2	5	15	2
II	Jun 19-Jul 15	0	0	7	10	1	13	7
III[c]	Jul 16-Oct 17	10.54	12	6	1	12	8	2
IV[d]	Oct 18-Nov 2	.71	0	4	8	0	4	8
I + III	(growth)		22	13	3	17	22	4
II + IV	(dormancy)		0	11	8	1	17	18

a. ↑: Increase in symptom severity; =: no change in symptom severity; ↓: decrease in symptom severity.

b. Buffalo gourd plant dormant without foliage..

c. If premature death from Erwinia–Fusarium root rot occurred, this period of growth extended only from July 16 to September 30.

d. Premature deaths from Erwinia–Fusarium root rot occurred from October 1 to October 17 but were included in this period because the plants exhibited symptoms of dormancy.

concentration has been studied with findings of both positive and negative correlations between these three variables in SMV- and CMV-infected plants. Bancroft (1958) found that symptoms and growth rate were not correlated due to symptom masking at high temperatures. Squash mosaic virus in pumpkin and squash (both C. pepo) grown at 15.5°C and 21°C had more severe symptoms and higher titer of virus over a period of time than plants grown at 26.5°C at which growth was optimum (Bancroft, 1958). Cheo and Pound (1952) found that symptoms of CMV in tobacco were directly correlated to virus concentration and were cyclic, the periodicity of which was affected by temperature.

If the virus is neither masked nor lethal to the plant, mosaic symptoms are commonly cyclic (Gibbs and Harrison, 1976). The decrease in virus concentration and symptoms may be due to the saturation of the host's ability to support replication of the virus. Nevertheless, growth produces a new niche for virus replication and after an incubation period the cycle starts anew (Cheo and Pound, 1952). There is some evidence for cyclic behavior in field-grown buffalo gourd; however, plants mechanically inoculated with SMV or CMV and grown in the greenhouse have remained uniformly symptomless for several months after the initial period of symptom expression.

Field Assays

Five of six field-grown buffalo gourd plants exhibiting symptoms in Assay 1 (Table 10) contained mechanically transmissible virus. Plant 5, the only plant observed exhibiting Type III symptoms, was also the only plant in this assay exhibiting symptoms from which no

Table 10. Field symptoms of buffalo gourd plants observed on July 4, 1979 (Period II) and symptoms exhibited by cucurbits inoculated with extracts of buffalo gourd plants, Assay 1

	Field Symptoms in Buffalo Gourd[a]				Symptoms in Cucurbits[b]					
Plant Number	Mosaic	Distortion	Leaf Size in cm	Vigor	'Sugar Pumpkin'		'Big Max' pumpkin		'Florida Giant' watermelon	
5	chlorotic	cupped	2-5	low	6/6[c]	ns	4/4	ns	6/6	ns
55	+	cupped	2-5	low	1/6	rl,m	4/4	ns	6/6	ns
63	+	+	7-10	moderate	2/6	rl,m	4/4	ns	6/6	ns
70	+	o	15	moderate	6/8	rl,m	4/4	ns	1/6	chlorotic, stunting
75	chlorotic	+, roll, enations	10-12	moderate	2/8	rl,m	2/4	m	6/6	ns
116	+	++	10	moderate	3/8	rl,m	3/4	rl	6/6	ns
78	o	o	12-15	moderate[d]	6/6	ns	4/4	ns	6/6	ns
79	o	o	25	high[d]	1/6	m	4/4	ns	6/6	ns
80	o	o	10	moderate[d]	6/6	ns	4/4	ns	6/6	ns
81	o	o	17	moderate	6/6	ns	4/4	ns	6/6	ns
82	o	o	25	high	6/6	ns	4/4	ns	6/6	ns

a. + = mild; ++ = severe; o = none. b. rl = recurved leaves; ns = no symptoms; m = mosaic.

c. Numerator = number of plants exhibiting symptom(s); denominator = total number observed.

d. In Period III these plants showed distortion and enations on new growth.

transmissible agent was obtained. Only one (79) of the six symptomless plants contained a transmissible virus, and this plant did exhibit symptoms during a later period of intense growth (Period III). Two other plants (78, 81) of this group also exhibited symptoms during Period III, but the titer may have been too low for mechanical transfer.

Two of five buffalo gourd plants chosen for their symptoms in Assay 2 contained mechanically transmissible virus particles (Table 11). Of the plants from which transfers were not successful, 29 and 30 had been previously shown to contain CMV and SMV, respectively. It may be concluded that inoculating cucurbits with extracts of buffalo gourd only once (as done in Assay 2) is not as effective as three times (as in Assay 1).

Four of five buffalo gourd plants randomly chosen to test the extent of virus infection in CAF Plot 1 exhibited symptoms. However, the one plant (83) that did not exhibit symptoms did contain a mechanically transmissible virus. The plant died from root rot, presumably caused by a Erwinia–Fusarium complex, before symptoms could have possibly been observed later in 1979. Although results from more samples are needed, it seems likely that most of the buffalo gourd plants in CAF Plot 1 were virus infected.

Extracts of plant 55 (Assay 1) and plant 28 (Assay 2) were inoculated onto diagnostic species. The results on Chenopodium amaranticolor and 'Xanthi' tobacco were similar to those observed from inoculation by 29V and thus the extracts appear to contain CMV. Perhaps less SMV symptoms were observed because these plants were assayed in the summer; whereas the first group of cuttings (29, 30,

Table 11. Field symptoms of buffalo gourd plants observed on July 4, 1979 (Period II) and symptoms exhibited by cucurbits inoculated with extracts of buffalo gourd plants, Assay 2

Plant Number	Field Symptoms in Buffalo Gourd[a]				Symptoms in Cucurbits[b]		
	Mosaic	Distortion	Leaf Size in cm	Vigor	'Sugar Pumpkin'	'Big Max' pumpkin	'Florida Giant' watermelon
28	+	+, enations	12	high	3/6[c] m	3/6 m	1/5 m
29	chlorotic	o	15	high	6/6 ns	6/6 ns	6/6 ns
30	+	+, roll	15	high	6/6 ns	6/6 ns	6/6 ns
50	+	+, roll, enations	15	high	3/6 ns	6/6 ns	6/6 ns
76	+	+	9	high	6/6 ns	6/6 ns	6/6 ns
83	o	o	15	high[d]	1/6 m	6/6 ns	6/6 ns
84	+	o	22	high[d]	6/6 ns	6/6 ns	6/6 ns
85	+	+	15	high	6/6 ns	6/6 ns	6/6 ns
86	o	o	20	moderate[e,f]	1/6 m	6/6 ns	6/6 ns
87	+	+, enations	15	high[f]	6/6 ns	6/6 ns	6/6 ns

a. + = mild; o = none. b. ns = no symptoms; m = mosaic.

c. Numerator = number of plants exhibiting symptom(s); denominator = total number observed.

d. Erwinia-Fusarium death occurred within one month.

e. Distortion appeared on new growth later in 1979. f. Many leaves per node.

33, 44) were taken for characterization in the fall. Squash mosaic virus infection is more extensive in the fall due to higher populations of cucumber beetles (Nelson and Tuttle, 1969). To explore the relative proportions of SMV and CMV in the field, a larger sample size would need to be characterized.

In summary, virus infection appears to be extensive in the Campbell Avenue Farm fields. Virus can be isolated from apparently symptomless plants, supporting previously reported correlations between environmental conditions, growth, and symptom expression. Some plants exhibiting symptoms did not appear to contain a mechanically transferable particle. More extensive characterization is needed to ascertain the relative proportions of SMV and CMV in Arizona buffalo gourd fields.

Seed Transmission

Greenhouse Tests

Many diseased plants do not flower, or if they do, the number of gourds is greatly reduced; therefore, 1978 AZ #1 reciprocal seed was probably largely from normal plants. In 1978 there were more symptomless plants in Campbell Avenue Farm Plot 1 than in 1979, when most of the plants exhibited symptoms. The proportion of plants infected with SMV, which is known to be seed transmitted in cucurbits (Frietag, 1956), contrasted to the proportion of plants infected with CMV is not known. The seed was tested because it has been sent worldwide.

Of 1,000 seedling, 3.5% appeared to have slightly deformed leaves at 3 weeks; however, new leaves appeared normal at 7 weeks (Table 12). No mosaic or chlorosis was observed. No rod-shaped, filamentous, or isometric viruses in high titer were observed when extracts were examined by electron microscopy. No symptoms were observed on the cucurbits or indicator plants after inoculation with extracts of abnormal buffalo gourd seedlings.

Consequently, it appears that a small amount of deformation of the first true leaves is normal and is probably due to physical pressure of the seed coat. However, to be absolutely certain that there is no seed-borne virus in Arizona #1 reciprocal seed, the cucurbits should be inoculated three times rather than only once with extracts of the abnormal gourd seedlings.

The identity of the virus present in the diseased plants, except for plant 30, is unknown; therefore, it is not known if seed transmission of SMV, CMV, CTV, or SLC is being tested. One seedling out of the 63 (1.6%) produced from seed from SMV-infected plant 30 exhibited mosaic symptoms but later became symptomless. However, to conclude that seed-borne SMV was involved, the virus would need to be isolated and characterized. Two seedlings exhibiting mosaic symptoms came from seed from other diseased plants (22, 200), but again isolation and characterization would be required to conclude that the mosaic was seed-borne.

Leaf deformation may be virus, seed coat, or genetically induced. Incidence of leaf deformation (or distortion) in seedlings from diseased plants range from 2.4% (plant 22) to 100% (plant 36) and

Table 12. Percentage of abnormal seedlings from seed of bulked Arizona #1 reciprocal and diseased buffalo gourd plants tested for presence of seed-borne virus

Plant Number	Symptom Type	Total Planted	Germination		Abnormal Seedlings				
						3 wk		7 wk	
			n	%	Total Symptoms	n	%	n	%
Arizona #1 reciprocal		1200	960	80	slight puckering of leaves	31	3.1	0	0
					arched veins	4	.4	0	0
22	II	50	42	84	mosaic	1	2.4	0	0
					distorted leaves, veinal irregularities	1	2.4	0	0
30-1	I (SMV)	167	27	16	slightly deformed leaves	4	15	0	0
30-2	I (SMV)	165	35	21	grossly deformed first true leaves	4	11	0	0
					minor deformation	1	<1	0	0
30-2	I (SMV)	50	2	4	light mosaic	1	50	0	0
36	I	113	7	6	puckered veins or slightly irregular margins	7	100	0	0
200	not tested	213	179	84	puckerings or physical deformity	11	6	0	0
					chlorosis	1	.6	0	0
					mosaic	1	.6	0	0
200	---	50	31	62	chlorosis	2	6	0	0

averaged 3.1% in Arizona #1 reciprocal seed. Curly top virus, which may induce leaf deformation in buffalo gourd, is not known to be seed transmitted (Keener, 1956). Seed transmission of SLV has not been tested. Further host range and serology studies are needed to determine whether virus infection induces leaf deformation.

The germination rates appeared quite variable, ranging from 4% to 84%. It may be significant that plant 30, which was known to be SMV infected, had the lowest germination rate.

Field Tests

Two percent (67 of 3500) buffalo gourd plants were flagged for leaf deformation, usually of the first true leaves. This appeared developmental, i.e., due to seed coat pressure. All plants outgrew the deformity by the end of the growing season and appeared normal. The percentage in field-grown plants was comparable to that observed in greenhouse tests (3.5%).

Crop Loss Measurement

Seed Yield

Only a quarter of the diseased buffalo gourd plants in the field produced flowers or subsequently gourds in 1979. If flowers were produced, they were often abnormal and much reduced in number compared to those of a healthy plant. Several plants that did not produce gourds in 1979 did produce gourds in 1978.

Subjectively, the proportion of diseased plants appeared to increase sharply between 1978 and 1979 in the Campbell Avenue Farm

Plot 1. A 90% reduction in seed yield was recorded. However, this marked reduction was due not only to virus but also partially to Erwinia-Fusarium root rot (Butler, 1980).

Flower and seed production in most plants is decreased or even entirely stopped by virus infection. The actual yield reduction depends on such factors as the strain of virus, the species and variety of plant, and, most importantly, when infection occurs in the life of the plant (Gibbs and Harrison, 1976). Cucumber mosaic virus has been known to cause an epiphytotic loss of cucumbers. In addition, in some areas yield losses have been so continuous year to year that cucumbers had to be replaced by other crops (Corbett and Sisler, 1964). Curly top virus induces small abnormal flowers as well as premature fruit ripening in melons and squash (Keener, 1956).

Root Starch Yield

There was no significant difference in the dry weight percentage of root starch between nine diseased and six healthy roots (Tables 13 and 14). Moreover, when means were compared using the two-tailed t test at the 5% level of significance, the t statistic equaled 1.25. The calculated value of t demonstrated that the two samples were not statistically significantly different (critical value of t = 2.160, 5% level of signifiance, 13 degrees of freedom).

The wide variation in root starch within the healthy or diseased populations appears not to be due to symptom type but to vigor of the roots (Table 13); the average dry weight percent of starch for plants exhibiting Type I symptoms is 50.93 and for Type II is 51.03.

Table 13. Percent dry weight of root starch in diseased and healthy buffalo gourd plants from Campbell Avenue Farm Plot 4, with description of symptoms

Plant Number	Symptoms, 10/7/79[a]		Average Leaf Length (cm)	Vigor	Symptoms Type	Starch % Dry Weight
	Mosaic	Distortion				
Diseased, Field-grown						
211	++	+	10	low	I	52.3
212	+++	++	7.5	high	I	65.2
214	o	+++	25	moderate	II	57.8
215	o	++	12.5	moderate	II	47.4
216	++	+	5	moderate	I	47.6
217	o	roll	12.5	low	II	47.9
218	++	wavy	5	low	I	37.5
219	++	++	5[b]	high	I	59.1
220	++	cupped	2.5	moderate	I	43.9
Healthy, Field-grown						
201	o	o	17.5	moderate		52.3
203	o	o	30	moderate		60.7
205	o	o	20	moderate		56.8
206	o	o	20	moderate		55.3
207	o	o	20	moderate		54.8
208	o	o	12.5	moderate		51.4

Table 13. Percent dry weight of root starch--Continued

Plant Number	Symptoms, 10/7/79[a]		Average Leaf Length (cm)	Vigor	Symptoms Type	Starch % Dry Weight
	Mosaic	Distortion				
Healthy, Greenhouse-grown						
203	o	o	(c)	low		26.1
205	o	o	(c)	low		25.0
206	o	o	(c)	low		31.2
208	o	o	(c)	low		39.9

a. +++ = severe; ++ = moderate; + = mild; o = none.

b. Many leaves per node.

c. Not measured.

Table 14. Mean and standard deviation of dry weight percent of root starch in diseased and healthy buffalo gourd plants. -- From data in Table 13

Plant Numbers	n	mean	SD
Diseased, field-grown			
211–220	9	51.44[a]	8.23
Healthy, field-grown			
201–208	6	55.22[a]	3.07
Healthy, greenhouse-grown			
	4	30.57	5.88

a. Calculated value of the t statistic in the two-tailed t test between means of diseased and healthy field-grown samples was 1.25; critical t = 2.160 at a 5% level of significance with 13 degrees of freedom.

However, roots of the two most vigorous plants (212, 219) had an average of 62.2% starch as opposed to 47.8% in the roots of remaining diseased plants, which showed moderate or low vigor. But plant 212, which showed the most prominent mosaic, had the highest percentage of starch and was also one of the most vigorous. Vigor appears to take precedence over symptom expression; however, a more significant conclusion could be drawn with a larger sample size. The low percentage of starch in the healthy adventitious roots transferred to pots in the greenhouse was presumably due to lack of space and overwatering, which caused an overall lack of vigor. The overall percentage of starch from both diseased and healthy field roots seems to compare favorably with roots assayed in the same month of 1976, 51–55% vs. 45%, respectively (Berry et al., 1978).

The general effect of virus infection has been shown to decrease that rate at which starch accumulates in the leaves and is translocated from leaves (as simple sugars) during the night (Gibbs and Harrison, 1976). More specifically, cucumber mosaic virus decreased the starch and saccharide content of cucumber leaves as a result of reduced CO_2 fixation and an increased rate of degradation. This is particularly critical in the later stages of infection, when the infected plants had less than half the starch of healthy controls (Sindelar, Makovcova, and Hanusova, 1980). Therefore, one would expect that in an environmentally controlled experiment with an adequate sample size and uniform seed source, a more significant difference in percentage of starch in roots of diseased and healthy buffalo gourd plants would be found.

Infectivity Assays

Of the 10 virus and strains inoculated to buffalo gourd seedlings, only SMV-2 has infected buffalo gourd and been recovered from it (Table 15). Unlike 29V, both strains of CMV from the American Type Culture Collection did not produce symptoms in buffalo gourd and no virus was recovered. Provvidenti et al. (1978) considered buffalo gourd resistant to CMV (New York strain) because it reacted with only local infection. It appears that 29V is an unusual strain of CMV that has developed the capacity to infect buffalo gourd.

Tobacco ringspot virus and WMV did not infect buffalo gourd. Provvidenti et al. (1978) also found buffalo gourd resistant to WMV and TRSV, although the latter produced local lesions. Provvidenti et al. found TmRSV (not tested in the study reported here) to produce a systemic mosaic and leaf distortion in buffalo gourd; the plants eventually recovered from the symptoms. Extracts of 'Sugar Pumpkin' infected with 29V were serologically tested with TmRSV antisera (ATCC, PVAS-174) with negative results.

Search for Disease Resistance

Of the 10 roots that appeared symptomless in Mesa during 1978, 6 roots died and the remaining 4 exhibited symptoms after transplanting to the Campbell Avenue Farm. Of the 12 roots that exhibited symptoms in Mesa, 3 died and 9 continued to exhibit symptoms after transplanting. It appears that the "symptomless" roots were weak and vigorless and therefore did not exhibit symptoms in Mesa. Subsequently, many

Table 15. Results of buffalo gourd seedling infectivity assays with 10 viruses

Virus Group	Virus	Isolate or Strain	Source	Host[b]	Indicator Plant[b]	Symptoms[a] on Indicator Plant	Symptoms[a] on Buffalo Gourd
Cucumovirus	CMV	PV-59	ATCC[c]	X	X	sm	r
		PV-242	ATCC	X	X	sm	r
Comovirus	SMV	2	Wisconsin	C	C	sm	mm
Nepovirus	TRSV	PV-157	ATCC	X	X	rs	r
Potyvirus	WMV	2	C. melo, Arizona	C	C	sm	r
	pepper mottle	Arizona	Datura, Arizona	A	A	stem necrosis	r
	potato virus Y	NC-57	Gooding[d]	X	X	vein clearing	r
	tobacco etch	PV-69	ATCC	X	Dm	sm	
					X	etch	r
Carlavirus	pea streak	PV-87	ATCC	Al	P	necrosis	
					Ca	LL	r
Bacilliform	alfalfa mosaic	Arizona	Mesa, AZ	Al	Ca	sm	r
	alfalfa mosaic	PV-92	ATCC	Al	Ca	sm	r

a. sm= severe systemic mosaic; mm = mild systemic mosaic; rs = ringspots; LL = local lesions; r = resistance.

b. A = Capsicum annum cv. Anaheim pepper; Al = Medicago sativa cv. Hayden alfalfa; C = Cucurbita pepo cv. Caserta squash or Sugar Pumpkin; Ca = Chenopodium amaranticolor; Dm = Datura metel; P = Pisum sativum cv. Progress #9 pea; X = Nicotiana tabacum cv. Xanthi.

c. American Type Culture Collection, Rockville, MD.

d. G. V. Gooding, North Carolina State University, Department of Plant Pathology, Raleigh.

died and others exhibited symptoms when they found more ideal growing conditions after transplanting to Campbell Avenue Farm.

The field symptoms of the Mesa plants were similar to those found in Tucson plants. Cucurbits inoculated with a diseased Mesa plant (116) exhibited symptoms identical to those in cucurbits inoculated with extracts of a CMV-infected Campbell Avenue Farm plant. The pumpkin cutting taken from Mesa was infected with SMV.

SUMMARY AND CONCLUSIONS

Striking viruslike symptoms on field-grown buffalo gourd have been observed from 1978 through 1981 in the Tucson and Phoenix areas of Arizona. Suspected pathogens are SMV, CMV, WMV, and CTV, which commonly occur on cucurbits in Arizona (Nelson and Tuttle, 1969), and possibly SLC (Flock and Mayhew, 1981). Squash mosaic virus and cucumber mosaic virus have been identified as components of this field syndrome. The CMV isolated from buffalo gourd is a strain able to infect gourd, unlike the other strains of CMV tested. Conclusive serological tests need to be performed. Watermelon mosaic virus has not been found occurring naturally, and buffalo gourd resists mechanical inoculation of WMV-2.

At this writing, the three types of naturally occurring disease syndromes cannot be correlated to the mosaic pathogens, either SMV or CMV. Other pathogens, either CTV, SLC, or other, may be responsible for the distortion-type symptoms, and these may mask differences between the mosaic caused by SMV and CMV. In the greenhouse, mosaic symptoms due to SMV or CMV are present for only a few weeks before the plant becomes symptomless. It may be hypothesized that their is either an interaction of the mosaic and distortion factors or an environmental condition (for example, one that produces more rapid growth) that causes the mosaic symptom to be expressed for longer periods of time in the field. The severity with which symptoms are expressed appears to be somewhat in response to the physiological state

of the plant; plants that are rapidly growing generally express more severe symptoms, and the converse is also true.

The virus disease syndrome does appear to be extensively present in second- and third-year plants in the field, resulting in a reduction and seed yield and perhaps starch yield; however, yield reduction remains to be quantified. Preliminary studies of seed transmission show that SMV is possibly seed transmitted in buffalo gourd. However, because the seed yield is reduced in diseased plants, the percent transmission in bulked seeds, if present, would be very low.

Currently the search for disease resistance has been unsuccessful; only plants that are symptomless due to reduced vigor have been found. Techniques of screening seedlings and inoculating mature plants must be explored. Remnants of the original germplasm collected from North America (Bemis, Berry, et al., 1978) could be screened for resistance.

It appears important to characterize the distortion factor and to identify the proportion of yield reduction that it causes. In addition, SMV- and CMV-infected seedlings must be planted in the field, preferably away from plants known to be carrying the distortion factor, symptoms observed, seed transmission tested, and yield loss of seed and starch determined. Depending on the outcome of these experiments, it may be more appropriate to search for plants resistant to the distortion factor rather than for those resistant to SMV or CMV.

Breeding for disease resistance may not be the only solution. The disease syndrome has rarely been observed on 1-year-old plants. Perhaps the disease can be avoided by growing the root as an annual

rather than as a biennial or perennial. This would also reduce the problem of volunteer plants acting as carriers for the disease between years. In addition, buffalo gourd plots could be isolated from other cultivated cucurbits, avoiding a potential source of inoculum and vectors. Infection may also be avoided if fields were isolated from dwellings whose perennial ornamentals harbor deleterious viruses, e.g., Vinca rosea serving as a reservoir for CMV.

Epiphytotics of wild plants under cultivation using normal agricultural practices are common. Disease itself is an ecological force that will eventually restore balance within the ecosystem (Cook, 1981). Reduction of vector populations may be achieved through biological control that could help restore balance and stability in the agricultural ecosystem.

As agricultural practices become more ecologically sound and the wisdom of biological control becomes more prevalent, epiphytotics will not have to be the force that rights the ecological balance. In the meantime, the potentially perennial buffalo gourd must be grown as an annual, or at most as a biennial, to avoid severe virus complex infection. However, for Cucurbita foetidissima to live up to its "promise for improving the quality of life in tropical areas" (National Academy of Science, 1975, p. v) for years to come, it must be integrated into an ecologically sound agricultural system.

APPENDIX

PREPARATION OF AGAR GEL PLATES

Combine in a 1-L flask:

3.75 g Special Nobel Agar (Difco)

4.50 g NaCl

400 ml H_2O (double-distilled, deionized).

Boil until agar is dissolved (stirring constantly)

Autoclave at 17 lb for 35 min.

When cool, add:

100 ml 0.1% sodium azide.

Pour 25 ml into each 15 mm × 100 mm sterile plastic petri dish.

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