Effects of Light Intensity, Photoperiod, and Temperature on Symptom Expression and Host and Virus Ultrastructure in *Saponaria vaccaria* Infected with Carnation Etched Ring Virus

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ABSTRACT

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The expression of symptoms caused by carnation etched ring virus (CERV) on *Saponaria vaccaria* was compared at different temperatures (21, 27, and 32 C), light levels (7,532, 16,140, and 22,596 lux [700, 1,500, and 2,100 ft-c] of cool-white fluorescent light with or without 1,076 lux [100 ft-c] of incandescent light), and photoperiods (8, 12, 14, and 16 hr/day). The temperature-induced differences in symptom expression also were related to temperature-induced differences in host cell and virus inclusion ultrastructure. Optimal conditions for rapid cytoplasmic inclusion formation, virus accumulation, and synchronous development of red local lesions were 27 C and 22,596 lux (2,100 ft-c) of fluorescent illumination for 14–16 hr/day. High temperature, low light levels, short photoperiods, and photoinduction of flowering decreased the red local lesion response in *S. vaccaria*. The appearance of symptoms and the rise in infectivity in inoculated plants were slower at 21 C than at 27 C. This was correlated with a slow rate of virus inclusion formation and virion accumulation at 21 C. At 32 C, chlorotic (not red) local lesions developed and a reversible, temperature-induced inhibition of cytoplasmic inclusion formation was found. However, nuclear virions and intranuclear membranes accumulated at 32 C and nuclear pores commonly contained virons. The formation of complex networks of interconnected chambers and expanded and extended plasmodesmata within the cell wall was temperature dependent and occurred extensively at 21 but not at 32 C. When epidermal strips were treated with fluorescein isothiocyanate-conjugated antiserum to normal virus inclusions, the abnormal inclusions formed at 32 C fluoresced.

Additional key words: bioassay, anthocyanin production.

Carnation etched ring virus (CERV) is a caulimovirus (10) that infects carnation (*Dianthus caryophyllus*). *Saponaria vaccaria* 'Pink Beauty' is a host for several carnation viruses, including CERV (3). However, the usefulness of Pink Beauty as a local lesion host for CERV has been limited because incubation periods may range from 10–30 days and a variety of symptoms can develop. Although variations may be due to genetic variation in the plant populations (3), environmental conditions also may cause the variations.

Controlled environmental studies were conducted to determine the effect of light intensity, photoperiod, and temperature on CERV symptom expression in Pink Beauty. The purpose of the study was to find optimal environmental conditions for rapid synchronous expression of an easily detected symptom in Pink Beauty and to relate variations in symptom expression to variations in the ultrastructure of infected leaves.

MATERIALS AND METHODS

Experiments were conducted in three Sherer Model CEL 37-14 controlled environment chambers illuminated with up to 16 40-W fluorescent tubes (GE F72T12, cool-white, 1,500 mA). Experimental conditions were: temperatures of 21, 27, and 32 C (±2 C); photoperiods of 8, 12, 14, and 16 hr of fluorescent light (FL), with or without 1,076 lux (100 ft-c) of incandescent light (IL) from several 40-W bulbs; and cool-white fluorescent light levels of 7,532, 16,140, and 22,596 lux (±2,152 lux) (700, 1,500, and 2,100 ft-c [±200 ft-c]) that will be referred to as low (L), medium (M), and high (H) light levels, respectively.

S. vaccaria seeds were stored at 4 C for at least 3 mo, then sown in a soil:peat:perlite mix (2:1:1, v/v), and lightly covered with sieved soil. Seeded pots were kept moist and held in the dark at 2 C for 2 wk. Seeds were drenched with Dexon and germinated under intermittent mist in the greenhouse. Seedlings were transplanted, placed under mist for 2 days, and transferred to the growth chambers within 5 days.

Plants were staked and maintained in each chamber at 27 C under high levels of fluorescent lighting (HFL) on a 12- or 16-hr photoperiod until two or three pairs of leaves had expanded. They were dusted with Carborundum and mechanically inoculated with the crude sap of fresh or frozen leaves of CERV-infected Pink Beauty ground in 0.05 M neutral Na_2HPO_4-KH_2PO_4 buffer. Uninoculated plants served as controls. Plant height, leaf weight, and flowering were measured to assess the influence of the environment on plant growth. The latent periods, the color intensity of local lesions, the amount of the inoculated leaf covered by lesions, and the number of leaves that developed local lesions were compared in the different environments to assess the environmental influence on symptom expression.

Electron microscopy. In four experiments, inoculated and systemically infected leaf samples from plants grown at 21, 27, and 32 C under 16 hr of HFL per day were fixed and embedded 11–14 and 18–19 days after inoculation to observe the effects of temperature on leaf cell and virus inclusion ultrastructure. Comparable leaf samples were taken from healthy plants in each environment.

In two additional studies, plants were grown at 21, 27, and 32 C under 16 hr of HFL per day. Leaf samples were taken for ultrastructure studies 14 days after inoculation. The plants at each temperature were then divided into three groups; one group was left at the previous growing temperature and two groups were moved to the two alternate temperatures for about 1 wk. Leaf samples were taken for ultrastructural observations.

The ultrastructure of leaves from infected plants grown at 27 C on an inductive (16 hr of HFL + IL per day) and a noninductive (16 hr of HFL per day) photoperiod also was compared in two experiments.

All tissues were fixed in a mixture of 2% glutaraldehyde and 1.5%

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acrolein in 0.05 M Na_2HPO_4-KH_2PO_4 buffer, pH 7.2, postfixed in buffered 1% osmium tetroxide, and embedded in Epon 812 (4). Ultrathin sections were double stained in an aqueous solution of 4% uranyl acetate and Reynolds' lead citrate.

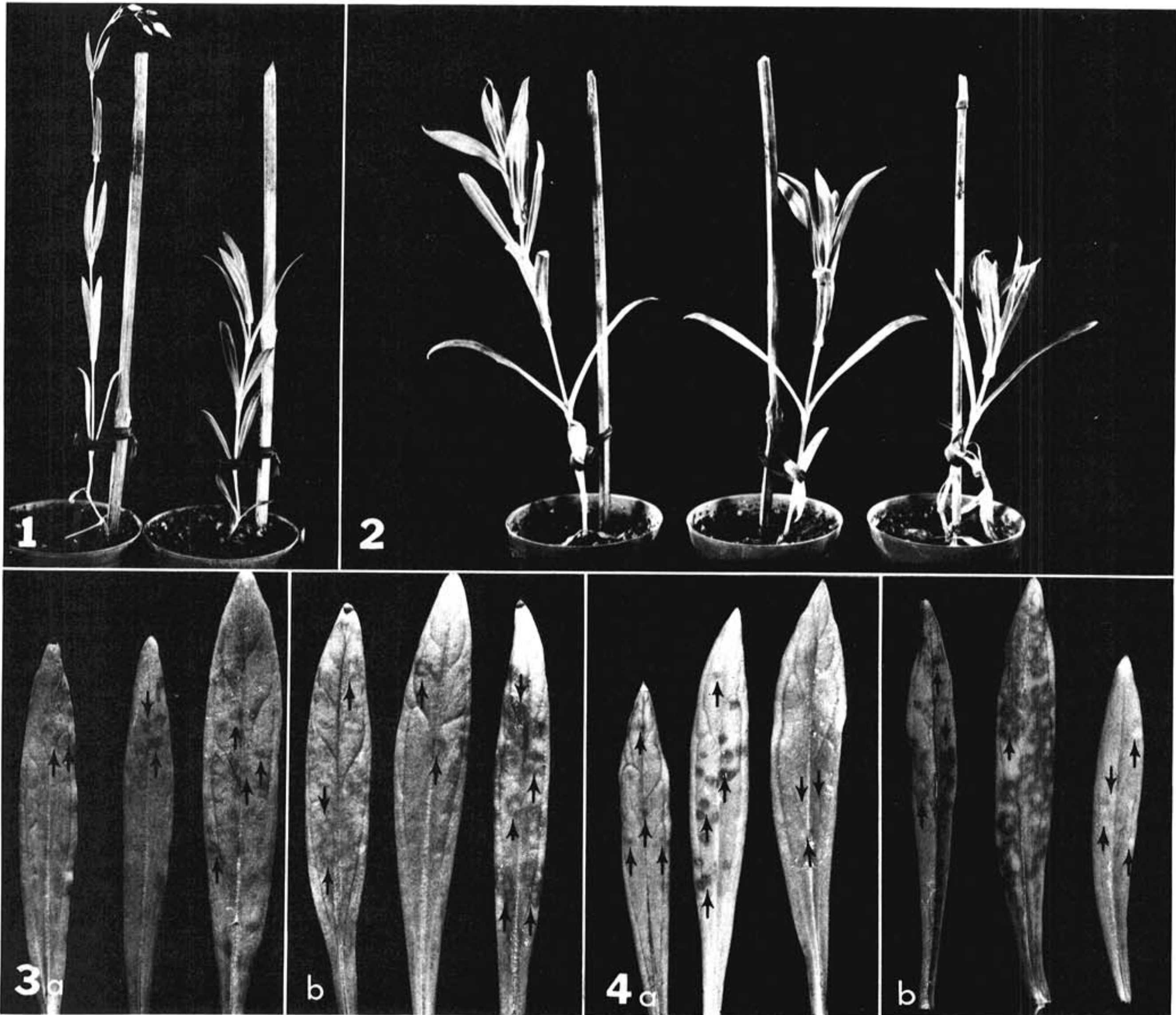
Temperature effects on infectivity and extractable inclusions. Infectivity dilution endpoints (DEP) were determined on extracts from plants grown at 21, 27, and 32 C under 16 hr of HFL per day. At 10, 14, and 18 days after inoculation, two sets of three inoculated leaves were randomly selected from plants at each temperature and frozen. Leaves were triturated in 3 ml of 0.05 M Na_2HPO_4-KH_2PO_4 neutral buffer and the sap was filtered through cheesecloth. Dilutions were made in the same buffer and assayed on *S. vaccaria* seedlings.

Inclusions from plants grown at 21, 27, and 32 C under HFL, were extracted and embedded as previously described (5). Inclusion extracts were also examined by light microscopy (5).

Fluorescent antibody staining of epidermal leaf strips. Rabbit globulin containing antibodies to extracted inclusions (5) was conjugated with fluorescein isothiocyanate (FITC) (6) and used to stain epidermal leaf strips (2) from inoculated and control leaves of plants grown at 21, 27, and 32 C under 16 hr of HFL per day. Stained epidermal cells were viewed with a Leitz Dialux microscope with a Ploemopak 2.3 fluorescence vertical illuminator that included a KP 490 exitation filter and a K 510 suppression filter. With this system cells could be examined by fluorescent and then light microscopy.

RESULTS

Effects of light intensity, photoperiod, and temperature on plant growth. Pink Beauty, a long-day plant, flowered under 16 hr of FL + IL daily illumination. If no IL was used or the FL + IL photoperiod was less than 14 hr, the plants remained vegetative (Fig. 1). Under noninductive photoconditions, stem elongation was decreased by increasing the light level (Fig. 2) and decreasing the temperature. Leaf size was increased by increasing the light,



Figs. 1–4. Comparisons of the effects of photoperiod, levels of illumination and temperature on plant growth and symptom expression of carnation etched ring virus-inoculated *Saponaria vaccaria*. **1,** Plants 15 days postinoculation that were grown at 21 C under medium levels of fluorescent plus incandescent (FL + IL) light on a 16-hr (left) and a 12-hr (right) photoperiod. **2,** Plants 13 days postinoculation that were grown at 27 C on a 12-hr photoperiod at low (left), medium (center), and high (right) levels of illumination. **3,** Comparisons of local lesions (←) at 27 C under high levels of illumination at **a,** 9 days and **b,** 12 days after inoculation of plants on a 12-hr (left) or a 16-hr (center) photoperiod of FL + IL light or a 16-hr photoperiod of FL only (right). On the 16-hr FL photoperiod local lesions appeared sooner and developed a darker color than on the 12-hr photoperiod. The number of local lesions was reduced by the 16-hr FL + IL photoperiod compared to the 12-hr photoperiod or the 16-hr FL photoperiod. **4,** Local lesions (←) at **a,** 10 days and **b,** 16 days after inoculation of plants growing on a 16-hr photoperiod of high level FL at 21 C (left), 27 C (middle), or 32 C (right). The appearance and enlargement of red local lesions were slower at 21 than at 27 C. Chlorotic, not red, local lesions developed at 32 C.

decreasing the temperature, and lengthening the photoperiod. Thus, high light at 21 or 27 C with 12 hr of FL + IL or 16 hr of FL were optimal for obtaining maximum leaf size and tissue for virus or inclusion purification. Growth of infected and healthy plants was similar, except that infected plants were slightly shorter (Table 1) when the environmental conditions favored severe symptom expression in inoculated plants.

Effects of light intensity, photoperiod, and temperature on symptom expression. *Light intensity.* The latent period decreased and the number of inoculated leaves that developed lesions, the number of lesions on a leaf (Tables 1 and 2), and the intensity of the red color of the lesions (Fig. 3) increased as the light levels were increased at 21 and 27 C. The effects of L, M, and H light were not compared at 32 C because plants under L light were chlorotic and lesions were difficult to detect.

Daylength. The length of the latent period was inversely related to the length of the photoperiod unless plants were photoinduced to flower. The number of leaves that developed local lesions, the number of local lesions on a leaf (Fig. 3), and the formation of systemic symptoms (Table 1) were suppressed under 16 hr of FL + IL. Photoinductive illumination for only 3 days prior to inoculation significantly reduced the number and color intensity of the local lesions.

Temperature. At 21 and 27 C, local lesions began as tiny red spots that enlarged into concentric red rings; at 32 C, local lesions began as chlorotic spots that enlarged and acquired faint red borders or formed concentric yellow and green rings (Fig. 4). At 21 and 27 C, the leaf pair immediately above the inoculated leaves curled under and the next one-to-three leaf pairs developed red veins and lines; at 32 C, a systemic leaf speckle appeared on even the apical leaves. Temperature also affected the latent period and plant susceptibility to infection (Table 3). The percentage of plants with local lesions at a given time after inoculation varied from experiment to experiment, but the latent period was always shorter at 27 and 32 C than at 21 C. The percentage of inoculated plants that became infected and the number of local lesions was greatest at 27 C. A synchronous appearance of local lesions was more nearly achieved at 27 and 32 C than at 21 C. At 21 C a nonspecific reddening of the veins and interveinal tissue occurred on inoculated and control leaves.

Electron microscopy. Temperature-induced alterations in the leaf ultrastructure were similar in healthy and inoculated plants. Leaves from plants grown at 22 and 27 C were large, thick, and deep green. This was correlated with the following ultrastructural characteristics of healthy and diseased leaf (Figs. 5–9) tissue: the mesophyll contained several layers of cells; cells in the leaves were large and rich in cytoplasm, ribosomes, and endoplasmic reticulum (ER); chloroplasts usually contained well-developed lamellae and grana networks and often starch granules but only a few small oil droplets; nuclei were often elongated (more so at 22 than 27 C) with large areas of densely stained and compact chromatin; and nucleoli often adjoined condensed chromatin and consisted of large areas of darkly stained matrix with granular particles (g areas), large areas of very densely stained matrix without granules (f areas), and pockets of lightly stained matrix within the f areas (Fig. 6) (11). At 27 C the f areas appeared as discrete thin bands around the pockets of lightly stained matrix.

Leaves from plants grown at 32 C were small, thin, and chlorotic. The ultrastructural characteristics (Figs. 10–13) of the tissue included the following: the mesophyll contained only a few cell layers; cells were small and contained only a thin watery-appearing stream of cytoplasm with a few ribosomes and little ER; many chloroplasts had poorly developed or distorted lamellae and grana and large oil droplets; some nuclei were large and rounded with chromatin that appeared less compact than that in nuclei of plants grown at 22 C; nucleoli seldom adjoined condensed chromatin and they consisted of a predominant g area and either no or only a few small f areas and pockets of lightly stained matrix; and often numerous, pilae-shaped, densely stained deposits lined the cell wall (Fig. 10) and caused bulging of the plasmalemma.

In the inoculated plants, temperature-induced alterations were observed in structures associated with virus replication and accumulation. Inclusions in leaf cells of plants grown for 11–14 days at 21 C usually consisted of small to massive aggregates of probodies with numerous vesicles containing a densely stained core, dictyosomes, and ER (Fig. 5a). In some inclusions, early signs of matrix accretion was evident (Fig. 5b). (Similar, but usually smaller, aggregates with fewer vesicles occurred at 7–10 days in local lesions in plants grown at 27 C). Nuclei seldom contained virions (Figs. 5b and 6); virus-induced intranuclear membranes were not observed. Virions were numerous in the cytoplasm and plasmodesmata. The plasmodesmata often were grossly modified

TABLE 1. Effect of illumination on postinoculation plant height and the rate of postinoculation symptom expression at 27 C in *Saponaria vaccaria* 'Pink Beauty' inoculated with carnation etched ring virus

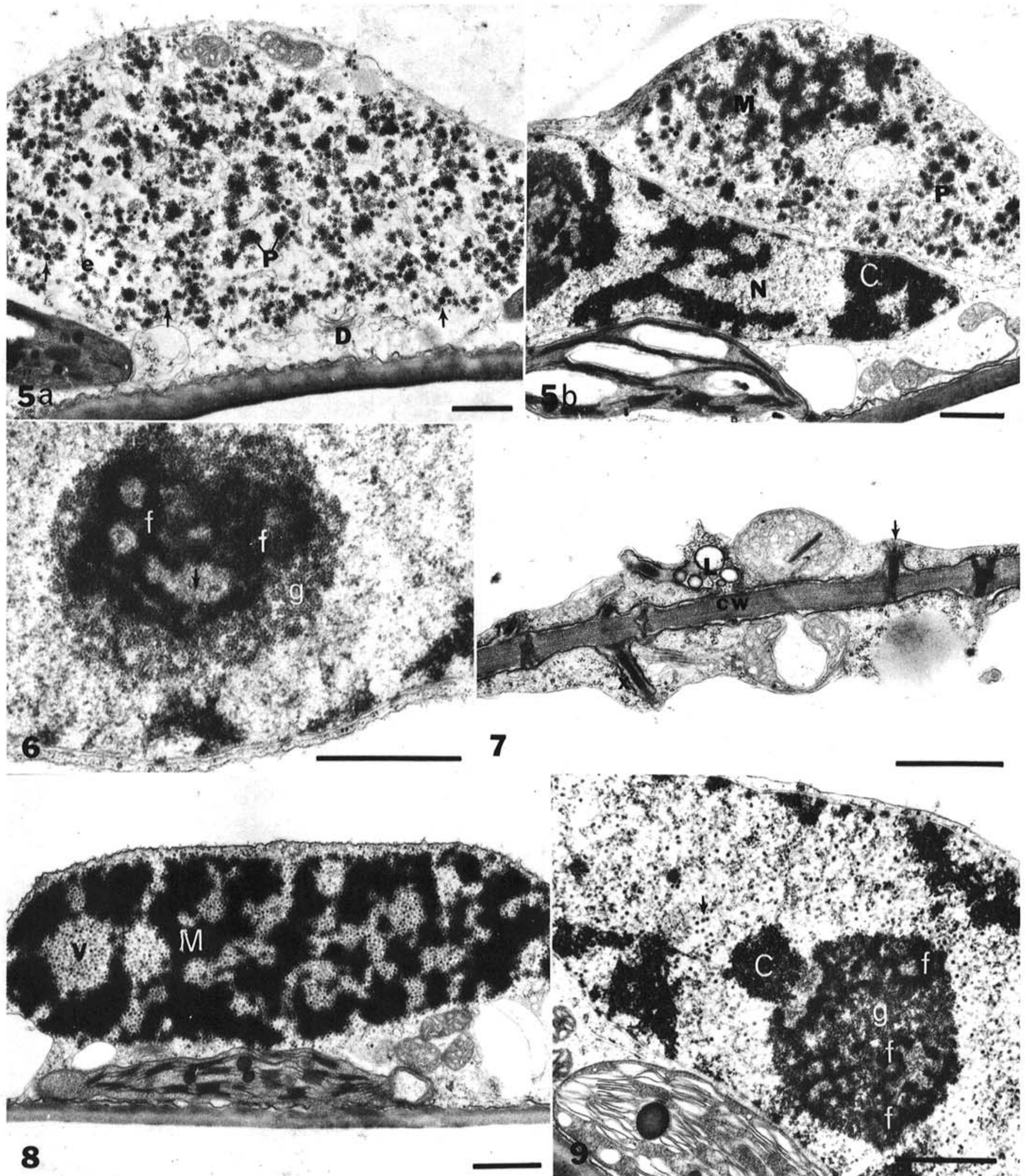
Illumination		Average height at 11 days (cm)		Plants with local lesions[b] (%)			Leaf symptoms 11 days postinoculation	
Intensity (lux[ft-c])	Time (hr/day) (fluor.–incand.)	Healthy[a]	Diseased[b]	8 days	9 days	11 days	Percent rated +++[c]	Systemic (avg no./plant)
23,672 [2,200]	16–0	10.0(2.9)[d]	9.7(2.5)	64	96	96	79	2
17,216 [1,600]	16–0	13.8(4.3)	11.9(2.2)	30	64	87	64	1
17,216 [1,600]	16–16[e]	21.5(3.4)	18.9(3.9)	30	47	84	37	0.4

[a]Approximately 30 plants in each treatment.
[b]Approximately 50 plants in each treatment.
[c]+++ = Local lesions covered two-thirds or more of the leaf (see Table 3).
[d]Numbers in parentheses indicate standard deviations.
[e]96% of the plants flowered.

TABLE 2. Effect of light intensity on plant height and symptom expression in carnation etched ring virus-inoculated *Saponaria vaccaria* grown at 27 C under 12 hr/day fluorescent + 1,076 lux (100 ft-c) incandescent light

Fluorescent illumination (lux [ft-c])	Average height after 14 days (cm)	Percentage of plants infected[a]					Percentage of leaves with symptoms at 17 days			Avg no. of symptomatic leaves per plant (no.)
		9 days	10 days	12 days	14 days	17 days	+[b]	++	+++	
23,762 [2,200]	11.2	43	71	100	100	100	0	58	42	5.2
13,988 [1,300]	14.7	20	43	85	86	100	0	80	20	5.3
8,608 [800]	17.0	5	15	50	70	90	56	35	9	3.9

[a]Plants inoculated = 20–25.
[b]Because sizes of inoculated leaves varied with position on the plant and the environmental conditions, and because local lesions appeared over a period of several days and then merged as they expanded into concentric rings, it was not feasible to count local lesions. Therefore, local lesion coverage was rated according to the proportion of the leaf covered: $\leqslant 1/3(+)$; $\leqslant 2/3(++)$; and $\geqslant 2/3(+++)$.



Figs. 5–9. Ultrathin sections of *Saponaria vaccaria* leaves from carnation etched ring virus-inoculated plants grown under a 16-hr photoperiod of high-level fluorescent light for 11–14 days at 21 C (Figs. 5–7) or 27 C (Figs. 8 and 9). **5a,** A large accumulation of probodies (p), virions, endoplasmic reticulum (e), dictyosomes (D) and vesicles with dense cores (←) that is typical of an early stage in the development of a virus inclusion. **5b,** A maturing inclusion with large accumulation of probodies and initial accretions of matrix (M) adjacent to a nucleus (N) with compact condensed chromatin (C). **6,** A nucleolus consisting of a peripheral matrix with granular particles (g) and a central area of densely stained matrix (f) around pockets of lightly stained matrix (←). **7,** Snoutlike projections (x) of widened, virion-containing (←) plasmodesmata that extend from the cell wall (CW) into the cytoplasm and associated lomasomes (L). **8,** A large virus inclusion with numerous virions (V) and accretions of dense matrix (M) typical of a late stage in the maturation of the inclusion. **9,** A portion of a nucleus with virions (←) in the nucleoplasm and a nucleolus that consists of a large g area (g) with the f areas (f) appearing as narrow bands around pockets of lightly staining matrix. Condensed chromatin (C) is adjacent to the nucleolus (Bars = 1 μm).

into complex networks of wide channels and chambers with expanded desmotubules (Fig. 7). The widened channels not only traversed the cell wall but some also ran parallel to it. Long snoutlike cell wall protrusions containing expanded plasmodesmata and desmotubules were common and sometimes were associated with lomasomes (Fig. 7).

Plants grown for 11–14 days at 27 C also contained probodies and aggregates of probodies like those at 21 C. However, they also contained large cytoplasmic inclusions 2–10 μm long with numerous associated virions (4) (Fig. 8). The nuclei sometimes contained virions (Fig. 9) and occasionally virions and intranuclear membranes. Virions occurred in the cytoplasm and in plasmodesmata. The plasmodesmata and surrounding cell wall protrusions were not as long as those in plants grown at 21 C and usually the plasmodesmata traversed the cell wall but did not run parallel to it.

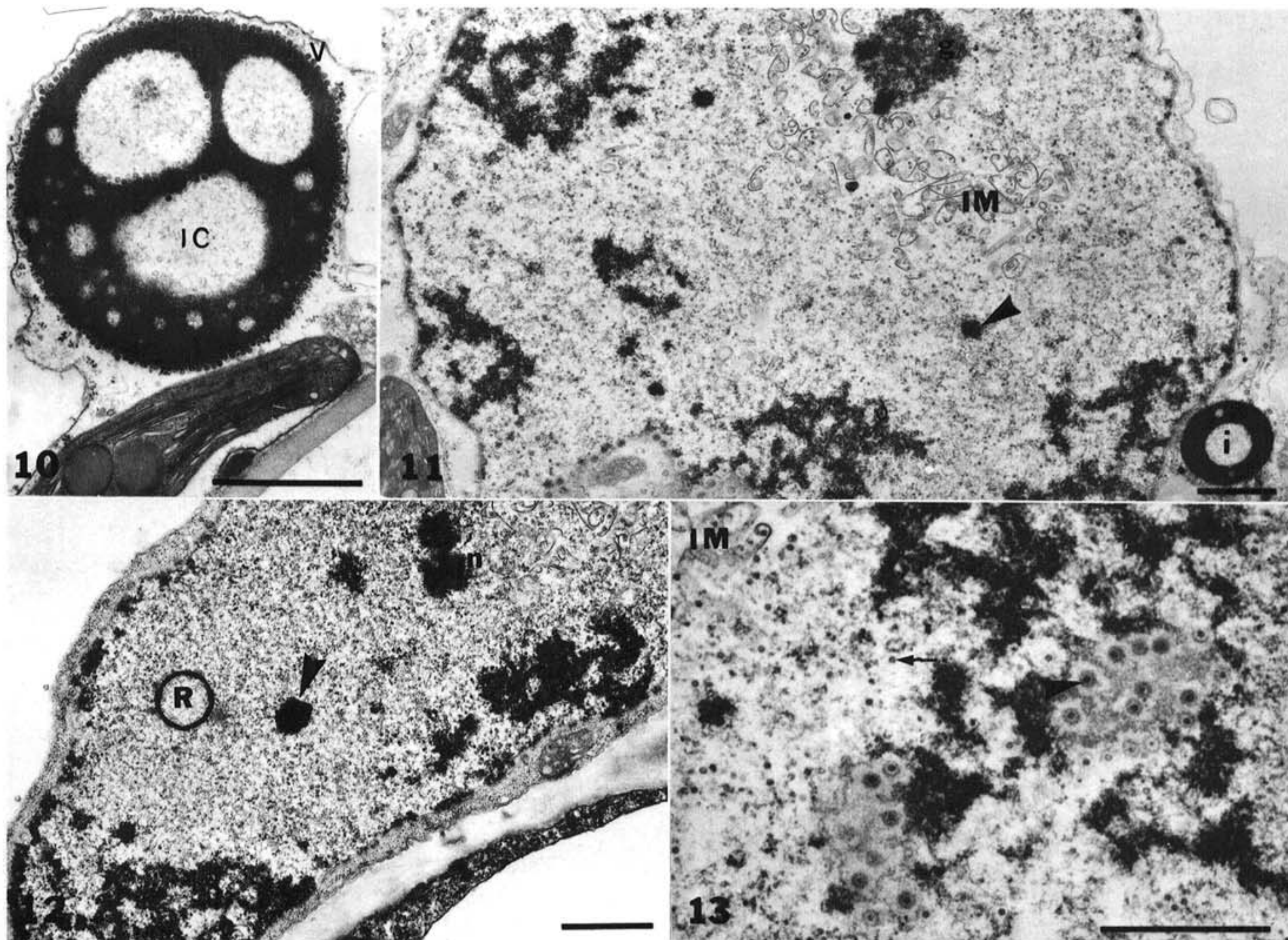
Leaf cells of plants grown at 32 C for 11–14 days contained several probodies but they were not aggregated in masses. Slightly larger spherical bodies (0.5–2.0 μm) of matrix with one or several large lacunae (Figs. 10 and 11) also were found. These inclusions may have been derived from limited enlargement of the individual probodies. Virions, if present, lined these inclusions and their lacunae. The lacunae often contained flocculent material. The nuclei often contained virions and intranuclear membranes (Figs. 11–13) and virions were commonly present in nuclear pores (Fig. 13). Nuclei also contained rings of densely stained material in the nucleoplasm and small scattered bodies of material resembling nucleolar fragments (Figs. 11 and 12). Few virions occurred in the cytoplasm but rarely in the plasmodesmata. Even short extended plasmodesmata were rare.

The ultrastructure of leaves sampled at 18–20 days postinoculation from plants grown at 21 C differed from that of those sampled at 11–14 days in that inclusions were more mature in the later samplings. The inclusions frequently resembled the mature, elongated, or round inclusions with numerous virions that were found in plants grown at 27 C for 11–14 days. Nuclei often contained many virions but intranuclear membranes were still rare and small when present. In plants grown at 27 C, a large proportion

TABLE 3. Effect of temperature on the rate of appearance of carnation etched ring virus-induced local lesions on *Saponaria vaccaria*[a]

Temperature (C)	% Infected Plants[b]		
	8 days	11 days	15 days
21	0	21	88
27	35	96	100
32	70	87	96

[a]Cultivar 'Pink Beauty' grown in a controlled environment chamber illuminated at 21,520 lux (2,000 ft-c) cool-white fluorescent light 16 hr/day. Days indicated are postinoculation observation times.
[b]Number of plants inoculated = 70–85.

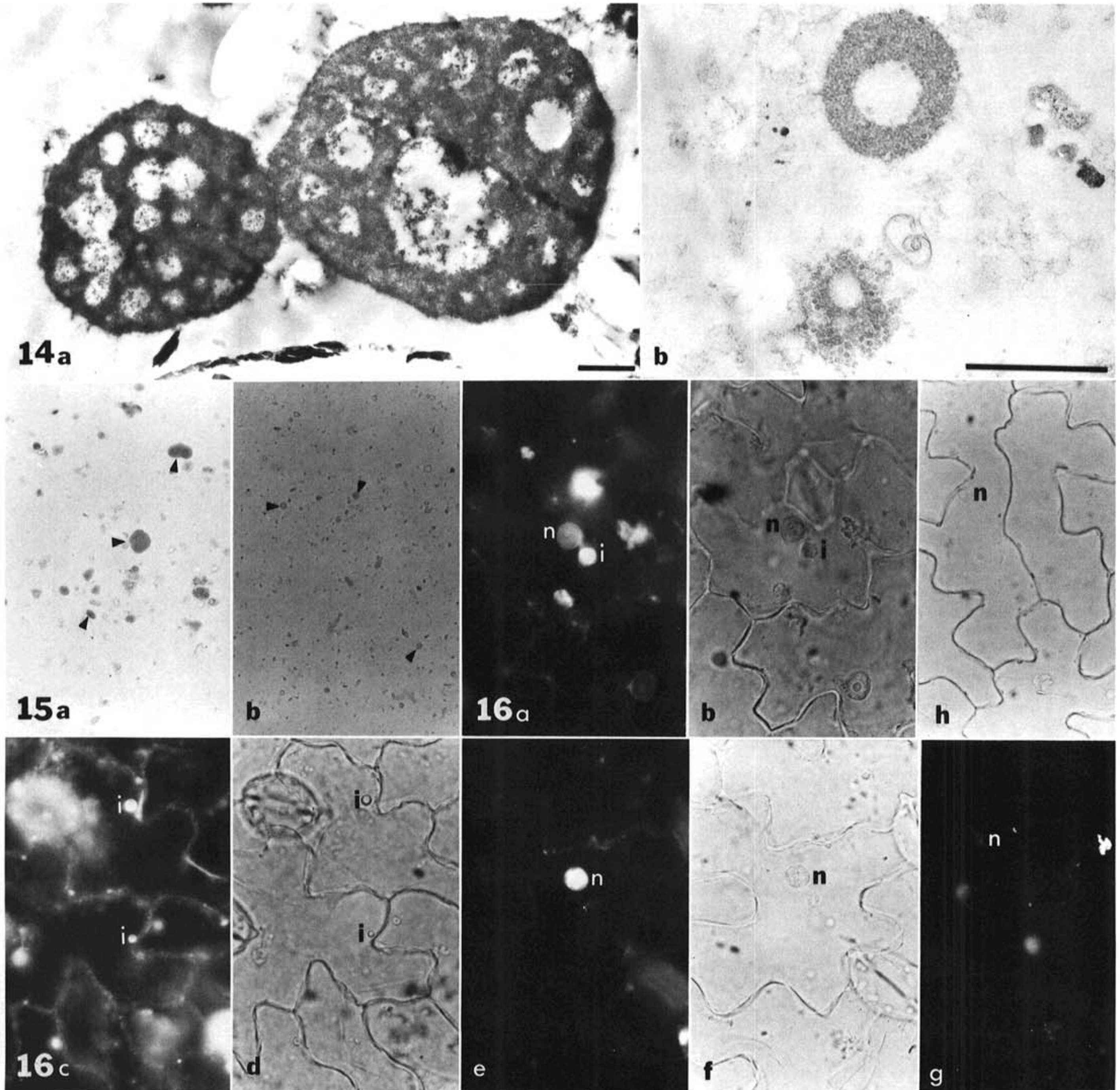


Figs. 10–13. Ultrathin sections of carnation etched ring virus-inoculated leaves from plants grown on a 16-hr photoperiod of high-level fluorescent light for 11–14 days at 32 C. **10,** A typical inclusion with some virions on the periphery of the inclusion and the internal lacunae (IC). A loosely aggregated, moderately staining material occurs within the lacunae. **11,** A nucleus containing virions, intranuclear membranes (IM) and scattered, small, densely stained bodies (◀) in the nucleoplasm. The small nucleolus is predominantly composed of a g area (g). A virus inclusion (i) is near the nucleus. **12,** A nucleus that contains a ring structure (R) in addition to a nucleolus (n), virions, intranuclear membranes, and dense bodies (◀). **13,** A portion of a nucleus that shows intranuclear membranes (IM) and virions (←), and virions within some nuclear pores (◀). (Bars = 1 μm).

of the inclusions were elongated or round; some consisted almost entirely of virions but few probodies were present. Many nuclei contained virions and large intranuclear membranes with an occasional virion in a nuclear pore. However, many cells were senescing. At 32 C, the larger inclusions were more numerous at 18–22 days than at 11–14 days after inoculation, but generally few cells contained inclusions and many cells had senesced. The number of virions per nucleus in leaves at the second sampling was greater at 21 and 27 C than at 32 C.

The ultrastructure of systemically infected tissue differed little from that of inoculated leaves if allowance was made for the time lag needed for translocation of the virus. At 32 C, however, small accumulations of virions were associated with many of the inclusions.

Effects of temperature change on developing inclusions. All plants sampled at 2 wk (prior to making the temperature shift) contained inclusions as previously described for plants grown at 21, 27, and 32 C. When plants grown at 21 and 27 C for 2 wk were transferred to 32 C for 1 wk, the inoculated leaves collapsed. The systemically infected, yellow-speckled leaves that subsequently



Figs. 14–16. Virus inclusions extracted from carnation etched ring virus (CERV)-infected leaves and in epidermal strips of *Saponaria vaccaria* plants grown under high-intensity fluorescent lighting for about 2 wk at 21, 27, and 32 C. **14,** Ultrathin sections of extracted inclusions from plants grown at **a,** 27 C and **b,** 32 C. Inclusions extracted from plants grown at 27 C were more numerous, larger, and contained more virions and matrix than the small, faintly staining inclusions extracted from plants grown at 32 C. **15,** Comparison of extracted inclusions (◀) from plants grown at **a,** 27 C and **b,** 32 C as observed in the light microscope when stained with phloxine (×230). **16,** Epidermal strips from CERV-infected (**a–f**) (×310) and healthy (**g** and **h**) (×230) *S. vaccaria* plants grown 13 days at 21 C (**a** and **b**) or 32 C (**c–h**) and reacted with FITC conjugated CERV inclusion antiserum. Areas are shown as they appeared in fluorescent microscopy (**a, c, e,** and **g**) and light microscopy (**b, d, f,** and **h**). In epidermal strips from leaves grown at 21 C, large inclusions (i) fluoresced brightly but the nuclei (n) showed little or no fluorescence (**a** and **b**). In epidermal strips from plants grown at 32 C small, often hollow inclusions (i) (**c** and **d**) fluoresced as did some nuclei (n) (**e** and **f**). Neither fluorescing nuclei (n) nor inclusionlike bodies were found in healthy epidermal strips (**g** and **h**). (Bars = 1 μm).

developed at 32 C contained inclusions similar to those in plants grown for 3 wk at 32 C. When plants grown at 32 C for 2 wk were transferred to 21 or 27 C for 1 wk, the inoculated leaves quickly developed red rings and spots at the sites of the existing chlorotic spots. Nuclei with virions and membranes, large masses of probodies, and circular and elongated inclusions with many virions occurred in the local lesions and in systemically infected leaves. The unique inclusions typical of the infection at 32 C were no longer present.

Effects of a photoinductive light period on cell and virus inclusion ultrastructure. The ultrastructure of inclusions in leaf samples from plants grown in an inductive photoperiod differed from that of inclusions in plants grown under a noninductive photoperiod only in that fewer inclusions and associated structures were found in induced plants. Occasionally structures resembling vesicles or dilated ER and ring structures of dense chromatinlike material were present in the nuclei of induced plants.

Temperature effects on infectivity and extractable virus inclusions. Dilution endpoint determinations of CERV infectivity in *S. vaccaria* were in agreement with ultrastructural observations and indicated that infectious virus was produced faster at 27 C than at 21 C. The infectivity in extracts from plants grown at 21 C for 18 days reached levels (DEP 10^{-3}) near those of plants grown at 27 C (DEP 10^{-4}) for 14 days. Infectivity at 32 C was detectable at 10 days (DEP 10^{-1}) but did not increase with time.

Inclusion extracts from infected tissue confirmed the quantitative and qualitative aspects of the ultrastructure observations (Figs. 14 and 15). Inclusions from plants grown at 32 C were small, often were only faintly stained (suggesting a loss of matrix during extraction), had one or more cavities, and were few in number (Figs. 14b and 15b). Those from plants grown at 21 and 27 C were larger, more numerous, contained much dense matrix, and had many virions (Figs. 14a and 15a).

Fluorescent antibody staining of leaf epidermal strips. Inclusions in FITC-stained epidermal strips fluoresced regardless of the temperature at which the plants had been grown (Fig. 16). Epidermal strips from plants grown at 32 C contained small, often doughnutlike, fluorescing bodies (Fig. 16c and d) while those in tissue grown at 21 and 27 C were large and round or elongated (Fig. 16a and b). Grainy appearing fluorescent areas in the cytoplasm also were commonly detected in plants grown at 21 C and were probably massive aggregates of probodies. The nucleoplasmic regions of some nuclei in infected, but not healthy, plants grown at 32 C (Fig. 16e and f) fluoresced strongly; in those grown at 21 and 27 C, however, only a weak nucleoplasmic fluorescence was detected in healthy and diseased leaves (Fig. 16g and h).

DISCUSSION

Illumination and temperature affected the efficiency with which CERV could be detected in *S. vaccaria*. Red local lesions were selected as an easily recognizable symptom of CERV infection. Optimal conditions for the production for these lesions on CERV-inoculated leaves were 27 C and 12 hr/day of FL + IL or 12–16 hr/day of FL at ($\geqslant$ 21,520 lux $\geqslant$ 2,000 ft-c). Under these conditions, some plants began to show lesions in 8 days and 100% showed symptoms in 10–12 days. Our data showed that if lower temperatures and shorter or less intense illuminations (or both) were used for growing plants, a larger number of plants had to be inoculated and the plants had to be held longer to insure detection of a positive sample in a bioassay test. Plants that had received a photoinductive treatment of sufficient length to initiate flowering were unsuitable as assay plants.

We also found that development of red or chlorotic lesions on CERV-inoculated leaves was partially controlled by temperature and photoregulation of plant anthocyanin production. High levels and long durations of illumination and lower temperatures, conditions optimal for CERV symptom expression, also favor anthocyanin production (9). Cold initiation may have caused the nonspecific reddening observed at 21 C. Photoinduction of flowering affected the quantity and color intensity of the red local lesions produced in Pink Beauty. Both of these effects may be a result of photohormonal regulation of flowering, anthocyanin production, (9) and virus accumulation (7), which are known but poorly understood.

The effects of temperature and illumination on infectivity and local lesion formation have been examined for many virus-host systems (7), but events at the cellular level (13) have rarely been identified. Environmentally induced variations in CERV symptom expression were accompanied by variations in both cell and virus inclusion ultrastructure. The low infectivity of extracts from plants grown at 32 C was correlated with a low concentration of virions within cells and a failure of the cytoplasmic inclusions to mature normally. The rate of inclusion formation and maturation, as well as symptom expression, was temperature dependent and slower at 21 C than at 27 C. Nuclear virions also appeared later in plants grown at 21 C than in those grown at 27 or 32 C.

These data are in agreement with our former proposal that virus assembly and/or synthesis can take place in *S. vaccaria* by two separate processes (4). It would now appear that the nuclear and cytoplasmic processes have slightly different temperature sensitivities. Similarly, cauliflower mosaic virus DNA can be transcribed to RNA, but with different efficiencies by RNA polymerases I, II, and III from wheat embryos (12). Polymerase II activity has been shown to be enhanced by light (8). Thus, polymerases (plant- or virus-coded) with different locations, photoregulations, and temperature optima (1) could provide a biochemical mechanism for achieving, environmentally, regulation of CERV replication.

Temperature also may affect other cellular functions that in turn affect virus replication. Differences in nuclear ultrastructure, particularly nucleolar ultrastructure, indicate that temperature may modify synthesis of messenger RNA (in the f areas) and ribosomal precursors (in the g areas) (11) in Pink Beauty. Temperature affected the formation of ER and the vesicles with densely staining centers that accumulated around the inclusions. Normal inclusions formed when plants grown at 32 C were placed at lower temperatures, which proved that temperature-induced inhibition of inclusion formation was reversible.

Viral translocation within the plant also was temperature dependent. Virion movement through nuclear pores and short plasmodesmata complexes may facilitate efficient spread of the relatively few virions formed at 32 C in Pink Beauty.

Although the results of this study do not determine how variations in symptom expression result from environmental regulation of plant metabolism or from the ability of the host to respond to infection (ie, production of anthocyanins, formation of modified plasmodesmata, production of inhibitors of virus replication, and modification of virus replication), they do show that high temperature does not merely inactivate or degrade virions in the cells. More importantly, the effects of high temperature are a basis for further investigations into the interaction of the two systems in the developing disease syndrome.

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