Photoacclimation was studied in tobacco leaves (Nicotiana tabacum cv Xanthi) infected with two strains of tobacco mosaic virus (TMV) and grown under different light and nitrogen nutrition regimes. Photosynthetic acclimation measured by the quantum yield and the maximum rate in saturating light of CO₂-saturated photosynthesis was impaired to a greater extent in tobacco leaves infected with TMV strain PV230 than in those infected with TMV strain PV42. Infection with TMV strain PV230 severely impaired photosynthetic acclimation at high light/low nitrogen and during transfer from low to high light. Expanding leaves showing chlorotic-mosaic symptoms had greatly reduced capacity to acclimate to high light compared with controls and with developed leaves without visible symptoms. We conclude that the failure of expanding leaves to acclimate was largely due to the destruction of chloroplasts in yellow areas of the tissue, accompanied by severe reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase levels, and corresponding reduction in photosynthesis on a leaf-area basis. When corrected for areas of healthy green tissue, photoacclimation of infected leaves was the same as that of controls. Visible symptom development was greatest in high light/low nitrogen treatments. In developed leaves without visible symptoms, virus accumulation, which was as extensive as in expanding leaves, accelerated senescence and impaired photoacclimation during transfer from low light to high light. Generally, infection with TMV strain PV42 did not impair photosynthetic acclimation and even enhanced it in some treatments, even though virus accumulated to the same concentration as in PV230-infected leaves. These data show that TMV does not simply impair photoacclimation in tobacco by competing with chloroplasts for leaf nitrogen reserves. Rather, specific properties of severe strains, such as PV230, which lead to visible symptom development and patchy loss of photosynthetic activity in expanding leaves as well as general acceleration of chloroplast senescence in developed leaves, contribute to impaired

Photoacclimation to HL, which involves concerted changes in chloroplast membrane and photosynthetic enzyme activities (Björkman, 1981; Chow and Anderson 1987a, 1987b), is particularly sensitive to nitrogen nutrition (Osmond, 1983; Ferrar and Osmond, 1986; Seeman et al., 1987; Evans, 1989). Nitrogen nutrition has large effects on the partitioning of nitrogen between chloroplasts and other components (Evans, 1988; Makino and Osmond, 1991), especially on the primary carboxylase of photosynthesis, Rubisco, which is the dominant sink for nitrogen during acclimation to bright light. It has been suspected for some time that biological factors such as virus infection may interfere with the nitrogen-dependent ability of plants to acclimate to bright light (Osmond, 1983). Early studies of Wildman et al. (1949) showed that following TMV infection Rubisco concentration in tobacco leaves was reduced. In such cases we might expect the ability of infected leaves to acclimate to HL to be impaired.

Shade populations of many herbaceous plants containing different viruses showed no overt visible symptoms, whereas sun-grown populations showed severe symptoms (Osmond et al., 1990). In this context it is significant that many virus infections lead to visible symptoms such as mosaic, striping, chlorosis, and vein clearing, which are a result of virus effects on Chl synthesis and disruption of chloroplast structure and function (Goodman et al., 1986). However, the complexity of virus interactions with host plants is such that few generalizations are possible. Even though it is widely accepted that pathogens alter the ability of infected plants to compete and survive in a specific environment, there are few studies in which host performance has been examined after infection (Ayers, 1984).

In this paper we quantitatively evaluate the effects of TMV strains PV230 (which causes severe chlorotic mosaic symptoms) and PV42 (which induces mild or masked symptoms) on the photoacclimatory responses and allocation of nitrogen to Rubisco and virus protein in leaves of tobacco (Nicotiana tabacum).
plants grown in sun and shade under different nitrogen nutrition regimes. Photoacclimation has been characterized in terms of light- and CO₂-saturated photosynthetic capacity as well as in terms of the efficiency of photosynthesis (quantum yield). We have focused on effects of virus infection on nitrogen partitioning (concentrations of soluble protein, Rubisco, and virus coat protein) in leaves of tissues of different ages and show that impaired photoacclimation following infection is greatest in expanding leaves in which visible symptoms develop. Development of visible symptoms is light- and nitrogen-dependent, and the role of photoinhibition in these processes is examined in subsequent reports (Balachandran and Osmond, 1994; Balachandran et al., 1994).

**MATERIALS AND METHODS**

**Plant Culture**

Seeds of *Nicotiana tabacum* cv Xanthi showing susceptibility to TMV were obtained from the U.S. Department of Agriculture Tobacco Research Laboratory (Oxford, NC, courtesy of Dr. Harvey Spurr). Seeds were germinated in vermiculite and gravel mix (1:1) and transplanted 2 weeks later to the same medium in 6-inch pots. Initially, the plants were grown under natural light conditions in the glasshouse of Duke University Phytotron (Durham, NC). Temperature in the glasshouse was maintained at 28°C during the day and 22°C at night. RH was maintained at about 70%. Half-strength Hoagland nutrient solution was supplied each day in the morning, and water was supplied in the evening.

In steady-state-light experiments, the inoculated plants were kept in a low-light growth chamber (LL, 150 μmol photons m⁻² s⁻¹) for 1 d following inoculation and then either transferred to a high-light growth chamber (HL, 1000–1200 μmol photons m⁻² s⁻¹) or kept in LL for up to 15 d. In transfer experiments (LL→HL), the plants were kept first in LL for 7 d and then in HL for another 7 d. In all cases, uninoculated control plants were grown in other chambers under comparable conditions. The growth chambers were maintained at 26°C day/22°C night temperatures with RH of 70%. Half-strength Hoagland nutrient solution was supplied each day in the morning, and water was supplied in the evening.

In steady-state-light experiments, the inoculated plants were kept in a low-light growth chamber (LL, 150 μmol photons m⁻² s⁻¹) for 1 d following inoculation and then either transferred to a high-light growth chamber (HL, 1000–1200 μmol photons m⁻² s⁻¹) or kept in LL for up to 15 d. In transfer experiments (LL→HL), the plants were kept first in LL for 7 d and then in HL for another 7 d. In all cases, uninoculated control plants were grown in other chambers under comparable conditions. The growth chambers were maintained at 26°C day/22°C night temperatures with RH of 70%. The plants were supplied with nutrient solution containing high NO₃⁻ (HN, 8.0 mM) or low NO₃⁻ (LN, 0.5 mM) concentration with nearly constant cation and anion composition. LN-treated plants received 1.0 mM PO₄³⁻, Mg²⁺, Ca²⁺, and Cl⁻, 3.0 mM SO₄²⁻, and 5.0 mM K⁺, and HN-treated plants received 1.0 mM PO₄³⁻, Mg²⁺, and Cl⁻, 3.0 mM SO₄²⁻, 4.5 mM Ca²⁺, and 9.0 mM K⁺. All plants were supplied with 13.6 μM Fe(EDTA), 10 μM Mn²⁺, 10 μM Zn²⁺, 10 μM Cu²⁺, 50 μM B⁺, 0.6 μM Mo³⁺, and 100 μM Na⁺. The pots were flushed with an excess of nutrient solution each morning and with deionized water each evening.

**Inoculation of TMV Strains**

When the plants were 30 to 40 d old and had three or four developed leaves, the youngest developed leaf was inoculated with either a severe TMV strain (PV230) or a masked strain (PV42), using carborundum as an abrasive. An inoculum (500 μL) containing between 0.6 and 0.8 mg mL⁻¹ virus protein was used. After 15 d, young, expanding leaves showed a range of chlorotic-mosaic symptoms. The fifth leaf above the inoculated leaf showed severe mosaic symptoms. Visible symptoms corresponded to the chlorotic mosaic pattern typical of a severe strain of TMV, i.e. to patches of white or yellow tissue within a normal green leaf. The third leaf above the inoculated leaf, which expanded to become fully developed during the experiment, remained visibly symptomless 15 d after infection. These leaves are described as developed leaves. A third class of leaves, those that were fully developed at the start of the experiments, was also examined, but data are not shown because they generally confirmed those for the developed leaves described above.

These experiments were repeated using the masked strain PV42. The same leaf categories were examined in plants inoculated with TMV strain PV42, but none showed visible symptoms.

**Photosynthetic Measurements**

Leaves of differing developmental stages, showing the differing visible symptomatology detailed above, were chosen for measurement of photosynthetic oxygen evolution and nitrogen partitioning. The light response of photosynthesis at CO₂ saturation was characterized by means of oxygen evolution measurements to determine Ps and apparent Qy using a leaf disc oxygen electrode system (model LD-2, Hansatech). After calibration of volume, the chamber containing the leaf disc was purged with water-saturated 10% CO₂ in air (at 25°C) until a steady oxygen signal was obtained. The chamber was closed, and the leaf was illuminated with 200 μmol photons m⁻² s⁻¹ until a steady rate of oxygen evolution was obtained. The leaf was then darkened, respiratory rate was determined, and the light response of oxygen evolution was measured during a preprogrammed sequence of PPFD light values up to 750 μmol photons m⁻² s⁻¹. Diffusers and a Flexiglas light pipe were used to obtain more uniform light distribution at light levels below 150 μmol photons m⁻² s⁻¹. This step was necessary to obtain reproducible Qy. The light source did not completely saturate photosynthesis, but rates in the range 600 to 750 μmol photons m⁻² s⁻¹ were usually not statistically different from each other. Figure 1 shows a typical light response of oxygen evolution in uninfected tobacco leaves and leaves infected with TMV strains PV230 and PV42. The Ps cited in data sets is that measured at the maximum light intensity. Data are shown as mean ± se (n = 3), with bars showing the highest se for each data set.

The apparent Qy was estimated from the initial slope of the light-response curve (Fig. 1), and maximum Qy was calculated after correction for absorptance. Total leaf A was measured on a 1-cm-diameter disc placed in a laboratory-built integrating sphere of the type described by Ehleringer (1981). A Flexiglas light pipe was used to conduct red light focused from the light-emitting diode to the leaf disc, which could be moved into and out of the light path. A was calibrated with matt black and white standards, using a LiCor (Lincoln, NE) 199B quantum sensor. The difference
Photoacclimation in Tobacco Infected with Tobacco Mosaic Virus

Figure 1. Light response of oxygen evolution in uninfected expanding tobacco leaves and leaves infected with TMV strains PV230 and PV42 grown under HL/HN conditions. O, Uninfected; O, PV230-infected; O, PV42-infected leaves.

between the signals obtained with sample and standards in and out of the light beam was used to calculate absorptance as follows

\[ \text{Absorptance} = 1 - \frac{B}{S/B - W} \]

where \( B \) is the measurement with black and \( W \) the measurement with white standards and \( S \) is the measurement with the sample.

To determine whether the reduction in photosynthetic rates in chlorotic-mosaic leaves reflected the reduced area of healthy green tissue, leaf discs were photocopied after photosynthetic measurements and the dark area (normal Chl content) was determined with a Delta-T area meter (model MK2).

Estimation of Protein and Chl Contents

Leaf tissue used for photosynthetic measurements (excluding midrib) was ground in a glass homogenizer in lithium phosphate buffer (50 mM) with iodoacetic acid (2.5 mM) and 2-mercaptoethanol (120 mM, pH 7.2) with a ratio of 1 g of tissue to 7 mL of buffer. The leaf homogenate was centrifuged at 10,000g for 15 min, and the supernatant was taken as the soluble fraction. Aliquots of the soluble fraction were treated with lithium dodecylsulfate (2%) to dissociate proteins. Aliquots of these preparations (10–15 \( \mu \)g) were loaded on SDS-PAGE minigels (Bio-Rad, 15% separating gel with Tris buffer, pH 8.8) and separated by electrophoresis for about 45 min at 30 mA at room temperature and then stained with Coomassie brilliant blue. After exhaustive destaining, blue bands corresponding to large and small subunits of Rubisco (55 and 14 kD) were cut out and the color was extracted with formamide; the \( A_{495} \) of the extract was then measured in a spectrophotometer as outlined by Makino et al. (1986). The amount of Rubisco protein in each subunit was estimated from standard curves constructed with BSA protein taken through the same procedure.

A densely staining band in the 17.5-kD region was evident in extracts from leaves infected with both strains of TMV. In uninfected controls there was almost no protein staining at 17.5 kD. The blank for the 17.5-kD band was taken from uninfected controls loaded at the same total protein concentration. The 17.5-kD band was identified as TMV coat protein using antisera raised against TMV coat protein (provided by Dr. Roger Beachy, Scripps Institute, La Jolla, CA). Coomassie blue-stained virus coat protein bands, which were cut from gels and calibrated against BSA, gave precise agreement with virus coat protein content of purified TMV samples measured using Bio-Rad assays (data not shown).

Chl content was measured using 150 \( \mu \)L of the initial extract in 650 \( \mu \)L of water and 3.2 mL of 100% acetone (80% final concentration). The centrifuged extract was measured at 645.6 and 663.6 nm in a spectrophotometer and the Chl was estimated using equations of Porra et al. (1989).

RESULTS

Chl Content and Visible Symptomatology

In expanding leaves, the HL and LN treatments led to significant reductions in Chl concentration in both steady-state and transfer experiments. Infection with the severe strains (PV230) reduced Chl content further (Table I). This reduction in Chl in leaves with mosaic patches was related to destruction of chloroplasts in the yellow areas; the green

| Table 1. Effects of virus infection, nitrogen nutrition, and light treatment on Chl content in expanding and developed tobacco leaves |
|---|---|---|---|---|
| Leaf Class | Chl Content |
| | LL | HL | LL \( \rightarrow \) HL |
| | LN | HN | LN | HN | LN | HN |
| Expanding leaves | Uninfected | 2.15 ± 0.02 | 2.84 ± 0.06 | 0.91 ± 0.02 | 2.28 ± 0.09 | 1.17 ± 0.05 | 1.74 ± 0.00 |
| | PV230 | 1.64 ± 0.08 | 1.46 ± 0.19 | 0.36 ± 0.13 | 1.05 ± 0.20 | 0.72 ± 0.17 | 0.52 ± 0.00 |
| Developed leaves | Uninfected | 1.94 ± 0.05 | 2.27 ± 0.26 | 0.79 ± 0.03 | 1.46 ± 0.18 | 0.97 ± 0.09 | 1.35 ± 0.00 |
| | PV230 | 1.80 ± 0.05 | 1.33 ± 0.13 | 0.64 ± 0.03 | 1.10 ± 0.19 | 0.87 ± 0.03 | 0.66 ± 0.00 |

Shown are means ± SD, n = 3 or 4.

mg g\(^{-1}\) fresh weight
areas of the mosaic retained Chl concentrations similar to controls. The mild strain (PV42) produced no symptoms in expanding leaves, and Chl concentrations were not affected by infection with the exception of HL/LN leaves, where it increased (data not shown).

Developed leaves of uninfected controls contained lower Chl than expanding leaves in all treatments. Although infection with PV230 did not produce typical chlorotic mosaic symptoms in these leaves, Chl concentration was reduced compared with controls (Table I). The masked strain (PV42) again had no effect, or led to a slight increase in Chl in HL/ LN leaves (data not shown).

Expanding Leaves
Photosynthetic Responses

Photosynthetic capacities and Qy for expanding leaves are presented in Table II. In uninfected expanding leaves, light-saturated photosynthesis (Ps) was not affected by nitrogen nutrition during growth under LL conditions, confirming results with other species (Osmond, 1983). However, in HL, Ps increased more under LN than under LN. Although Qy in LN-grown leaves was less than that in LN-grown leaves, it did not differ between LL and HL treatments. Acclimation to HL in expanding leaves of transferred plants was evidently complete within 7 d of growth with either LN or LN nutrition, because Ps and Qy reached values comparable to those of leaves of plants grown at steady-state HL. However, Ps in LN leaves was about 35% less than that in LN leaves, confirming that nitrogen nutrition affected the ability of expanding tobacco leaves to acclimate to HL levels.

Infection with TMV strain PV230 reduced Ps and Qy in all treatments in expanding tobacco leaves (Table II). This reduction is more pronounced in HL than in LL treatments. Thus, the effect of virus infection was influenced by growth light level. A significant observation is that Ps is similar in LN-treated leaves either in LL or in HL. Only with LN treatments is there an increase in Ps in the HL treatments.

HN treatment reduced the effects of virus infection on photosynthetic characteristics in expanding leaves in both HL and LL treatments. However, during LL→HL transfer, increased nitrogen supply to the plant did not ameliorate the effect of virus infection in expanding leaves; indeed, values remained below those of LL-grown leaves.

These changes in photosynthetic properties of expanding leaves reflect the extent of symptom development (Osmond et al., 1990). Table III shows that if area-based Ps is corrected for the extent of yellow areas in mosaic leaves grown at HL/ LN, Ps did not differ from that of uninfected leaves, suggesting that chloroplasts in yellow regions were nonfunctional in photosynthesis.

In general, infection with the masked strain PV42 did not affect photosynthesis (Table II), a finding consistent with earlier observations (Osmond et al., 1990). The only significant interactions following infection were an unexpected increase in photosynthesis in LL/HN treatments and incomplete acclimation during LL→HL transfer experiments.

Soluble Protein Fractions

In uninfected expanding leaves under LL, Rubisco comprised about 25 to 30% of soluble protein in both LN and HN treatments (Fig. 2). However, in HL Rubisco increased 4-fold with LN, rising to more than 50% of total soluble proteins. Thus, the requirement for an increase in Rubisco protein for acclimation of photosynthesis to HL (Table II) was met under HN conditions. The pool of other soluble proteins generally did not increase in response to nitrogen or light treatment.

Table III. Correction of photosynthetic rates for chlorotic mosaic areas in three different expanding tobacco leaves infected with TMV strain PV230

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Area of Green Tissue cm²</th>
<th>Maximum Photosynthesis μmol O₂ m⁻² s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosaic leaf I</td>
<td>5.1</td>
<td>9.6</td>
</tr>
<tr>
<td>Uninfected</td>
<td>10.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Mosaic leaf II</td>
<td>2.6</td>
<td>9.8</td>
</tr>
<tr>
<td>Uninfected</td>
<td>10.0</td>
<td>13.9</td>
</tr>
<tr>
<td>Mosaic leaf III</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Uninfected</td>
<td>10.0</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Effect of virus infection, nitrogen nutrition, and light treatment on photosynthetic parameters in expanding tobacco leaves

Table II. Effects of virus infection, nitrogen nutrition, and light treatment on photosynthetic parameters in expanding tobacco leaves

<table>
<thead>
<tr>
<th>Leaf Class, Virus Treatment</th>
<th>Photosynthetic Parameter</th>
<th>Light and Nitrogen Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL</td>
<td>HN</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ps</td>
<td>10.9 ± 0.9</td>
<td>11.1 ± 0.2</td>
</tr>
<tr>
<td>Qy</td>
<td>85 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>PV230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ps</td>
<td>6.5 ± 0.4</td>
<td>9.1 ± 0.9</td>
</tr>
<tr>
<td>Qy</td>
<td>61 ± 1</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>PV42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ps</td>
<td>12.1 ± 0.8</td>
<td>15.3 ± 0.8</td>
</tr>
<tr>
<td>Qy</td>
<td>83 ± 2</td>
<td>92 ± 3</td>
</tr>
</tbody>
</table>

a Ps, Light- and CO₂-saturated photosynthesis; μmol O₂ evolved m⁻² s⁻¹.  
Qy, Quantum yield; mol O₂ mol⁻¹ photons (x1000).
In expanding leaves of tobacco infected with TMV strain PV230, Rubisco content was invariably reduced in all light and nitrogen regimes (Fig. 2). The reduction in Rubisco due to infection was greater in HL and during LL→HL transfer than in LL. Other soluble proteins were also reduced following infection under most treatments. Accumulation of virus coat protein was highest at HL/HN, and coat protein often accumulated to the same concentration as Rubisco. Thus, infection with TMV strain PV230 either inhibited the increase in Rubisco needed for acclimation or accelerated degradation of Rubisco.

Expanding leaves infected with TMV strain PV42 contained as much coat protein as leaves infected with PV230, but Rubisco content was usually less reduced than that of other soluble proteins (Fig. 2). Even though Rubisco was reduced by infection with PV42 in HL/HN, photosynthetic acclimation to HL was not impaired (Table II).

These comparative experiments suggest that the effect of PV230 on Rubisco content is due to reduced synthesis and/or accelerated degradation of Rubisco in the yellow areas of the chlorotic mosaic leaves.

Developed Leaves

Photosynthetic Responses

In older leaves both Ps and Qy were reduced compared to uninfected expanding leaves (cf. Tables II and IV). The Ps in developed leaves was lower than in expanding leaves in both nitrogen treatments, and the extent of acclimation during the LL→HL treatments was much reduced, especially in the LN treatments. Qy declined quite markedly in LN treatments, especially in the LL→HL treatments. These results suggest that the extent of acclimation to HL conditions in tobacco leaves is dependent on leaf age, and that nitrogen limitation predisposes developed tobacco leaves to poor photoacclimation under HL conditions, as observed in other species (Ferrar and Osmond, 1986).

Unlike expanding leaves, developed leaves showed no visible symptoms 15 d after infection with TMV strain PV230, yet virus infection led to similar, but quantitatively smaller,

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**Table IV. Effects of virus infection, nitrogen nutrition, and light treatment on photosynthetic parameters in developed tobacco leaves**

<table>
<thead>
<tr>
<th>Leaf Class, Virus Treatment</th>
<th>Photosynthetic Parameter</th>
<th>Light and Nitrogen Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LL LN HN</td>
</tr>
<tr>
<td>Uninfected</td>
<td>Ps</td>
<td>8.8 ± 1.4 9.8 ± 0.1 11.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Qy</td>
<td>75 ± 5 83 ± 1 73 ± 2 85 ± 2</td>
</tr>
<tr>
<td>PV230</td>
<td>Ps</td>
<td>7.4 ± 0.3 8.2 ± 1.5 10.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Qy</td>
<td>76 ± 4 78 ± 8 63 ± 5 76 ± 2 35 ± 1 47 ± 4</td>
</tr>
<tr>
<td>PV42</td>
<td>Ps</td>
<td>10.8 ± 0.9 11.9 ± 0.3 9.9 ± 1.6 17.9 ± 0.6 11.0 ± 0.7 13.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Qy</td>
<td>82 ± 2 86 ± 4 70 ± 5 79 ± 2 73 ± 2 76 ± 1</td>
</tr>
</tbody>
</table>

*a* Ps, Light- and CO₂-saturated photosynthesis; μmol O₂ evolved m⁻² s⁻¹. *b* Qy, Quantum yield; mol O₂ mol⁻¹ photons (x1000).
reductions in \( P_s \) and \( Q_y \) when compared with uninfected leaves. The effect of virus infection was most marked during LL→HL transfer; developed leaves of plants grown with either LN or HN treatments failed to acclimate and displayed extremely low values of \( P_s \) and \( Q_y \).

Infection with TMV strain PV42 did not affect photosynthetic capacity, but \( Q_y \) was slightly reduced compared with uninfected leaves. Like expanding leaves infected with PV42, developed leaves from HN plants also showed slow acclimation during LL→HL transfer conditions. Interestingly, infected leaves of plants grown in LN retained higher photosynthetic activity during transfer than did control, uninfected leaves.

**Soluble Protein Fractions**

In uninfected developed leaves, differences in the concentrations of soluble protein and Rubisco showed trends with respect to light and nitrogen treatments similar to those observed in uninfected expanding leaves, but absolute levels of Rubisco were only 50 to 60% of those in expanding leaves (Fig. 3). During LL→HL transfer, developed leaves also showed smaller increases in Rubisco, consistent with incomplete photosynthetic acclimation (Table IV).

Infection with TMV strain PV230 did not affect Rubisco content at LL, consistent with minimal effects on photosynthesis (Table IV), but the content of other soluble proteins was reduced (Fig. 3). In HL and transfer treatments, Rubisco content was reduced to a larger extent in LN than in HN treatments, but other soluble proteins were unaffected by infection (cf. expanding leaves in LN treatments). Photosynthesis of infected leaves did not acclimate under these conditions (Table IV).

In developed leaves infected with TMV strain PV42, virus coat protein accumulated to the same extent or at higher concentrations than in those infected with PV230, with practically no effect on Rubisco, although other soluble proteins were usually reduced. In one instance, during transfer in LN treatments, infected leaves showed increases in Rubisco and other soluble proteins compared with uninfected leaves. Consistent with this, these leaves showed higher photosynthetic acclimation than uninfected controls.

**DISCUSSION**

The dimensions of the effects of TMV on photosynthesis and photoacclimation in tobacco are very complex. The above results clearly establish that TMV strain PV230 produces severe effects on photosynthetic characteristics and nitrogen balance of tobacco leaves that interfere with photoacclimation, whereas TMV strain PV42 does not. Expanding leaves with mosaic symptoms showed impaired photoacclimation to a greater extent than either developed or mature leaves following infection with TMV strain PV230. These experiments support the conclusions of other studies in which no clear association was observed between symptom expression, virus coat protein accumulation, and virus effect on photosynthesis (Fraser et al., 1986; Van Kooten et al., 1990).

That the capacity of expanding leaves of tobacco plants to acclimate to HL conditions was impaired by infection with TMV strain PV230 was evident in both steady-state HL and LL→HL experiments. In these experiments most of the effect of infection on photosynthesis can be associated with symptomatology.

This conclusion is based on our observation (Table III) that when yellow, nonphotosynthetic areas of the mosaic symptoms were discounted, rates per unit area were comparable to those of control, uninfected tissue. Thus, in expanding leaves, \( P_s \) and \( Q_y \) remain higher in HN than in LN treatments because HN sustains a larger area of functional green tissue, consistent with the observations of Weathers and Pound (1954), who found that addition of nitrogen could reduce symptom severity.

Destruction of chloroplasts in yellow, chlorotic-mosaic tissues of expanding leaves has been previously associated with higher concentrations of virus in chlorotic areas (Atkinson and Matthews, 1970; R.A.J. Hodgson, personal communication). It is commonly believed that remaining green areas are resistant to further infection (i.e., they are effectively "cross-protected"; Murakishi and Carlson, 1976; Van Loon, 1987). Thus, we would expect that those green areas that escape infection would show photosynthetic parameters comparable to controls (Table III; Osmond et al., 1990). This is supported...
by the observation by Van Loon et al. (1990) that isolated chloroplasts show the same electron transport activity (on a Chl basis) whether they are extracted from symptomless leaves (1–4 d after infection) or from mosaic leaves (15–18 d after infection).

In symptomless developed leaves infection led to the accumulation of virus coat protein, often at levels equal to or in excess of those in leaves with symptoms. The presence of the virus led to reduced soluble protein and Rubisco levels but had only minor effects on photosynthetic parameters in steady-state treatments. However, marked inhibition of photoacclimation was observed during LL—HL transfer even though the effect of virus on Rubisco content was about the same as in the steady state.

Thus, our comparison of expanding and developed leaves shows that effects of PV230 on photosynthesis differ depending on the stage of leaf development at the time of infection and the nature of the photoacclimatory stress. In expanding leaves infection leads to virus replication in restricted areas of the leaf, and this replication is accompanied by destruction of chloroplasts and symptom development. The extent of this patchiness and its effects on photoacclimation are greatest in the first infected expanding leaves, in LL—HL transfer experiments, and in LN treatments. In developed leaves infection leads to virus replication to the same extent as in expanding leaves, without symptomatology, but with a general acceleration of Chl loss and especially pronounced interference in photoacclimation during LL—HL transfer experiments.

Contrary to the above observations with PV230-infected leaves, infection with TMV strain PV42 induced an apparent delay in senescence, characterized by higher Ps in expanding and developed leaves in LL treatments, and the maintenance of photoacclimation capacity in LN transfer experiments, especially in developed leaves. These effects were clearly related to the maintenance of high Rubisco concentrations in these leaves, at levels comparable to or greater than those in uninoculated controls, independent of the amount of virus protein present in the leaves.

Enhanced or unaffected photosynthetic rates following virus infection have been documented in several host-virus systems by other techniques (Zaitlin and Jagendorf, 1960; Platt et al., 1979; Montalbini and Lupattelli, 1989). It has been suggested that virus replication could be sustained for a longer time if photosynthetic assimilation was stimulated following infection, especially under LL conditions. Indeed, in several treatments PV42 infection led to much higher TMV coat protein levels in tobacco leaves than were found following PV230 infection. In a sense, TMV strain PV42 resembles some animal viruses (such as myxo viruses) in which replication of the virus proceeds without significant effects on host cell protein synthesis (Choppin, 1964).

Our data suggest that the consequences for the chloroplast depend on the time and stage of leaf and chloroplast development at which infection occurs. In our experiments, mosaic symptoms were seen only in leaves that were less than 2.0 cm in length at the time of infection, as observed previously (Atkinson and Matthews, 1970; Gianinazzi et al., 1977).

The patches of chlorosis that characterize the symptoms of TMV PV230 could arise for several reasons. If meristematic cells were infected by the virus, subsequent cell division would result in a population of cells carrying the virus, all of which would be susceptible to impaired photosynthesis. In expanding, as well as in developed, leaves the virus also spreads from cell to cell through plasmodesmata (Esau and Cronshaw, 1967; Hull, 1989) to produce expanding patches of tissue with increasing chloroplast malfunctions as virus replication continues. In the expanding leaves studied here, systemic infection that leads to symptom development probably involves both paths for the spread of the virus.

Van Loon et al. (1990) proposed that virus infection of meristematic tissues that coincides with chloroplast development from proplastids is primarily responsible for symptom formation. Presumably, the extent of symptom development responds to nitrogen status because HN status promotes more rapid and robust chloroplast development in expanding leaves. Also the extent of symptoms responds to light intensity because symptom development involves light-dependent destruction of chloroplasts.

It remains unclear just how virus replication, coincident with chloroplast development, should lead to destruction of chloroplasts and symptom expression. TMV strain PV230 is located almost exclusively in chlorotic regions of the infected chlorotic-mosaic leaves (R.A.J. Hodgson, personal communication). One of the most striking effects of TMV strain PV230 is the association of virus coat protein with chloroplast thylakoids and specifically with PSII reaction centers (Reinero and Beachy, 1986; Hodgson et al., 1989). The inhibitory effects of this binding on PSII electron transport (Hodgson et al., 1989; Reinero and Beachy, 1989) could lead to the light-dependent destruction of chloroplasts in infected cells and to symptom development. This conclusion is consistent with our observations based on fluorescence imaging, reported in a subsequent paper (Balachandran et al., 1994).

We suspect that in developed leaves, which were symptomless in our experiments, the interactions with PV230 were due primarily to spread through plasmodesmata. Van Loon et al. (1990) proposed that virus infection of these nonmeristematic cells interferes with maintenance processes in mature chloroplasts, enhancing senescence. Our data are consistent with this hypothesis. However, the bulk of our evidence argues against a simple competition for leaf nitrogen resources between TMV protein synthesis and chloroplast protein synthesis as the mechanism for impaired photoacclimation. The masked strain PV42 can accumulate to the same extent as PV230, in the same treatments, without impairing photoacclimation.

These studies show that strain-specific effects due to infection with TMV strain PV230 are major components of the failure of photoacclimation in expanding leaves of tobacco. However, on a whole-plant basis, it does not matter whether the failure to acclimate to HL is due to patchiness, to the loss of photosynthetic area associated with symptom development in expanding leaves, or to enhanced senescence in symptomless green tissues of developed leaves. Both processes tend to be exaggerated by HL and LN and both involve biological interactions that accelerate photoinhibition and impair photoacclimation.
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