Acquired Systemic Susceptibility to Infection by Tobacco Mosaic Virus in *Nicotiana glutinosa* L.

By R. S. S. FRASER, S. A. R. LOUGHLIN AND R. J. WHENHAM

Biochemistry Section, National Vegetable Research Station, Wellesbourne, Warwick CV35 9EF, U.K.

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SUMMARY

In *Nicotiana glutinosa* L. formation of local lesions on lower leaves inoculated with tobacco mosaic virus increased the susceptibility of the upper leaves to infection in a subsequent inoculation. The increase in susceptibility was detected as an increase of up to 3.5-fold in the number of lesions produced on the upper leaf and a corresponding increase in the amount of virus RNA synthesized. The concentration of endogenous abscisic acid in the upper leaves was negatively correlated with susceptibility to infection. This acquired systemic susceptibility to infection in *N. glutinosa* is in direct contrast to the acquired systemic resistance to infection reported to occur in hypersensitive varieties of *Nicotiana tabacum* under similar conditions. Mechanisms which might be involved in the acquisition of systemic resistance or susceptibility are discussed.

INTRODUCTION

The phenomenon of acquired systemic resistance has been extensively described in plants reacting to virus infection by formation of necrotic local lesions. If lower leaves are inoculated and develop lesions, the upper leaves become to some extent resistant to a subsequent challenge inoculation. This resistance is recorded as a reduction in lesion size, lesion number or both. The effect has been described in several host-virus combinations (Gilpatrick & Weintraub, 1952; Ross, 1961, 1964; Loebenstein, 1963; Nagaich & Singh, 1970; Kassanis et al. 1974). However, most studies have dealt with acquired resistance to tobacco mosaic virus (TMV) in varieties of *Nicotiana tabacum* L. such as Xanthi-nc (Kassanis & White, 1974, 1975) and Samsun NN (Ross, 1966; van Loon & van Kammen, 1970) containing the N gene (Holmes, 1938) for local lesion formation. It has been suggested that alteration in host protein synthesis (Kassanis et al. 1974; van Loon & van Kammen, 1970) or growth regulator metabolism (Balazs et al. 1977) may be involved in the acquisition of systemic resistance.

We have studied the systemic effects of local lesion formation in *Nicotiana glutinosa* L., a species which shows the local lesion reaction to TMV, and is the original source of the N gene (Holmes, 1938). In contrast to the acquired systemic resistance found in hypersensitive *N. tabacum*, we find that local lesion formation in the lower leaves of *N. glutinosa* increases the susceptibility of the upper leaves to infection in a subsequent inoculation.

Application of abscisic acid (ABA) to leaves of hypersensitive tobacco has been shown to increase the number of lesions formed as a result of subsequent inoculation with TMV (Balazs et al. 1973). In this paper we show that at the time of the challenge inoculation, the
level of endogenous ABA in *N. glutinosa* leaves showing acquired systemic susceptibility was lower than in control leaves. The implications of these results for the mechanisms involved in acquired systemic resistance and susceptibility are discussed.

METHODS

**Host plants and viruses.** *Nicotiana glutinosa* L. plants were grown in John Innes no. 2 compost in 12.5 cm diam. pots. The plants were kept in a glasshouse under natural lighting. The temperature was maintained at 16 °C at night; during the day it rose to 20 to 25 °C depending on the season.

Tobacco mosaic virus strains *vulgare* and *flavum* (originally obtained from the Max Planck Institut für Biologie, Abteilung Melchers, Tübingen, West Germany) were multiplied in the systemic host *Nicotiana tabacum* cv. Samsun. Infected leaf material was stored at −20 °C. Inocula were prepared by grinding infected leaf material in 50 mM-sodium phosphate buffer, pH 7.0, using a pestle and mortar. The homogenate was filtered through muslin and diluted with phosphate buffer as required.

*N. glutinosa* plants were given a first inoculation when 15 to 20 cm tall, with five to eight fully-expanded leaves. Three lower expanded leaves on each plant were lightly dusted with 400-mesh Carborundum and inoculated by rubbing with virus suspension. Control plants were sham-inoculated by rubbing with sterile phosphate buffer, or with ground, frozen healthy leaf material at the same final dilution in phosphate buffer as the virus inoculum. Leaves were washed with water after inoculation.

Seven to ten days after the primary inoculation, the lesions on the inoculated leaves were counted. Three upper expanded leaves on each plant were lightly dusted with 400-mesh Carborundum and inoculated by rubbing with virus suspension (the 'challenge' inoculation). Lesions on the upper inoculated leaves were counted 7 days later. Statistical tests of differences between lesion numbers in treatments were by t-test, using transformations for lesion numbers with small or very low means described by Kleczkowski (1949, 1955).

Lesion diam. were measured on the upper, challenge-inoculated leaves 7 days after inoculation, using a stereoscopic microscope with × 50 magnification. One randomly-chosen diam. of each of 7 lesions on each of 18 leaves was measured, giving a total sample of 126 lesions for each treatment.

TMV accumulation in challenge-inoculated upper leaves was measured 7 days after inoculation, using a stereoscopic microscope with × 50 magnification. One randomly-chosen Fraser & Whenham (1978).

Most experiments were done with intact plants. However, in some experiments indicated below, the plants were decapitated up to one week before primary inoculation.

**Abscisic acid determination.** The endogenous abscisic acid (ABA) content of upper leaves of *Nicotiana glutinosa* was measured at the time of the challenge inoculation, 7 days after primary inoculation of the lower leaves. For each treatment, seven plants were used for ABA determination; seven similarly-treated plants received the challenge inoculation.

Ten to 20 g fresh weight of upper leaves were homogenized in 150 ml ice-cold 80% (v/v) methanol using an Ultra-Turrax homogenizer. DL-cis-trans-2-14C-abscisic acid (5 nCi; sp. act. 11.1 mCi/mmol; Radiochemical Centre, Amersham, U.K.) was added to the homogenate to allow estimation of the percentage recovery of ABA after purification and to facilitate location of ABA on chromatograms.

ABA in the homogenate was partially purified by successive acid/base partitioning between water and dichloromethane (Zabadal, 1974). ABA was further purified by chro-
matography on 3MM paper developed with isopropanol/ammonia/water (8:1:1, v/v). ABA was located on the chromatogram in a spark chamber radiochromatogram scanner (Birchover Instruments Ltd) and eluted with 80% methanol. After methylation with ethereal diazomethane (Schlenk & Gillerman, 1960), the ABA was rechromatographed on thin layer plates of silica gel 60 F$_{254}$ (Merck) multiply-developed with hexane/ethyl acetate (1:1, v/v).

ABA was located on the chromatogram in the spark chamber. The fraction containing the ABA was scraped off the plate and ABA was eluted with ether. The extract was dried, then taken up in 50 μl ethyl acetate. Samples of 5 μl were mixed with 10 ml scintillator [60%, v/v, toluene; 40%, v/v, 2-methoxyethanol, containing 5 g/l 2(4'-t-butylphenyl)-5-(4'-biphenylyl)-1,3,4-oxadiazole]. Disintegrations per minute of the $^{14}$C-ABA in the extract, hence the percentage efficiency of the ABA extraction, were measured by scintillation counting. The counting efficiency of each sample was determined by internal standardization, by addition of a known activity of $^{14}$C-toluene (Radiochemical Centre, Amersham).

Total ABA content of the extract was measured by gas chromatography. Samples of 5 μl of the extract were chromatographed on a column containing 5% SE-30 coated on 80- to 100-mesh Chromosorb W AW-DCMS. The column temperature was 200 °C; the carrier gas was oxygen-free nitrogen at a flow rate of 40 ml/min. The chromatograph was equipped with a flame-ionisation detector. Cis- and trans-methyl abscisate were identified on chromatograms by co-chromatography with authentic cis- and trans-methyl abscisate (ABA from Sigma Chemical Co., methylated as above) and by combined gas chromatography-mass spectrometry.

ABA was measured by the peak area of cis- plus trans-methyl abscisate on the gas chromatograph trace, calibrated by chromatography of known amounts of pure cis- and trans-ABA methylated as above, and corrected to 100% recovery using the $^{14}$C-ABA recovery measurements.

**RESULTS**

**Acquired systemic susceptibility**

Fig. 1 shows that when *Nicotiana glutinosa* plants were previously inoculated on the lower leaves, the number of lesions produced on subsequent inoculation of the upper leaves was greater than in control plants. Primary inoculation of the lower leaves thus made the upper leaves in some way more susceptible to infection by TMV. Khuruna & Hidaka (1977) have shown that the non-infected leaves of TMV-inoculated *N. glutinosa* contain an extractable factor which, if mixed with TMV suspension, will increase the number of lesions formed by that inoculum when applied to a fresh *N. glutinosa* plant. We do not know at present whether our in vivo acquired systemic susceptibility involves the operation of such a factor.

The degree of acquired systemic susceptibility produced depended on the lesion density on the lower, previously inoculated leaves. Generally, the more concentrated the virus suspension used to inoculate the lower leaves, the greater the increase in susceptibility to infection of the upper leaves (Fig. 1). If the mean number of lesions per upper challenged leaf is plotted against mean number of lesions per lower inoculated leaf for individual plants and the data are then grouped on the basis of lower leaf lesion numbers, then an approximately linear relationship is obtained between log challenged-leaf lesions and log lower-leaf lesions (Fig. 2).

In the experiment shown in Fig. 1 and 2, the range of dilutions of inoculum used for the lower leaves was sufficiently wide to produce all possible responses in the lower leaves.
Fig. 1. Acquired systemic susceptibility to infection by TMV in leaves of *Nicotiana glutinosa*. Three lower leaves on each plant were inoculated with sterile phosphate buffer (control) or with various dilutions of TMV strain *vulgare*; (b) shows the mean numbers of lesions which developed in each treatment. Seven days after the primary inoculation, three upper leaves on each plant were challenge-inoculated with TMV *vulgare* in sap diluted 1:10^4. Lesion numbers on the upper leaves (a) were counted 7 days later. (*) and (**) indicate values significantly different from the control at \( P = 0.01 \) and \( P = 0.001 \), respectively. Data shown in (a) and (b) are means ± s.e. mean; (c) shows mean lesion diam. on the challenge-inoculated leaves. The vertical bar is the least significant difference (LSD) between treatments at \( P = 0.05 \).

The most dilute inocula produced a few lesions on most leaves inoculated, but failed to induce any lesions on some. The most concentrated inocula produced complete necrotic collapse of all inoculated lower leaves within 7 days of inoculation and thus stimulated the maximum possible expression of the hypersensitive reaction.

No lesions were found on the upper leaves of any plants when these leaves received no challenge inoculation, or when they were sham-inoculated with sterile phosphate buffer.
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Fig. 2. Acquired systemic susceptibility to infection by TMV in upper leaves of *Nicotiana glutinosa*: the relation between lesion numbers on the lower and upper leaves. For individual plants, the mean number of lesions on the upper, challenge-inoculated leaves was plotted against the mean number on the lower, previously inoculated leaves. The data were then grouped on the basis of lower leaf lesion numbers. The categories for grouping were controls (no lower leaf lesions); plants with lesions on the lower leaves were grouped in 4's in ascending order of lesion number.

Table 1. **TMV RNA concentration and number of local lesions formed on challenge-inoculated upper leaves**

<table>
<thead>
<tr>
<th>Inoculum used for lower leaves</th>
<th>TMV RNA (µg/g upper leaf)</th>
<th>Lesions per half upper leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMV</td>
<td>10.1 ± 0.8</td>
<td>1222 ± 76</td>
</tr>
<tr>
<td>Sterile phosphate buffer</td>
<td>3.1 ± 0.5</td>
<td>331 ± 40</td>
</tr>
</tbody>
</table>

* Plants were inoculated on three lower leaves with TMV strain *flavum* in sap diluted 1:10³, or with sterile phosphate buffer (control). Seven days later the three upper leaves of all plants were inoculated with TMV strain *flavum* in sap diluted 1:10⁵. Lesion numbers and TMV RNA contents were measured 7 days after the second inoculation. All values are means ± s.e. mean. The differences between the treatments are significant at $P = 0.01$.

Thus the increase in number of lesions formed on the upper leaves of plants previously inoculated on the lower leaves was not the result of systemic spread of virus from the lower inoculated leaves.

Table 1 shows amounts of TMV RNA found in upper inoculated leaves of plants which were showing acquired systemic susceptibility and control plants. Because of the restriction of virus multiplication by local lesion formation, TMV will only multiply to very low concentrations in leaves of *N. glutinosa*. It was therefore necessary to use a high concentration of inoculum for the upper, challenge-inoculated leaves in this experiment, to produce high lesion numbers and thus comparatively high and measurable amounts of TMV RNA. It is clear that considerably more TMV RNA was present in leaves showing acquired systemic susceptibility than in the control leaves. The increased number of lesions found on leaves of plants made more susceptible to infection by a previous inoculation was thus paralleled by a similar increase in virus accumulation.

This result, plus the absence of any lesion formation on uninoculated upper leaves, allows us to exclude the possibility that the increased number of lesions formed on upper leaves of plants after primary inoculation of the lower leaves was due to the formation of 'non-viral lesions', such as have been reported to form on uninoculated parts of *N. glutinosa* plants kept under continuous light (Shimomura & Ohashi, 1975).
Table 2. *Acquired systemic susceptibility in Nicotiana glutinosa at various times of year* *a*

<table>
<thead>
<tr>
<th>Date of challenge inoculation</th>
<th>TMV strain</th>
<th>Lesions per half leaf</th>
<th>Degree of acquired systemic susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control plants</td>
<td>Previously inoculated plants</td>
</tr>
<tr>
<td>29 Sept. flavum</td>
<td>331 ± 40</td>
<td>1222 ± 76</td>
<td>3.69</td>
</tr>
<tr>
<td>29 Sept. vulgare</td>
<td>338 ± 50</td>
<td>1092 ± 59</td>
<td>3.23</td>
</tr>
<tr>
<td>10 Feb. flavum</td>
<td>3.5 ± 0.7</td>
<td>9.4 ± 1.7</td>
<td>2.68</td>
</tr>
<tr>
<td>28 Feb. flavum</td>
<td>13.8 ± 1.6</td>
<td>38.7 ± 6.6</td>
<td>2.80</td>
</tr>
<tr>
<td>28 Mar. flavum</td>
<td>68 ± 15</td>
<td>118 ± 30</td>
<td>1.76</td>
</tr>
<tr>
<td>1 June vulgare</td>
<td>62 ± 9</td>
<td>139 ± 17</td>
<td>2.23</td>
</tr>
<tr>
<td>16 June vulgare</td>
<td>31 ± 4</td>
<td>44 ± 5</td>
<td>1.40 NS</td>
</tr>
<tr>
<td>16 June vulgare</td>
<td>40 ± 4</td>
<td>68 ± 9</td>
<td>1.72</td>
</tr>
</tbody>
</table>

* Seven days before challenge inoculation of the upper leaves, plants were inoculated on three lower leaves with sterile phosphate buffer (control plants) or with the TMV strain stated (previously inoculated plants). All plants were challenge inoculated on three upper leaves. The degree of acquired susceptibility is the mean number of lesions per upper half leaf on previously inoculated plants divided by the lesion number on leaves of control plants. Except where marked NS, differences between treatments are significant at \( P = 0.05 \). All values are means ± s.e. mean.

**Reproducibility of acquired systemic susceptibility**

To determine whether the acquired systemic susceptibility in *N. glutinosa* was a reproducible phenomenon, we examined its occurrence under various experimental and environmental conditions. Table 2 is a summary of several experiments. It is clear that acquired systemic susceptibility could be observed at all seasons, and therefore over a wide range of natural lighting conditions. The effect occurred both with a common (*vulgare*) strain of TMV and with the yellow strain *flavum*.

The increase in lesion number on the upper leaves of plants showing acquired systemic susceptibility ranged from 1.4- to 3.7-fold. In no case did we observe any significant reduction in lesion number on the upper leaves as a result of prior inoculation of the lower leaves.

The numbers of lesions produced on the challenge-inoculated leaves varied considerably in different experiments, as a consequence of the different dilutions of challenge inocula used. There was some tendency for the highest levels of acquired systemic susceptibility to be associated with the highest absolute lesion numbers on the challenged leaves, but in some experiments high levels of acquired susceptibility were found in upper leaves with low lesion numbers (Table 2).

Acquired systemic susceptibility was observed equally in young plants and in old plants well into the flowering phase. Decapitation reduced the absolute numbers of lesions in all treatments, but also reduced the relative increase in susceptibility caused by previous inoculation of the lower leaves (Table 3). However, decapitated plants still showed statistically significant acquired systemic susceptibility.

Sham-inoculating the lower leaves of control plants with diluted, healthy leaf material homogenate rather than sterile phosphate buffer made no difference to the level of acquired susceptibility obtained. The susceptibility induced is thus unlikely to have been associated with any effect of a normal plant component present in the homogenate of infected leaf used as primary inoculum for the virus treatments.

When *Nicotiana tabacum* plants carrying the *N* gene for resistance to TMV (Holmes, 1938) were grown in the same glasshouse, the classical acquired systemic resistance was observed. Plants on which three lower leaves had been previously inoculated with TMV
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Table 3. Effects of decapitation on acquired systemic susceptibility in Nicotiana glutinosa*

<table>
<thead>
<tr>
<th>Treatment of plants</th>
<th>Lesion number per challenged upper half leaf</th>
<th>Degree of acquired systemic susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control plants</td>
<td>Previously inoculated plants</td>
</tr>
<tr>
<td>Intact</td>
<td>62 ± 9</td>
<td>113 ± 13</td>
</tr>
<tr>
<td>Decapitated</td>
<td>49 ± 5</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>Intact</td>
<td>62 ± 9</td>
<td>139 ± 17</td>
</tr>
<tr>
<td>Decapitated</td>
<td>24 ± 5</td>
<td>47 ± 8</td>
</tr>
</tbody>
</table>

* Two experiments are shown. For the first, plants were decapitated immediately before the primary inoculation. For the second, plants were decapitated one week before the primary inoculation. Three lower leaves on each plant were inoculated with sterile phosphate buffer (control plants) or with TMV strain *vulgare* (previously inoculated plants). The degree of acquired systemic susceptibility induced by primary inoculation of the lower leaves was calculated as explained in the legend to Table 2. With the exception of the value marked NS, differences between treatments are significant at P = 0.05.

showed approx. 50% reduction in mean lesion diam. and lesion number on the challenge-inoculated upper leaves compared to controls (our unpublished results). Thus the acquired systemic susceptibility displayed by *N. glutinosa* appears to be a feature of the host species, rather than the environment.

Acquired systemic susceptibility and lesion size

Fig. 1 shows mean lesion diam. in upper, challenge-inoculated leaves showing various degrees of acquired systemic susceptibility. It is clear that increase in lesion numbers on the leaves of plants showing increased susceptibility was associated with a small, though statistically significant reduction in mean lesion diam. The largest decrease in mean lesion diam. was 16% of the control and was obtained when the lower leaves had been inoculated with an inoculum rather more dilute than that required to induce the highest level of acquired susceptibility.

Endogenous abscisic acid and acquired systemic susceptibility

Balazs *et al.* (1973) have shown that when tobacco leaves are treated with high concentrations of exogenous ABA, their susceptibility to infection by tobacco mosaic virus increases, resulting in an increased number of lesions on the treated leaves. To determine whether the acquired systemic susceptibility in *N. glutinosa* was related in any way to endogenous levels of ABA, we measured endogenous ABA concentrations at the time of the challenge inoculation. Plants were previously inoculated on the lower leaves with different concentrations of virus suspension, to produce a range of lesion densities, or with sterile phosphate buffer as controls. At the time of the challenge inoculation, plants in each treatment were split into two groups. Upper leaves of one group were challenge inoculated and used to determine the level of acquired systemic susceptibility induced by the treatment. Upper leaves of the other group were used for ABA determinations.

The concentration of ABA in upper leaves of control (sham-inoculated) plants varied from 25 to 65 ng/g fresh weight, depending on the experiment and environmental conditions. Inoculation of the lower leaves resulted in a reduction in the concentration of ABA in the upper leaves at the time of the challenge inoculation 7 days later. Fig. 3 shows the collected results of replicate experiments. All ABA values are expressed as percentages of the ABA
Fig. 3. Endogenous abscisic acid concentrations in *Nicotiana glutinosa* leaves showing acquired systemic susceptibility. Data from two separate experiments are shown (○) and (●). ABA levels were measured in the upper leaves 7 days after sham-inoculation of the lower leaves (controls) or after inoculation of the lower leaves with various concentrations of TMV strain *flavum*. Upper leaves on plants treated in parallel were challenge-inoculated at the same time as the ABA determination. Lesions that developed on the challenged leaves were counted, and the increase in lesion number on leaves showing acquired susceptibility relative to the controls was calculated.

concentration in the sham-inoculated control for that experiment. Generally, the higher the level of acquired systemic susceptibility recorded in the upper leaves, the lower their ABA concentration (relative to the control) at the time of the challenge inoculation. Thus we find a negative correlation between endogenous ABA concentration and susceptibility to infection by TMV, in contrast to the result of Balazs *et al.* (1973). However, in considering the differences between the two sets of results, it should be remembered that Balazs *et al.* (1973) used ABA concentrations vastly in excess of those occurring naturally.

**DISCUSSION**

In this paper, we have shown that inoculation of the lower leaves of *Nicotiana glutinosa* with TMV alters the response of the upper leaves to a subsequent inoculation with TMV. This alteration takes two forms: the lesions formed on the challenged upper leaves are slightly reduced in size, and are considerably more numerous, than on similarly treated leaves of control plants. It is likely that separate mechanisms underly these two changes in response induced by the initial local lesion formation on the lower leaves.

Ross (1966) found that prior inoculation of Samsun NN tobacco with TMV resulted in a reduction of up to 80% in the size of lesions formed on subsequent challenge-inoculation of previously uninoculated parts of the plant. This reduction in size has been interpreted as evidence for systemic acquired resistance. Balazs *et al.* (1977) however have suggested that the reduction in lesion size is a consequence of failure of tissue to turn necrotic, not a reduction in the amount of virus multiplication in the challenge-inoculated leaf. In our experiments, the maximum reduction in lesion diam. was only 16%. Clearly, any resistance
mechanism which operates by reducing the size of lesions in the challenged leaf has very much less activity in *N. glutinosa* than in hypersensitive *Nicotiana tabacum*.

Many reports of acquired systemic resistance in hypersensitive *Nicotiana tabacum* show that the number of lesions formed on the challenged leaf is reduced as a result of prior inoculation of the lower leaves with TMV (Ross, 1966; Kassanis et al. 1974). Treating the challenged or lower leaves with polyanions such as polyacrylic acid before challenge inoculation also reduces the number of lesions formed (Gianinazzi & Kassanis, 1974; Kassanis & White, 1974, 1975; Cassells et al. 1978). Our results showing acquired systemic susceptibility in *N. glutinosa* are thus the direct opposite of the acquired systemic resistance found in hypersensitive varieties of *N. tabacum*. Our results show that the presence of the N gene and its activity in local lesion formation do not inevitably lead to acquired resistance as the overriding response. This raises the question of what mechanisms are involved in the acquisition of systemic susceptibility or resistance.

Two classes of mechanism may be proposed. For acquired systemic resistance, it has been suggested that a specific antiviral mechanism operates against infection or virus multiplication, to reduce lesion number or size (Ross, 1966; Kassanis et al. 1974). Certain new proteins (‘b’ proteins) become detectable in the resistant leaves after primary virus inoculation or polyacrylic acid treatment (van Loon & van Kammen, 1970; Kassanis et al. 1974; Antoniw & Pierpoint, 1978). It has been suggested that these ‘b’ proteins may be involved in the resistance reaction, perhaps in a manner analogous to interferon in animals (Gianinazzi & Kassanis, 1974).

Clearly, our finding of acquired systemic susceptibility cannot be explained on the basis of any such specific resistance mechanism. Acquired susceptibility does not, however, in itself exclude the possibility that an interferon-like mechanism might operate in *N. glutinosa*: the resistance mechanism could be obscured by a separate mechanism stimulating lesion formation. An interferon-like mechanism could be responsible for the slight reduction in lesion diameter observed in leaves showing increased susceptibility as measured by increased lesion numbers (Fig. 1). van Loon & van Kammen (1970) did find new proteins in non-infected parts of *N. glutinosa* after lesion formation; these proteins were in some ways similar to the ‘b’ proteins of *N. tabacum*. However, ‘b’ proteins have not yet been shown to have any direct antiviral action (Antoniw & Pierpoint, 1978), and van Loon (1975) was able to induce acquired resistance in Samsun NN tobacco with mercuric chloride without the occurrence of ‘b’ proteins. The existence and function of an interferon-like antiviral mechanism in the response of the plant to a second inoculation thus remain to be established. The acquired systemic susceptibility shown by *N. glutinosa* suggests that induction of an antiviral mechanism is unlikely to give a complete explanation of the altered response to the challenge inoculation.

An alternative model may offer an explanation of both acquired systemic resistance and susceptibility. Increases or decreases in lesion numbers might arise from changes induced in the metabolic or physiological state of the host which alter the susceptibility of sites on the challenged leaf to infection or lesion development. Cassels et al. (1978) have shown in Xanthi-nc tobacco that the resistance to TMV induced by polyacrylic acid is associated with increased leaf water stress. They suggest that the loss of turgidity may reduce the susceptibility of surface sites to infection by TMV. Experimental support for this hypothesis comes from their observation that treatment of leaves with an anti-transpirant abolishes the polyacrylic acid-induced resistance (Cassells et al. 1978).

Our results with *N. glutinosa* are also consistent with the idea that alterations in leaf water status affect susceptibility to infection. Abscisic acid levels are known to increase with
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increasing leaf water stress (ZabadaI, x974; Beardsell & Cohen, 1975). We consistently found that leaves with acquired susceptibility to infection had lower ABA concentrations than control leaves (Fig. 3), suggesting that such leaves had lower water stress and greater turgidity than control leaves. This may explain their enhanced susceptibility to infection.

It remains to be seen how local lesion formation on the lower leaves of tobacco plants can alter the water relations of the upper leaves. However, cytokinins (Livné & Vaadia, 1965; Biddington & Thomas, 1978) and abscisic acid (Cummins et al. 1971; Zabadal, 1974) are known to affect transpiration and stomatal opening. The demonstration that formation of local lesions on lower leaves can alter the ABA concentrations (Fig. 3) and cytokinin concentrations (Balazs et al. 1977) in uninfected upper leaves provides the basis of a possible mechanism.

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