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"Hypothesis to explain how viruses induce production of local lesions in plant tissues"

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Foreword

This unpublished hypothesis was written in 1969-1970. It was started at the Scottish Crop Research Institute, Dundee, Scotland, and completed at the Virology Department, Medical School, University of Birmingham, England. Roger Jones's interest in producing a hypothesis to explain how viruses induce local lesions in plant tissues was stimulated by his Ph.D. studies on the use of different regimes of light and temperature to manipulate the formation of *Potato mop-top virus* lesions. These studies were done in controlled environment cabinets in naturally-infected tubers of potato cv. Arran Pilot, and in leaves of Xanthi-nc tobacco and *Chenopodium amaranticolor*. They were written up in his Ph.D. thesis along with the local lesion hypothesis. A manuscript describing these studies and the local lesion hypothesis was prepared for, and presented at, a Meeting of the British Association for the Advancement of Science held at Durham in 1970, but only a brief abstract was eventually published (Jones, 1971). The studies on potato tubers and tobacco leaves subsequently formed part of two publications (Harrison & Jones, 1971a,b). The results obtained with *C. amaranticolor* and the local lesion hypothesis were never published outside the Ph.D. thesis (Jones, 1970).

This unpublished manuscript from 1970 represents a worthwhile contribution to the scientific literature. Although it involved speculation and extrapolation and might have been considered somewhat 'oversimplified' so as to achieve a 'unitary' working explanation, the local lesion hypothesis is still of potential benefit to other researchers. This is because the question of what underlying mechanism is responsible for the production of plant virus local lesions remains unanswered to this day. We therefore decided to publish the hypothesis as the second in a series of historical bulletins containing unpublished manuscripts by members of our family. Since the main contribution to emphasise from the manuscript was the local lesion hypothesis, we have made this the principal text of the Bulletin and moved the text covering the results of the controlled environment experiments to an appendix. As mentioned above, of the controlled environment results, only

the *C. amaratacolor* data remain unpublished outside the Ph.D. thesis. Subject to this alteration involving moving the data from the experiments into an appendix, the original manuscript is published in full in this Bulletin without any attempt to bring it up to date by reference to more recent relevant scientific publications since 1970. This is because the author's research on plant viruses has been on other topics since that time and he has been unable to follow developments in this field in sufficient detail. This publication should therefore be considered as a historical document.

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July 2007

Note: This article is available online at the MacDougall-Jones Family History web site at: http://www.geocities.com/macdougalljones/ and by e-mail from Susan MacDougall at smacdougall@actewagl.net.au

Hypothesis to explain how viruses induce production of local lesions in plant tissues

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Manuscript written in 1970

Summary

A hypothesis is described to explain how viruses induce production of localised lesions in infected plant tissues. In the hypothesis, whether or not infection causes local lesions to form depends on how much it depletes the reserves of energy and reducing potential available in affected cells. The reserves of energy reside mainly in ATP. Those of reducing potential reside in $NADH_2$ and $NADPH_2$, along with compounds like ascorbic acid, cysteine and reduced glutathione. The key points of the hypothesis are: (i) death results from breakdown of localisation of phenols to the cell vacuole; (ii) this breakdown occurs when levels of energy and reducing potential are insufficient to maintain localisation; (iii) virus infection decreases the amounts of energy and reducing potential within cells mainly through specific interactions between individual host and virus genes that upset nuclear control of mitochondrial (or chloroplast) DNA, and thereby respiration (or photosynthesis), and through stimulating the activity of enzymes that synthesise and oxidise phenols, thereby causing the formation of an oxidation-reduction balance centred around newly synthesised phenols, so draining the reducing potential available in the cytoplasm; (iv) the host-virus interactions occur only in newly invaded cells because the virus gene product(s) involved is (are) inactive in cells invaded later; (v) stimulation of enzymes that synthesise and oxidise phenols occurs in all infected cells and is a non-specific response to stress; (vi) chlorotic local lesions develop instead of necrotic ones when amounts of energy and reducing potential in newly invaded cells are decreased enough to cause breakdown of chlorophyll but insufficiently to cause breakdown of phenol localisation, the presence of a

host-virus interaction affecting transcription of chloroplast DNA also being required; and (vii) environmental factors affect the availability of energy and reducing potential in newly invaded cells, thereby influencing whether local lesions form. Although speculation and extrapolation are involved, this can serve as a simplified, unitary, working hypothesis to help explain the underlying mechanisms responsible for the production of localised viral lesions in infected plant tissues.

Introduction

The research on use of different light and temperature regimes to manipulate formation of PMTV lesions described in the Appendix of this Bulletin, Jones (1970) and Harrison & Jones (1971a,b), stimulated the author to form a hypothesis to explain how viruses induce local lesions in plant tissues. In this hypothesis, whether or not virus infection results in production of local lesions depends on how much it depletes the reserves of energy and reducing potential in cells. The reserves of energy are mainly in ATP and those of reducing potential in $NADH_2$ and $NADPH_2$ plus compounds such as ascorbic acid, cysteine and reduced glutathione. The main points of the hypothesis are given in Table 1. A summarized account of the hypothesis is provided in this Bulletin, while a more detailed description was given in the original Ph.D. thesis (Jones, 1970).

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* The following abbreviations are used: -

ADP	- Adenosine diphosphate
ATP	- Adenosine triphosphate
DNP	- 2,4-dinitrophenol
DNA	- Deoxyribonucleic acid
RNA	- Ribonucleic acid
NAD	- Nicotinamide-adenine dinucleotide
$NADH_2$	- Reduced nicotinamide-adenine dinucleotide
NADP	- Nicotinamide-adenine dinucleotide phosphate
PMTV	- *Potato mop-top virus*
TMV	- *Tobacco mosaic virus*
N.	- *Nicotiana*
C.	- *Chenopodium*

Table 1 *Summary of hypothesis to explain the production of lesions in virus-infected plant tissues*

1. Death results from breakdown of localisation of phenols to the cell vacuole.
2. Breakdown of localisation occurs when levels of energy and reducing potential are insufficient to maintain localisation.
3. Virus infection decreases the levels of energy and reducing potential in cells, mainly:
    (a) through specific interactions between individual host and virus genes that upset nuclear control over transcription of mitochondrial (or chloroplast) DNA, and thereby upset respiration (or photosynthesis).
    (b) by stimulating the activity of enzymes that synthesise and oxidize phenols, thereby causing the formation of an oxidation-reduction balance centred around newly synthesized phenols while they are available to oxidases in the cytoplasm *(i.e.,* before they become localised), so draining the reducing potential available in the cytoplasm.
4. The host-virus interactions occur only in newly invaded cells because the virus gene product(s) involved is (are) active in them but not in cells invaded earlier.
5. Stimulation of enzymes that synthesise and oxidize phenols occurs in all infected cells and is a non-specific response to stress, perhaps due to ethylene formation.
6. Chlorotic local lesions develop instead of necrotic ones when levels of energy and reducing potential in newly invaded cells are decreased insufficiently to cause breakdown of phenol localisation but sufficiently for breakdown of proteins (including chlorophyll) to occur. The presence of a host-virus interaction affecting transcription of chloroplast DNA is also required.
7. Environmental factors influence whether local lesions form by affecting the availability of energy and reducing potential in newly invaded cells.

1. 'Death results from breakdown of localisation of phenols to the cell vacuole.'

Plant cells contain reserves of phenolic compounds and oxidases potentially able to convert them to toxic quinones, but the two are kept apart, the phenols in the vacuole (Frey-Wyssling & Mühlethaler, 1965) and the oxidases in the cytoplasm (Davies *et al.*, 1964). When localisation of phenols *via* the tonoplast breaks down, they meet and death inevitably results.

2. 'Breakdown of localisation occurs when levels of energy and reducing potential are insufficient to maintain localisation'.

Energy is always required to maintain localised high concentrations of compounds in cells, phenols in this instance, against concentration gradients (Beevers, 1961). When insufficient energy is available localisation breaks down. The availability of energy is itself determined by that of reducing potential, through oxidative phosphorylation.

The breakdown of localisation can be prevented by supplementing reserves of energy and reducing potential. Formation of necrotic TMV lesions in inoculated leaves of *N. glutinosa* was markedly inhibited when ascorbic acid, reduced glutathione or cysteine were fed to the tissue in high concentrations, and similar results were obtained when ascorbic acid was supplied to tobacco leaves inoculated with *Tobacco necrosis virus* (Farkas *et al.*, 1960). White (1969) also inhibited formation of viral necrotic lesions with ascorbic acid. At least with ascorbic acid, the inhibition does not seem to be caused by effects on virus multiplication because the virus content of ascorbic acid treated leaves was not very much less than that of control leaves in the work of Farkas *et al.* (1960) and was little affected in that of White (1969).

3(a) 'Virus infection decreases the levels of energy and reducing potential in cells through specific interactions between individual host and virus genes that upset nuclear control over transcription of mitochondrial (or chloroplast) DNA'

Whether or not lesions develop is determined primarily by the genetic constitutions of the virus and host in question. Hadwiger & Schwochau (1969) suggested a 'gene for gene interaction' mechanism to explain the hypersensitive response of some host plants to products released by micro-organisms. Similar 'gene for gene interactions' could occur between individual virus and host genes, exact matching of a virus gene and a gene in the host being required for formation of lesions. Such a mechanism would explain, for example, the different responses of the tobacco cvs Samsun NN and Samsun to manual inoculation with preparations of the type strain of TMV, because these cultivars differ only by a single gene determining whether infection will or will not cause the formation of necrotic local lesions (Holmes, 1938). It would also explain why the tomato aucuba mosaic strain of TMV produces necrotic local lesions in inoculated leaves of white burley tobacco whereas the type strain does not (Smith, 1957).

There is evidence that virus infection can upset the control cell nuclei exert over mitochondria and chloroplasts. This is as follows:

(i) Mitochondria of *N. clevelandii* change greatly in appearance when cells become infected with isolates of *Tobacco rattle virus* which cause necrotic lesions (Harrison *et al.*, 1970).

(ii) Mitochondria multiply in cells of *N. glutinosa* leaves inoculated with TMV (Weintraub *et al.*, 1964).

(iii) The absence of greening in newly virus-invaded cells of PMTV-infected cv. Arran Pilot potato tubers exposed to light suggests that differentiation of protoplastids into chloroplasts is inhibited in them.

(iv) Chloroplasts isolated from tobacco leaves infected with TMV have a decreased capacity for both nucleotide incorporation into RNA and amino acid incorporation into protein (Hirai & Wildman, 1969).

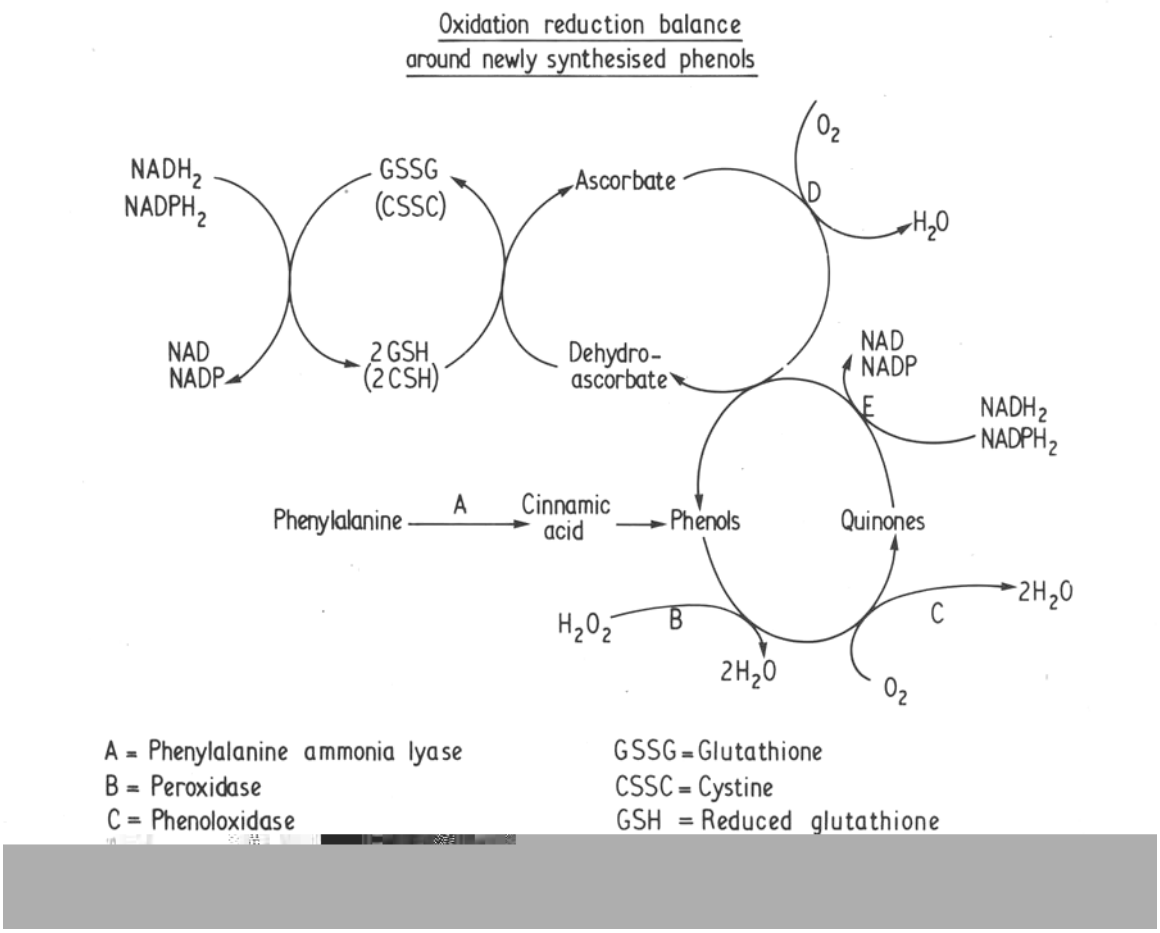
(v) Actinomycin D, an inhibitor of DNA dependant RNA polymerase, induces chlorotic mosaic symptoms in healthy tobacco leaves which closely resemble symptoms of TMV infection (Hirai & Wildman, 1967, 1969) suggesting that both produce symptoms by repressing transcription of chloroplast DNA, thereby preventing synthesis of new chlorophyll molecules.

(vi) The effect of Actinomycin D on light-induced chloroplast differentiation (Smillie *et al.,* 1963) is comparable with that of PMTV; both prevent chloroplast formation, suggesting that they both 'switch off" protoplastid DNA. Both mitochondria and chloroplasts have their own DNA, transcription of which seems under the control of nuclear DNA. Alteration in the regulation of mitochondrial DNA as a result of a host-virus interaction could decrease respiration. Similarly, alteration in regulation of chloroplast DNA could decrease photosynthesis. In either instance less energy and reducing potential would then be available in affected cells. However, it is difficult to see how decreasing photosynthesis alone could deplete levels of energy and reducing potentially sufficiently to cause necrosis unless absolutely no reserves of starch or other substances, which can be used in respiration to produce them, are present.

3(b) 'Virus infection decreases the levels of energy and reducing potential in cells by stimulating the activity of enzymes that synthesise and oxidise phenols'.

Phenolic compounds accumulate in virus-infected cells (Andreae, 1948; Hirae, 1956; Holden 1957; Martin & Morel, 1958; Best, 1968), and at least one enzyme concerned with their synthesism, phenylalanine ammonia lyase, has been shown to be stimulated by virus infection (Farkas & Szirmai, 1969). Also stimulated are the enzymes peroxidase and phenoloxidise (Farkas *et al.,* 1960; Suseno & Hampton, 1965). Oxidation of newly synethesised phenols by these two oxidases before the phenols become localised in the vacuole could deplete the level of reducing potential in affected cells as shown in the scheme in Fig. 1; peroxidase and phenoloxidase oxidise phenols to quinones and then these are immediately reduced back to phenols at the expense of reserves of reducing potential. Depletion of reducing potential would automatically decrease production of ATP. Obviously, the extent to which oxidation of newly synthesised phenols occurs will be much greater in cells in which their rate of localisation is slow. Localisation would be expected to be particularly slow in cells already depleted of ATP by the host-virus interaction.

Fig. 1



Legend to Fig. 1:

Oxidation-reduction balance centred around newly synthesized phenols while they are available to oxidases in the cytoplasm.

4. 'The host-virus interactions occur only in newly invaded cells because the virus gene product(s) involved is (are) inactive in those invaded earlier'.

The work with PMTV showed that when lesions form symptoms develop only in newly invaded cells (see Appendix). Host-virus interactions could be present in these but not in cells which have been invaded for some time. Viruses of vertebrates produce two classes of proteins, early and late. The early proteins are normally enzymes and are produced only in newly invaded cells. The late ones, commonly coat proteins, are produced some time after infection. By analogy, the virus genes involved in host-virus interactions might produce only early proteins, and be inactive in cells which have been infected for some time.

5. 'Stimulation of enzymes that synthesis and oxidise phenols occurs in all infected cells and is a non-specific response to stress, perhaps due to ethylene formation.'

Accumulation of phenols and stimulation of oxidases occurs both in healthy cells at the onset of senescence and in younger cells as a non-specific response to any factor causing stress, and both seem to occur in virus-invaded cells regardless of the period for which they have been infected. Ethylene can stimulate synthesis of such enzymes as phenylalanine ammonia lyase, phenoloxidase and peroxidase (Stahmann *et al.,* 1966; Imaseki *et al.,* 1968) and is produced both in response to conditions of stress and at the onset of senescence. Nakagaki *et al.* (1970) showed its production by leaves in response to infection with TMV. Conditions of stress may therefore stimulate ethylene formation which in turn may stimulate enzymes which synthesise and oxidise phenols.

'Chlorotic lesions develop when levels of energy and reducing potential in newly invaded cells are decreased insufficiently to cause breakdown of phenol localisation but sufficiently to cause breakdown of proteins'.

Ultimately, chlorotic symptoms obviously result from breakdown of chlorophyll. Proteins are normally continuously being broken down and replaced within cells. Their breakdown would be favoured by shortage of energy and reducing potential because both are required for new synthesis. Chlorophyll breakdown would therefore be promoted under such conditions. Gene interactions affecting chloroplast DNA could further favour its breakdown by repressing new synthesis.

6. 'Environmental conditions influence whether symptoms develop by affecting the availability of energy and reducing potential in newly invaded cells'.

The experiments with PMTV showed that light promotes lesion production. This did not seem to be through any affect on photosynthesis, because light induced necrotic lesions in potato tubers which lacked differentiated chloroplasts (see Appendix). It may occur through stimulation of the light-induced enzyme phenylalanine ammonia lyase, which catalyses the initial reaction involved in diversion of aromatic compounds into phenolics and controls the rate at which this diversion occurs (Zucker, 1965). This enzyme is induced by blue light (Engelsma, 1967) which also promotes formation of *Arabis mosaic virus* symptoms in *N. clevelandii* and *Potato virus X* symptoms in Samsun tobacco (Casper, 1968), and seemed to promote production of PMTV lesions in *C. amaranticolor* (and possibly also in Xanthi-nc tobacco) more than did other wavelengths (see Appendix). Stimulation of phenylalanine ammonia lyase by light in invaded cells could increase the concentration of phenols available to oxidases in them and thereby, in the newly invaded ones, cause increased depletion of reserves of energy and reducing potential, over and above that already caused by the host-virus interaction. Depletion to levels insufficient for the maintenance of localisation of phenols would result in necrosis.

Determination of PMTV lesion type, necrotic or chlorotic, in *C. amaranticolor* leaves by light (see Appendix) can be explained in terms of photosynthesis. Less light was required for development of necrotic lesions than for formation of chlorotic ones. Where chlorotic lesions developed, enough photosynthesis

might have occurred in cells adjacent to those infected with virus to prevent death of the infected ones by raising the levels of energy and reducing potential in them above the critical level below which localisation of phenols breaks down. Thus passage of sugars from adjacent into infected cells might, through respiration, replenish reserves to levels above that required to maintain localisation. Reserves might not be replenished sufficiently, however, to prevent breakdown of proteins (including chlorophyll), hence the formation of chlorotic symptoms.

Formation of virus lesions is inhibited with many host-virus combinations when plants kept in the light are put at higher temperatures. Sunderland & Merrett (1965) found that the response to DNP of Xanthi-nc tobacco leaves inoculated with TMV and kept at 35ºC was different from that of leaves kept at 25ºC and showing a dense cover of necrotic local lesions. At 35ºC the oxygen uptake of infected leaves was stimulated by DNP, resulting in a similar maximum oxygen uptake to that obtained when DNP was added to healthy leaves. At 25ºC there was little or no stimulation of oxygen uptake. Thus at 35ºC, but not at 25ºC, oxygen uptake of infected leaves seemed to be controlled by phosphorylation, as in healthy leaves at both temperatures. This suggests that non-mitochondrial oxidases may not be active at the higher temperature. At 25ºC necrotic lesions form whereas at 35ºC they do not. Lack of lesion production at higher temperatures may therefore be due to lack of activity of non-mitochondrial oxidases. If so, depletion of energy and reducing potential through oxidation of newly synthesised phenols by phenoloxidase and peroxidase would not occur at the higher temperatures, which might explain why lesions are not produced at these temperatures.

In potato tubers in which PMTV was spreading, lesions were induced by lowering the storage temperature and this also induced parallel changes in sugar content (see Appendix). If the mitochondria of newly invaded cells were affected by a host-virus interaction, they might be able to supply the cells with enough energy and reducing potential to remain alive only when large amounts of sugar are available for respiration.

Appendix

The influence of light and temperature on formation of Potato mop-top virus *lesions*

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Summary

Potato mop-top virus (PMTV) characteristically produces lesions consisting of concentric necrotic rings. Controlled environment cabinets were used to study their formation in naturally-infected tubers of cv. Arran Pilot potato, and in leaves of Xanthi-nc tobacco and *Chenopodium amaranticolor* infected by manual inoculation of sap.

Naturally-infected potato tubers kept in darkness at constant temperatures (18°, 13° or 4°C) developed no lesions. However, brown necrotic rings developed at the boundary of the zone invaded by virus when tubers kept initially at 18° were moved to 13°C and when others kept at *c.* 17° were moved to 4°C. In contrast, no lesions formed when tubers kept at 13° were moved to 18° or when others at 4° were moved to 13°C. But when tubers kept at 4° in the dark were transferred to 13°C in the light they produced rings and white weals at their surface. The rings formed at the edges of the weals, on the side nearest to the original site of infection; light failed to stimulate chlorophyll formation in the weals. Induction of rings may be related to changes in sugar content; on transfer from 18° to 13°C, the concentration of reducing sugars in healthy Arran Pilot tubers reached a minimum after 3 days, exactly the time need for rings to appear in PMTV-infected tubers.

In inoculated leaves of Xanthi-nc tobacco at 14°C in the light (including continuous light), PMTV lesions formed initially as necrotic spots or small rings; these later became surrounded by a series of necrotic rings of increasing diameter. In general, the time needed for successive rings to appear at 14°C was increased by decreasing either the light intensity or the photoperiod. Lesions rarely developed at 14°C in darkness or 22°C in light, but when plants were transferred from these conditions to 14°C in light necrotic spots or rings developed. Their size depended on the interval between inoculation and transfer, and on the conditions during this period. Rings developed when inoculated plants were transferred from 22°C in light to 14°C in darkness, but not when the order of treatments was reversed. The process of lesion formation therefore includes an early phase requiring light and a later phase requiring low temperature. Necrosis occurred only in cells that were newly infected when conditions became favourable for lesion development.

C. amaranticolor plants kept at 14°C after leaf inoculation with PMTV produced lesions which were either chlorotic spots or necrotic rings, or were symptomless, depending upon the photoperiod, the light intensity and the type of controlled environment cabinet used. Symptomless infection occurred when the plants received too little light, but light with a larger blue content seemed to favour lesion development more than that with a smaller blue component. Concentric necrotic rings formed in the lower, or shaded upper, leaves of plants receiving adequate continuous light, and chlorotic lesions in the unshaded upper leaves. Light thus determines whether lesions will form and whether they will be necrotic or chlorotic, the first effect apparently being influenced by light quality as well as by the amount of light supplied.

Introduction

The symptoms of *Potato mop-top virus* (PMTV) in naturally-infected potato plants in the field and in artificially-infected test plants in the glasshouse are greatly affected by environmental conditions. Controlled environment cabinets were used to try to define the conditions under which lesions develop in tubers of Arran Pilot potato, and in inoculated leaves of two test

plants, Xanthi-nc tobacco and *Chenopodium amaranticolor* Coste & Reyn. The work described in this Appendix is presented in greater detail elsewhere (Jones, 1970; Harrison & Jones 1971a, b).

Materials and methods

Two types of controlled environment cabinets, A and B, were used. Type A held temperature to within 0.5°C of the mean and were lit by a combination of Philips warm white fluorescent tubes and tungsten filament lamps. Type B held temperature to within 1°C of the mean and were lit by F48T 12/CW/VHO fluorescent tubes augmented by tungsten filament lamps.

The infected tubers used were grown in soil heavily infested with the virus-carrying fungus vector *Spongospora subterranea* (Wallr.) Lagerh. (Jones & Harrison, 1969; Jones, 1970); virus-free seed tubers were planted. Test plants were reared in glasshouses at about 20°C and infected by rubbing their leaves with preparations containing PMTV isolate T (Harrison and Jones, 1970; Jones, 1970) in presence of an abrasive. After inoculation, they were kept in the shade in the glasshouse for up to 5 hours before transfer to the cabinets, in which they were spaced such that they did not greatly shade one another.

Experiments with potato tubers

Arran Pilot tubers infected with PMTV from the soil by its vector fungus develop lesions consisting of brown necrotic rings. The rings usually extend into the tuber flesh and their centres mark the initial sites of infection. At harvest time, distinct rings are present only in tubers at or near the soil surface.

In the first experiment, tubers were dug, washed, and three comparable lots of 40 each selected (after discarding any with symptoms). These were left on a glasshouse bench for 2-3 days and then put in darkness at 5°, 13° and 18°C, one lot/condition. Rings developed within 2 wk in nearly half of the tubers at

5°C and 13°C but in only two of those at 18°C. In contrast, hardly any rings developed in tubers bagged straight after they were dug, put at 13°C within a few hr and examined after 2 months. The 2-3 days in the glasshouse therefore seemed to have pre-disposed tubers to produce rings. During this period the tubers were both exposed to light and to higher temperatures than they would otherwise have experienced. Similarly, tubers at the soil surface are exposed to light and experience higher temperatures than do those deeper down in the soil. Hence, experiments were done to see whether these two factors would promote ring formation.

Tubers kept in darkness developed rings only if they were moved from higher to lower temperatures. None developed when they were kept at constant temperature or when they were moved from lower to higher temperatures. Table 2 summarises the results of an experiment which showed this. The tubers used already had one ring induced by transfer from a glasshouse at *c.* 17°C to 4°C. They were divided into three comparable samples, two of which were put at 18° and one at 13°C. No rings developed. After 4 wk, one of the samples at 18° was moved to 13°C. Rings developed within 1 wk (Fig. 2), but there were still no symptoms in the other two samples. After 10 wk, the sample which had been at 13°C throughout was moved to 18°C. No rings developed until it was moved back to 13°C again 2 wk later.

Light also induced ring formation. When tubers, stored in the dark at 4°C, were exposed to light in a cabinet at 13°C, rings and white weals developed (Table 3) but the rings did not extend into the tuber flesh. No rings or white weals formed in control tubers. The weals were zones in which light failed to stimulate chlorophyll production. Rings formed at their inside edges (Fig. 3), ie. on the side of the weal nearest to the original site of infection of the tuber. Because rings form only at boundaries of virus invaded zones, the inside edge seems to represent the point to which virus had spread at the time of transfer, and the white weal itself tissue invaded after transfer. Light stimulates chlorophyll production by causing protoplastids to differentiate into

Table 2. *Effect of transferring tubers between 18° and 13°C in the dark on development of a second ring**

Storage history	Total no. of tubers	Tubers with second rings after:				
		4 wk	5 wk	10 wk	12 wk	13 wk
18°C throughout	6	0	0	0	-	-
18°C for 4 wk, then 13°C	6	0	5	5	-	-
13°C for 10 wk+ 18°C for 2 wk+ 13°C for 1 wk	6	0	0	0	0	4

* First ring present in all tubers, induced by transfer from *c.* 17°C to 4°C.

Tubers left at 4°C for 2 months, then moved to 18°C or 13°C.

Fig. 2 *Potato tubers with second PMTV rings*

Legend to Fig. 2:

Three potato tubers with second PMTV rings induced simultaneously by transfer from 18ºC to 13ºC. The first-formed rings (marked black) were also all induced at the same time as one another.

Table 3 *Effect of exposure to light on development of tuber symptoms*

Tubers transferred from darkness at 4°C* to:-	Total no. of tubers	Surface symptoms		Arcs in flesh
		White weals	Pigmented rings	
13°C/darkness	10	0	0	0
13°C/light (500 ft. candles, 8 hrs/day)	15	10	6	0

* Tubers had been at 4°C for at least 2 wk.

Duration of experiment: 3 wk.

Fig. 3 *Ring formation in potato tubers after exposure to light in a growth cabinet at 13°C*

Legend to Fig. 3:

Surface of an infected potato tuber exposed to light at 13°C for 2-3 wk. Note greening, white weal, and thin pigmented line at inside edge of weal.

Tubers stored in the dark at 4° C before exposure to light.

chloroplasts (Smillie *et al.,* 1963). This process seems to be inhibited in newly PMTV-invaded tuber cells.

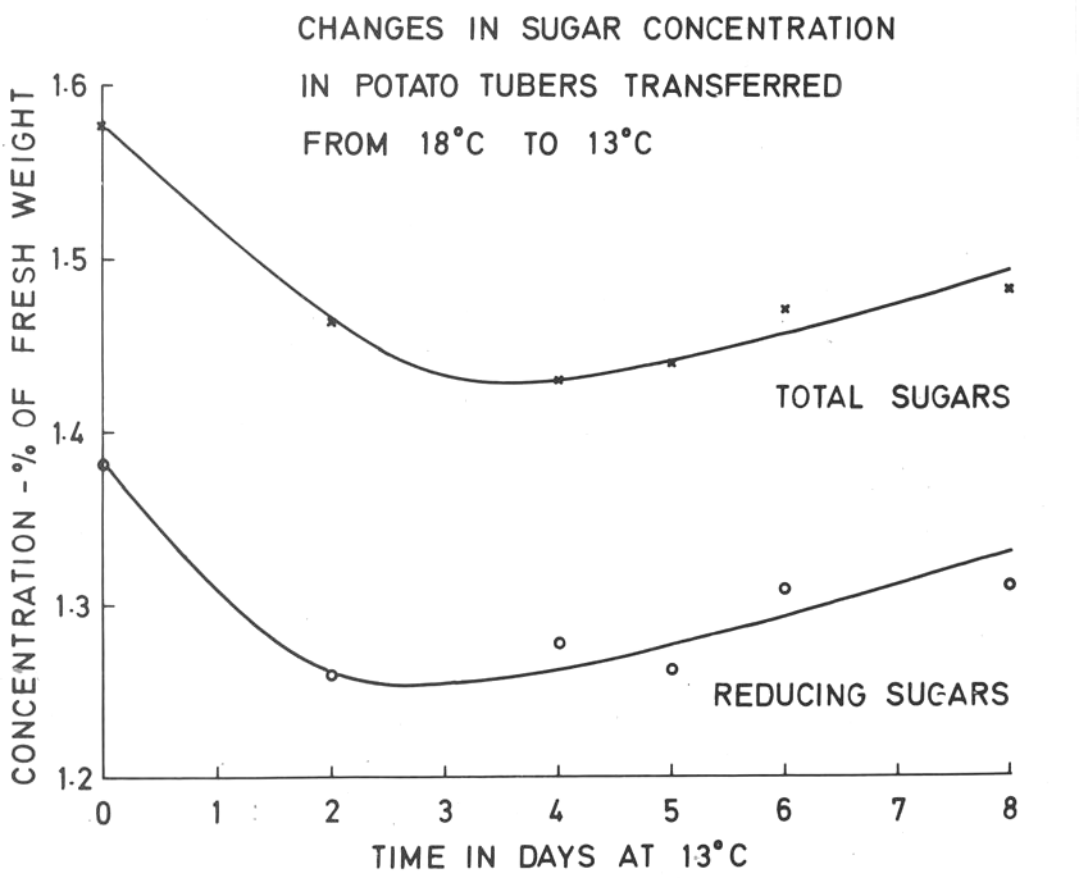
That rings form only at the boundary of the virus invaded zone was shown by tests done on tubers in which rings had just been induced by transfer from higher to lower temperature. Small samples of tissue were taken from the newly-induced rings themselves and from 5-10 mm on either side of them, and inoculated to leaves of *C. amaranticolor*. Samples from rings and from behind them gave lesions, but no virus was detected in samples from in front of rings.

The sugar content of tubers is greatly affected by alterations in storage temperature (Burton, 1966). The effect of transfer from higher to lower temperature on this was therefore examined. Twelve comparable samples of ten washed, healthy Arran Pilot tubers each, were placed at 18°C in the dark. After 10, 12, 14 and 16 days samples (two/day) were moved to 13°C, and on the 18th day dry ice was used to freeze both these and the two which still remained at 18°C. Thus there were two samples left at 18°C throughout and two frozen 2, 4, 5, 6 and 8 days after transfer. Each was analysed for its total sugar and reducing sugar contents using methods described in Burton (1969). Reducing sugars decreased initially, and then increased again (Fig. 4). They reached their lowest point after 3 days, which coincided exactly with the time taken for rings to appear in PMTV-infected tubers transferred from 18°C to 13°C. This suggests that induction of rings by lowering storage temperature may result from shortage of reducing sugars.

Experiments with Xanthi-nc tobacco

In winter in the glasshouse, PMTV lesions in inoculated leaves of Xanthi-nc tobacco form initially as necrotic spots. These become surrounded by successive series of concentric necrotic rings as the virus moves away from the original infection site. In summer, the lesions appear initially as rings rather than spots, or infection is symptomless.

Fig. 4 *Reducing sugar contents in potato tubers*



At 22°C in the light infection was symptomless, like that occurring in the glasshouse in hot summer conditions; faint ill-formed rings formed at 18°C in the light. At 14°C in light, lesions like those that formed in the glasshouse in winter developed, and the time needed for the successive rings to appear was related to light intensity and photoperiod. In general, the rings took longer to appear when either was decreased, and, in contrast to the situation in tubers, they still formed under constant conditions (Table 4; Fig. 5).

Infection was symptomless at 22°C in darkness. At 14°C in the dark infection was normally also symptomless, but a few lesions did occasionally form, appearing 2-3 days later than those in leaves exposed to light at 14°C. Keeping plants at 14°C in the dark for 2 or 4 days before inoculation had no effect on the number or time of appearance of lesions at 14°C in light.

When plants were moved from conditions in which infection was symptomless to 14°C in the light, necrotic lesions appeared. In plants transferred from 22°C in the light, the diameters of the lesions (rings) produced depended upon the duration of the period between inoculation and transfer (Table 5). Lesions formed after transfer from 22° or 14°C in the dark expanded little in diameter.

With type B cabinets, plants transferred from 14° in the dark to 14°C in light (continuous, 400 ft candles) developed rings instead of spots when the interval between inoculation and transfer was longer than one day. However, for the lesions all to be rings with type A cabinets, a longer interval (>6 days) was necessary between inoculation and transfer to light (8 hr photoperiod, 500 ft candles). Because the light in type A cabinets contained more blue than that in type B cabinets, this behaviour may result from differences in light quality rather than photoperiod or light intensity.

The virus content of inoculated leaves at 14°C in light was greater than that of leaves in conditions in which infection was symptomless. However, the virus content of leaves soon increased when plants were moved from conditions in

Table 4 *Effect of photoperiod and light intensity on lesion development in Xanthinc tobacco leaves at 14°C*

Lighting conditions		Days needed for appearance of symptoms		
Photoperiod (hr/day)	Light intensity (ft candles)	Central spot	First ring	Second ring
Exp. 1 (Type A cabinets)				
8	170	5-6	8-9	12
8	500	4-5	7	10
24	170	5-6	7	11
24	500	4-5	6	10
Exp. 2 (Type B cabinets)				
8	350	5-6	8	14
8	700	4-5	7	12
24	350	4-5	6	8

Fig. 5 *Ring formation on a Xanthi-nc tobacco leaf*

Legend to Fig. 5:

Lesions consisting of a central necrotic spot and two concentric necrotic rings in a Xanthi-nc tobacco leaf 11 days after inoculation. Leaf was from a plant kept at 14°C in continuous light (350 ft candles).

Table 5 *Diameters of necrotic rings produced in Xanthi-nc tobacco leaves on transfer of plants to light at 14°C*

Treatment before transfer *	Exp. 1	Exp. 2
	Lesion diameter (mm)[+]	
14°C light (control)	1.8 (3)	1.5 (3)
14°C dark, 2 days	2.3 (4)	-
14°C dark, 4 days	2.3 (6)	-
14°C dark, 6 days	3.0 (9)	-
22°C light, 2 days	-	2.3 (4)
22°C light, 3 days	-	4.3 (5)
22°C light, 4 days	-	6.3 (6)
22°C dark, 2 days	3.0 (4)	2.1 (5)
22°C dark, 3 days	2.7 (5)	2.2 (6)
22°C dark, 4 days	2.4 (8)	2.2 (8)

* Type B cabinets; where supplied, light (400 ft candles) was continuous.

[+] Figures are mean values for thirty lesions; figures in parentheses are numbers of days needed for lesions to appear.

which no symptoms developed to 14°C in light. For example, in one experiment plants were kept at 14°C in two cabinets, one with and the other without light. The virus content of leaves in the dark was small but that of leaves in light, moved from light to dark or moved from dark to light was greater (Table 6). (Note: this paragraph contains virus content data provided by B.D. Harrison.)

When plants were transferred between environmental conditions of 22° in the light and 14°C in the dark, lesions (rings) developed on transfer from 22° to 14°C, but not on transfer from 14° to 22°C. This suggests that the process of lesion formation includes an early phase in which light is required, and a later phase requiring low temperature. Support for this idea came from experiments in which plants held at 14° in the light were transferred either to 14°C in the dark or to 22°C in the light before lesions formed in their leaves; a temperature of 22°C prevented lesion formation at any time before symptoms appeared, whereas darkness did so only if applied soon after inoculation.

A few lesions developed in leaves of plants kept after inoculation in darkness at 18°C, and also when plants kept in darkness after inoculation were transferred from 14° to 18°C, but not when the transfer was in the reverse direction. Lesions developed when plants were transferred from 18° in darkness to 14°C in light (Fig. 6). Thus, in contrast to the situation in tubers, no lesions formed when infected plants were moved from higher to lower temperatures in the dark.

When PMTV lesions form in Xanthi-nc tobacco leaves, only newly invaded cells die, as they do in tubers. When small samples of tissue were tested by inoculation to leaves of other Xanthi-nc plants, virus was detected in those from newly induced rings and from the green tissue inside them, but not in those taken from areas of the leaf outside the rings.

Table 6 *Effect of light on accumulation of virus in Xanthi-nc tobacco leaves at 14°C*

Conditions after inoculation*	Infectivity of leaf extracts (diluted 1/10) after:		Symptoms
	6 days	12 days	
Dark	+4	6	None
6 days dark + 6 days light	7	225	Rings in 10 and 13 days
6 days light + 6 days dark	270	400	Spots in 3 days, extra ring in 6 days
Light	236	323	Spots in 3 days, successive rings in 6, 10 and 13 days

* Type B cabinets; where supplied, light (400 ft candles) was continuous.

\+ Total lesions in eight half-leaves of Xanthi-nc tobacco.

Note: the data in this table were provided by B.D. Harrison, and are not from Jones (1970).

Experiments with *Chenopodium amaranticolor*

In winter in the glasshouse, PMTV lesions in inoculated *C. amaranticolor* leaves consist of a series of concentric necrotic rings. In summer, they are faint chlorotic blotches, or infection is symptomless.

At 14°C in light, leaves responded to inoculation in various ways depending on photoperiod, light intensity and type of growth cabinet used (Table 7). In type A cabinets, necrotic rings formed with 8 hour days at 500 ft candles, but infection was symptomless with 170 ft candles at this photoperiod. In type B cabinets, 8 hr days at both 350 and 700 ft candles produced little or nothing in the way of symptoms. But distinct lesions formed at 350 ft candles with continuous lighting in type B cabinets; the lesions in the upper leaves were chlorotic spots and those in the lower leaves necrotic rings. Hence, a certain amount of light is required before lesions are produced and this requirement was greater in type B than in type A cabinets. Because the lights in type A emitted more blue than those in type B, blue wavelengths may promote formation of lesions in leaves of *C. amaranticolor.*

When plants in a type B cabinet lit continuously at 350 ft candles were allowed to shade the upper leaves of other plants, necrotic rings instead of chlorotic spots developed in the shaded leaves (Figs 7 & 8). This shows that light and not age of leaf determines whether lesions will be chlorotic or necrotic.

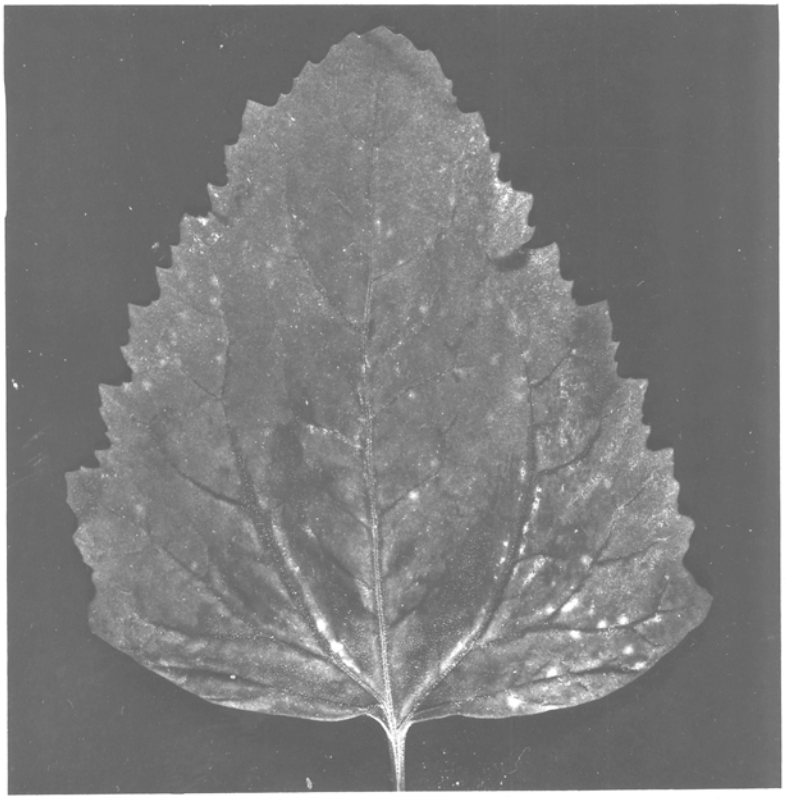
When plants were kept at 14°C in the dark after inoculation, no necrotic rings or chlorotic spots formed. When they were moved to type B cabinets lit continuously (350 ft candles), however, chlorotic rings developed in their upper leaves (Fig. 9). By contrast, in plants kept in continuous light (350 ft candles) after inoculation and moved to 14°C in the dark shortly before symptoms appeared, the lesions in the upper leaves were necrotic rings. Thus lesions developed if plants were supplied with light and were necrotic rather than chlorotic in upper leaves unless the light was present at the time of lesion appearance.

Table 7. *Effects of different light regimes on lesion development in* C. amaranticolor *leaves at 14°C.*

Photoperiod (hr/day)	Light intensity (ft candles)	Symptoms	
	Type A cabinets	*Upper and lower leaves*	
8	500	Necrotic rings, 5-6 days Second rings, 8 days	
8	170	None*	
	Type B cabinets	*Upper leaves*	*Lower leaves*
24	350	Chlorotic spots, 4-5 days	Nectrotic rings, 6 days
8	350	None*	None*
8	700	A few chlorotic spots, 8 days	None*

* Traces of symptoms appeared on either day 10 or day 11.

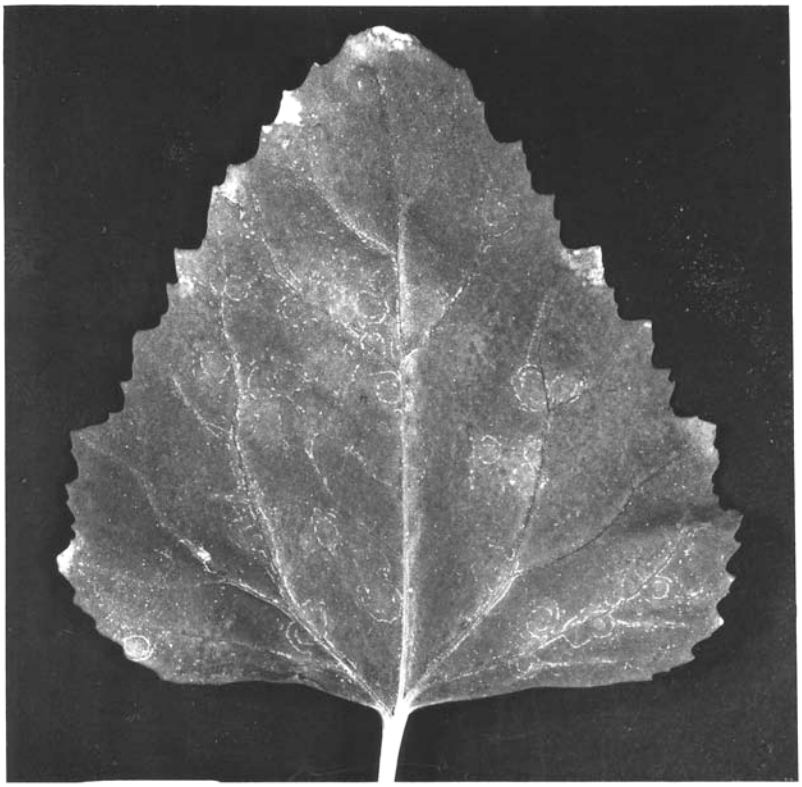
Fig. 7 C. amaranticolor *leaf lesions in the form of chlorotic spots*



Legend to Fig. 7:

Upper leaf of *C. amaranticolor* from a plant kept at 14°C in continuous light (350 ft. candles) for 5 days after inoculation. Lesions are chlorotic spots.

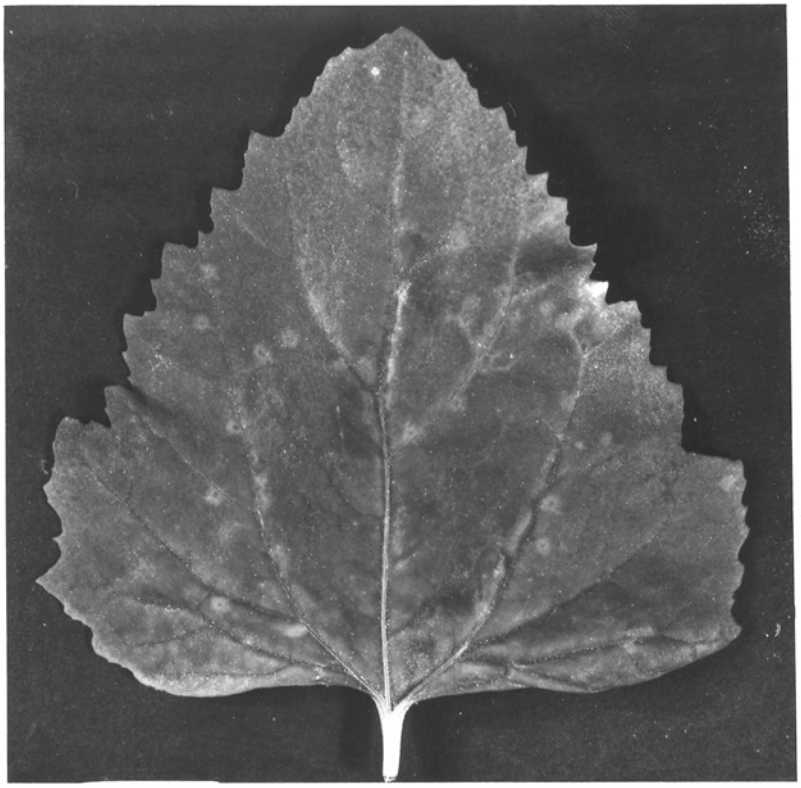
Fig. 8 C. amaranticolor *leaf lesions in the form of necrotic rings*



Legend to Fig. 8:

Upper leaf of *C. amaranticolor* from a plant kept at 14°C in continuous light (350 ft. candles) for 11 days after inoculation but shaded by taller plants. Lesions are concentric necrotic rings.

Fig. 9 C. amaranticolor *leaf lesions in the form of chlorotic rings*



Legend to Fig. 9:

Leaf of *C. amaranticolor* removed 11 days after inoculation from a plant kept at 14°C in the dark for 7 days followed by 4 days at 14°C in continuous light (350 ft. candles). Lesions are chlorotic rings.

These results show that light has two quite different effects on lesion production in *C. amaranticolor*. It determines whether or not they will form and whether they will be necrotic or chlorotic. The second effect seems to depend on the amount of light supplied, but the first also seems influenced by light quality.

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