Plant growth regulators and virus infection: A critical review

R.S.S. FRASER and R.J. WHENHAM

Biochemistry Section, National Vegetable Research Station, Wellesbourne, Warwick CV35 9EF, United Kingdom

Key words: Plant growth regulators, plant virus infection, control of host-virus interaction, resistance to virus disease, chemotherapy of virus disease

Abstract. Virus infection can severely inhibit plant growth and distort development. This article reviews changes in plant growth regulator metabolism caused by infection. In general, virus infection decreases auxin and gibberellin concentrations and increases abscisic acid concentration. Ethylene production is stimulated in necrotic or chlorotic reactions to infection, but not where the virus spreads systemically without necrosis. While these broad trends are true for most host-virus combinations studied, several situations are recorded where the virus had other effects on growth substance concentration. Cytokinin changes do not show any common pattern: both increases and decreases after infection have been reported.

The extent to which virus-induced changes in growth substance concentration could be responsible for observed alterations in host growth and development is discussed. While changes in abscisic acid, gibberellin and ethylene production seem potentially important, the experimental evidence does not provide conclusive proof for control of growth by these changes.

The numerous investigations of effects of exogenous regulators on virus multiplication and pathogenesis are reviewed. Different regulators, or the same regulator applied at different times or concentrations, had very diverse effects, and in some cases did significantly alter virus multiplication and pathogenesis. However, such studies seem to have yielded disappointingly little understanding of the biochemistry of the host-virus interaction, and the possible involvement of growth substances in this.

Possible uses of plant growth regulators in chemotherapy of virus disease, and their possible involvement in natural or induced resistance mechanisms are discussed.

Introduction

Virus infection can cause serious inhibition of plant growth and loss of yield (21, 51, 90). Developmental processes may also be deranged, giving rise to distorted plants or bizarre growth forms such as tumours and phyllody (60, 75, 90). These alterations in host growth might be controlled in part by virus-induced alterations in growth regulator metabolism.

Some virus infections result in accumulation of very large amounts of viral nucleoprotein. For example, tobacco mosaic virus (TMV) can multiply in tobacco or tomato until it forms 1% of the leaf fresh weight (38, 44). In the synthesis of this vast amount of 'foreign' nucleoprotein, some 75% of the host capacity for synthesis of RNA and protein is diverted to production of viral

Plant Growth Regulation 1: 37–59 (1982). 0167–6903/82/0011–0037 $3.45.

products (39, 43). The means by which the virus takes over these anabolic activities of the host so effectively are not fully understood, but might involve alterations in controls of transcription and translation involving growth regulators.

Many cases are known where plants have genetically-controlled resistance to a virus normally infecting that species, or where apparent resistance may be induced in susceptible plants by a prior infection or chemical treatment. In no case do we have a complete understanding at the biochemical level of how these resistances operate, but early evidence indicates that plant growth regulators could be involved in certain resistance-like mechanisms.

An alternative approach to the study of virus infection and its interaction with growth regulator metabolism has been to alter the growth regulator metabolism of plants artificially by supply of exogenous regulators. Some of this work has been done in the hope of discovering chemical treatments which might interfere with the development of pathogenesis, and so be of potential use in the chemotherapy of virus disease.

In this article we review the effects of virus infection on metabolism of endogenous growth regulators, and discuss whether the changes could be the cause of altered host growth and metabolism. We consider the effects of exogenous regulators on virus multiplication and pathogenesis, and possible roles of growth regulating compounds in chemotherapy and resistance.

To our knowledge this is the only recent review of this subject. Other, earlier reviews (32, 80, 105, 121) have covered not only virus infections, but also the extensive literature on alterations in growth regulator metabolism caused by fungal and bacterial infections, which are beyond our scope here. Unlike viruses, fungi and bacteria may also produce or metabolize growth regulators themselves during infection.

Effects of virus infection on endogenous growth regulator concentrations

Plant viruses are very diverse in form. They range in size and complexity from the viroids, covalently closed circles of naked RNA with a sequence length of 350–450 nucleotide residues (54), to large complex particles such as clover wound tumour virus (WTV) containing up to 16 large, double-stranded RNAs and several types of protein (110). The majority are rod-shaped or isometric particles with a single type of protein and a genome size of 5–15 kilobases (51).

The effects of virus infection on their host plants are equally diverse. Many, such as TMV and turnip yellow mosaic virus (TYMV) can spread systemically through the host and invade and multiply in virtually all living tissue. Others, such as tobacco necrosis virus (TNV) or tobacco rattle virus (TRV) are normally restricted to necrotic lesions which form around the infection sites. Some aphid-transmitted viruses are restricted to the phloem (139). With this great diversity of form and pathogenic effect, it is reasonable to expect that

different viruses in different hosts could have very diverse effects on growth regulator metabolism. Furthermore, the extreme severity of symptoms caused by many viruses could give rise to changes in growth regulators which are secondary responses to the symptoms, and not directly involved in control of the host-virus interaction.

Measurement of endogenous growth regulator concentration in plants is difficult; most occur at very low concentration, in the presence of substances which interfere with the final assay. Many studies of the effects of virus infection on growth regulator metabolism have used bioassays. These methods tend to be only semi-quantitative, lack specificity and do not give unequivocal identification of the growth regulator being measured. If available, physical methods of assay such as gas chromatography or combined gas chromatography-mass spectrometry are much to be preferred, but these must be accompanied by adequate internal standardisation and control experiments to establish that possible losses of growth regulators during extraction and purification are corrected for.

Auxins

The earliest experiments on effects of infection on growth regulators were with auxins in plants showing conspicuous stunting. Most reports indicated a reduction in auxin activity after infection, e.g. with tomato spotted wilt virus (TSWV) (53), potato leaf roll virus (PLRV) (61, 85, 130) and TMV (103, 104). Beet curly top virus (BCTV) reduced auxin concentration in susceptible lines of tomato, *Phaseolus vulgaris* and *Beta vulgaris* (127). In systemically infected tobacco, TMV decreased the concentration of indoleacetic acid (IAA) and phenylacetic acid (PAA) by up to 95%; concentrations of their precursors tryptophan and phenylalanine were increased (107). Dramatic reductions in leaf auxin content in these experiments were observed within 24 h of inoculation, when only a small proportion of leaf cells would be infected. If these results are confirmed, they would suggest that reduction in auxin concentration is induced by some factor which spreads through the leaf faster than the virus.

One possible mechanism of reduced auxin activity after infection could involve increased activity of IAA oxidase, which has been reported in cowpea mosaic virus (CPMV) infection (79). Several other host-virus combinations have been shown to have increased activity of peroxidase, which has IAA oxidase activity (49, 83, 134, 149). There do not appear to have been any attempts to reverse virus-induced stunting in infected plants showing reduced auxin concentration by supply of exogenous auxin.

Infection has not only been reported to cause reduced auxin concentration. In barley yellow dwarf virus (BYDV)-infected barley, auxin concentration was unchanged although the infected plants were severely stunted (115). In lupins infected with pea mosaic virus (PMV) (151); potato in the initial stages of potato virus X (PVX) infection (62) and tobacco forming local lesions after

TMV infection (148), increased levels of auxin activity were found. Basipetal transport of ^{14}C-IAA was inhibited in stem sections from BCTV-infected tomato showing apical symptoms (128) although this was associated with detectable phloem damage (109). In contrast, an increased rate of auxin transport was found in stem sections of tomato after infection with aster yellows virus (AYV) (142) although this pathogen is now known to be a mycoplasma (51). It is very likely that effects of virus on long-distance movement of growth substances may be indirect, and non-specific in that rates of movement of other small molecules may be similarly altered.

Abscisic acid

The effects of virus infection on abscisic acid (ABA) concentration reported so far have been varied. Measurements of the β-inhibitor complex of growth-retarding substances, of which ABA is a major component (132), showed unchanged activity after TMV infection of tobacco (132), but increased activity in PMV-infected lupin (151) and rice tungro virus (RTV)-infected rice (95). In experiments where ABA was measured by physical methods, infection of cucumber with cucumber mosaic virus (CMV) was reported to cause no change (9), but other workers reported a three-fold increase (1).

A decrease in ABA concentration in TMV-infected tobacco was reported (107) but in this study only the very early stages of infection were examined. Other workers reported increases (10, 156). In our experiments (156) we used the White Burley variety of tobacco containing the *N'* gene. This gives either a localised necrotic reaction or allows systemic spread of virus, depending on the strain of TMV used. We found a two- to six-fold increase in ABA concentration with systemic infection, which persisted throughout the period of active virus multiplication. The local lesion strain of TMV caused a much larger increase in ABA, up to 20-fold. This increase was associated with the lesions rather than the interlesion areas, and first became detectable at the time of lesion appearance. The timing made it unlikely that ABA increase was a primary cause of lesion formation; it was more likely to have been a secondary consequence of necrosis.

Experiments in which ABA was sprayed onto healthy plants to produce an increase in internal concentration similar to that caused by systemic virus infection suggested that the virus-stimulated rise in ABA could be a major cause of the reduced growth of infected plants (156). Both TMV and ABA reduced leaf growth by inhibition of cell division, and had no effect on cell expansion. Experiments with the local lesion strain and radiolabelled ABA showed that ABA was transported from the necrotic, inoculated leaves to upper, healthy leaves, in sufficient quantity to explain the observed inhibition of growth of the upper leaves (40). An analogous situation was reported by Fraser & Matthews (36) who found that inoculation of cotyledons of Chinese cabbage with TYMV caused a rapid transient inhibition of leaf initiation at the seedling apex, long before infectious virus could be shown to have invaded

the apex. Although the translocated factor was not identified, ABA is a likely candidate.

The means by which TMV infection stimulates ABA production are not yet clear. In the necrotic reaction, it is possible that water loss from necrotic tissue and ABA release from disrupted chloroplasts is responsible. In systemic, non-necrotic infection, an early effect of TMV is to alter chloroplast metabolism, including chloroplast protein synthesis, chloroplast ribosomal RNA synthesis and stability (37, 59) and the properties of the chloroplast membrane proteins (74). It is possible that this could allow release of ABA from chloroplasts thus de-repressing its further synthesis within the chloroplast (58), although the role of the chloroplast in ABA synthesis has recently been questioned (57).

Systemic TMV infection causes the plant to synthesise large amounts of TMV RNA, and also causes an early transient stimulation of cytoplasmic ribosomal RNA synthesis (39). Treatment of healthy or TMV-infected leaf discs with sufficient exogenous ABA to cause an increase in internal ABA concentration similar to that caused by virus infection also stimulated synthesis of both host and viral RNAs (155). It may therefore be speculated that the increase in ABA concentration caused by TMV could play a role in stimulating the observed increases in RNA synthesis after infection. This report of stimulation of RNA synthesis by ABA is in contrast to most reports showing an inhibition. Most other studies however have been with embryonic tissue and in the presence of other growth regulators (20, 152).

Although ABA is biochemically one of the simpler growth regulators, its metabolism includes two complications which must be taken into account when assessing data from the effects of virus infection. First, it is readily metabolised to bound forms including the glucosyl ester (91) and other glycosides (92, 120). The metabolic relationships and controls of interconversions between the free acid and bound forms are not fully understood, but the concentration of bound ABA may exceed that of the free acid in the leaf (153, 156), so any study which measures only free acid may present an incomplete picture. Secondly, ABA may either be sequestered in the chloroplast, or released to other parts of the cell. The presence of these reservoirs of spatially or chemically sequestered ABA might mask metabolically significant changes in active ABA concentration caused by virus infection. It is perhaps relevant that in tobacco grown under water stress conditions, and so having a high endogenous ABA concentration, TMV infection caused no further increase (R J Whenham, unpublished).

Gibberellins

The visible, and often dramatic stunting of shoot growth associated with virus infection also led to studies of gibberellic acid content. In cucumber seedlings infected with CMV, hypocotyl elongation was inhibited. Bailiss (8) reported that this was associated with a reduction in concentration of gibberellins A1 and A3 but found no qualitative difference in gibberellins between healthy .

and infected plants. In another study of the same system, gibberellin content was also found to be reduced after infection (1) but this was accompanied by increases in ABA concentration (1) and in ethylene production (89). Ben-Tal and Marco (18) reported that there were qualitative changes in gibberellin content after infection, and suggested that the pattern of gibberellin degradation was altered. They were unable to conclude whether the decreased gibberellin concentration was the cause of the stunting or merely a consequence of disease symptoms without a controlling role. Infection of barley with BYDV caused stunting and decreased gibberellin concentration (115).

Some reports have shown that virus-induced stunting may be partly prevented by treating plants with exogenous gibberellins, for example with severe etch virus (SEV) in tobacco (133), or with corn stunt virus (CSV), AYV and WTV infections (86). These experiments provide at best circumstantial evidence that virus-induced reduction in endogenous gibberellin concentration was responsible for stunting. Healthy cucumber seedlings were shown to respond more to applied gibberellin than CMV-infected, stunted seedlings (34, 87), raising the possibility that although applied gibberellin may stimulate elongation, this may be quite separate from the means by which virus infection inhibits elongation. There have been several reports that inhibition of stem growth by virus infection is associated with a reduction in cell division rather than cell expansion (7, 27, 120, 158); application of exogenous gibberellin has been reported to stimulate cell division rather than cell expansion in some species, but both processes in others (reviewed in Reference 52).

Gibberellin concentration was reported to be unaltered by TMV infection of tobacco (22) and by tomato aspermy virus (TAV) in tomato (7).

Cytokinins

Tobacco ringspot virus (TRSV) infection of *Nicotiana glutinosa* or cowpea was reported to reduce cytokinin activity (71, 140). In contrast, TMV and CMV infections increased cytokinin activity in tobacco (137, 138). Chromatograms of extracts from both healthy and systemically-infected plants showed peaks of cytokinin activity in bioassay, corresponding to zeatin and zeatin riboside. Activity of both was higher in the infected tissue, which also contained two cytokinin activities not present in the healthy leaves. Inoculation of the tobacco cultivar Xanthi-nc, which restricts TMV to necrotic local lesions, was also reported to cause an increase in cytokinin activity (135).

All these studies used bioassay to quantify cytokinin activity. This has two shortcomings: accurate estimation of quantitative changes in cytokinin concentration after infection is difficult, especially as different cytokinins have different bioactivities in the assay system. Secondly, identification of the chemical nature of the cytokinin activity by bioassay of chromatographically-fractionated extracts is far from unequivocal. There is a clear need for accurate estimation and characterization of cytokinin activities in healthy and infected tissue using rigorous physical methods.

An interesting and provocative connection between TMV and cytokinins

was the suggestion that TMV RNA may itself contain residues with cytokinin bioactivity (135, 136). The major active components were tentatively identified by bioassay after paper or Sephadex LH20 chromatography as zeatin and isopentenyladenine and their respective derivatives. It was suggested that each TMV RNA molecule, 6340 nucleotide residues long, contained between 5 and 17 cytokinin-active nucleosides. As TMV RNA can reach 400 μg/g fresh weight in a successful infection (38), this represents a cytokinin concentration equivalent to 300–1050 ng/g; much higher than the normal leaf level of 1–10 ng/g (76) or the amount found in tRNA (55).

We have recently attempted to confirm the presence of cytokinin nucleosides in TMV RNA by a newly developed and highly sensitive gas chromatographic procedure, using permethylated cytokinins and a nitrogen-specific detector (154). We failed to detect any cytokinin nucleoside known to occur in RNA by this method or by soybean callus bioassay (157). As the gas chromatographic method would have detected as little as 1 cytokinin nucleoside per 10 TMV RNA molecules, it suggests that cytokinins are either absent, or present in such small amounts as to be of questionable biological significance.

Ethylene

Generally, ethylene production appears to be increased by infection leading to necrotic or chlorotic reactions, but not by viruses which multiply systemically without necrosis. Ethylene production was stimulated 3 to 13 h before appearance of local lesions after TMV (106) and TNV (48) infection of tobacco, but the largest increases occurred after lesions appeared. A sudden burst of ethylene production has also been reported before appearance of TMV lesions (33). *Physalis floridana* leaves infected with potato virus Y (PVY) showed increased rates of ethylene production at the onset of necrosis (113), while cowpea leaves infected with CMV or CPMV also showed increased ethylene production (66, 79). Tobacco leaves systemically-infected with TMV did not have increased rates of ethylene production (15, 48, 98) except in very late stages of infection in senescent leaves (98). In beet, ethylene production was unchanged during leaf yellowing caused by infection with beet mosaic (BMV) and beet mild yellows virus (BMYV); in contrast a necrotic infection with the fungus *Cercospora beticola* caused increased ethylene production (70).

These observations raise the question of whether ethylene is actively involved in promoting the necrotic response; the evidence is contradictory. In tobacco reacting hypersensitively to TMV infection, an inhibitor of ethylene synthesis, succinic acid 2,2′-dimethylhydrazide, did not alter lesion size (106). Ethylene production in TMV-infected leaves was also inhibited by treatment with aminoethoxyvinylglycine, but lesions still formed (33). In *Tetragonia expansa*, bean yellow mosaic virus (BYMV) normally causes chlorotic lesions. These lesions became necrotic when plants were exposed to ethylene, while in plants exposed to CO_2, an inhibitor of ethylene action,

development of both necrosis and chlorosis was prevented (10). When tobacco leaves were pricked with needles moistened with the ethylene-releasing compound 2-chloroethylphosphonic acid (Ethrel, ethephon), necrotic spots similar to virus-induced lesions were produced (146). Changes in protein constitution and peroxidase isoenzyme patterns induced by ethephon and TMV necrosis were similar. Treatment of inoculated leaves with ethephon reduced the size of lesions formed (146). These experiments provide evidence for a role of ethylene in control of the necrotic response, but do not prove that ethylene and TMV induce necrosis by the same mechanism. It seems likely that much of the ethylene production associated with virus-induced necrosis may be a consequence of non-specific wounding effects. Ethylene synthesis occurs at the plasmalemma (78); it is interesting in this connection that some of the early effects of virus infection include altered membrane permeability (74) and increased activity of a membrane-bound ATPase (64).

Virus-stimulated ethylene production appears to occur without necrosis in CMV-infected cucumber seedlings. Ethylene production increased at the time of appearance of chlorotic lesions on cotyledons (88). It was suggested that the increased ethylene production, coupled with an increase in resistance of cotyledons to gaseous diffusion, may have been responsible for the marked epinasty of cotyledons seen after infection. Exogenous ethylene also caused epinasty of cotyledons on healthy plants (77). Ethylene production was also shown to be enhanced in hypocotyls of infected seedlings; experiments with application of ethylene or ethephon to healthy seedlings suggested that the CMV-induced ethylene production could be important in the suppression of hypocotyl elongation after infection (89), but is probably not the sole cause. Removal of ethylene by oxidation with potassium permanganate only temporarily stimulated growth of infected plants (89).

Although most studies of changes in endogenous growth substances after infection have been concerned with individual growth substances, there have been some attempts to interrelate changes induced in several growth substances (87). Perhaps the best studied system is CMV-infection of cucumber. Inhibition of hypocotyl growth in infected plants was associated with decreased gibberellin content (1, 8); increased ABA content (1) and increased ethylene production (89) all of which could have contributed to the stunting. A careful and intensive study of the timings of changes, coupled with application of physiologically reasonable concentrations of exogenous growth regulators, might establish which changes were important in control of growth, and which were secondary effects. However, until we have a deeper understanding of how growth substances may interact in growth control in the healthy plant, the question will remain difficult to answer.

Effects of exogenous growth regulators on virus multiplication and pathogenesis

We considered above some experiments where exogenous growth regulators

were applied to healthy plants, to mimic changes in regulator concentration caused by virus, or applied to infected plants to rectify a virus-induced reduction in growth regulator concentration. These experiments were designed to test the involvement of virus-induced changes in regulator concentration in control of host growth. In this section we are concerned with the effects of exogenous growth regulators on virus multiplication and development of the pathogenesis.

Senescence: ABA and ethylene

There have been several reports that susceptibility to virus is increased by treatments which promote leaf senescence. Infiltration of tobacco leaf discs, or injection of attached leaves with ABA caused small increases in size and number of lesions resulting from infection with TMV, and a small increase in the amount of infectious virus produced (10, 14). Unfortunately, the data were not analysed for statistical significance. The highest ABA concentrations used (10 and 100 μg/ml) were phytotoxic and with these methods of application which favour high ABA uptake, probably caused increases in internal ABA concentration far beyond normal physiological concentrations (155). In a separate study, we found that spraying intact plants with ABA at concentrations sufficient to produce a physiologically reasonable increase in internal ABA concentration caused statistically significant reductions in lesion size and number after TMV infection (42).

Ageing tobacco leaves on intact plants do not accumulate high concentrations of ABA (156). Old, flowering plants of the cv. Xanthi-nc formed very many fewer lesions when inoculated than young plants (41). Multiplication of TMV in the systemic cv. Samsun was much greater in leaves inoculated when young than in those inoculated when mature and fully expanded (38). These results suggest that there is no correlation in the intact plant between senescence and susceptibility to infection, and that the apparent increased susceptibility by treatment with high ABA concentration (10, 14) may have been nonspecific effects of chemical damage, bearing little relation to any action of ABA in vivo.

Treatment of Xanthi-nc tobacco leaves with ethrel caused changes similar to senescence, and was reported to increase susceptibility to TMV infection, measured variously as small increases in lesion number, diameter and TMV multiplication (10, 13). In a separate study, treatment with ethylene did not increase TMV multiplication or lesion formation (98). A third report showed that ethephon treatment reduced lesion size (145, 146). Ethrel did not increase TMV multiplication in the systemic host *Physalis floridana* (25). The weight of the evidence is therefore against any increase in susceptibility to virus infection after ethylene-induced senescence.

Changes similar to senescence may also be induced by detaching leaves. The effects on susceptibility to TMV were complex, but did not provide consistent evidence that induced senescence enhances susceptibility to TMV (97, 99).

Exogenous regulators and the local lesion response

Many investigators have sought to analyse the interaction of exogenous growth regulators with virus multiplication and host response by studying the effects of regulators on local lesion formation. This experimental system yields data readily, but is biologically extremely complex; consequently the effects reported are varied and sometimes contradictory.

In Samsun NN tobacco, a local lesion host for TMV, treatment with high (near phytotoxic) concentrations of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2, 4-D) greatly promoted lesion expansion, while lower doses inhibited expansion (123, 147). Infected protoplasts from tobacco plants with the *N* gene for local lesion formation did not become necrotic (101). Cultured in the absence of 2, 4-D, protoplasts from *N* gene plants supported much lower virus multiplication than those from systemic hosts lacking the *N* gene (82). However, addition of 2, 4-D at 1 μg/ml, the normal concentration used in protoplast culture, increased the amount of virus infectivity produced in protoplasts from *N* gene plants, and reduced it in those from non-*N* gene plants, so that they were equal. The difference between the two genotypes, and the differential effect of 2, 4-D, were apparently eliminated in protoplasts from plants which had received high levels of nutrient, or those which were inoculated with high virus concentrations. These factors, and the effects of a range of 2, 4-D concentrations, would require further study before it can be concluded that 2, 4-D is interacting with the mechanism normally inhibiting virus spread in *N* gene plants. Treatment of cucumber with 2, 4-D increased the number of lesions formed on inoculation with TMV (18).

TMV lesion size on young expanding leaves of *N* gene tobacco plants was also reduced by other synthetic (α-naphthylacetic acid) (NAA) and natural auxins (IAA, PAA) (147). Unlike 2, 4-D, these compounds did not cause increased lesion size at high concentration. Auxins also failed to restrict lesion size in older leaves; detached leaves, or those with the 'acquired systemic resistance' phenomenon (112). Clearly the interaction of auxin with other factors in leaf development and metabolism is complex and influences its ability to alter lesion development. It has been suggested that auxin-stimulated ethylene production could be responsible for inhibition of lesion growth (147).

Reports of exogenous cytokinins altering lesion development are legion. Kinetin reduced lesion number and virus infectivity produced in TMV-infected discs of *N. glutinosa* (67). With the same host, others reported inhibition of lesion formation but stimulation of virus production by various cytokinins (93). The latter workers also reported that lesion growth was either inhibited or stimulated by two particular cytokinins. Aldwinckle subsequently pointed out that these were both benzyladenine masquerading under two synonyms (3). Kinetin at various concentrations was reported to increase both size and number of lesions formed on detached leaves (35); preliminary experiments indicated that zeatin had a similar effect.

In TMV-infected Xanthi-nc tobacco, kinetin did not reduce the number of local lesions, but did reduce their necrotisation to the extent where they became invisible to the naked eye (17). Virus multiplication in contrast was not inhibited by kinetin. Kasamo and Shimomura (63) suggested that the epidermis and underlying tissues of tobacco leaves have different responses to kinetin as it affects the necrotic reaction. As TMV initially multiplies in epidermal cells then spreads later to the mesophyll, they suggested that this might explain the different effects reported, especially the effects of time of application which are discussed in more detail below. In *N. glutinosa* and *Datura stramonium*, application of cytokinins both increased and decreased lesion number, depending on conditions (116).

Kinetin reduced the size and number of lesions caused by TSWV on petunia leaf strips and greatly reduced multiplication of this virus in tomato leaves (118). Benzyladenine reduced lesions on petunia and *N. rustica* caused by TSWV. Supplied before inoculation of *N. rustica* it reduced production of virus infectivity; if supplied after inoculation it increased it (4).

In cowpea, kinetin reduced the number of lesions formed by TRSV by 75% (71). Infectivity of the same virus produced in *N. glutinosa* was markedly reduced if plants were treated with high concentrations of kinetin 9 days before inoculation but not if treated 3 days before (140).

The general conclusions from these results are that while the most common effect of cytokinin appears to be to reduce lesion number and size, this may or may not be associated with a reduction in the virus multiplication within the lesion, ie. there may be inhibition of necrotisation rather than of virus multiplication. Time of application relative to inoculation appears to be important in determining the effect of cytokinins and could be the cause of their ability to increase as well as decrease lesion number and size. There seem to be no reports yet of cytokinins allowing a virus normally restricted to local lesions to spread systemically.

Exogenous regulators and the systemically-infected host

A less complicated approach to study the effects of exogenous growth regulators on virus multiplication and pathogenesis has been to apply them to plants which permit systemic multiplication of virus. The results are then unobscured by the complex inhibitory effects of the local lesion reaction on virus multiplication.

The auxins NAA and indolebutyric acid (IBA) retarded appearance of TMV mosaic symptoms on tobacco, but only when applied at near-phytotoxic concentrations (100). These auxins were also reported to inhibit TMV multiplication in tissue culture (72, 73). IAA reduced TMV multiplication in young, expanding tobacco leaves, but only by about 30% (147). In contrast, 2, 4-D was reported to stimulate TMV multiplication in *Physalis floridana* (25) and in tobacco (117). The latter author also reported that IAA increased TMV multiplication. TMV normally causes only a subliminal infection in

cotton; an interesting report was that 2, 4-D, and several other growth regulators, considerably increased the level of multiplication (26).

Kinetin either increased or decreased the infectivity of tomato aucuba mosaic virus (TAMV) produced in tobacco leaves, depending on the time of application (30). The most effective suppression was when kinetin was supplied from 4 weeks before inoculation. On a much shorter time scale, application of benzyladenine to tobacco leaves before or 1 minute after inoculation with TMV reduced the production of viral infectivity; application 5–120 min after inoculation enhanced infectivity (3). Kinetin, kinetin riboside, isopentenyl adenosine and benzyladenine all increased the infectivity of TMV produced in systemically-infected tobacco or *N. rustica* plants (93), but all reduced production of infectivity in tissue cultured from infected plants (94). In CMV-infected meristematic cultures of *N. rustica,* kinetin at various concentrations had little effect on infectivity, or slightly stimulated it (124). Low concentrations of kinetin or benzyladenine increased the TMV infectivity produced in tomato leaf discs; high concentrations reduced it (11). Kinetin increased TYMV multiplication in floated leaf discs of Chinese cabbage (19) but inhibited TMV and PVX multiplication in tobacco and *Datura stramonium* (111).

The effects of exogenous auxins and cytokinins on multiplication of viruses causing systemic infection are therefore highly varied; clearly concentration and time of application are again major factors determining the effect. It should be noted that in no case has there been any demonstration of an effect of the growth regulator directly on the virus replication process as such, though generally these effects have not been sought in suitable in vitro experimental systems. Ralph et al. (108) found no effect of zeatin or benzyladenine on initiation of protein synthesis or rate of polypeptide chain elongation in a wheat germ cell-free system programmed with TMV RNA. It may be that the effects of exogenous auxins and cytokinins on virus multiplication are indirect consequences of primary effects on other aspects of host metabolism.

Some general comments on the design of experiments to study effects of auxins and cytokinins on local lesion and systemic virus infections are pertinent. The majority of tests have measured virus multiplication by infectivity bioassay, by assessing the number of local lesions produced on a suitably reacting second host. This has some disadvantages: without considerable elaboration, the method is not really accurate enough to discriminate small differences in virus concentration (17) yet many of the reported effects of exogenous growth regulators are indeed small. With a few exceptions, much of the published work does not include proper statistical analysis, with appropriate transformation of raw data (68). Furthermore, treatment of plant tissue with growth regulators might well have an indirect influence on the specific infectivity of the virus produced (number of lesions induced per unit weight of virus), especially at high or phytotoxic concentrations. Thus a reduced infectivity in crude extracts might not reflect a reduction in virus con-

centration. For these reasons some physical measurement of virus multiplication is desirable before claiming an effect of a growth regulator on multiplication.

Many experiments with exogenous auxins and cytokinins have been done with potentially unphysiological concentrations, but generally there have been no measurements of amounts of uptake or alteration in internal concentration caused by treatment. The vast majority of experiments with cytokinins have used kinetin and benzyladenine, which do not occur naturally in leaves, and whose metabolism might be quite different from naturally-occurring compounds. It would perhaps be interesting to test the forms of cytokinins occurring naturally in the tissue being studied.

Many experiments have been done with detached leaves or leaf discs. While this is a useful experimental approach, excision in itself does induce metabolic changes, especially in long-term experiments (2). These might influence the response of tissue and virus to exogenous growth regulators and make conclusions inapplicable to the intact plant.

Chemotherapy

The ability of exogenous growth regulators to delay or reduce the severity of visible symptoms of virus infection, and in some cases to inhibit virus multiplication, has stimulated research on possible methods of chemotherapy. One approach was to screen chemicals already in use in agriculture, which were known to be taken up and to spread systemically. The fungicide thiabendazole (TBZ) reduced the susceptibility of sugar beet to yellowing viruses (114). Part of the effect was attributed to a reduced ability of the aphid vectors to colonise treated plants, but an effect on susceptibility of treated plants to virus was also possible. Methyl benzimidazol-2yl carbamate (MBC), the water decomposition product and fungitoxic principle of the benomyl fungicides (28), inhibited formation of visible symptoms of TMV on tobacco and beet western yellows virus (BWYV) in lettuce (143). Other studies showed that the multiplication of TMV in tobacco was strongly inhibited (46), and that MBC treatment completely prevented the normal inhibition of plant growth resulting from TMV infection (47). MBC doses giving over 90% inhibition of TMV multiplication were about one-fiftieth of those required to produce phytotoxic effects and growth inhibition (47). However, the multiplication of virus was inhibited, not abolished, and MBC-treated plants did eventually accummulate high concentrations of TMV after several months.

Both TBZ and MBC have activity in cytokinin bioassays (125, 141). We found that MBC had no direct inhibitory effect on TMV RNA synthesis. It was ineffective in suppression of TMV multiplication when supplied to mature leaves, but caused a persistent inhibition of TMV multiplication lasting well through leaf maturity and senescence, if it entered the leaf while still very young. This suggests that the inhibitory effect of MBC on TMV multiplication was an indirect one through altered leaf development (47). One possibility is that the cytokinin activity of MBC maintains the leaf in some juvenile state

unsuitable for virus multiplication. In many cases juvenile tissues such as meristems are known to support little or no virus multiplication (29, 129, 131). MBC was found to be ineffective against several other viruses (11, 143).

An alternative approach to chemotherapy has been to screen chemicals such as virazole (synonym ribavirin; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (126) which have activity against animal viruses. Virazole was effective in elimination of various viruses from plant tissue cultures (24, 122, 124) and reduced virus multiplication in intact plants (56, 69, 117). Its mode of action in plants is not understood, but it is known to have cytokinin-like activity: it stimulated growth of tissue cultures at low concentration and suppressed root formation (124). High concentrations of kinetin in the growth medium antagonised the antiviral action of virazole (124). Pulse labelling experiments failed to show any direct and specific inhibitory effect of virazole on TMV RNA or coat protein synthesis in infected tobacco protoplasts (Fraser & Gerwitz, unpublished results). These results raise the possibility that virus eradication by virazole might depend on a mechanism involving its cytokinin activity. The interaction of its antiviral activity with exogenous kinetin further suggests that knowledge of the effects of cytokinins on virus multiplication and virazole would be required before this type of chemotherapy could be fully exploited.

Plant growth regulators and resistance to virus disease

Developmental or spatial resistance

In systemic infection, certain areas of the plant may be partially or completely resistant to invasion by the virus. Typically these areas include shoot and root meristems (29, 129, 131). Embryos or the entire seed may also not be invaded (102). No explanation is yet available for the apparent resistance of these tissues to infection; a possibility which deserves study is that it is related to their particular and unusual growth regulator status.

A related type of resistance is found when leaves are invaded at a very early stage of development, for example with TMV or TYMV. Certain cells become infected, and give rise to yellow, diseased tissue as the leaf expands. Other groups of cells remain uninfected, and by division give rise to 'green islands' which remain substantially virus-free (6, 81, 96). These green islands have higher chlorophyll concentration than healthy leaves, and may be analogous to meristematic tissue in that they are resistant to infection because they are suspended in some juvenile non-infectible state. It has been reported that they have higher cytokinin concentration than the surrounding infected tissue (135). The possible role of endogenous growth regulators in maintaining the virus-free state of the dark green islands clearly deserves further investigation.

Constitutive resistance

Many cases are known where particular varieties of a species can contain a gene or genes conferring resistance or tolerance to a virus normally affecting

that species. In no case do we have a full understanding of the biochemistry of gene action; superficially the various mechanisms appear to be very diverse.

BCTV caused auxin concentration to fall equally in susceptible and virus-resistant varieties of tomato, *Phaseolus* bean and beet, although the resistant varieties showed less severe symptoms and lower virus multiplication (127). In tomato, a TMV-tolerant variety was found to have higher concentrations of gibberellins and cytokinins in its sap than a susceptible variety (119). The relationship of this finding to the expressed tolerance is not clear, but in view of the experimental evidence that exogenous growth regulators can suppress symptoms and virus multiplication, a mechanism of tolerance based on endogenous growth regulators deserves further study.

Induced resistance

When plants form necrotic lesions after inoculation with virus, the response of uninoculated parts to a second or challenge inoculation can be altered. Lesions formed after the challenge inoculation are often smaller and less numerous than those formed on previously uninoculated control plants. This effect, referred to as 'induced' or 'acquired systemic resistance' has been reported in several host-virus combinations (112). In tobacco varieties forming lesions after TMV infection, at least 4 new host-coded proteins are detectable in parts of the plant showing acquired systemic resistance (50, 150). It has been suggested that these 'pathogenesis-related' proteins may be involved in the resistance, perhaps in a way analogous to interferon in animals (65). However, whether these proteins play any part in the apparent resistance has recently been questioned (41, 42).

An alternative explanation of acquired resistance is based on changes in host growth regulator metabolism induced by the first inoculation, and proposes separate mechanisms controlling altered lesion number and lesion size in the second inoculation. Leaves showing acquired resistance were reported to have higher cytokinin content than control leaves (16, 137). The increases were not great, and the types of cytokinin involved were not identified, but the reports are consistent with other evidence that exogenous cytokinin can reduce lesion size by inhibiting necrotisation (12, 93, 118). This is supported by evidence that the amount of virus multiplication in small lesions on leaves with acquired resistance is as great as in the larger lesions of control leaves (16, 40).

Alteration in lesion number as a consequence of the first inoculation appears to bear an inverse relationship to induced changes in leaf abscisic acid concentration. 'Resistant' leaves of *N. tabacum* formed fewer lesions in the second inoculation, and had higher ABA concentration at the time of inoculation than comparable leaves on control plants (40, 156). In experiments with radiolabelled ABA, it was shown that the increased ABA concentration of upper leaves was at least partly due to transport of ABA from the lower, lesion-bearing leaves. In *N. glutinosa* the converse situation applied. A primary

inoculation of lower leaves with TMV reduced ABA concentration of uninoculated upper leaves, but increased the number of lesions they formed on subsequent challenge inoculation (45). The reduction in number of lesions formed when healthy *N. tabacum* plants were sprayed with ABA before inoculation is also consistent with this correlation (42). Whether ABA is the direct control of altered susceptibility to infection, or involved indirectly through an effect on leaf water relations and mechanical susceptibility of tissue to inoculation remains to be established. Van Loon (146) has shown that ethephon treatment induces effects similar to acquired systemic resistance and has suggested that ethylene production by lower, lesion-forming leaves could be responsible for the 'resistance' of upper leaves.

It is interesting in connection with the proposed growth regulator explanation of acquired systemic resistance that growth regulators have also been reported to induce synthesis of the pathogenesis-related proteins in healthy plants (5, 146).

Conclusions

There is considerable evidence that virus infection can cause major changes in growth regulator concentrations. Some of these changes have been shown to be of potential importance in the control of host growth after infection; others appear to be secondary effects. There is very little information on how virus infection causes alteration in host growth regulator metabolism. The possible involvement of chloroplasts in synthesis or compartmentalization of some growth substances (31, 58, 84) and the observed early effects of infection on chloroplast membranes and metabolism (37, 59, 74) suggest further lines of study.

There is little understanding of how virus-induced changes in host growth regulators might cause alterations in growth, development and metabolism. This point reflects the paucity of our current knowledge of how growth regulators work in healthy plants (144). Virus infections might indeed be a useful experimental approach to the study of growth regulator function and metabolism.

Experiments with exogenous growth regulators have suggested that they can influence virus multiplication and pathogenesis. To date they have given disappointingly little insight into how growth regulators may affect multiplication and symptom development. Early work on chemotherapy and involvement of growth regulators in mechanisms of resistance raises the possibility of ultimately developing new antiviral strategies based on manipulation of endogenous growth regulators.

References

1. Aharoni N, Marco S and Levy D (1977) Involvement of gibberellins and abscisic acid in the suppression of hypocotyl elongation in CMV-infected cucumbers. Physiol Plant Pathol 11: 189–194

2. Aharoni N and Richmond AE (1978) Endogenous gibberellin and abscisic acid content as related to senescence of detached lettuce leaves. Pl Physiol 62: 224–228
3. Aldwinckle HS (1975) Stimulation and inhibition of plant virus replication *in vivo* by 6-benzylaminopurine. Virology 66:341–343
4. Aldwinckle HS and Selman IW (1967) Some effects of supplying benzyladenine to leaves and plants inoculated with viruses. Ann Appl Biol 60: 49–58
5. Antoniw JF, Ritter CE, Pierpoint WS and Van Loon LC (1980) Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. J gen Virol 47: 79–87
6. Atkinson PH and Matthews REF (1970) On the origin of dark green tissue in tobacco infected with tobacco mosaic virus. Virology 40: 344–356
7. Bailiss KW (1968) Gibberellins and the early disease syndrome of aspermy virus in tomato (*Lycopersicon esculentum* Mill.). Ann Bot 32: 543–552
8. Bailiss KW (1974) The relationship of gibberellin content to cucumber mosaic virus infection of cucumber. Physiol Plant Pathol 4: 73–79
9. Bailiss KW (1977) Gibberellins, abscisic acid and virus-induced stunting. In: Kiraly Z ed. Current Topics in Plant Pathology, a Symposium, pp 361–373: Budapest, Akadémiai Kiadó
10. Bailiss KW, Balazs E and Kiraly Z (1977) The role of ethylene and abscisic acid in TMV-induced symptoms in tobacco. Acta Phytopathol. Acad Sci Hung 12: 133–140
11. Bailiss KW, Cocker FM and Cassells AC (1977) The effect of Benlate and cytokinin on the content of tobacco mosaic virus in tomato leaf discs and cucumber mosaic virus in cucumber cotyledon discs and seedlings. Ann Appl Biol 87: 383–392
12. Balazs E, Barna B and Kiraly Z (1976) Effect of kinetin on lesion development and infection sites in Xanthi-nc tobacco infected with TMV: single cell local lesions. Acta Phytopathol Acad Sci Hung 11: 1–9
13. Balazs E and Gaborjanyi R (1974) Ethrel-induced leaf senescence and increased TMV susceptibility in tobacco. Z für Pflanzenkrankheiten und Pflanzenschutz 81: 389–93
14. Balazs E Gaborjanyi R and Kiraly Z (1973) Leaf senescence and increased virus susceptibility in tobacco: the effect of abscisic acid. Physiol Plant Pathol 3: 341–346
15. Balazs E Gaborjanyi R Toth A and Kiraly Z (1969) Ethylene production in Xanthi tobacco after systemic and local virus infection. Acta Phytopathol Acad Sci Hung 4: 355–358
16. Balazs E Sziraki I and Kiraly Z (1977) The role of cytokinins in the systemic acquired resistance of tobacco hypersensitive to tobacco mosaic virus. Physiol Plant Pathol 11: 29–37
17. Bawden FC (1950) Plant Viruses and Virus Diseases. Waltham Mass: Chronica Britanica Company.
18. Ben-Tal Y and Marco S (1980) Qualitative changes in cucumber gibberellins following cucumber mosaic virus infection. Physiol Plant Pathol 16: 327–336
19. Berridge MV and Ralph RK (1969) Some effects of kinetin on floated Chinese cabbage leaf discs. Biochem Biophys Acta 182: 266–269
20. Bex JHM (1972) Effects of abscisic acid on nucleic acid metabolism in maize coleoptiles. Planta 103: 1–10
21. Broadbent L (1964) The epidemiology of tomato mosaic virus VII. The effect of TMV on tomato fruit yield and quality under glass. Ann Appl Biol 54: 209–224
22. Bundagyan EG Lohznikova VN Goddin MI and Chailakhyan MK (1963) On the effect of gibberellin-like substances on TMV. Proc Acad Sci Armen SSR 36: 111–116
23. Cassells AC, Barnett A and Barlass M (1978) The effect of polyacrylic acid treatment on the susceptibility of *Nicotiana tabacum* cv. Xanthi-nc to tobacco mosaic virus. Physiol Plant Pathol 13: 13–22
24. Cassells AC and Long RD (1980) The regeneration of virus-free plants from cucumber mosaic virus and potato virus Y infected tobacco explants cultured in the presence of virazole. Z Naturforsch 35c: 350–351
25. Cheo PC (1969) Effect of 2,4-dichlorophenoxyacetic acid on tobacco mosaic virus infection. Phytopathology 59: 243–244

26. Cheo PC (1971) Effects of plant hormones on virus replicating capacity of cotton plants infected with tobacco mosaic virus. Phytopathology 61: 869–872
27. Chessin M (1957) Growth substances and stunting in virus infected plants. Proc Third Conf Potato Diseases Lisse-Wageningen pp 80–84
28. Clemons GP and Sisler HD (1969) Formation of a fungitoxic derivative from benlate. Phytopathology 59: 705–706
29. Crowley NC and Hanson J (1960) The infection of apical meristems of tomato roots with tobacco mosaic virus after treatment with ethylenediaminetetra-acetic acid. Virology 12: 603–606
30. Daft MJ (1965) Some interactions of kinetin and temperature on tobacco leaves infected with tomato aucuba mosaic virus. Ann Appl Biol 55: 51–56
31. Davey JE and Van Staden J (1981) Cytokinins in spinach chloroplasts. Ann Bot 48: 243–246
32. Dekhuijzen HM (1976) Endogenous cytokinins in healthy and diseased plants. In Heitefuss R and Williams PM, eds. Encyclopedia of Plant Physiology New Series Vol 4 pp 526–559 Berlin: Springer Verlag
33. De Laat AAM Van Loon LC and Vonk CR (1981) Regulation of ethylene biosynthesis in virus-infected tobacco leaves. I. Determination of the role of methionine as the precursor of ethylene. Pl Physiol 68: 256–261
34. Fernandez TF and Gaborjanyi R (1976) Reversion of dwarfing induced by virus infection: effect of polyacrylic and gibberellic acids. Acta Phytopathol Acad Sci Hung 11: 271–275
35. Fletcher RA, Quick WA and Phillips DR (1968) Effect of kinetin on senescence and tobacco mosaic virus infection in leaves of *Nicotiana glutinosa*. In: Wightman F and Setterfield G, eds. Biochemistry and Physiology of Plant Growth Substances, pp 1447–1456. Ottawa: Runge Press
36. Fraser L and Matthews REF (1981) A rapid transient inhibition of leaf initiation induced by turnip yellow mosaic virus infection. Physiol Plant Pathol 19: 325–336
37. Fraser RSS (1969) Effects of two TMV strains on the synthesis and stability of chloroplast ribosomal RNA in tobacco leaves. Molec Gen Genet 106: 73–79
38. Fraser RSS (1972) Effects of two strains of tobacco mosaic virus on growth and RNA content of tobacco leaves. Virology 47: 261–269
39. Fraser RSS (1973) The synthesis of tobacco mosaic virus RNA and ribosomal RNA in tobacco leaves. J gen Virol 18: 267–279
40. Fraser RSS (1979) Systemic consequences of the local lesion reaction to tobacco mosaic virus in a tobacco variety lacking the *N* gene for hypersensitivity. Physiol Plant Pathol 14: 383–394
41. Fraser RSS (1981) Evidence for the occurrence of the 'pathogenesis-related' proteins in leaves of healthy tobacco plants during flowering. Physiol Plant Pathol 19: 69–76
42. Fraser RSS (1982) Are 'pathogenesis-related' proteins involved in acquired systemic resistance of tobacco plants to tobacco mosaic virus? J gen Virol 58: 305–313
43. Fraser RSS and Gerwitz A (1980) Tobacco mosaic virus infection does not alter the polyadenylated messenger RNA content of tobacco leaves. J gen Virol 46: 139–148
44. Fraser RSS and Loughlin SAR (1980) Resistance to tobacco mosaic virus in tomato: effects of the *Tm-1* gene on virus multiplication. J gen Virol 48: 87–96
45. Fraser RSS Loughlin SAR and Whenham RJ (1979) Acquired systemic susceptibility to infection by tobacco mosaic virus in *Nicotiana glutinosa* L. J gen Virol 43: 131–141
46. Fraser RSS and Whenham RJ (1978) Inhibition of the multiplication of tobacco mosaic virus by methyl benzimidazol-2-ylcarbamate. J gen Virol 39: 191–194
47. Fraser RSS and Whenham RJ (1978) Chemotherapy of plant virus disease with methyl benzimidazol-2yl-carbamate: effects on plant growth and multiplication of tobacco mosaic virus. Physiol Plant Pathol 13: 51–64
48. Gaborjanyi R Balazs E and Kiraly Z (1971) Ethylene production, tissue senescence and local virus infection. Acta Phytopathol Acad Sci Hung 6: 51–55
49. Ghabrial SA and Pirone TP (1967) Physiology of tobacco etch virus-induced wilt of tabasco peppers. Virology 31: 154–162

50. Gianinazzi S Martin C and Valee J–C (1970) Hypersensibilite aux virus, temperature et proteins solubles chez le *Nicotiana tabacum* Xanthi n.c. Apparition de nouvelles macromolecules lors de la repression de la synthesis virale. Compt Rend 270D 2383–2386
51. Gibbs A and Harrison BD (1976) Plant Virology: the Principles. London: Arnold
52. Goodwin PB (1978) Phytohormones and growth and development of organs of the vegetative plant. In Letham DS, Goodwin PB and Higgins TJV eds, Phytohormones and Related Compounds: a Comprehensive Treatise. Vol 1 pp 205–264. Amsterdam: Elsevier/North-Holland
53. Grieve BJ (1943) Studies on the physiology of host-parasite relations. 4. Some effects of tomato spotted wilt virus on growth. Aust J Exptl Biol Med Sci 21: 89–101
54. Gross HJ Domey H Lossow C Jank P Raba M Alberty H and Sanger HL (1978) Nucleotide sequence and secondary structure of potato spindle tuber viroid. Nature 273, 203–208
55. Hall RH (1968) Cytokinins in the transfer RNA: their significance to the structure of tRNA. In Wightman F and Setterfield G eds. Biochemistry and Physiology of Plant Growth Substances pp 47–56. Ottawa: Runge Press
56. Hansen JA (1979) Inhibition of apple chlorotic spot virus in *Chenopodium quinoa* by ribavirin. P Dis Reptr 63: 17–20
57. Hartung W Heilmann B and Gimmler H (1981) Do chloroplasts play a role in abscisic acid synthesis? Plant Sci Letters 22: 235–242
58. Heilmann B, Hartung W and Gimmler H (1980) The distribution of abscisic acid between chloroplasts and cytoplasm and the permeability of the chloroplast envelope. Z Pflanzenphysiol 97: 67–78
59. Hirai A and Wildman SG (1969) Effect of TMV multiplication on RNA and protein synthesis in tobacco chloroplasts. Virology 38: 73–82
60. Holmes FO (1964) Symptomatology of viral diseases in plants. In Corbett MK and Sisler HD eds. Plant Virology pp 17–38. Gainsville, University of Florida Press
61. Jahnel H (1939) Wuchsstoffuntersuchungen an abbaukranken Kartoffeln. Phytopath. Z 12: 312–317
62. Jaros J (1963) Studies on the phases of development of healthy and virus X, Y and X- and Y-infected potatoes. Acta Biol Cracov Ser Bot 6: 75–86
63. Kasamo K and Shimomura T (1977) The role of the epidermis in local lesion formation and the multiplication of tobacco mosaic virus and its relation to kinetin. Virology 76: 12–18
64. Kasamo K and Shimomura T (1978) Response of membrane-bound Mg^{2+}-activated ATPase of tobacco leaves to tobacco mosaic virus. Pl Physiol 62: 631–634
65. Kassanis B Gianinazzi S and White RF (1974) A possible explanation of the resistance of virus-infected tobacco plants to second infection. J gen Virol 23: 11–16
66. Kato Y (1976) Ethylene production during lipid peroxidation in cowpea leaves infected with CMV. Ann Phytopathol Soc Jpn 43: 587–589
67. Kiraly Z and Szirmai J (1964) The influence of kinetin on tobacco mosaic virus production in *Nicotiana glutinosa* leaf discs. Virology 23: 186–188
68. Kleczkowski A (1955) The statistical analysis of plant virus assays: a transformation to include lesion numbers with small means. J gen Microbiol 13:91–98
69. Kluge S and Marcinka K (1979) The effects of polyacrylic acid and virazole on the replication and component formation of red clover mottle virus. Acta Virol 23: 148–152
70. Koch F, Baur M, Burbe M and Elstner EF (1979) Ethylene formation by *Beta vulgaris* leaves during systemic (Beet mosaic virus and beet mild yellowing virus, BMV + BMYV) or necrotic (*Cercospora beticola* Sacc.) diseases. Phytopath Z 98: 40–46
71. Kuriger WE and Agrios GN (1977) Cytokinin levels and kinetin-virus interactions in tobacco ringspot virus-infected cowpea plants. Phytopathology 67: 604–609
72. Kutsky R (1952) Effects of indolebutyric acid and other compounds on virus concentration in plant tissue cultures. Science 115: 19–20
73. Kutsky RJ and Rawlins TE (1950) Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue cultures, as revealed by a spectroscopic method. J Bacteriol. 60: 763–766

74. Ladygina ME Grishkova VP and Alyoshina NV (1979) Membrane proteins of chloroplasts of intact and TMV-infected tobacco plants. Biokhimiya 44: 1635–1642
75. Lee CL and Black LM (1955) Anatomical studies of *Trifolium incarnatum* infected by wound tumour virus. Am J Bot 42: 160–168
76. Letham DS (1978) Cytokinins. In Letham DS Goodwin PB and Higgins TJV eds, Phytohormones and related Compounds: a comprehensive treatise. Vol 1 pp 205–264. Amsterdam: Elsevier/North-Holland
77. Levy D and Marco S (1976) Involvement of ethylene in epinasty of CMV-infected cucumber cotyledons which exhibit increased resistance to gaseous diffusion. Physiol Plant Pathol 9: 121–126
78. Lieberman M (1979) Biosynthesis and action of ethylene. Ann Rev Pl Physiol 30: 533–591
79. Lockhart BE and Semancik JS (1970) Growth inhibition, peroxidase and 3-indoleacetic acid oxidase activity, and ethylene production in cowpea mosaic virus-infected cowpea seedlings. Phytopathology 60: 553–556
80. Loebenstein G (1972) Localization and induced resistance in virus-infected plants. Ann Rev Phytopathol 10: 177–206
81. Loebenstein G, Cohen J, Shabtai S, Coutts RHA and Wood KR (1977) Distribution of cucumber mosaic virus in systemically-infected tobacco leaves. Virology 81: 117–125
82. Loebenstein G, Gera A, Barnett A, Shabtai S and Cohen J (1980) Effect of 2,4-dichlorophenoxyacetic acid on multiplication of tobacco mosaic virus in protoplasts from local-lesion and systemic-responding tobaccos. Virology 100: 110–115
83. Loebenstein G and Linsey N (1963) Effect of virus infection on peroxidase activity and C_6/C_1 ratios. Phytopathology 53: 350
84. Loveys BR (1977) The intracellular location of abscisic acid in stressed and non-stressed leaf tissue. Physiol Plant 40: 6–10
85. Lucas H (1939) Weitere Untersuchungen über den Wuchsstoffhaushalt abbaukranker Kartofeln. Phytopathol. Z 12: 334-350
86. Maramorosch K (1957) Reversal of virus-caused stunting in plants by gibberellic acid. Science 126: 651–652
87. Marco S (1978) Changes in hormone balance in relation to the diseases produced by viruses. Proceedings of the 3rd International Congress of Plant Pathology, München, 1978 p. 19
88. Marco S and Levy D (1979) Involvement of ethylene in the development of cucumber mosaic virus-induced chlorotic lesions in cucumber cotyledons. Physiol Plant Pathol 14: 235–244
89. Marco S Levy D and Aharoni N (1976) Involvement of ethylene in the suppression of hypocotyl elongation in CMV-infected cucumbers. Physiol Plant Pathol 8: 1–7
90. Matthews REF (1970) Plant Virology. New York and London: Academic Press
91. Milborrow BV (1970) The metabolism of abscisic acid. J exp Bot 21: 17–29
92. Milborrow BV (1980) Regulation of abscisic acid metabolism. In Skoog F, ed. Plant Growth Substances (1979), pp 262–273. Berlin and Heidelberg: Springer Verlag
93. Milo GE and Srivastava BIS (1969) Effect of cytokinin on tobacco mosaic virus production in local lesion and systemic hosts. Virology 38: 26–31
94. Milo GE and Srivastava BIS (1969) Effects of cytokinins on tobacco mosaic virus production in tobacco pith tissue cultures. Virology 39: 621–623
95. Mohanty SK, Anjanejulu A and Sridhar R (1979) Physiology of rice tungro virus disease: involvement of an abscisic acid-like substance in susceptible host-virus interaction. Physiol Plant 45: 132–136
96. Murakishi HH and Carlson PS (1976) Regeneration of virus-free plants from dark green islands of tobacco mosaic virus-infected tobacco leaves. Phytopathology 66: 931–932
97. Nakagaki Y and Hirai T (1971) Effect of detached leaf treatment on tobacco mosaic virus multiplication in tobacco and bean leaves. Phytopathology 61: 22–27
98. Nakagaki Y Hirai T and Stahmann MA (1970) Ethylene production by detached leaves infected with tobacco mosaic virus. Virology 40: 1–8

99. Nakagaki Y and Matsui C (1971) Effect of bean leaf detachment on susceptibility to tobacco mosaic virus infection. Phytopathology 61: 354–356
100. Nichols CW (1952) The retarding effect of certain plant hormones on tobacco mosaic symptoms. Phytopathology 42: 579–580
101. Otsuki Y Shimomura T and Takebe I (1972) Tobacco mosaic virus multiplication and expression of the *N* gene in necrotic responding tobacco varieties. Virology 50: 45–50
102. Owusa GK, Crowley NC and Francki RIB (1968) Studies of the seed transmission of tobacco ringspot virus. Ann Appl Biol 61: 195–202
103. Pavillard J (1952) Researches sur la croissance des plantes virosees; virus et auxines. Compt Rend 235: 87–88
104. Pavillard J and Beauchamp C (1957) La constitution auxinique de tabac sains on atteints de maladies à virus; présence et role de la scopoletine. Compt Rend 244: 1240–1243
105. Pegg GF (1976) Endogenous auxins in healthy and diseased plants. The involvement of ethylene in plant pathogenesis. Endogenous gibberellins in healthy and diseased plants. Endogenous inhibitors in healthy and diseased plants. In Heitefuss R and Williams PM, eds. Encyclopedia of Plant Physiology New Series Vol 4 pp 560–616. Berlin: Springer Verlag
106. Pritchard DW and Ross AF (1975) The relationship of ethylene to formation of tobacco mosaic virus lesions in hypersensitive responding tobacco leaves with and without induced resistance. Virology 64: 295–307
107. Rajagopal R (1977) Effect of tobacco mosaic virus infection on the endogenous levels of indoleacetic, phenylacetic and abscisic acids of tobacco leaves in various stages of development. Z Pflanzenphysiol 83: 403–409
108. Ralph RK, Wojcik SJ and Airey P (1980) *In vitro* plant protein synthesis and cytokinins. Plant Sci Letters 18: 237–247
109. Rasa EA and Esau K (1961) Anatomic effects of curly top and aster yellows viruses on tomato. Hilgardia 30: 496–515
110. Reddy DVR and Black LM (1973) Electrophoretic separation of all components of the double-stranded RNA of wound tumour virus. Virology 54: 557–562
111. Reunov AV Reunova GD Vasilyeva LA and Reifman VG (1977) Effect of kinetin on tobacco mosaic virus and potato virus X replication in leaves of systemic hosts. Phytopathol Z 90: 342–349
112. Ross AF (1961) Systemic resistance induced by localized virus infections in plants. Virology 14: 340–358
113. Ross AF and Williamson CE (1951) Physiologically active emanations from virus-infected plants. Phytopathology 41: 431
114. Russel GE (1968) Some effects of spraying with thiabendazole on the susceptibility of sugar beet to yellowing viruses and their vector *Myzus persicae* (Sulz.). Ann Appl Biol 62: 265–272
115. Russell SL and Kimmins WC (1971) Growth regulators and the effect of BYDV on barley (*Hordeum vulgare* L.). Ann Bot 35: 1037–1043
116. Schuster G (1972) Umwelt und Versuchsanordnung als modifizierende Faktoren der Wirkung von Kinetin auf die Ausbildung von Viruslokalläsionen. Arch Pflanzenschutz 8: 89–102
117. Schuster G (1976) Wirkung von 1-β-D-ribofuranosyl-1,2,4,-triazole-3-carboxamide (Virazole) auf die Vermehrung systemischer Viren in *Nicotiana tabacum* 'Samsun'. Bericht des Instituts für Tabakforschung 23: 21–36
118. Selman IW (1964) The effect of kinetin on infection of petunia and tomato leaves with tomato spotted wilt virus. Ann Appl Biol 53: 67–76
119. Selman IW and Yahampath ACI (1973) Some physiological characteristics of two tomato cultivars, one tolerant and one susceptible to tobacco mosaic virus. Ann Bot 37: 853–865
120. Sembdener G, Datter W, Kefeli VI and Kutacek M (1980) Abscisic and other naturally occurring plant growth inhibitors. In: Skoog F ed. Plant Growth Substances 1979, pp. 254–261. Berlin and Heidelberg, Springer Verlag
121. Sequeira L (1973) Hormone metabolism in diseased plants. Ann Rev Plant Physiol 24:353–380

122. Shepard JF (1977) Regeneration of plants from protoplasts of potato virus X-infected tobacco leaves. II. Influence of virazole on the frequency of infection. Virology 78: 261–266
123. Simons TJ Israel HW and Ross AF (1972) Effect of 2,4-dichlorophenoxyacetic acid on tobacco mosaic virus lesions and on the fine structure of the adjacent cells. Virology 48: 502–515
124. Simpkins I Walkey DGA and Neely H (1981) Chemical suppression of virus in plant tissue cultures. Ann Appl Biol 99: 161–169
125. Skene KGM (1972) Cytokinin-like properties of the systemic fungicide benomyl. J Hort Sci 47: 179–182
126. Smith RA (1980) Mechanisms of action of ribavirin. In Ribavirin, Eds. Smith RA and Kirkpatrick R pp 99–118. London: Academic Press
127. Smith SH McCall SR and Harris JH (1968) Alterations in the auxin levels of resistant and susceptible hosts induced by the curly top virus. Phytopathology 58: 575–577
128. Smith SH McCall SR and Harris JH (1968) Auxin transport in curly top virus-infected tomato. Phytopathology 58: 1669–1670
129. Smith SH and Schlegel DE (1964) The distribution of clover yellow mosaic virus in *Vicia faba* root tips. Phytopathology 54: 1273–1274
130. Söding H and Funke H (1941) Über den Wuchsstoffhaushalt abbaukranker Kartoffeln. Phytopathol Z 13: 351–363
131. Solberg RA and Bald JG (1963) Distribution of a natural and an alien form of tobacco mosaic virus in the shoot apex of *Nicotiana glauca* Grah. Virology 21: 300–308
132. Steadman JR and Sequeira L (1969) A growth inhibitor from tobacco and its possible involvement in pathogenesis. Phytopathology 59: 499–503
133. Stein DB (1962) The developmental morphology of *Nicotiana tabacum* 'White Burley' as influenced by virus infection and gibberellic acid. Amer J Bot 49: 437–443
134. Suseno H and Hampton RE (1966) The effect of three strains of tobacco mosaic virus on peroxidase and polyphenoloxidase activity in *Nicotiana tabacum*. Phytochemistry 5: 819–822
135. Sziraki I and Balazs E (1975) The effect of infection by TMV on cytokinin level of tobacco plants, and cytokinins in TMV RNA. In Kiraly Z, ed. Current Topics in Plant Pathology, pp 345–352. Budapest, Akadémiai Kiadó
136. Sziraki I and Balazs E (1979) Cytokinin activity in the RNA of tobacco mosaic virus. Virology 92: 578–582
137. Sziraki I Balazs E and Kiraly Z (1980) Role of different stresses in inducing systemic acquired resistance to TMV and increasing cytokinin levels in tobacco. Physiol Plant 16: 277–284
138. Sziraki I and Gaborjanyi R (1974) Effect of systemic TMV infection on cytokinin level of tobacco leaves and stems. Acta Phytopathol Acad Sci Hung 9: 195–199
139. Takanami Y and Kuho S (1979) Enzyme assisted purification of two phloem-limited plant viruses: tobacco necrotic dwarf and potato leaf roll. J gen Virol 44: 153–159
140. Tavantzis SM Smith SH and Witham FH (1979) The influence of kinetin on tobacco ringspot virus infectivity and the effect of virus infection on the cytokinin activity in intact leaves of *Nicotiana glutinosa* L. Physiol Plant Pathol 14: 227–233
141. Thomas TH (1974) Investigations into the cytokinin-like properties of benzimidazole-derived fungicides. Ann appl Biol 76: 237–241
142. Ting W-P and Gold AH (1967) Effects of aster yellows virus infection on transport through plant stem sections. Virology 32: 570–579
143. Tomlinson JA, Faithfull EM and Ward CM (1976) Chemical suppression of the symptoms of two virus diseases. Ann Appl Biol 84: 31–41
144. Trewavas A (1981) How do plant growth substances work? Plant, Cell and Environment 4: 203–228
145. Van Loon LC (1976) Hormone-mediated changes in symptom expression in virus-infected tobacco plants during growth and senescence. Abstracts of 9th International Conference on Plant Growth Substances, Lausanne. 1976 pp 412–414

146. Van Loon LC (1977) Induction by 2-chloroethylphosphonic acid of viral-like lesions, associated proteins and systemic resistance in tobacco. Virology 80: 417–420
147. Van Loon (1979) Effects of auxin on the localization of tobacco mosaic virus in hypersensitively-reacting tobacco. Physiol Plant Pathol 14: 213–226
148. Van Loon LC and Berbee AT (1978) Endogenous levels of indoleacetic acid in leaves of tobacco reacting hypersensitively to tobacco mosaic virus. Z Pflanzenphysiol 89: 373–375
149. Van Loon LC and Geelen JLMC (1971) The relation of polyphenoloxidase and peroxidase to symptom expression in tobacco var. 'Samsun NN' after infection with tobacco mosaic virus. Acta Phytopathol Acad Sci Hung 6: 9–20
150. Van Loon LC and Van Kammen A (1970) Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. Samsun and Samsun NN. II. Changes in protein constitution after infection with tobacco mosaic virus. Virology 40: 199–211
151. Van Steveninck RFM (1959) Factors affecting the abscission of reproductive organs of yellow lupins (*L. luteus* L.). III Endogenous growth substances in virus-infected and healthy plants and their effects on abscission. J exp Bot 10: 367–376
152. Walbot V Clutter M and Sussex I (1975) Effects of abscisic acid on growth, RNA metabolism and respiration in germinating bean axes. Pl Physiol 53: 125–127
153. Weiler EW (1980) Radioimmunoassays for the differential and direct analysis of free and conjugated abscisic acid in plant extracts. Planta 148: 262–272
154. Whenham RJ (1981) Gas chromatography of cytokinins. Rep natn Veg Res Stn for 1980: 27–28
155. Whenham RJ and Fraser RSS (1980) Stimulation by abscisic acid of RNA synthesis in discs from healthy and tobacco mosaic virus-infected tobacco leaves. Planta 150: 349–353
156. Whenham RJ and Fraser RSS (1981) Effect of systemic and local-lesion-forming strains of tobacco mosaic virus on abscisic acid concentration in tobacco leaves: consequences for the control of leaf growth. Physiol Plant Pathol 18: 267–278
157. Whenham RJ and Fraser RSS (1982) Does TMV RNA contain cytokinins? Virology 118: 263–266
158. Yerkes WD (1960) Interaction of potassium gibberellate and a stunting bean virus on beans, *Phaseolus vulgaris.* Phytopathology 50: 525–527