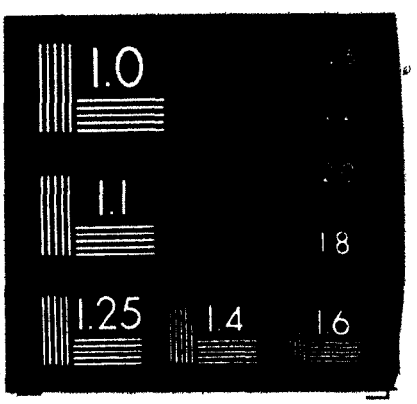

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STUDIES ON THE DWARFING INDUCED BY BARLEY YELLOW DWARF VIRUS IN *HORDEUM VULGARE* L. C.I. 666

by

Sonia Russell

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Dalhousie University January, 1972

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ABSTRACT

The dwarfing of Black Hulless barley (Hordeum vulgare L. C.I. 666) infected with barley yellow dwarf virus is investigated. Estimation of cell number in the third leaf blade of infected plants showed a marked decrease in mitotic activity. There was no decrease in mean cell size. Bioassay of endogenous gibberellins indicated that there was a significant decrease, in infected plants, of a substance corresponding to gibberellic acid. Bioassay of endogenous auxins revealed similar levels of these compounds were present in infected and healthy plants.

Black Hulless barley C.I. 666 was found to be susceptible to the growth retardant (2-chloroethyl)-trimethyl ammonium chloride (CCC), which has been shown to inhibit the biosynthesis of gibberellins in both higher plants and fungi. Estimation of cell number in the third leaf blade indicated that a significant decrease in mitotic activity occurred in treated plants. There was a small decrease in mean cell size. Bioassay of endogenous gibberellins indicated a significant decrease in a substance corresponding to gibberellic acid.

Application of gibberellic acid to BYDV-infected and CCC-treated plants reversed the dwarfing, but the

response was found to be due to increased cell elongation. There was no significant difference between the response of healthy plants, CCC-treated plants, and BYDV-infected plants.

Ethiopian barley C.I. 2376, which has been shown to be resistant to BYDV-infection, was found to be similarly resistant to CCC-treatment.

It is suggested that CCC and BYDV both induce dwarfing in Black Hulless barley by an inhibition of meristematic activity which is associated with diminished levels of endogenous gibberellins. The decrease in mitotic activity may be the result of decreased gibberellin biosynthesis, but is not reversed by exogenous gibberellic acid.

The following abbreviations are used in the text.

AMO 1618,	4-hydroxy-5-isopropyl-2 methylphenyl trimethyl ammonium chloride, 1-piperidine carboxylate
B9,	N-dimethylaminosuccinamic acid
BYDV,	barley yellow dwarf virus
CCC,	(2-chloroethyl) trimethyl ammonium chloride
DNA,	deoxyribonucleic acid
GA_3,	gibberellic acid
IAA,	indole-3-acetic acid
IAN,	indole-3-acetonitrile
Phosphon D,	2,4-dichlorobenzyl-tributylphosphonium chloride
RNA,	ribonucleic acid
TMV,	tobacco mosaic virus
TNV,	tobacco necrosis virus
TYMV,	turnip yellow mosaic virus
TWEEN 20,	polyoxyethylene sorbitan monooleate

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"Virus-disease revealed by research workers in artificial ways .... comes rather doubtfully into the category of natural history, unless of course one regards the virologist as an ecological factor to be considered along with the weather....."

C.H. Andrewes

INTRODUCTION

Virus infections can give rise to many and varied symptoms in the infected host plant. Tobacco mosaic virus, for example, causes the production of necrotic spots, called local lesions, on inoculated leaves of Nicotiana glutinosa (Holmes, 1932) while sugar cane mosaic virus produces a short stripe mottled pattern of discrete streaks in infected leaves of the sugar cane (Liu, 1950), and potato plants infected with potato witch's broom virus have purple tops and a bushy appearance (Smith, 1937) quite different from uninfected plants. Although the symptoms of virus infection are diverse, interference with the normal mode of growth of a plant is one of the commonest signs, e.g. cocoa swollen shoot virus, strain B, produces swellings on the branches and roots of the cocoa plant (Posnette, 1947) and rubus stunt virus causes dwarfing of the loganberry plant (Prentice, 1950). It is not uncommon for infected plants to be highly deformed, e.g. anemone alloiophylly virus-infected anemone nemorosa plants have misshapen leaves, thickened stems, suppressed flowers, and a spreading habit (Smith, 1937). However, one of the commonest growth deformations appears to be the stunting of the host plant, and this may not be associated with any other growth disorder. The naming of a large number of plant viruses demonstrates this

e.g. alfalfa dwarf disease virus, tobacco yellow dwarf virus, and sugar cane stunting disease virus, to name only a few.

Disturbances of normal growth patterns are very common in plant pathology. The fungus Synchytrium endobioticum attacks potato plants to produce warts on the tubers, Corynebacterium fascians infects many plants producing a syndrome of flattened stems and misshapen leaves called fasciation, and many smut diseases cause dwarfing of the host plants e.g. Tilletia contraversa produces dwarf bunt of wheat.

Investigation of some of these diseases has shown a clear involvement of plant growth regulators. Daly and Inman (1958) showed that the rapidly elongating hypocotyls of Carthamus tinctorius infected by Puccinia carthami contained up to ten times as much auxin equivalents as the more slowly growing uninfected hypocotyls. They also showed that the growth rate of healthy hypocotyls, in vitro, increased in response to exogenously applied IAA. Similarly, wart tissue in potatos infected with S. endobioticum, and tumor tissue from many plants infected with many different organisms, have been shown to contain greatly increased levels of indole auxins (e.g. Turian and Hamilton, 1960). A similar type of involvement was shown in the bakanae effect in rice by Kurosawa in 1926. The rice seedlings, which

elongated more, and more rapidly, than normal seedlings were shown to be infected with Fusarium moniliforme, and the fungus was then shown to produce, in vitro, a substance which promoted the elongation of uninfected rice seedlings. This growth promoting substance was characterised as gibberellin, and more recent work has shown that the gibberellins are a widespread group of natural growth regulators in higher and lower plants. This subject is dealt with more fully in a later section of this introduction.

In many cases the infectious agent can be clearly shown to produce the growth promoting agent, as in the bakanae effect, but in many cases the relationship may not be so clear. Setty and Wheeler, 1968, showed that the galled roots of tomato plants infected with root-knot nematodes contained more auxin than uninfected roots, but that the concentration was unaffected. They also showed that the nematode larvae infesting the roots do not contain enough auxin to account for the extra found in galled roots. Infected roots were shown to contain more free amino acids, including tryptophan, than healthy roots, and it is suggested that the nematodes secrete proteolytic enzymes which hydrolyse plant proteins. It is further suggested that the free tryptophan produced in this way reacts with endogenous phenolic acids to produce auxin, which promotes gall formation. Proof of

this type of reaction chain is not easy to obtain, but all the evidence that is presented suggests that it is a valid mechanism.

It has not been possible to relate all growth deformations to changes in growth regulator levels or concentrations. The auxin levels of all plants infected with the curly top virus were shown to be significantly lower than in healthy control plants, but there was little symptom expression in resistant hosts (Smith, McCall and Harris, 1968).

In general, a pathogen causes a disruption in the normal growth and development of the host plant by interference in the metabolic processes of the plant rather than by its physical presence (Wood, 1967). This disruption may occur in the specific sphere of growth regulation, as has been demonstrated in certain syndromes described above. However, the interference may be in more general metabolic processes, so that no relationship will be established between growth regulators and the pathogen.

Non-viral parasites can induce metabolic changes in host plants in several different ways. Most simply the parasite may itself synthesise, and excrete into the plant, a normal growth regulator, or its antagonist, and thus directly alter the level of growth regulator in the plant. However, there are very many ways in which

the synthetic products of a parasite could be involved in altering the effective growth regulator level at its site of action. Viral pathogens are particularly interesting because the size of the viral genome renders the virus incapable of synthesising any growth regulators, per se. However, the genome of all viruses contains more information than is required to code for the coat protein, (Matthews, 1971) and the other product, or products, of viral synthesis could be involved in the alteration of effective growth regulator levels.

Barley yellow dwarf virus was used in this study because it causes dwarfing as a primary symptom of infection, and by a suitable choice of growing conditions this may be the sole sign of infection (Oswald and Thung, 1955).

I. BARLEY YELLOW DWARF VIRUS

A. THE STRUCTURE OF THE VIRUS

BYDV was first isolated from oats, and partially purified, by Rochow and Brakke in 1964, and they described it as a dense polyhedral particle about 30 nm in diameter. They obtained a similar particle from BYDV-infected aphids. These particles had the same sedimentation coefficient on sucrose density-gradient centrifugation,

migrated with the infectious entity in sephadex gel filtration, and were absent from uninfected oats, and so were assumed to be the virus. Jensen, in 1969, demonstrated the presence of densely staining spherical particles, about 24 nm in diameter, in certain phloem cells in the leaves and roots of BYDV-infected barley, and was unable to find similar particles in healthy barley. Although the particles described by Jensen are smaller than those isolated by Rochow and Brakke there are no other contradictions. Jensen puts forward several possible explanations for this discrepancy. The samples used by Rochow and Brakke were air dried on collodion-coated specimen grids and it is possible that surface tension may have produced some degree of flattening during the drying process. Also, these specimens were tungsten shadowed which may have increased the apparent diameter. Jensen stained his sections with lead citrate and points out that if the outer layer of the virus capsid does not absorb the stain this would lead to an under-estimation of the diameter of the virus. He also suggests that if the virus particle was cut above or below the centre it would appear smaller than it really was. However, his photographs show many virus particles and it seems statistically unlikely that all, or even most of them, would be cut in this way. Palival and Sinha (1970) demonstrated the virus, in both infected plant

and aphid, with a diameter of 22 nm to 25 nm. These figures would tend to confirm the diameter proposed by Jensen.

B. TRANSMISSION OF THE VIRUS

BYDV is not mechanically transmissible (Oswald and Houston, 1953) but is transmitted by several species of aphids. It has been suggested that virus instability is a factor in preventing mechanical transmission of a virus. Heagy and Rochow (1965) showed that the thermal inactivation point of BYDV is between 65°C and 70°C, and they conclude that the virus is stable, and that failure to transmit BYDV mechanically must be due to some other factor. Since many viruses lose infectivity in the range 50-60°C it seems reasonable to conclude that BYDV exhibits above average thermal stability, but it seems unwarranted to extrapolate this to a conclusion of general stability.

Aphid-transmitted viruses can be divided into two classes, persistent and nonpersistent (Watson, 1938; Watson and Roberts, 1939 and 1940). The nonpersistent virus survives in the vector for only a short period, is acquired during a short acquisition probe on an infected plant, and does not persist through a molt.

This type of virus is frequently termed stylet-borne, which suggests that the insect is acting as a passive carrier of the virus, but the picture is not, perhaps, as simple as this. A particular vector will vary in its transmission efficiency depending on the virus strain involved, and although in some cases there is completely independent transmission of different viruses on the same stylet, in other cases one virus is only transmitted in the presence of another virus.

The persistent virus is ingested by the insect, passes through the hemocele into the salivary glands and is transmitted, after a latent period of some days, from the salivary gland during feeding by the insect. It is retained through the insect molt, and since the skin, foregut, hindgut and stylets are discarded during the molt the persistent virus is either in the midgut or lodged within the aphid body. BYDV is a persistent, or circulative, virus in several species of aphids. Palival and Sinha (1970) recovered BYDV from different regions in infected M. avenae and found that the gut was the best source of inoculum, and the salivary gland was the worst. There was no infectivity in the brain. They found that the level of infectivity in the gut increased with the length of the acquisition feed, but that for any given length of feed the infectivity remained at the same level for 6 hours to 24 hours and

then declined. They concluded that the virus does not multiply in the gut of the aphid, although they did not exclude the possibility that it may multiply, to a limited extent, in some other tissue.

Oswald and Houston (1953) showed that five different species of grain-infesting aphids were vectors of BYDV: Macrosiphum avenae, Rhopalosiphum maidis, R. prunifoliae, M. dirhodum, and Toxoptera graminum. Not all these species were equally effective vectors however, for T. graminum infected only 37% of infested barley plants while R. prunifoliae infected 100% under the same conditions. Subsequent work has revealed many other aphid species which act as vectors, but the actual list of vectors is confused since different names are given to the same aphid by different workers. Kennedy, Day and Eastop (1962) named ten vectors of BYDV, but R. padi, M. avenae and R. maidis appear to be the most important vectors in the field (Slykhuis, 1967).

Rochow (1960) observed that a clone of T. graminum obtained in Florida failed completely to transmit an isolate of BYDV which other clones of the same species, obtained from Wisconsin and Illinois, transmitted 100%. The greenbugs from Florida were shown to have a slightly different beak tip from the other two clones, but no other anatomical differences were found, and Rochow suggested that there were different strains of aphid.

Similar specialisation was shown using clones of R. padi from New York and Kansas (Rochow and Eastop, 1966).

Rochow (1961) suggested that there are different strains of BYDV, as well as different strains of aphid. This concept was based on the results of transmission tests using M. avenae and R. padi in which he found one BYDV isolate which was transmitted 100% by M. avenae and not at all by R. padi, and a second BYDV isolate in which the reverse was true. On this basis he found four different strains of BYDV. Gill (1968) showed the existence of 22 different isolates, obtained in Manitoba in one year, on a similar experimental basis. The terms isolate and strain seem to be synonymous in their work. Smith (1963) suggested that it was best to assume that there is only one BYDV which differs in adaptation for transmission by several aphid species. It seems abundantly clear that there are different strains, isolates, or adaptations of BYDV existing in nature and for simplicity these will now be referred to as strains, although Rochow himself (1965) pointed out that there was little basis for the use of the term except in virus-vector relationships. It is also evident that different strains predominate in different geographic regions (Rochow, Jedlinski, Coon and Murphy, 1965). Rochow surveyed the strains of BYDV found near Ithaca, New York, over a ten year period and found that there was a shift in

predominance of one strain during this time. Gill (1968) found a similar, annual, variation in Manitoba.

Vector specificity

Vector specificity can result from two different causes; either an inability of the aphid to acquire the virus from an infected plant, or the inability of the aphid to transmit the virus. Using a technique of aphid injection Rochow (1961b) showed that aphids can acquire a strain of BYDV which they do not transmit. He also showed (1965) that vector specificity was apparently lost after oat plants were doubly infected with two strains of BYDV and was at a loss to explain this phenomenon. Later, Rochow and Ball (1967) showed serological differences between different strains of BYDV, using the technique of Gold and Duffus (1967), and this was the first validation of the concept of different strains. All viruses produce antibodies when injected into suitable animals, mainly as a result of the antigenic properties of the surface layer of the virus. In a small virus like BYDV, which has no envelope, the surface layer is the coat protein. There is experimental evidence that turnip yellow mosaic virus RNA is involved in the antigneic reaction produced by TYMV since TYMV protein is less immunogenic than the whole virus (Marbrook and

Matthews, 1966). It is possible that many plant viral RNAs, including BYDV-RNA, enhance the immunogenic role of protein, but there is no proof of this. The antibodies, therefore, are mainly a reaction to the coat protein, and the production of two different antibodies to two different isolates of BYDV indicates differences in the coat protein of the two isolates. The difference could be as small as the replacement of a single amino acid residue, provided this is near the surface of the virus. If vector specificity is associated with the coat protein then loss of vector specificity after double infection could be a result of the coating of the viral RNA in the "wrong" coat protein. This is only speculation, however.

Site of infectivity

When aphids probe for any length of time, the stylet frequently penetrates the phloem, and anatomical studies of BYDV-infected cereals show that phloem degradation is the first sign of infection (Esau, 1957a). It has been concluded, therefore, that BYDV is transmitted directly into the phloem and is then transported in the sieve tubes. If this is so, the rate of translocation of virus should be equivalent to the rate of movement of other sieve tube translocates, 1.5 cm/h, and although this is true in most of the plant it is not true for the

movement of the virus out of the inoculated leaf, (Gill, 1968b) which has been shown to be relatively slow. Gill suggests that the aphid introduces the virus into the phloem parenchyma rather than into the sieve tubes, and that viral passage from parenchyma to sieve tube is slow. In fact, the rate of movement of BYDV out of inoculated leaves is similar to that of mechanically transmitted virus, which suggests very strongly that the two occur in the same manner.

Until recently it was thought that virus never invaded the zone of tissue near shoot and root tip, and one method of obtaining virus-free plant tissue has been the culture of excised apical meristems. However Walkey and Webb (1968) demonstrated the presence of cucumber mosaic virus in Nicotiana rustica apical meristems, strawberry latent ringspot virus in Chenopodium amaranticolor apical meristem, and cherry leaf roll virus in the apical meristem of several host plants. All these viruses are small isometric particles, similar to BYDV, and it is possible that a similar technique would reveal BYDV in the apical meristem of cereals.

C. REPLICATION OF THE VIRUS

The replication of bacterial and animal viruses has been studied, and detailed knowledge obtained about the sequence of events in these hosts. The processes are so similar that a great deal was inferred about the replication of plant viruses, and although the assumption that virus replication is basically the same in all host types seems rash, more recent work seems to show that this is indeed so, as is described below.

Uncoating of the virus

The first stage in the multiplication of any virus, after it has penetrated the cell, is thought to involve the uncoating of the nucleic acid core, a process which is clearly essential before the second stage, replication of the nucleic acid core, can occur. The necessity for this stage is more clearly understood than the mechanism, and until recently there was no definitive evidence that this stage did occur with plant viruses. Infection of plants with TMV produces symptoms only after a delay, which is considerably shortened by use of naked TMV-RNA as inoculum (Fraenkel-Conrat, Singer, Veldee, 1958). This time lag was interpreted as the time necessary to uncoat the nucleic acid, but Kassanis (1960)

found that tobacco necrosis virus and TNV-RNA produced the first lesions stimultaneously on infected host plants, although TNV-RNA produced lesions at a faster rate than TNV. He suggested that some particles are stripped immediately they penetrate the cell, but that the whole process takes some hours. Bawden & Kleczkowski (1960) disputed these conclusions and showed that TMV and TNV both survive on the surface of a leaf much longer than the naked RNA, so that the delay could be otherwise explained. However, there now appears to be direct evidence for the uncoating of TMV in tobacco leaves (Reddi, 1966; Hirashima and Hirai, 1969; Shaw, 1970). Shaw (1970) showed that some viral RNA was released within minutes of inoculation, but that this was noninfectious due to degradation. He also followed the liberation of protein subunits from the virus, and found no correlation between this and the appearance of RNA. He concluded that most virus particles lose some protein units very rapidly.

Assuming that uncoating does occur with all plant viruses, its mechanism is unclear. The time lag which occurs after infection with pox viruses, before symptoms appear, has been shown to be sensitive to inhibitors of protein synthesis, (Jocklick, 1964), and this suggested that a protein, the uncoating enzyme, was synthesised during this period; but how the enclosed genome is translated into its own uncoating enzyme is not made clear.

Synthesis of RNA

After the lag period which immediately follows inoculation there is a considerable increase in the nucleic acid content of infected material. Since nearly all plant viruses, and all the small, isodiametric viruses resembling BYDV, are RNA-containing, only the replication of this type of virus will be discussed here. The replication of animal and bacterial virus RNA has been studied in detail and the sequence is clearly documented. First the viral RNA forms an association with ribosomes, and acts as template for the synthesis of an RNA polymerase called replicase. A second frequent occurrence in animal cells is the cessation of host RNA and protein synthesis, but this is not always so. The viral RNA then acts as template for the synthesis of complementary RNA, replicase acting as the enzyme in a not clearly defined manner. The complementary strands then act as template for the parental-type RNA, again utilising replicase. The syntheses are thought to involve the formation of double-stranded intermediates, the replicative form (RF) and the replicative intermediate RI (Hofschneider and Hausen, 1968) although it has also been suggested that these are artefacts of the extraction process (Fraenkel-Conrat, 1970).

There is a great deal of evidence that a similar

sequence occurs in plant cells. Babos (1969) and Babos and Shearer (1969) demonstrated the synthesis of a rapidly labelled RNA in association with plant ribosomes in TMV-infected tobacco leaves. Bradley and Zaitlin (1971) showed that TMV-infected tissue contains a viral replicase, and in vitro experiments with this TMV-RNA replicase produced no single-stranded TMV-RNA, but two high molecular weight structures which are sensitive to ribonuclease. It is suggested that these are double-stranded RNA forms. Bové, Bové and Mocquot (1968), Ralph and Wojcik (1969) and Jackson, Mitchell and Siegel (1971) also claim to have isolated a double-stranded plant viral RNA. Yasuda and Hirai (1964) followed the incorporation of tritiated uracil, using microautoradiography, and found a large increase in RNA synthesis in the nuclei of TMV-infected cells compared to uninfected cells. Smith and Schlegel (1965) using actinomycin D, which suppresses DNA-dependent RNA synthesis, and a similar technique with ^{3}H-uridine and microautoradiography, showed RNA synthesis in the nucleoli of clover yellow mosaic virus-infected root tips of Vicia faba but not in uninfected root tips. This suggests that the virus utilises the normal cellular apparatus for RNA synthesis. However there is some evidence which conflicts with this. DeZoeten and Schlegel (1967) found evidence to suggest that broad bean mottle virus caused RNA synthesis in the

Golgi apparatus and endoplasmic reticulum. Ushiyama and Matthews (1970) found RNA synthesis in cytoplasmic spaces associated with clumped chloroplasts in chinese cabbage infected with turnip yellow mosaic virus. These vesicles were always found in TYMV-infected plants and never in healthy plants. Ushiyama and Matthews (1970) also suggest that actinomycin D may not suppress all DNA-dependent RNA synthesis so that post-actinomycin D RNA synthesis is not necessarily viral RNA synthesis. Since implication of the nucleolus as the site of viral RNA synthesis has usually involved the use of actinomycin, these results may require further confirmation. It is possible, of course, that not all plant viruses replicate in the same manner. Some may use the normal host cell mechanism for RNA synthesis and some involve other mechanisms. Despite the large amount of similarity between many viral reactions there is reason for all to be the same.

Synthesis of coat protein

The third stage in viral replication involves the translation of viral RNA and the synthesis of viral protein. In vitro synthesis using an E. coli cell-free system and f2 coliphage RNA as messenger produced a protein product resembling the coat protein of the f2

coliphage. However, with the same system using TMV RNA as messenger no product resembling TMV coat protein was obtained (Aach, Funatsu, Niremberg, and Fraenkel-Conrat, 1964). It was, perhaps, overoptimistic to expect plant viral RNA to act as messenger in a bacterial protein synthesising system, since the initiating factors might well be different, but in vitro plants systems proved to be impractical. In vivo studies have not revealed much information either, except that viral protein synthesis appears to occur in unspecified regions of the cytoplasm. It has been assumed by many workers that viral RNA is translated in the cytoplasm, and viral protein synthesised, using the normal cellular apparatus, in place of normal plant proteins. This theory would explain the interruption of normal cellular metabolic processes and the synthesis of viral protein, but is so far unproven.

Assembly of virus

The final stage in viral replication is the investing of RNA in coat protein. In vitro studies with the RNA and protein of small viruses, like BYDV, suggest that this is a spontaneous process, occurring rapidly when the concentration of RNA and protein reach a suitable level. The site of assembly is not so clear. Studies involving microscopy suggest TMV assembly in the

cytoplasm (e.g. Shalla, 1964). However it has also been claimed that TMV is assembled in the chloroplasts and the nucleus. These claims were based on the results of a cell fractionation technique and Matthews (1970) suggests that the organelles were contaminated by virus during the extraction procedure. Certainly, since all the evidence suggests that both components are present in the cytoplasm, the cytoplasm seems a likely location for assembly. However, there is convincing evidence that pea enation mosaic virus is synthesised in the nucleus (Shikata & Maramorosch, 1966).

D. MACROSCOPY OF BYDV-INFECTED PLANTS

Most plant viruses are discovered as a result of investigation of a plant which does not appear normal. Unfortunately widely differing causes can produce very similar symptoms, so that diagnosis is never easy. According to Oswald and Houston (1953) BYDV-infected plants were probably observed several years before these workers fully investigated the abnormal plants, and showed that the symptoms were transmissible by certain species of aphids, and therefore certainly a result of infection. No doubt the fact that nutritional deficiencies can cause leaf yellowing and stunting prevented earlier investigation, and it was not until the symptoms reached epiphytotic

dimensions, and caused large financial losses, that investigation was begun.

Age of plant and number of infesting aphids

The first plants examined were barley, as the name of the virus testifies, but the virus attacks other cereals, in particular oats and wheat. In the field, the severity of infection depends on the age at which the plant is infected, younger plants showing far more damage than mature ones. Oswald and Houston (1953) state that severity of the disease is wholly dependent on plant age when infected, but Smith (1967) showed that the number of aphids infecting a plant was also a significant factor. He found that higher numbers of aphids could produce as severe symptoms in older plants as small aphid numbers in seedlings. Jones and Catherall (1970) found that the degree of dwarfing of the plant was proportional to the number of aphids used to infest the plant.

Susceptibility

Oswald and Houston (1953) showed that different varieties of a particular cereal exhibit different degrees of susceptibility to the virus, regardless of the time of infection. They classified all varieties they tested

into four different reaction groups: highly resistant, tolerant, susceptible, and extremely susceptible, and found that all four groups showed a diminished response to the virus with increasing age at infection. Smith (1967) tested susceptibility to BYDV in other cereal varieties, and found that some varieties exhibited more resistance at the one leaf stage than at later stages of development, e.g. Fulghum oats. Both these workers measured susceptibility to the virus in terms of grain yield, which is probably more meaningful than a system based on leaf yellowing (Catherall and Hayes, 1967), although the latter has the advantage of speed.

Discoloration of leaves

The first sign of infection, according to Oswald and Houston (1953) is always a colour change in the leaves. Barley leaves turn a brilliant yellow, oat leaves a yellow-green which changes through red-brown to red, and wheat exhibits a darker green on outer leaves, followed by chlorosis of new leaves. In barley and oats the coloration starts at the tip and progresses down the leaf. Although the symptoms are fully described by Oswald and Houston there is plenty of variation. Leaf coloration appears to depend on illumination (Oswald and Thung, 1955), and we found Black Hulless barley C.I. 666, grown with less than 2,000 ft C illumination, exhibited dwarfing as the first sign of infection, followed much

later by a red colour at the leaf tip which turned yellow. Still less illumination prevented any discoloration.

As well as discoloration of leaves there is a change in leaf texture, and infected plants appear more erect due to stiffness of the leaves.

Dwarfing of plants

Plants also exhibit stunting, with shortened internodes, and dwarfed leaves, and decreased heading. The susceptibility of a plant is perhaps most obvious in its degree of stunting, but plants infected when fully grown obviously exhibit no stunting. The degree of dwarfing seems to depend on the growing conditions of the plant, and fast growing barley plants exhibit more resistance to the virus-induced dwarfing than slow growing plants, although the grain yield is less than normal (Catherall and Hayes, 1966).

The roots of the plant are as retarded as the aerial parts, and in drought the abbreviated root system is unable to reach water.

Tillering of plants

BYDV affects the tillering of host plants, but the effect varies with species. The tillering is

stimulated in barley (Oswald and Houston, 1953) but is suppressed in wheat (Oswald and Houston, 1953; Gill, 1967).

Plant breeding for BYDV-resistance

Susceptibility tests on barley show that many varieties, mainly from Ethiopia, are extremely tolerant, although not immune, to BYDV (Schaller, Rasmusson and Qualset, 1963). This tolerance is genetically controlled, and in Ethiopian barley only one gene, Yd2, is involved (Rasmusson and Schaller, 1959; Schaller, Qualset, Rutger, 1964). The whole picture is somewhat confused by the fact that environmental conditions seem to alter expression of genetically-controlled tolerance (Jones and Catherall, 1970) and so far there has been little success in plant breeding for BYDV tolerance.

E. MICROSCOPY OF BYDV-INFECTED PLANTS

The microscopy of BYDV-infected Gramineae was studied extensively by Esau (1957a; 1957b), who found that the primary symptom of infection was phloem degeneration, and that secondary effects may develop in other tissues. In the normal development of healthy Gramineae the first vascular elements in large vascular bundles differentiate

while the leaf is elongating and are then destroyed during the subsequent elongation processes leaving a lacuna. The parenchyma is not obliterated in this way. In BYDV-infected plants the obliteration of the first-formed vascular elements occurs earlier than in healthy plants, and is associated with necrosis. Frequently the surrounding parenchyma cells undergo a similar obliteration. Esau called BYDV-induced destruction necrotic obliteration, to distinguish it from normal developmental obliteration. The two processes differ in several respects. Necrotic obliteration is associated with the accumulation of safranin-staining material in the lumina of affected cells, and this is totally absent from control plants. Esau suggests that this material is wound gum. Developmental obliteration is limited to the protophloem and protoxylem elements of the larger vascular bundles, later-developed vessels not being subject to the stresses of leaf-elongation. Necrotic obliteration, however, affects phloem which is differentiated at any stage, so that all vascular bundles may be affected. The development of the whole bundle is inhibited in a highly susceptible host plant, and the bundle may be totally collapsed. The staining reaction of the phloem parenchyma is altered in BYDV-infected plants and Esau suggests that the deep staining of the protoplasts is a sign of incipient necrosis. Similar depth of staining is sometimes seen

in xylem parenchyma.

The degeneration of phloem elements, associated with an accumulation of safranin-staining material in the lumina of cells, the deeper-staining reaction of the protoplasm of some phloem parenchyma, and the collapse of seive elements and associated parenchyma cells also occurs in the roots of BYDV-infected plants.

Esau compared the structure of several BYDV-infected species and found that necrotic obliteration was more pronounced in barley than in wheat. Comparing the stunting effects of BYDV, Oswald and Houston (1953) found that wheat was more damaged than barley. Esau does not attempt to explain this lack of correlation and simply points out that a very small number of plants was used in her study.

Esau observed an inhibition of leaf initiation by the apical meristem in several BYDV-infected species, and she interpreted this as a reduction in meristematic activity, but was unable to show this in barley or wheat since the apices were no longer vegetative at the time of inspection. Confirmation of reduced meristematic activity was obtained in these species by the observation that the vascular elements mature in BYDV-infected plants at a shorter plastochronic distance from the apex than in controls. Apart from an increase in vacuolation in immature cells, no changes were observed in the meristematic

regions of infected plants, except in barley where the initiation of the floral apex was delayed in severely infected plants, and the cells increased in size. This produced thickened leaves.

F. METABOLISM IN BYDV-INFECTED PLANTS

It has long been recognised that the symptoms of virus infection must reflect a derangement of cellular physiology and plant pathologists have attempted to correlate the two by investigation of photosynthetic rates, transpiration rates and any other metabolic processes capable of comparison and measurement. As knowledge of cellular processes has advanced the aim has been to compare specific biochemical reactions in infected and control plants, and a large amount of information has been collected. Unfortunately none of it appears to relate directly to the symptoms of the infection. The physiological pattern usually associated with viral infection includes decreased photosynthetic activity, increased respiration rate, accumulation of soluble nitrogen compounds, increased polyphenoloxidase activity, and decreased activity of growth regulating substances (Diener, 1963). None of these findings is specific to viral infection, and several are produced by nutritional

deficiencies and adverse growing conditions. It seems likely that these are the result, not the cause of the more specific, primary changes caused by the virus.

Photosynthesis

The rate of photosynthesis in BYDV-infected plants was examined by Orlob and Arny (1961) and Jensen (1968) and both found a marked decrease. Jensen found the rate diminished to 12%-20% of the control value, depending on the time after inoculation, and Orlob and Arny found a value of 60% of that of healthy controls.

Respiration

Orlob and Arny (1961) observed that the respiration rate in BYDV-infected plants rose in the early stages of infection and then declined to values lower than normal after 39 days. Jensen (1968) found a similar increase, but did not take any measurements later than 21 days after inoculation, so that there is no confirmation of the reverse trend which Orlob and Arny observed. It is possible that this decline was a result of the general inhibition of metabolic processes observed in highly susceptible varieties infected with BYDV, or just the characteristic decline in activity of aging leaves, which process is hastened by viral infection. Jensen (1968b)

observed that there is an increase in percentage dry matter in BYDV-infected plants and when he calculated the respiration rate per unit dry weight there was no significant difference between infected plants and controls. The significance of this is not clear at the present state of knowledge.

Carbohydrate metabolism

Watson and Mulligan (1960) observed an accumulation of starch and soluble carbohydrates in BYDV-infected leaves. This was confirmed by Orlob and Arny (1961) who stated that the degree of carbohydrate accumulation can be correlated with the macroscopic symptoms, although they gave no figures to substantiate this claim and no indication of how they assessed the macroscopic symptoms quantitatively. The accumulation of carbohydrate materials is common [redacted] plants infected with yellow-type diseases but the [redacted]ason for this phenomenon is not known. Jensen (1968) suggested that a decrease in translocation would account for both accumulation of carbohydrate and increase in dry matter, but Orlob and Arny (1961) found no reduction in translocation in BYDV-infected leaves. Jensen, 1969, himself rejected the idea of reduced translocation as the basis of the physiological derangment of the plant since he

found only a small number of damaged sieve tubes in badly-stunted plants. Jensen obviously assumes that only physical damage to the cell will prevent translocation and ignores the possibility of biochemical control. However, all seem to be agreed that decreased translocation is not involved in the production of virus-induced symptoms.

Jensen (1969) correlated the levels of carbohydrate in the infected leaf with respiration rate, and suggested that the increased respiration rate is a result of carbohydrate accumulation. He failed to find any correlation between carbohydrate levels and rates of photosynthesis and concluded that a simple feedback mechanism was not in action, although there might be some indirect, or complex, relationship.

Nitrogen metabolism

Orlob and Arny (1961) and Jensen (1969b) both showed that BYDV-infection altered nitrogen metabolism in the leaves, but the results were contradictory since Orlob and Arny found a reduction in both total nitrogen and protein nitrogen whereas Jensen found an increase in total nitrogen and in non-protein nitrogenous compounds. Since both workers used the same host plant, Black Hulless barley C.I. 666, and similar methods for determination

of total nitrogen (semimicro-Kjeldahl) and protein nitrogen (rupture of cells followed by centrifugation and protein precipitation with trichloracetic acid) it is difficult to explain this discrepancy. The results described by Orlob and Arny are similar to the results obtained by Watson and Watson (1953) working with beet yellows virus, and they suggested that the reduced levels of nitrogen-containing compounds could be the result of destruction of the photosynthetic centres. It has been shown that the nitrogen content of the yellow part of variegated leaves is lower than that of the green part (McKee, 1958). The nitrogen content of chloroplasts is largely protein, and the figures given by Orlob and Arny would suggest that it is only the protein-nitrogen which is reduced during BYDV-infection. However in Jensen's experiments soluble-protein nitrogen is not reduced, and may be increased, during the course of infection. Jensen's figures show a significant rise in nitrogenous compounds excluding soluble protein, i.e. in insoluble protein, nucleic acids, and low molecular weight amines and amides. It has been suggested that nitrogen is translocated in the plant in the form of glutamine (Bollard, 1956) and Jensen suggests that an accumulation of glutamine in the leaves, as a result of impaired translocation, would explain the observed rise in nitrogenous material. However Jensen (1969) withdrew his suggestion of impaired

translocation, so that the involvement of glutamine may no longer be postulated, and it would seem essential to confirm the changes in nitrogenous-material levels in BYDV-infected cereals before indulging in any further speculation.

Enzyme activities

Orlob and Arny (1961) observed a decrease in catalase activity and a rise in peroxidase activity in BYDV-infected plants. Farkas and Kiraly (1958) demonstrated low catalase activity in virus-infected plants, but many conditions alter the activities of both these enzymes so that the effects are not at all viral specific.

Plant growth regulators

Orlob and Arny (1961b) investigated the effects of plant growth regulators on BYDV-infected plants and found that application of gibberellic acid partially overcame the dwarfing effect of the virus, while application of indole-3-acetic acid caused a slight increase in growth of dwarfed plants, and a decrease in the yellowing of leaves. They suggested that IAA might increase the tolerance of the plant towards the virus, in a mechanism not specified.

PLANT GROWTH AND DEVELOPMENT

A. MORPHOLOGY OF BARLEY GROWTH

The growth and differentiation of the plant shoot is determined by the growing point of the stem. In the young barley plant the growing point is hemispherical and surrounded by leaf initials, or primordia, and developing leaves (Bonnett, 1935). The leaf initials are seen as single transverse ridges on the growing point, or apex. Bonnett showed that all the leaves which will be present on the mature stem are observable as developing leaves, or transverse ridges, by the time the second leaf is well grown. At this stage the growing point begins to elongate, and differentiation of the flower spike begins. The first sign of spike differentiation is the appearance of double ridges on the growing point. The period between germination and the appearance of double ridges is termed the vegetative phase of development (Nicholls and May, 1963), and its duration depends on the growing conditions of the barley. Nicholls and May (1963) studied the development of Prior "A" and C.I. 5611 barley and observed that the vegetative phase was completed in under ten days when plants were exposed to continuous illumination, but that it lasted more

than 20 days when plants were only illuminated for an eight hour period during the 24 hour cycle. When plants were given this reduced lighting schedule and were also subjected to water stress the vegetative phase was even longer.

Nicholls and May (1963) correlated the length of the apex, which they measured from shoot tip to the base of the ridge immediately above the uppermost leaf, with the number of primordia present. They found that the growing point elongated during the vegetative phase but that there was no increase in interprimordial distance. They attributed the increase in length of the apex during this phase of growth solely to the addition of new primordia.

The transition from the vegetative phase of growth to the second stage is shown by the elongation of the internodes and the differentiation of spikelet structures (Bonnett, 1935). Bonnett showed that the upper ridge of each pair grows more rapidly and forms the spikelet, and he suggests that the internode of the rachis develops from the lower ridge. The upper ridge differentiates to form two glume initials on the sides of each spikelet. A ridge then forms across the spikelet and differentiates into the lemma, and this is followed by the differentiation of the anther, the pistil and finally the awn. Bonnett calls this period

of spikelet differentiation, and maturation in readiness for pollination, the second phase of development. Nicholls and May (1963) found it convenient to divide Bonnett's second phase of development into two stages. The stage starting with the appearance of double ridges and ending with the appearance of stamen initials they called the spikelet phase. Correlating the apex length with interprimordial distance they showed that during the spikelet phase the increasing apical length was due to the acropetal addition of new primordia, as during the vegetative phase. The appearance of stamen initials signalled the transition to a period of increasing interprimordial distance. They called this the elongating phase. The original work of Nicholls and May (1963) was in some disagreement with that of Bonnett (1935) who found a changeover to elongating internodes at the end of the vegetative phase. However, Nicholls and May (1964) presented more results which showed that there was indeed some elongation of the primordia during the spikelet phase, although they regarded this as a minor component of apical elongation.

Nicholls and May (1964) further investigated the spikelet and elongating phases of growth and found that the number of cells in a 40 µm internode segment was constant during the spikelet phase and increased during

the elongating phase. They also showed that mean cell length was at a maximum when stamen initials first appeared. They suggested that the cells in the pith region of rapidly elongating internodes undergo synchronous division.

Nicholls and May (1963) compared the rates of increase of apical length under different light regimes, with and without water stress, and found that the vegetative and spikelet phases of growth were both influenced by light conditions but not by water stress.

B. THE ANATOMY OF BARLEY GROWTH

The growing point of the shoot is called the apical meristem and is described as consisting of small, approximately isodiametric, thin-walled cells which are rich in cytoplasm (Kaplan, 1937). Meristematic cells are frequently said to be non-vacuolated (e.g. Sachs, 1965) but in 1932 Zirkle showed that many meristems contain vacuolated cells, although the vacuoles are small, and dispersed throughout the cytoplasm. Reeve (1948) also showed that meristems contain conspicuously vacuolated cells. In fact, although meristematic cells are always relatively undifferentiated, considerable differences exist in the meristematic cells of different species in regard to

size, shape, wall thickness, and nuclear size (Esau, 1965). All the cells which form the mature plant shoot originate in the apical meristem.

During the vegetative phase of growth of monocotyledons, including barley, small regions of meristematic tissue are laid down at the base of each leaf insertion (Lehman, 1906) and these are termed intercalary meristems. The growth of the leaf is a result of cell division in the meristem at the leaf base, and, during the elongation phase of growth, internode elongation is a result of cell division in the intercalary meristem (Sharman, 1942). Anatomically, the intercalary meristem differs from the apical meristem. Although both contain undifferentiated, densely-staining cells the intercalary meristem also contains some vascular tissue, without which the vascular system of the plant would not be continuous. The vascular elements in the internodes of Sorghum vulgare have been shown to be capable of extension (Artschwager, 1948) and thus do not inhibit the elongation of the internode. Thus, although the apical meristem is the original source of all cells in the mature monocotyledonous plant multiplication of these cells occurs mainly in the intercalary meristems.

Examination of the growth of most dicotyledonous plants shows a similar type of picture. The apical

meristem produces the initial cells which make up the plant, but the multiplication of these cells occurs some distance below the meristem (Grisebach, 1843). This region is now called the subapical meristem. Several points of similarity exist between the intercalary and subapical meristems. Both contain differentiated tissue. Both exhibit maximum activity at the phase of stem elongation, and both act independently of the apical meristem. Sachs (1965) states that "the entire zone of subapical meristematic activity is an intercalary meristem in its own right".

The role of the subapical meristem has been investigated in many plants and greater subapical meristematic activity was demonstrated in tall varieties of tomatoes than in dwarf varieties (Bindloss, 1942). Similar results were obtained from a comparison of long-shoot and short-shoot development in Gingko (Gunckel and Wetmore, 1946) and from normal and dwarf peach seedlings (Ledbetter, 1960). Sachs, Bretz, and Lang (1959) showed increased subapical meristematic activity in rosette plants which were elongating as a result of either gibberellin treatment or long-day treatment. In short, stem growth in response to environmental manipulation, normal development or exogenous chemical treatment is associated with increased activity of the subapical meristem; likewise inhibition

of stem growth for genetic or environmental reasons is associated with a lack of activity in the subapical meristem.

The role of the intercalary meristem during vegetative growth of monocotyledonous plants has not been examined at length but Fisher (1970) has shown that increased growth of the shoot of Cyperus alternifolius is associated with increased mitotic activity in the intercalary meristem and increasing length of the meristem. Cell elongation is also increased during the stem elongation process.

C. PHYSIOLOGY OF BARLEY GROWTH

The growth of a plant is a process involving the synthesis of new material within the plant, an irreversible increase of the plant as described by Whaley (1961). This process usually involves an increase in both size and mass of the plant, and growth of a higher plant is often correlated with increasing size of the plant, or size of a part of the plant. In many plants length of the shoot has proved a very useful measure of plant growth.

Early experiments in the field of plant growth involved the excision of various plant parts. Went (1941) showed that removal of leaves from developing

pea plants reduced internode elongation and it was similarly shown that defoliated Hyoscyamus niger plants do not form elongate stems (Sachs, 1965). Paal in 1918 (Sachs, 1965) showed that removal of the coleoptile tip inhibited coleoptile growth, and Kaldewey (1957) showed that decapitated Fritillaria stalks do not elongate. Grisebach (1843) showed that the presence of the flower bud was essential for the elongation of the flower stalk. The obvious conclusion from experiments of this type is that one or more substances are produced in the excised organs which regulate the meristematic activity of the stem and scape.

The first growth regulator to be isolated was auxin (Went, 1928) and this was shown to reverse the dwarfing induced in Bellis perennis by removal of the stem apex (Uyldert, 1928). However, auxin is not equally effective in reversing the dwarfing of all shoots. Dwarf peas treated with auxin elongate, but are still considerably smaller than control peas (Von Abrams, 1953).

The growth of a plant involves two distinct processes, namely the production of new cells and the elongation of the daughter cells. Inhibition of one, or both of these processes will obviously produce a dwarfed stem. Examination of the flower stems of Fritillaria showed that there were fewer cells in the

dwarfed scapes than in control scapes (Kaldewey, 1957). However, in the dwarfed shoot of many woody plants Tammes in 1903 found no diminution in cell number, and only cell elongation appears to be inhibited in the dwarfed shoot (Sachs, 1965). Likewise, stimulation of cell division or cell elongation promotes stem elongation. Examination of auxin-treated dwarfed scapes of Fritillaria revealed that auxin promotes cell division to only a small extent and that dwarfing is reversed by increased cell elongation.

The second growth regulator, gibberellin, was isolated in 1926 by Kurosawa, and this was shown to reverse dwarfing in very many species. Dwarf bean plants, for example, are indistinguishable from normal beans after treatment with gibberellin. Examination of the gibberellin-treated dwarfs showed that cell division was markedly stimulated by gibberellin, although cell elongation was also promoted to a lesser extent.

The two growth regulators, therefore, are very different in their mode of action in stimulating stem elongation, and further investigation showed that both exert other influences on the growth and development of the plant. These other effects are frequently mutually antagonistic. Gibberellins, for example, promote maleness in the flowers of cucumber (Galun, 1959) while auxins promote femaleness in the same plant.

D. GIBBERELLINS

The gibberellins were defined in 1954, as a class of naturally occurring compounds which exert, in low concentration, an effect on the growth and development of plants, (van Overbeck, Tukey, Went, and Muir, 1954). In 1961 these gibberellins first isolated were shown to possess a gibbane skeleton, Figure 1.1 (Cross, Grove, McClosky, MacMillan, Moffatt, and Mulholland, 1961).

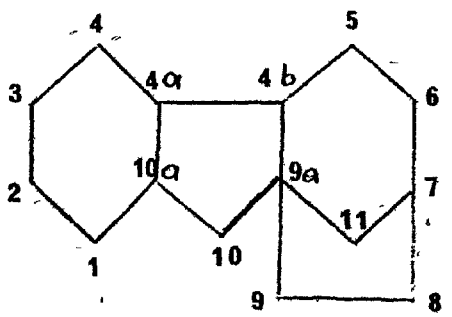


Figure 1.1. The structure and numbering of gibbane.

Paleg (1965) classified the thirteen gibberellins known in 1964 on the basis of the substituent groupings present at the 7 and 8 positions of the gibbane skeleton, and the presence, or absence, of a lactone configuration on the A ring.

In 1968, however, Rowe suggested that gibberellane (Figure 1.2) should be regarded as the parent compound of all gibberellins. The number of gibberellane

corresponds to that of other cyclic deterpenes, and is used by Lang (1970) in his review of the structure and metabolism of gibberellins.

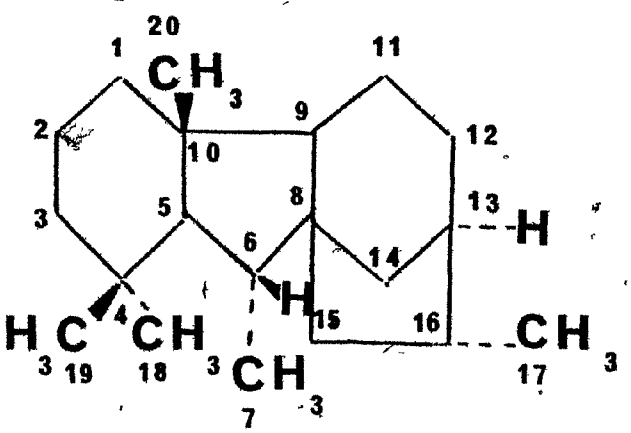


Figure 1.2. Structure and numbering of gibberellane. (Broken lines indicate bonds lying below the plane of the ring system, wedges indicate bonds lying above this plane.)

A system of A numbers was introduced (MacMillan and Takahashi, 1968) in the naming of gibberellins and by 1970 29 naturally-occurring, fully characterised gibberellins were thus numbered (Lang, 1970). Although the structures of these gibberellins are well-established, the stereochemistry is only assumed in many cases.

Lang divides all known gibberellins into two major classes depending on whether they have 19 or 20 carbon atoms. Those with 19 carbon atoms have a single carboxylic acid grouping at C-7 of the gibberellane molecule and a lactone ring. Those with 20 carbon atoms

have two carboxylic acid groupings, at positions 7 and 18, and may have an aldehyde grouping or a third carboxylic acid grouping at C-20. A complete list of known gibberellins, with their structural formulae is given in Appendix 1.

Biosynthesis of gibberellins

The biosynthesis of gibberellins has been established in both higher plants and fungus. Birch, Rickards, and Smith (1958) showed that both acetate and mevalonate are incorporated into gibberellic acid and suggested that the biosynthesis followed the normal route for cyclic diterpenoids (Birch, Rickards, Smith, Harris and Whalley, 1959). Cross, Galt, and Hanson (1964) showed that (-)-kaurene was a precursor of GA_3, and Graebe, Dennis, Upper and West (1965) elucidated the several stages involved in the synthesis of (-)-kaurene from acetate. They also showed that both (-)-kaurene, and its precursor, geranylgeranyl pyrophosphate, were incorporated into gibberellins. Shechter and West (1969) showed that the cyclisation of geranylgeranyl pyrophosphate to (-)-kaurene involved the formation of copalyl pyrophosphate as intermediate, and that this also is incorporated into the gibberellins. Dennis and West (1967) showed the incorporation of

kaurenal into gibberellins, and Geissmann, Verbiscar, Phinney and Cragg (1966) showed the incorporation of kaurenoic acid. The complete synthesis is shown in Appendix 2.

Sites of Synthesis

Three major sites of gibberellin biosynthesis have been identified (Lang, 1970). Lockhart (1957) showed that application of GA_3 reversed the effects of decapitating pea seedlings and suggested that gibberellins are synthesised in the stem tip of pea plants. Kuraishi and Muir (1964) disputed these results, but Jones and Phillips (1966), using a diffusion technique, showed that gibberellin synthesis certainly occurs in the young leaves in the apical bud of sunflower plants. Jones and Lang (1968) also showed the synthesis of gibberellins in pea shoot tips, and Stoddart and Lang (1968) obtained the same results using young leaves of red clover. It is now accepted that young leaves in the stem apex are an important site of gibberellin biosynthesis (Lang, 1970). Jones and Phillips (1966) also showed that gibberellin biogenesis occurs in the root tips of sunflowers. This is recognised as an important site of synthesis in other plants (Lang, 1970). The third site of gibberellin

biosynthesis is in developing seeds, and this has been demonstrated in many plants (e.g. Corcoran and Phinney, 1962; Baldev, Lang, and Agatep, 1965).

Translocation of Gibberellins

Gibberellins have been demonstrated in sieve tubes (Kluge, Reinhard, and Ziegler, 1964) and in xylem (Phillips and Jones, 1964) using several techniques (Lang, 1970). Bowen and Wareing (1969) showed that there is a direct exchange between the two systems, probably involving the phloem and xylem ray cells. The role of this circulating gibberellin is not known. Bowen and Wareing suggested that it may be supplying required gibberellins to the cambium, but Lang (1970) suggests that the circulating levels are unnecessarily high for this. Bowen and Wareing also suggested that the transport may be for inactivation or storage, and it has been shown that inactive forms of gibberellins, often called bound, conjugated, or butanol-soluble gibberellins, occur in large amounts in immature seeds of several species (Barendse, Kende, and Lang, 1968).

The translocation of gibberellins from the sites of synthesis, ie. root and shoot tips, to the vascular system is a slow process which probably occurs by diffusion.

The Physiological Effects of Gibberellins

The application of gibberellins to many plant systems causes an observable effect. For example, growth is promoted in many species and flowering is induced in certain long-day plants, as described in an earlier section of this thesis, the biosynthesis of α-amylase in the aleurone layer of cereal grain is stimulated (Paleg, 1960a and b), as is the binding of invertase to cell walls in artichoke tubers (Edelman and Hall, 1964). The nature of the response clearly depends on the plant, the developmental stage of the plant, and the organ of the plant under consideration.

Not all gibberellins are equally effective in producing all effects (Brian, Hemming, and Lowe, 1964). GA_3 does not induce flowering in Silene (Michniewicz and Lang, 1962) while GA_7 is very active in producing this effect, and GA_5 does not promote the growth of dwarf pea stem as GA_1 and GA_3 do (Kende and Lang, 1964). The activities of 26 gibberellins in 9 plant assays have been compared (Crozier, Kuo, Durley, and Pharis, 1970) and an attempt was made to correlate structure and activity but little success was obtained.

The role of GA_3 in the bolting of rosette plants was investigated by Sachs, Bretz, and Lang (1959) and Sachs and Kofranek (1963). They showed that the

gibberellin-induced bolting of Samolus parviflorus and Hyoscyamus niger was associated with an increase in mitotic activity of the subapical meristem. They also showed that subapical meristematic activity in Chrysanthemum morifolium was stimulated by GA_3. The mean cell length in Hyoscyamus niger plants treated with GA_3 was the same as that in untreated plants, and it was concluded that GA_3 is not involved in cell elongation in this plant. Purvis (1960) showed that application of GA_3 increased the growth rate of winter rye, and Barbat and Ochesanu (1963) showed that exogenous GA_3 was associated with an increase in length of the growing point in winter wheat. Nichols and May (1964) showed that the highest levels of endogenous gibberellin-like substances were found, in barley, during the period of internode elongation. They also showed (Nichols and May, 1963; Nichols and May, 1964) that internode elongation was largely the result of increased cell division, although cell length was increased to a small extent. Arney and Mancinelli (1966) showed that GA_3 stimulated both cell division and cell elongation in dwarf Meteor pea. Lockhart (1956) showed that red light inhibited both cell division and cell elongation in dark grown Alaska pea and Pinto bean, and that GA_3 reversed both effects. It is possible that GA_3 induces cell elongation in some plants but not in all, however

the role of GA_3 in cell elongation appears to be small, even when it can be demonstrated. Brian and Hemming (1958) showed that excised pea internode responded to GA_3 to only a small extent, and several workers have stated that full expression of the growth-promoting effects of GA_3 depends on the presence of an active, or potentially-active, meristem (Purves and Hillman, 1958; Brian, 1959).

The mechanism of action of gibberellins in promoting stem elongation is not clear. Konishi (1954) demonstrated that auxin was destroyed at a slower rate in bolting Silene armeria plants and attributed the stem elongation to higher auxin levels. Nitsch and Nitsch (1959) showed an increase in endogenous auxins in bean shoots four hours after GA_3-treatment, and Galston and McCune (1961) showed a decrease in extractable IAA-oxidase and peroxidase in GA_3-treated dwarf peas and dwarf corn. Halevy (1963) likewise demonstrated a GA_3-dependent decrease in peroxidase and IAA-oxidase activity in cucumber seedlings. Kuraishi and Muir (1963) demonstrated a forty-fold increase in the auxin levels of GA_3-treated Hyoscyamus niger plants just before the stem started to elongate. It seems certain that GA_3-treatment produces higher levels of endogenous auxin in many plants, although the mechanism whereby the auxin level is increased is not clear. Kogl and Elema

(1960) reported a three-fold increase in the concentration of polyhydroxycinnamic acids in peas after GA_3-treatment. These compounds are oxidase inhibitors, and their action would increase auxin concentration. Brian and Hemming (1958) suggested that GA_3 stimulated auxin-biosynthesis, and Sastry and Muir (1965) showed that GA_3-treated Avena coleoptile tips converted tryptophan to IAA more rapidly than untreated coleoptile tips. Valdovinos and Sastry (1968) showed that the GA_3-stimulated synthesis of IAA involved the formation of tryptamine as an intermediate. It is possible, therefore, that the auxin levels are increased as a result of both increased biosynthesis and decreased degradation; the latter possibly due to the effect of inhibitors on the normal degradative pathway. However, Bolduc, Cherry, and Blair (1970) report that GA_3 promotes the activity of IAA oxidase.

It has also been shown that at least in some cases, active auxin is required for GA_3-induced symptom expression (Cleland, 1964; Kefford, 1962). However, many GA_3-stimulated responses, including stem elongation, cannot be produced by application of auxin, and Sachs (1965) states that both GA_3 and IAA are required for stem elongation, and that the primary role of gibberellin is not to increase the physiological level of auxin. Cleland (1969) states that gibberellin acts in some unknown manner in the activation of the subapical

meristem, rather than through any alteration in the auxin levels of the plant.

Paleg (1960) showed that GA_3 stimulated the synthesis of α-amylase in the aleurone layer of cereal grain. Katsumi and Fukuhara (1969) showed that GA_3-treatment promoted α-amylase activity in the first leaf sheath of the d_5 mutants of maize, and that some, at least, of the enzyme was produced in the leaf sheath itself. Chrispeels and Varner (1967) showed that GA_3 promotes the synthesis of ribonuclease, and Cohen, Leshem, and Pinsky (1969) showed that GA_3 promotes the synthesis of proteases in germinating lucerne seeds, although GA_3 is not the only factor involved in this system. Harmey and Murray (1968) demonstrated that GA_3 promoted the synthesis of peroxidase in barley endosperm. Broughton and McComb (1971) found that the GA_3-stimulated increase in levels of amylase and β-fructofuranosidase activity in pea internode paralleled the increase in growth of the internode. They also found no GA_3-stimulated increase in cellulase and pectinesterase activities, and concluded that GA_3 functions by providing more substrate for cell metabolism and wall synthesis.

Varner, Ram Chandra, and Chrispeels (1965) showed that the GA_3-induced synthesis of α-amylase was sensitive to protein synthesis inhibitors, and hence concluded that GA_3 promoted de novo synthesis of α-amylase. They also

showed that the process was sensitive to Actinomycin D, an inhibitor of DNA-dependent RNA synthesis, and suggested that GA_3 acts as a gene derepressor, in a method not explained, causing the synthesis of new messenger RNA which codes for α-amylase. Paleg (1965) states that RNA-synthesis inhibitors are not effective in preventing GA_3-promoted synthesis of α-amylase unless isolated aleurone is used, but he does not dispute that GA_3 acts at the gene level.

Carlisle, Osborne, Ellis, and Moorhouse (1963) showed that GA_3 was active in a locust moulting assay. Although the activity was small compared to that of ecdysone, the insect moulting hormone, it was suggested that the two compounds were functioning in the same manner in the assay. Since ecdysone has been shown, cytologically, to function at the gene level, this has been used to strengthen the argument of a gene derepressor function for gibberellins. Bamberger (1971) showed that GA_3 destabilises portions of native DNA, and that this occurs using physiological concentrations of GA_3. Fellenberg (1969) suggests that substances which destabilise native DNA are capable of initiating RNA synthesis, and Bamberger (1971) suggests that GA_3 acts by promoting RNA synthesis.

Ormrod and Williams (1960) demonstrated that GA_3 increased the content of RNA in clover and Broughton

(1968) demonstrated a GA_3-dependent increase in RNA in etiolated dwarf-pea seedlings. Ecklund and Moore (1968) investigated the quantitative changes in gibberellins and RNA associated with the senescence of the shoot apex in peas, and found a decrease in both endogenous gibberellins and RNA. However, they were unable to conclude that the changes in RNA level were the result of changes in GA_3 concentration. Paulson and Beevers (1970) showed that GA_3-treatment of etiolated barley leaf segments increased the level of endogenous RNA. They also showed that GA_3-treatment increased the incorporation of radioactivity from ^{32}P-orthphosphate in RNA, and concluded that GA_3 promoted the synthesis of RNA. Jarvis, Frankland, and Cherry (1968) found a similar increase in RNA synthesis in GA_3-treated hazel seeds.

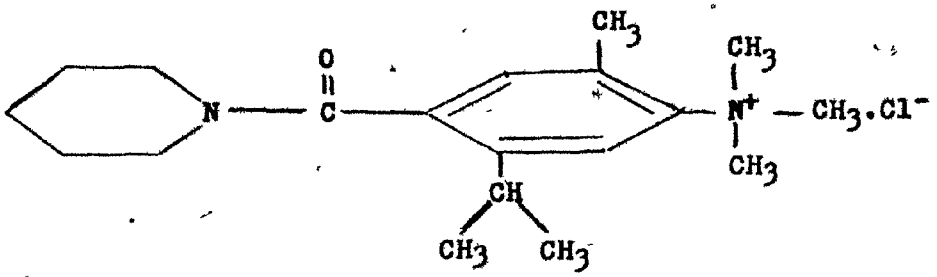
Key (1969) states that the simplest explanation of the results obtained in these experiments "is that GA_3 is involved in the regulation of the synthesis of specific RNAs (transcriptional control). These RNAs would then serve as templates for the synthesis of the proteins required for the physiological response in question. The evidence .... is at best indirect and fragmentary." Overbeek (1966) suggests that there may be several sites of primary hormonal action, some of

which might be at the translational level, while others are at the transcriptional level, and still others are not mediated by any mechanism involving DNA, RNA or protein synthesis.

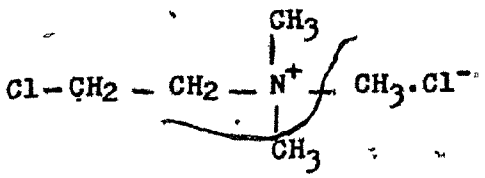
E. GROWTH RETARDANTS

The growth retardants are synthetic compounds which retard cell division and cell elongation and thus regulate, physiologically, plant height (Cathey, 1964). The more important of these compounds are AMO-1618, CCC, Phosphon D, and B9 (Lang, 1970), the chemical names and structures of which are given in Figure 1.3.

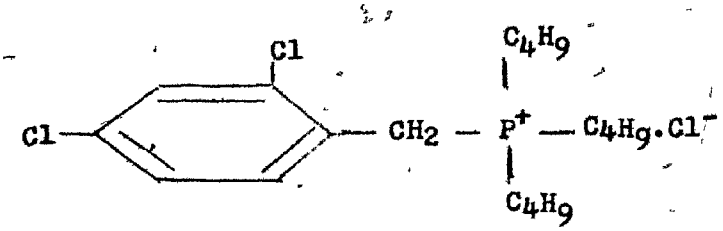
Structurally these compounds are very different, and their action is highly selective (Cathey, 1964). AMO-1618 is active in only a small number of plants whereas CCC and B9 are effective dwarfing agents in a wide range of plants (Cathey, 1964). It is impossible to predict the response of any given plant to a particular retardant since related plants may exhibit widely differing responses to the same compound. Tolbert (1960) showed that wheat was very sensitive to CCC, but subsequent work has shown that other economically important Gramineae are so much less sensitive that they can be considered unresponsive (Cathey, 1964). There appear to



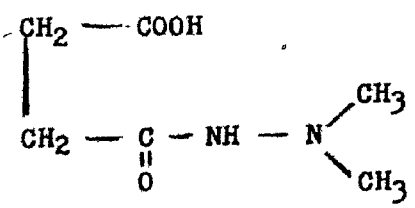
AMO-1618. 4-hydroxyl-5-isepropyl-2-methylphenyl trimethyl ammonium chloride, 1-piperidine carboxylate.



CCC. (2-chloroethyl) trimethyl ammonium chloride.



Phosphon-D. 2,4-dichlorobenzyl-tributylphosphonium cloride.



B-9. N-dimethylaminosuccinamic acid.

Figure 1.3. Chemical structures and names of growth retardants.

be considerable differences in the responsiveness of different varieties of the same species (Tolbert, 1960). Humphries (1969) states that barley does not respond to CCC, but we found that Black Hulless barley C.I. 666 was significantly retarded by this compound (Russell and Kimmins, 1972). A similarly confused picture is observed in the responses to other retardants (Cathey, 1964). Humphries (1969) suggests that the failure of a plant to respond to CCC may be because the compound does not penetrate the plant.

The picture is even more confused since some plants respond to CCC by increased growth. Monselise, Goren, and Halevy (1966) reported promotion of stem growth in lemons, and similar reports have been made for Begonia (Heide, 1969), peas (Adedipe, Ormrod, and Maurer, 1968), gladiolus (Halevy and Shilo, 1970) and barley (Humphries, 1969). Lang (1970) tentatively suggests that CCC may be broken down rapidly in these plants and thus provide additional nitrogenous material which stimulates growth nutritionally. However, most plants respond to CCC by an inhibition of internode elongation and, to a lesser degree, of leaf enlargement.

Investigation of the nature of the stunting has shown that in some cases there is an inhibition of cell elongation (Wheaton, 1960), but that in most cases the dwarfing is a result of a reduction in cell division

(Sachs, Bretz, and Lang, 1959).

The application of growth retardants produces other physiological effects. CCC and B-9 induce floral initiation in Rhododendron (Stuart, 1962) and CCC promotes the production of female flowers in cucurbitaceous plants, (Ghosh and Bose, 1970). Metabolic changes have also been observed as a response to treatment with growth retardants. Halevy (1962) demonstrated an increase in the peroxidase and catalase activities of AMO-1618-treated cucumber seedlings and Wheaton (1960) demonstrated an increase in pectin methylesterase activity in AMO-1618-treated cucumber seedlings.

Tolbert (1960) showed that the stunting effects of growth retardants are usually antagonised by the application of GA_3. Kende, Ninnemann and Lang (1963) showed that AMO-1618 and CCC inhibited the biosynthesis of GA_3 by Fusarium moniliforme without inhibiting the growth of the fungus. Zeevaart (1966) demonstrated a similar inhibition of gibberellin biosynthesis in CCC-treated higher plant. Dennis, Upper, and West (1965) showed that AMO-1618 inhibited the formation of kaurene from mevalonate by Echinocystis endosperm, and caused accumulation of geranylgeraniol. Using the same system Dennis and West (1967) showed that AMO-1618 did not inhibit the conversion of kaurene to GA_3. Cross and Myers (1969) showed that AMO-1618 and CCC both inhibit

the synthesis of kaurene and GA_3 in Fusarium moniliforme. More recently, Schechter and West (1969), using a cell free preparation of Fusarium moniliforme, showed that AMO-1618 and CCC inhibited a single reaction in the biosynthetic pathway, namely the conversion of geranyl-geranyl pyrophosphate to copalyl pyrophosphate. Robinson and West (1970) found the same results using a cell free preparation of castor bean seedlings. Phosphon D was shown to inhibit this step, and the subsequent conversion of copalyl pyrophosphate to kaurene.

While it is established that AMO-1618, CCC, and Phosphon D are capable of selective inhibition of gibberellin biosynthesis it is not suggested that this is the only role for these compounds (Harada and Lang, 1965; Baldev, Agatep, and Lang, 1965). However, in the many cases where the retardant effects are reversed by the application of exogenous GA_3, the effects of the retardant have been attributed to an inhibition of gibberellin biosynthesis.

MATERIALS AND METHODS

A. CARE OF APHIDS AND GROWTH OF PLANTS

A virus-free clone of cherry-oat aphids (Rhopalosiphum padi L.) was established by transferring one adult to an isolated barley leaf for 24 h. The new born nymphs were collected and transferred to a fresh barley plant and allowed to develop there. This procedure was repeated until aphids were obtained which produced no symptoms on barley plants after a five day infestation period. Healthy aphids were maintained on Herta barley (Hordeum vulgare L. var Herta) grown in steam sterilised soil in well-drained eight-inch plastic pots, watered daily with tap water. The pots were kept in a cage at 68°C (± 2°C) 30" below four 48" Sylvania 40 W cool white fluorescent strip lights. Every week a fresh pot of barley was introduced into the cage and the aphids allowed to transfer to it. Regular inspection was made for any parasitised aphids so that these could be removed, however none appeared during the period of the investigation.

Aphids were infected with barley yellow dwarf virus (BYDV) by a three-day inoculation feeding period on Black Hulless barley (Hordeum vulgare L. var C.I. 666)

infected with a moderately severe strain of the virus. The aphids were given an inoculation feeding period on either a caged intact plant, or an isolated leaf supported in a test-tube with its basal end immersed in water.

Black Hulless barley plants grown individually in steam sterilised soil in three-inch pots were used as the test plants, except where otherwise mentioned. The plants were maintained at 18°C in a growth chamber with a 12 h period of 2,500 ft C of mixed incandescent and fluorescent illumination per 24 h. Plants to be inoculated were caged with four adult aphids on the eighth day after planting, and control plants were caged similarly, without aphids. After three days all plants were sprayed with 0.25% malathion and returned to the growth chamber until required.

B. SELECTION OF PLANTS FOR EXPERIMENTS

Seven days after seeds were sown plants were chosen for experimental use on the basis of the height of the unexpanded first leaf blade above the coleoptile tip. This was generally between 60 mm and 45 mm. Plants were measured and assigned into pairs, triplets, or the correct number for the particular experiment

to be done. One plant was then selected randomly from each group for a particular treatment. In this way the mean height of the plants undergoing all treatments was the same as that of the controls.

C. DETERMINATION OF DRY WEIGHT

The material was cut into small pieces and heated to constant weight at 98°C (± 1°C) in a hot air oven.

D. DETERMINATION OF CELL NUMBER

Cells were counted according to the method of Brown and Rickless (1949). The third leaf blade was excised, weighed, and macerated in 10 ml of 5% chromic acid (CrO_3) for three days at room temperature. The macerate was forced through a pasteur pipette several times and then mixed on a Vortex mixer for two minutes. Ten samples of each homogenate were counted using a haemocytometer. The remainder of the plant was used to determine dry matter from which the dry weight of the leaf was calculated.

E. EXTRACTION OF AUXINS

Endogenous auxins were extracted from frozen material in two different procedures using different solvents.

(a) Equal weights of aerial parts of healthy plants, plants infested with healthy aphids, and infected plants were extracted for 1 h at 0°C with ether, using 5 ml per g fresh weight. The ether was purified according to the method of Larsen (1956) and redistilled immediately before use. The ether was decanted and the residue extracted for a further 1 h with an equal volume of ether. The combined ether fractions were reduced to 20 ml under vacuum, at a temperature not exceeding 40°C, and were then extracted three times with an equal volume of 1 M $NaHCO_3$. The combined ether layers were evaporated to dryness, at a temperature not exceeding 40°C, and the residue assayed as neutral auxins.

The aqueous layers were adjusted to pH 3.0 with 5 N H_3PO_4 and extracted three times with an equal volume of ether. The combined ether layers were evaporated to dryness, as before, and assayed as free acidic auxins.

The plant material left after the ether extractions was treated at 100°C for 1 hr with N KOH, using 2.5 ml per g fresh weight. The mixture was cooled

to room temperature, adjusted to pH 3.0 with 5 N HCl and extracted twice with an equal volume of ether at 0°C for 1 h. The ether layers were partitioned with alkali as before, adjusted to pH 3.0 and re-extracted with ether. The ether fractions were evaporated to dryness as before and assayed as bound auxins. A flow sheet of the procedure is given in Figure 2.1.

(b) The second extraction procedure for auxins was that of Baillis and Wilson (1967). Equal weights of the aerial parts of healthy plants and plants infested with BYDV-infected aphids were frozen at -15°C for 8 h. The tissue was then homogenised in a Waring blender, at slow speed, for two minutes with 80% methanol, using 10 ml per g fresh weight. The mixture was left at 3°C for 16 h and then filtered. The residue was reextracted with an equal volume of 80% methanol for a further 6 h. The combined filtrates were reduced to the aqueous phase on a rotary evaporator, at temperature not exceeding 40°C, and left for 18 h at 3°C. The aqueous extract was filtered through celite, adjusted to pH 3.0 with 5 N HCl and extracted four times with an equal volume of freshly redistilled ethyl acetate. The extract was reduced to half bulk, under vacuum, and extracted four times with half volume 1% $NaHCO_3$. The aqueous fractions were combined, adjusted to pH 3.0 with 5 N HCl and

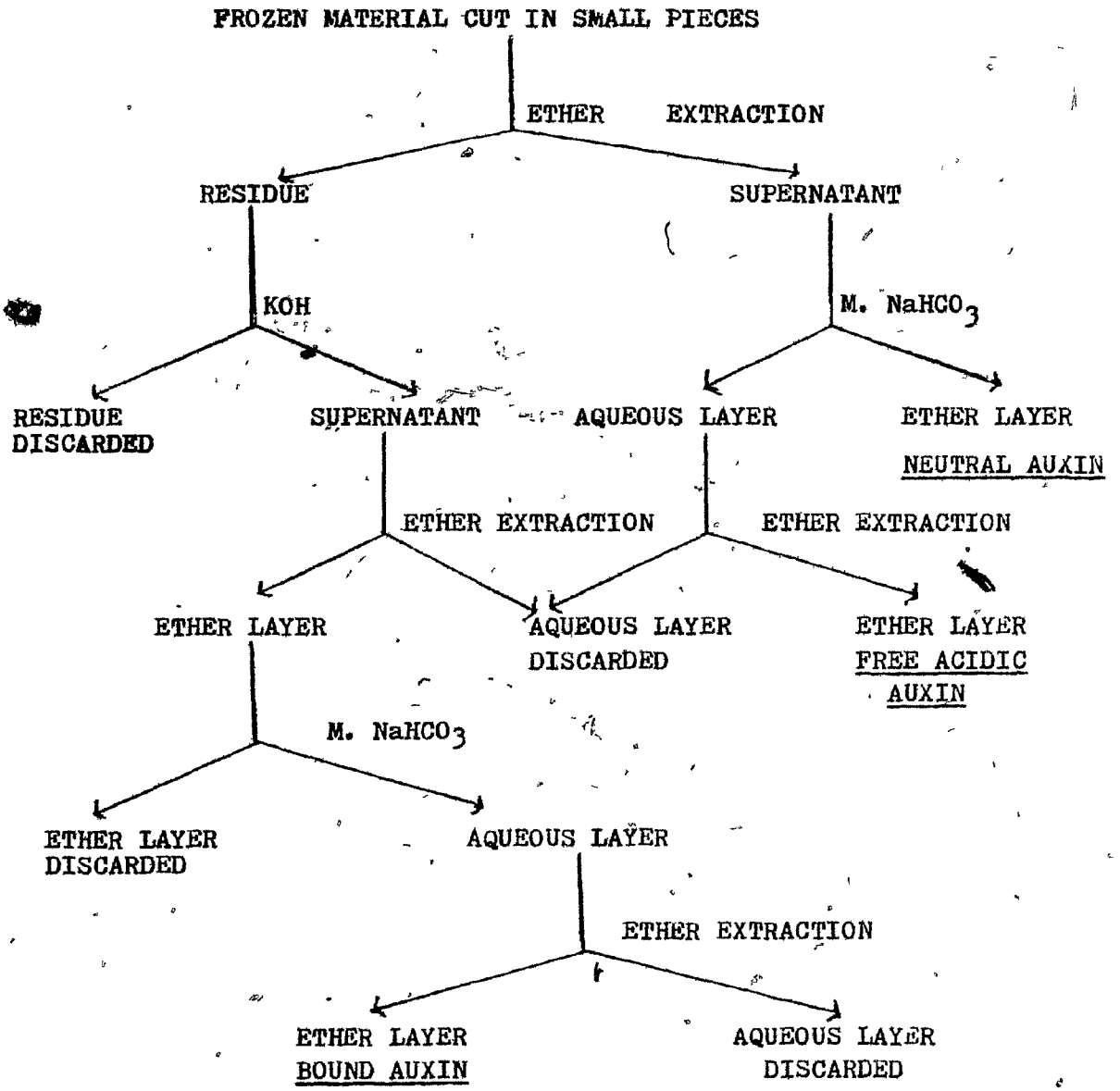


FIGURE 2.1. EXTRACTION PROCEDURE (a) FOR AUXINS

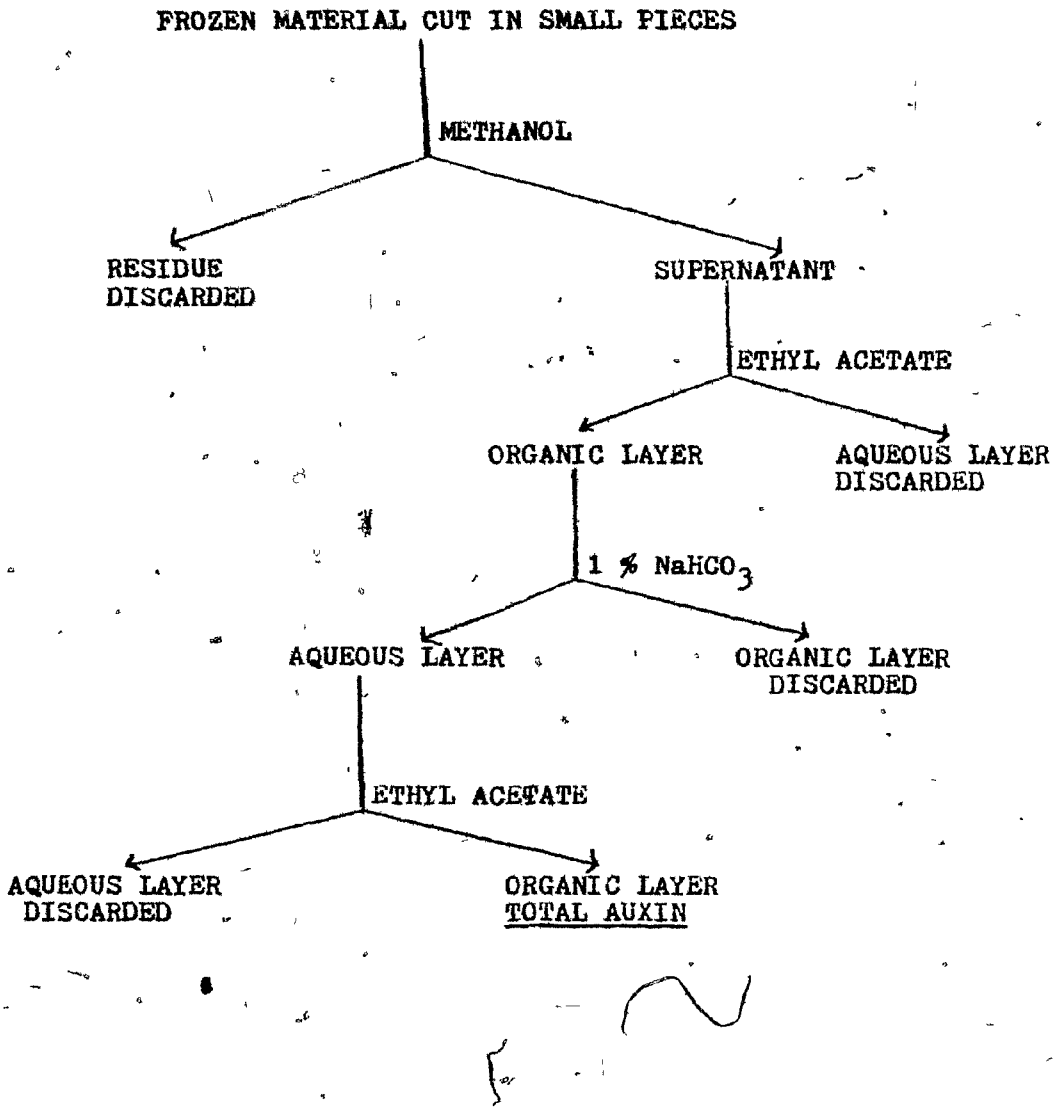


FIGURE 2.2. EXTRACTION PROCEDURE (b) FOR AUXINS

reextracted four times with half volume of ethyl acetate. The ethyl acetate extracts were reduced to dryness, as before. No fractionation of the extract was performed and the single extract was assayed as total auxins. A flow sheet of the procedure is given in Figure 2.2.

F. EXTRACTION OF GIBBERELLINS

Endogenous gibberellins were extracted from frozen material according to the second extraction procedure used for auxins, except that the pH was adjusted to 2.5 prior to extraction with ethyl acetate. A blank extraction was performed simultaneously in all experiments.

G. CHROMATOGRAPHY OF AUXINS

All residues from the extraction procedure were dissolved in 300 µl of redistilled ethyl acetate and streaked on prepoured plates of silica gel G.F. (E. Merck, Darmstadt). The solvent system was isopropanol:28% ammonia:water (8:1:1) and chromatograms were developed at room temperature in the dark. The solvent front was allowed to travel 10 cm from the line of application.

A marker spot of indole-3-acetic acid (Sigma Chemical Co.) was included on chromatograms of free and bound auxins and a marker spot of indole-3-acetonitrile (Sigma Chemical Co.) was used with neutral auxins. These were detected as areas of UV absorbance against the fluorescent background of the plates, using UV illumination from a Harmer chromalite with Woods filter (254 nm).

H. CHROMATOGRAPHY OF GIBBERELLINS

The residue obtained from the extraction procedure was dissolved in 200 µl of redistilled ethyl acetate and chromatographed on prepoured plates of silica gel G.F. using isopropanol:water (4:1 v/v) as the solvent system. The chromatograms were developed at room temperature and the solvent front allowed to travel 10 cm from the point of application. A reference spot of GA_3 (Sigma Chemical Co.) was included and detected by spraying with concentrated sulphuric acid:ethanol (5:95 v/v) and heating at 110°C for 10 minutes. Authentic GA_3 was visualised as a grey-green spot.

I. BIOASSAY OF AUXINS

Auxin activity in each cm of the chromatograms was assayed by the method of Nitsch and Nitsch (1956) using Genesee wheat seedlings with coleoptiles between 20 and 25 mm long. Four mm sections of coleoptile were cut three mm from the tip and starved by floating in distilled water for three hours. Auxin standards were used with each bioassay, the IAA dissolved in ethanol and diluted to the required volume with 0.01 M citrate-phosphate buffer pH 5.0 containing 2% sucrose. Material from the chromatograms was similarly assayed in the same sucrose-containing buffer. A minimum of ten coleoptile sections was used to test the activity of each cm of the chromatographic plate, and a control was included to ascertain the lack of growth promotion in the material of the thin layer. The final length of the coleoptile sections was determined using a binoccular microscope fitted with a micrometer eye piece after incubating for 24 h in the dark at 28°C.

J. BIOASSAY OF GIBBERELLINS

The gibberellin activity of each cm of the unsprayed portion of the chromatographic plate was assayed using the lettuce hypocotyl bioassay of Frankland

and Wareing (1960). Lettuce seeds (var. Grand Rapids) were germinated under constant light at 28°C and seedlings used when the radicles were 5 mm long. GA_3 standards were used with each bioassay and a minimum of ten seedlings was used to determine the activity of each cm of the plate. A control was used to ascertain the lack of growth promotion in the material of the thin layer. The final length of the lettuce hypocotyl was determined to the nearest mm after incubating the seedlings at 28°C for a minimum of 40 h.

K. APPLICATION OF EXOGENOUS GIBBERELLIC ACID

GA_3 was applied to plants at a concentration of 10 ppm in an aqueous solution containing 0.05% polyoxyethylene sorbitan monooleate (TWEEN 20). Control plants were sprayed with water containing Tween 20. Plants were sprayed with an atomiser and approximately 0.75 ml was applied to each plant.

L. APPLICATION OF GROWTH RETARDANTS

Plants were treated with growth retardants in two different ways.

(a) The plants were sprayed with an aqueous solution containing an appropriate concentration of the retardant and 0.05% Tween 20. Control plants were sprayed with water containing 0.05% Tween 20. All plants received approximately 0.75 ml.

(b) The retardant in suitable concentration in an aqueous solution was added to the soil around the roots of the plant using a pipette. The volume used is indicated in the appropriate experiments. Control plants were similarly treated with water.

M. ASSAY OF ENDOGENOUS ANTIGIBBERELLIN ACTIVITY

The aerial parts of virus-infected plants were weighed, cut into small pieces, and homogenised at high speed in a Virtis "23" blender for one minute with 2 ml per g fresh weight of 0.01 M phosphate buffer, pH 7.0. The homogenate was strained through triple thickness cheesecloth. A similar extract was prepared from control plants.

Two pieces of Whatman no. 1 filter paper were placed in a 6 cm petri dish containing a 1.5 ml standard solution of GA_3, or water, and 0.5 ml of strained plant extract. A minimum of ten lettuce seedlings, as used

in the bioassay of GA_3, was added to each petri dish. The final length of lettuce hypocotyl was measured as in the GA_3 bioassay.

RESULTS

A. GROWTH AND DEVELOPMENT OF BARLEY C.I. 666

In order to obtain data on the growth and development of barley C.I. 666 100 seeds were planted and the seedlings were measured weekly (Fig. 3.1). Under the conditions described in the previous section the first leaf blade was open and measurable two weeks after planting and subsequent measurements showed that the leaf blade opened at its fully expanded size. A new leaf blade opened, fully expanded, at weekly intervals thereafter. The fresh weight and dry weight of a small sample of seedlings were also determined weekly (Fig. 3.2) and both were found to increase in approximately linear fashion after three weeks. The dry matter was relatively constant, at nine percent of the fresh weight, at all times.

B. GROWTH AND DEVELOPMENT OF BYDV-INFECTED BARLEY SEEDLINGS

The stunting effect of BYDV was first observed in the third leaf blade of infected seedlings before any yellowing occurred (Plate 3.1, Fig. 3.3). At this

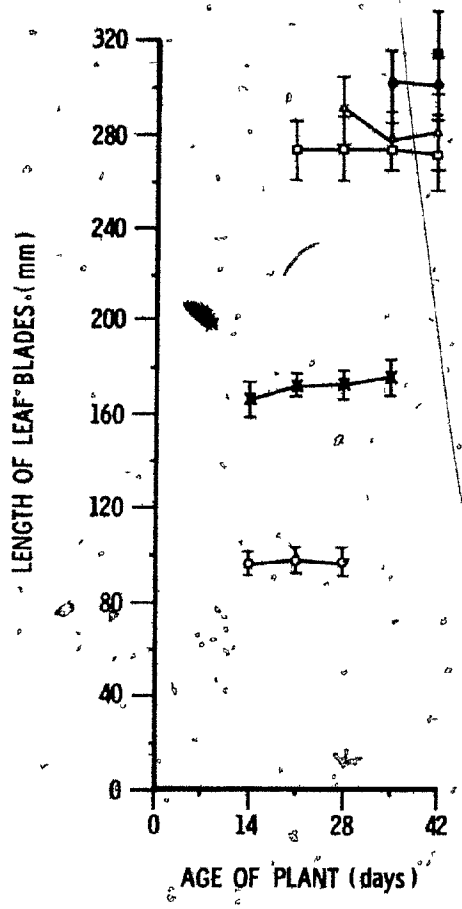


Figure 3.1. The development of barley C.I. 666.

first leaf blade
second leaf blade
third leaf blade
fourth leaf blade
fifth leaf blade
sixth leaf blade

vertical line indicate the 1 per cent fiducial limits

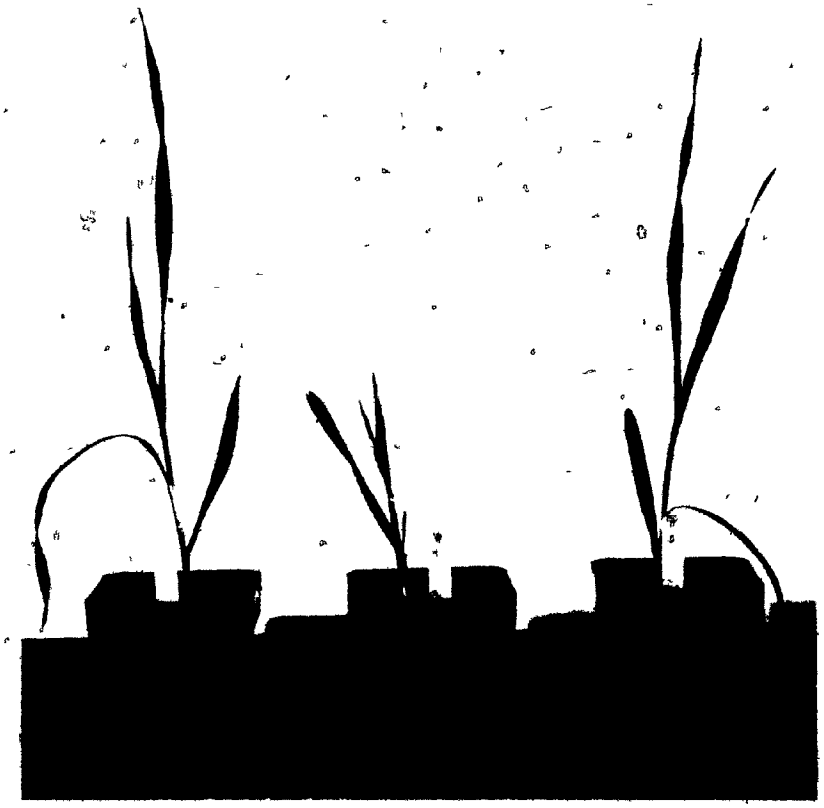


Plate 3.1. 24 day old barley seedlings (X $\frac{1}{3}$)

left, 15 days after infestation with healthy aphids.
centre, 15 days after infestation with BYDV-infected aphids.
right, control plant.

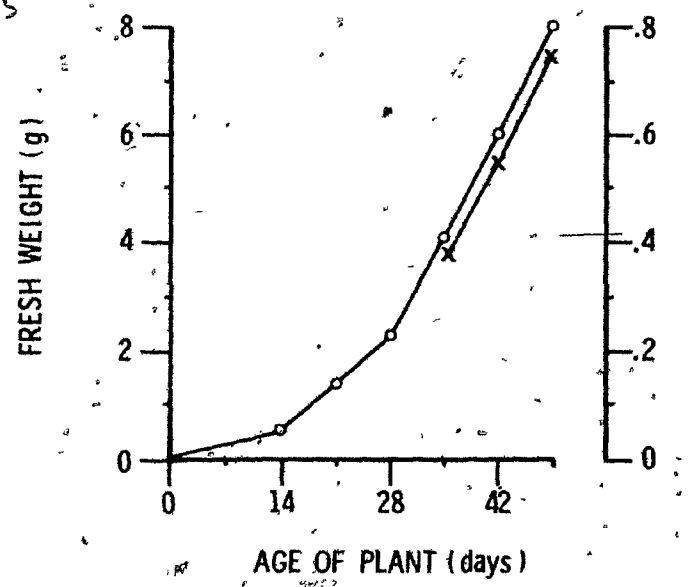


Figure 3.2: The fresh and dry weight of barley C.I. 666.

○——○ fresh weight

X——X dry weight

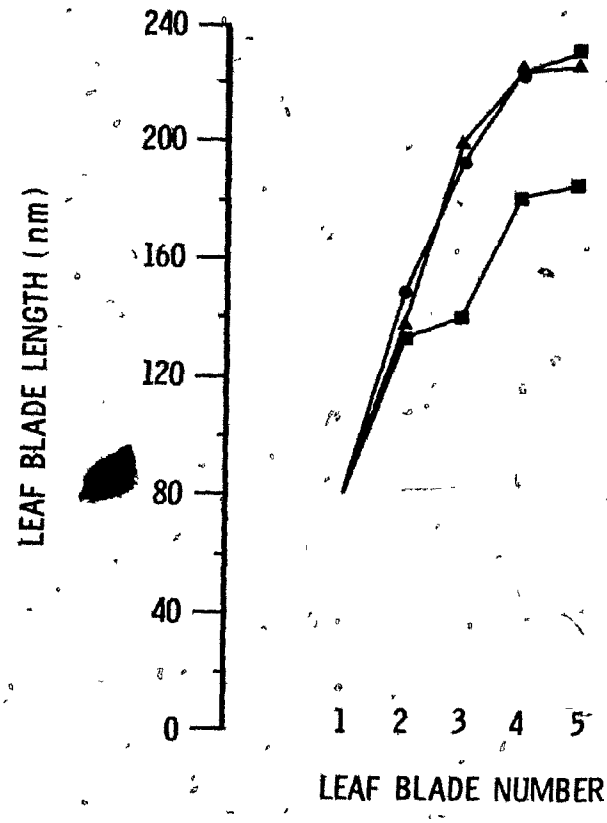


Figure 3.3. The length of barley leaves.

●——● healthy plants
▲——▲ plants infested with healthy aphids
■——■ plants infested with infected aphids.

Leaf blades #3, #4, #5 of plants infested with infected aphids were significantly shorter, at the 1% level, than corresponding leaf blades of healthy plants and plants infested with healthy aphids.

stage the fresh weight of the infected plant was significantly lower than that of control plants, and the percentage dry matter greater (Table 3.1). Healthy aphids were observed to have no effect on the size of the plants, fresh weight or dry weight.

TABLE 3.1. The effect of BYDV on fresh weight, dry weight and percentage dry matter of barley C.I. 666.

	C	HA	IA
Fresh weight (g)	0.649	0.710	0.468*
Dry weight (g)	0.08	0.08	0.054*
Percentage dry weight	11.1	11.1	12.8

C , healthy plants
HA, plants infested with healthy aphids
IA, plants infested with infected aphids
* , significantly different from HA and C at 1% level.

C. EFFECT OF BYDV-INFECTION ON ENDOGENOUS GIBBERELLINS

Plants were assayed 11 and 17 days after infestation with aphids and gibberellin-like activity was found at Rf 0.6-0.8 in all plant extracts. This corresponded to the position of the authentic GA_3

marker. No growth promoting activity was found in the material obtained from the blank extraction and no difference was found between the extracts obtained from 20 and 26 day old plants (Fig. 3.4). The chromatographic distribution of gibberellin-like activity was the same in extracts from plants infested with healthy aphids and those from uninfested plants. In extracts from infected plants, the distribution was qualitatively similar but significantly less activity was present.

The growth of lettuce seedlings at varying concentrations of authentic GA_3 was determined simultaneously with the bioassay of plant extracts, and a standard curve was constructed (Fig. 3.5). Using this standard curve it was possible to calculate the growth stimulant concentration, as GA_3 equivalents, in healthy and infected plant extracts. This was done using only the Rf values which caused growth promotion which was significantly higher, at the 1% level, than that caused by water controls (Table 3.2).

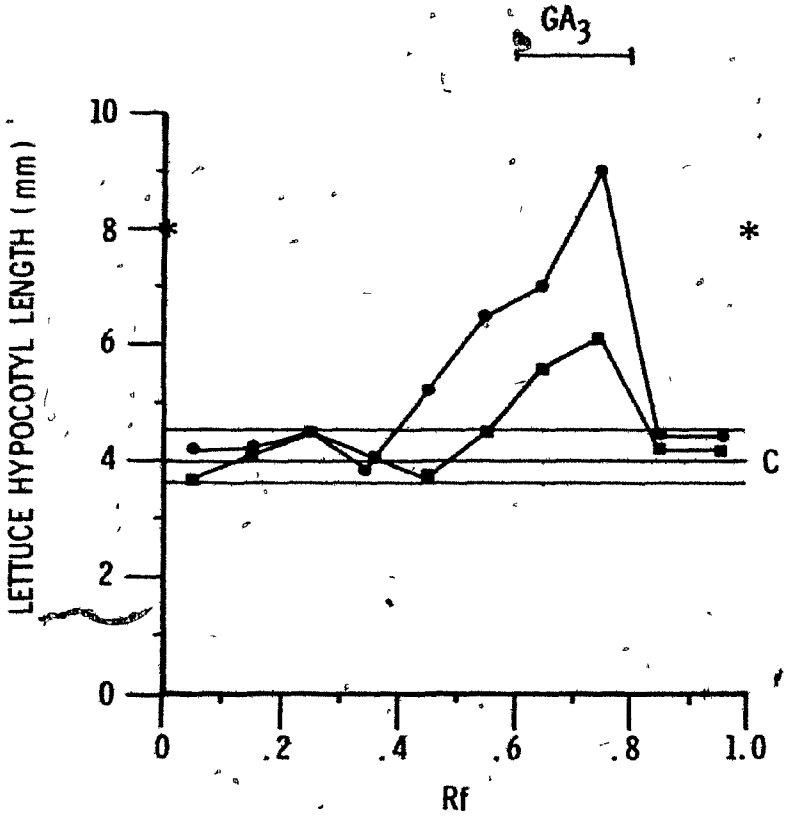


Figure 3.4. The lettuce hypocotyl bioassay of extracts of 38 g fresh weight of barley.

●——● healthy plants

■——■ BYDV-infected plants

* response of hypocotyls to 10^{-1} mg/L GA_3.

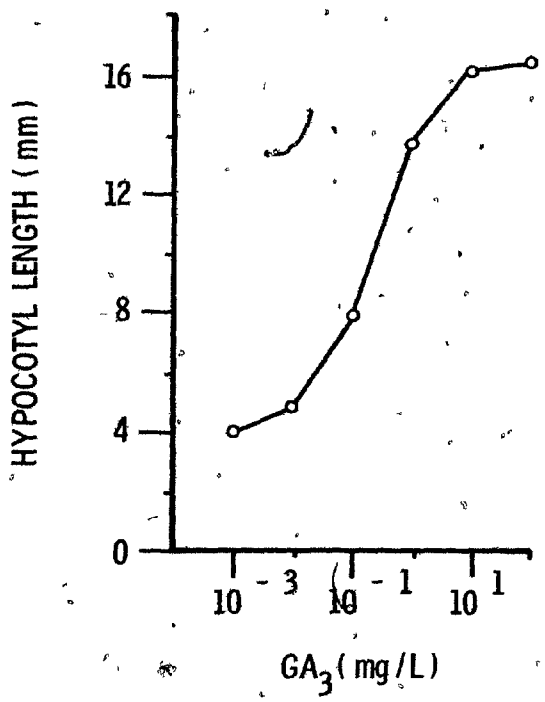


Figure 3.5. Relationship between lettuce hypocotyl length and GA_3 concentration, determined simultaneously with the bioassay of plant extracts.

TABLE 3.2. The growth stimulant concentration of healthy and BYDV-infected plant extracts.

	IA	C
GA_3 equivalents (μg/Kg dry wt.)	58	345

C, healthy plants; IA, BYDV-infected plants

It was not possible to compare the endogenous gibberellin-like activity of plants before the onset of dwarfing, so that no causal relationship can be inferred. However, a reduction in endogenous gibberellins was clearly associated with BYDV-induced dwarfing.

D. EFFECT OF BYDV-INFECTION ON ENDOGENOUS AUXINS

Auxins were extracted 20 and 30 days after infestation with aphids and the ether extract fractionated, as described in the previous section, into free acid auxins, neutral auxins and bound auxins. Bioassay of the neutral auxin fraction revealed no growth promotion, from which it was concluded that neither healthy barley nor BYDV-infected barley contain neutral auxins (Fig. 3.6). In both the free acid fraction and the bound acid extract growth promotion was detected at Rf 0.4-0.6,

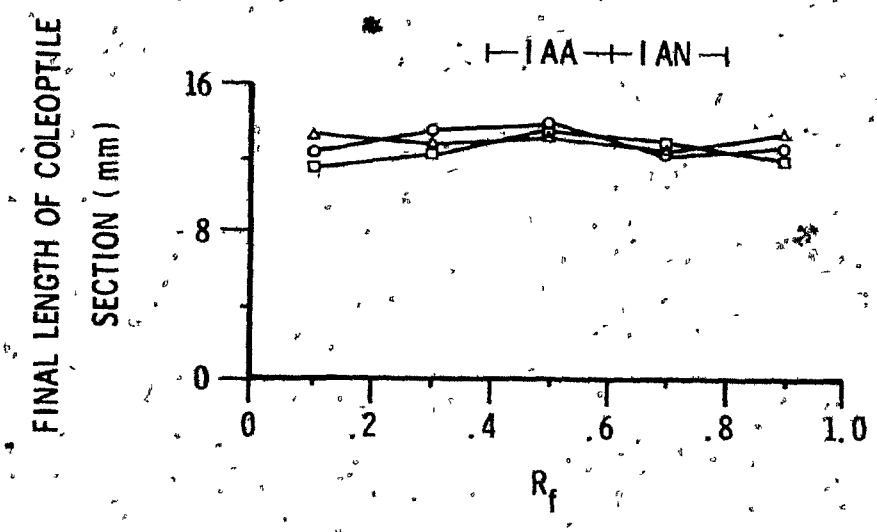


Figure 3.6. Genesee wheat coleoptile straight growth bioassay of neutral fraction of ether extract of 40 g fresh weight of barley.

○——○ healthy plants

□——□ plants infested with healthy aphids

△——△ BYDV-infected plants.

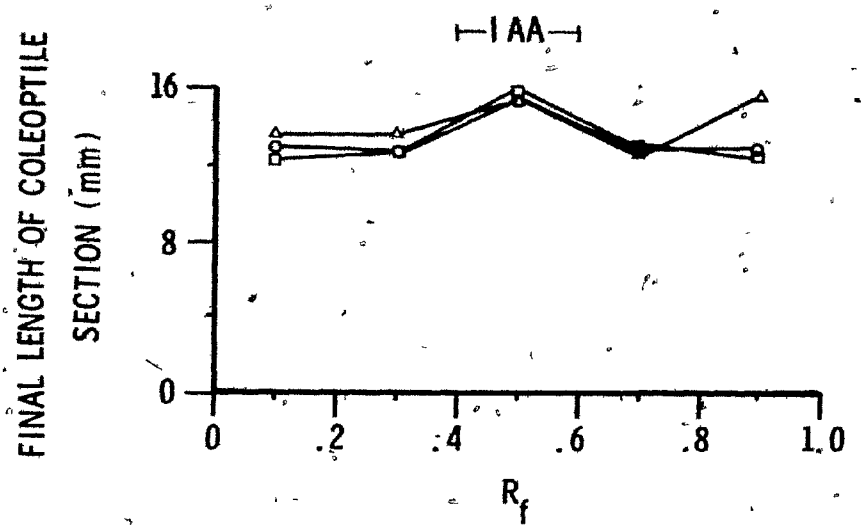


Figure 3.7. Genesee wheat coleoptile straight growth bioassay of free acid fraction of ether extract of 40 g fresh weight of barley.

○—○ healthy plants

□- -□ plants infested with healthy aphids

△——△ BYDV-infected plants.

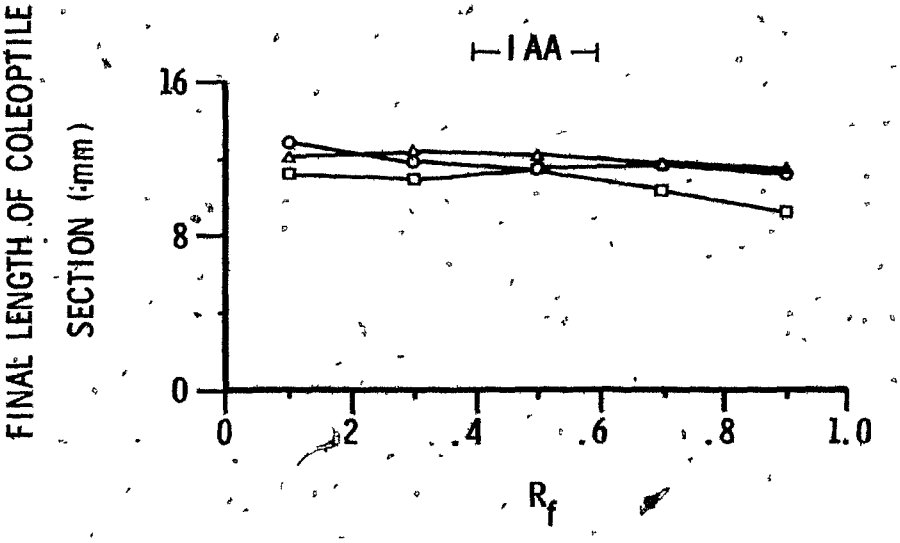


Figure 3.8. Genotee wheat coleoptile straight growth bioassay of bound acid fraction of ether extract of 40 g fresh weight of barley.

○——○ healthy plants

□——□ plants infested with healthy aphids

△——△ BYDV-infected plants.

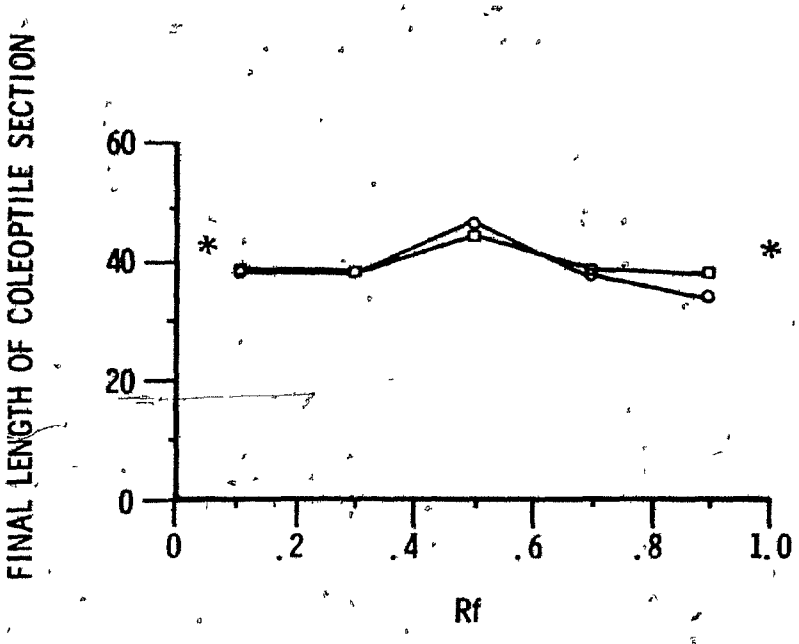


Figure 3.9. Genesee wheat coleoptile straight growth bioassay of ethyl acetate extracts of 40 g fresh weight of barley.

○——○ healthy plants

□——□ BYDV-infected plants

* response of coleoptiles to 10 ug/L/AA

corresponding to the Rf of the IAA marker. The chromatographic distribution of the auxin-like activity was the same in extracts from healthy plants, plants infested with healthy aphids, and plants infested with BYDV-infected aphids, and no quantitative difference was observable (Fig. 3.7 and Fig. 3.8). The auxin-like activity extracted was very low, and for this reason a second extraction procedure was attempted, using ethyl acetate. In this case considerably greater auxin-like growth promotion was obtained, but there was no significant difference between the growth promotion obtained from healthy and infected plant extracts (Fig. 3.9).

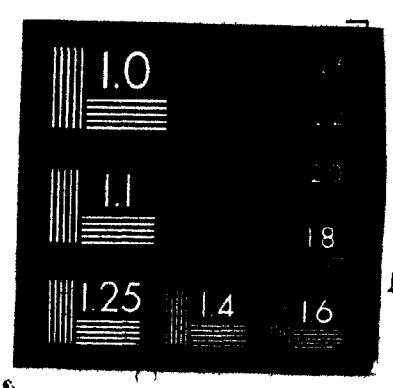
Since there was no significant decrease in extractable auxins from infected plant it was concluded that BYDV-induced dwarfing of barley is not mediated by reduced levels of endogenous auxins.

E. EFFECT OF BYDV-INFECTION ON CELL NUMBER

The third leaf blade of BYDV-infected barley plants is fully expanded before any yellowing symptoms develop on the plant, and this provides a suitable system for the investigation of cell number. In order to determine the relationship between the number of

2 2

OF/DE



cells present and the dimensions of the leaf blade a preliminary experiment was done using only healthy plants. From these results it was observed that the number of cells is directly proportional to the fresh weight of the leaf blade, Figure 3.10. No similar relationship was observed between the number of cells and leaf blade length.

The number of cells in healthy and BYDV-infected third leaf blades was determined by the same procedure and the results of one such experiment are shown in Figure 3.11. The total number of cells in BYDV-infected third leaf blades was significantly reduced indicating diminished meristematic activity. There was no significant difference in the number of cells per unit fresh weight or dry weight, implying that the cell dimensions were not significantly altered.

F. MECHANISMS OF REDUCTION OF ENDOGENOUS GIBBERELLIN LEVELS

If BYDV-induced dwarfing of barley results primarily from a reduction in endogenous gibberellin levels there are two possible mechanisms by which this may be effected. The synthesis of gibberellins may be depressed, and their removal may be stimulated. These

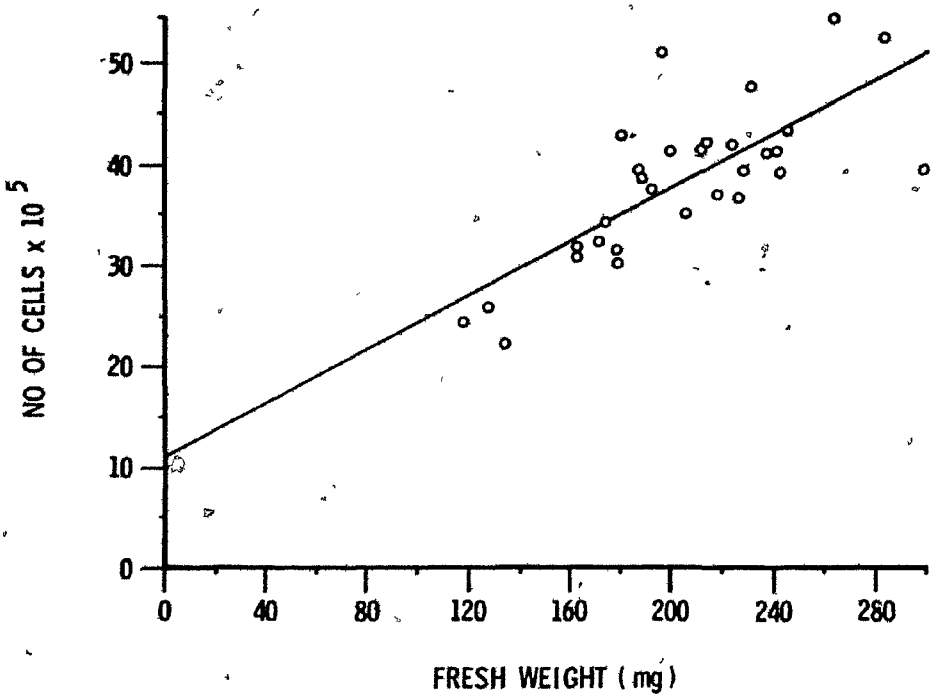


Figure 3.10. The relationship between the mean number of cells in the third leaf blade of healthy barley plants and the fresh weight of the leaf blade.

The correlation coefficient of the 1° regression line is .77243, ($n = 28$), $P = 1\%$.

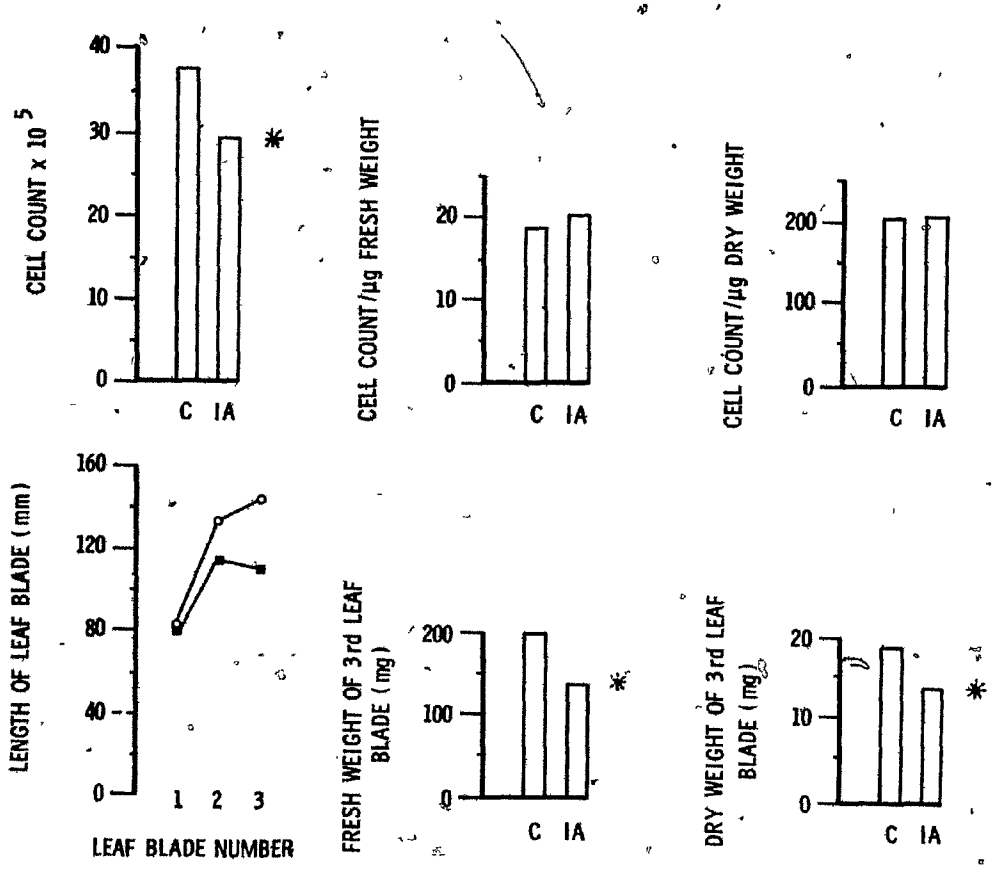


Figure 3.11. Effect of BYDV-infection on mean number of cells in third leaf blade of barley.

○——○ , IA, BYDV-infected plants

■——■ , C , healthy plants

Each value is the mean obtained using 31 plants.

*Significantly different from C at 1 per cent level.

mechanisms could function independently or together. It was decided to investigate these two possibilities.

G. EFFECT OF BYDV-INFECTION ON ENDOGENOUS LEVELS OF GIBBERELLIN-DESTROYING ACTIVITY

While a great deal of detailed knowledge exists on the biosynthesis of gibberellins in lower and higher plants there appears to be little information on the biodegradation of these compounds. It has been shown, however, that higher levels of extractable gibberellins exist in young leaves than in older leaves (Radley, 1958) and it does not seem unreasonable to suggest that there exists, in those plants which contain gibberellins, a normal mechanism for their inactivation, or removal. Any increased activity in this normal metabolic pathway would result in a lowering of the endogenous gibberellin concentration. There are several possible mechanisms whereby virus infection could influence the activity of a normal metabolic pathway, and these are discussed in aother section of this thesis. It is also theoretically possible for virus infection to promote new metabolic pathways within a host plant, and lowered levels of endogenous gibberellins could be obtained by the activity of a virus-induced gibberellin-degradative

pathway. The mechanisms by which new metabolic pathways may be produced in a host plant by virus infection are discussed elsewhere in this thesis. In the present section the attempt is only to show that virus infection could produce diminished levels of gibberellins in barley by promoting the breakdown or removal of gibberellin, and that this could be brought about in several different ways. However, if BYDV does promote this type of activity then it should be possible to demonstrate the presence of more gibberellin-destroying activity in BYDV-infected barley than in healthy plants, and this demonstration should be independent of the pathways involved.

Preliminary experimentation was done using healthy plants only. An extract of the aerial parts of the plants was made using 2 ml of 0.01M pH 7.0 phosphate buffer per g fresh weight, as described in the previous section. Serial dilutions of the strained extract were prepared using the same phosphate buffer. A standard GA_3 bioassay was performed, as described in the previous section, adding 1 ml of either buffer, plant extract or diluted plant extract to the petri dishes. The final length of the lettuce hypocotyl was shown to be dependent on the concentration of plant extract (Figure 3.12). These results confirm the presence of an endogenous antigibberellin system in

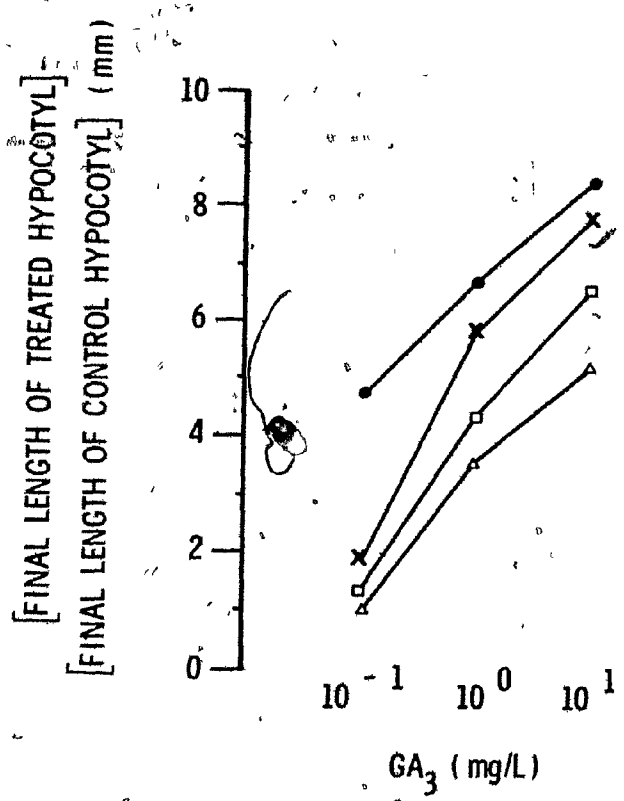


Figure 3.12. Relationship between lettuce hypocotyl length and GA_3 in the presence of healthy plant extract.

●——● in presence of 1 ml buffer
X---X in presence of 1 ml 1/100 plant extract
□——□ in presence of 1 ml 1/10 plant extract
△——△ in presence of 1 ml undiluted plant extract.

barley. Similar results were obtained using plants which were 10 days old, 21 days old, and 36 days old.

Since similar results were obtained with all three concentrations of GA_3 it was decided to assay plants in the presence of 10 mg GA_3 per litre and to express the results in terms of percentage reduction in hypocotyl length caused by the extracts.

BYDV-infected and healthy plants were used when 20 days old. At this stage dwarfing was pronounced in BYDV-infected plants but no difference was found between extracts, Figure 3.13. These results were obtained from three separate experiments, so that different degrees of dwarfing were observed as a result of minor variations in physical conditions. Similarly, the percentage reduction in hypocotyl length varied with each experiment due to slight differences in maturity of the lettuce seedlings used in the bioassay. However there was never any significant difference between healthy and infected plant extracts assayed simultaneously.

It was concluded that endogenous antigibberellin activity was not involved in BYDV-induced dwarfing of barley.

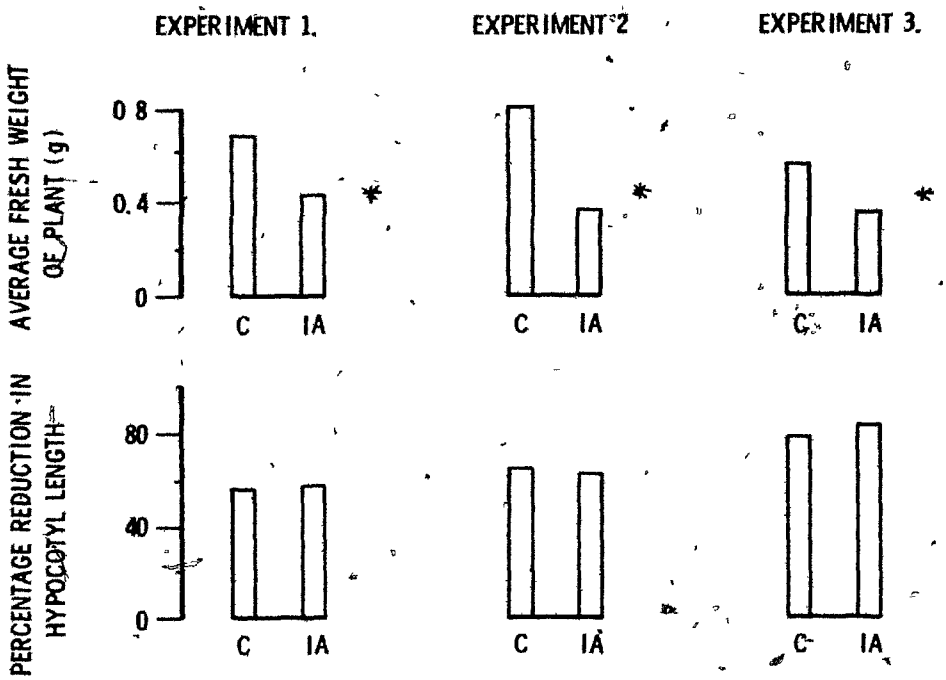


Figure 3.13. Effect of BYDV-infection on endogenous antigibberellin activity of barley plant extracts.

C, healthy plant extract
IA, BYDV-infected plant extract
Each plant extract prepared from a minimum of 6 plants.
*Significantly different at the 1 per cent level.

H. EFFECT OF GROWTH RETARDANTS ON THE GROWTH OF BARLEY PLANTS

The synthesis of gibberellins was investigated using antigibberellins. Since these compounds are highly specific and the sensitivity of any cultivar of a plant to a particular antigibberellin cannot be predicted, a preliminary experiment was performed to find if any antigibberellin was effective in dwarfing barley C.I. 666. The growth retardants were applied by spraying, in the concentrations given in Table 3.3, to seven day old seedlings. The leaf blades were measured weekly and dwarfing was first observed in the third leaf blade in plants treated with CCC. No effect was observed in plants treated with AMO 1618, and only CCC was used in subsequent experiments.

Plants sprayed with CCC exhibited some chlorosis near the base of the leaf blade and the experiment was repeated, applying CCC as a root drench. This method of treatment prevented chlorosis, and the results were otherwise unaltered. In all subsequent experiments CCC was applied as a root drench.

The dwarfing was found to be dependent on the amount of CCC applied, Figure 3.14, Plate 3.2, and directly proportional to the logarithm of amount of CCC applied, Figure 3.15. This was convincing evidence that

TABLE 3.3. The effect of growth retardants CCC and AMO 1618 on leaf blade length, fresh weight, and percentage dry matter of barley C.I. 666.

	AMO 1618	C	CCC
length of 3rd leaf blade (mm)	221	215	171*
fresh weight (g)	-	3.03	2.71+
% dry weight	-	10.5	9.7

C, plants sprayed with 0.75 ml water containing 0.05% TWEEN 20.

CCC, plants sprayed with 0.75 ml solution of CCC, 3 mg/ml, containing 0.05% TWEEN 20.

AMO 1618, plants sprayed with 0.75 ml solution of AMO 1618, 10^{-3} mg/L, containing 0.05% TWEEN 20.

*CCC significantly different from C at 1% level.

+CCC significantly different from C at 5% level.

the response was directly due to the growth retardant and not to any physical effects of the method of application.

I. EFFECT OF CCC ON CELL NUMBER

Eight day old seedlings were treated with 3 mg CCC, applied as a root drench, and the number of cells in the third leaf blade of treated plants was found to be significantly lower than in control plants, Figure 3.16.

There was a significant increase in the number of cells per unit fresh weight in treated plants, implying that these cells were smaller than in untreated plants. It was concluded that CCC inhibits cell division and cell expansion in barley.

J. EFFECT OF CCC ON ENDOGENOUS GIBBERELLINS

6 mg CCC was applied as a root drench to 8 day old seedlings and plants were assayed for endogenous gibberellins 17 days after treatment. Gibberellin-like activity was found at the Rf corresponding to the authentic GA_3 marker and no growth promoting activity

Plate 3.2. The effect of CCC dosage on barley seedlings (X½)

A - untreated plant
B - 10^{2} mg CCC applied in 1 ml solution
C - 10 mg CCC applied in 1 ml solution
D - 10^{0} mg CCC applied in 1 ml solution
E - 10^{-1} mg CCC applied in 1 ml solution.

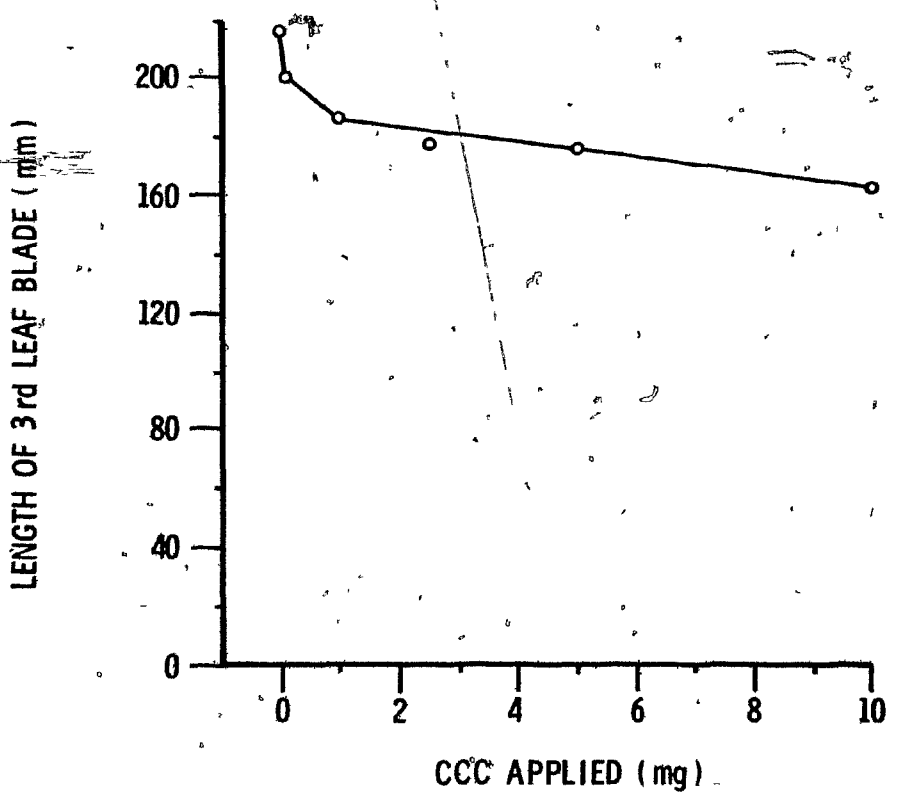


Figure 3.14. Relationship between length of third leaf blade and amount of CCC applied, as a root drench, in 1 ml solution.

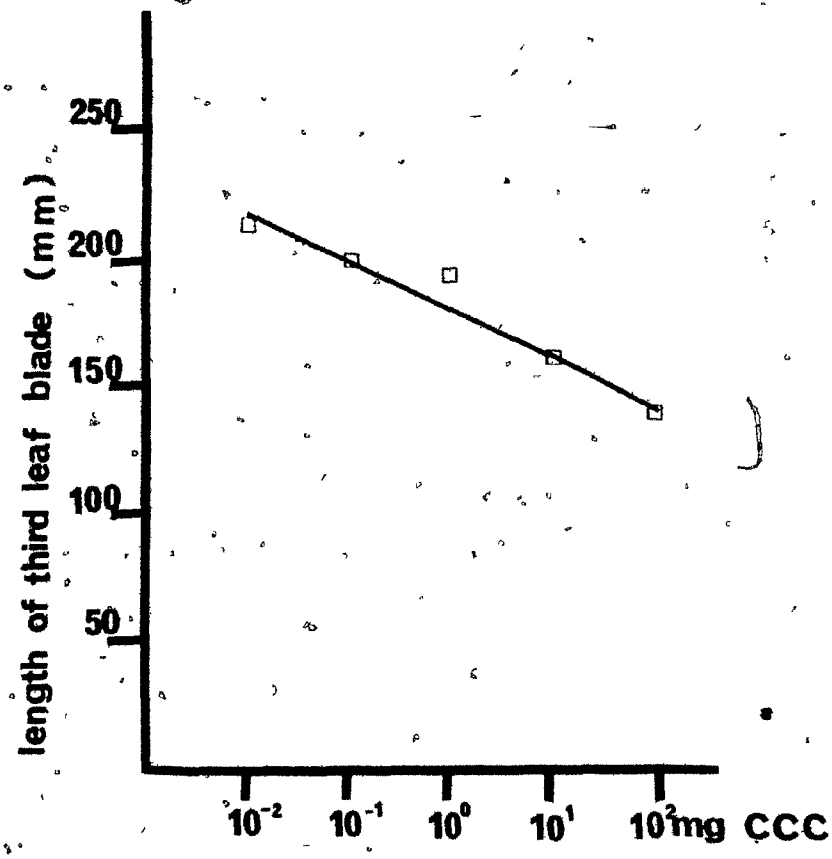


Figure 3.15. Relationship between length of third leaf blade and log dose CCC, applied in 1 ml solution, as a root drench.

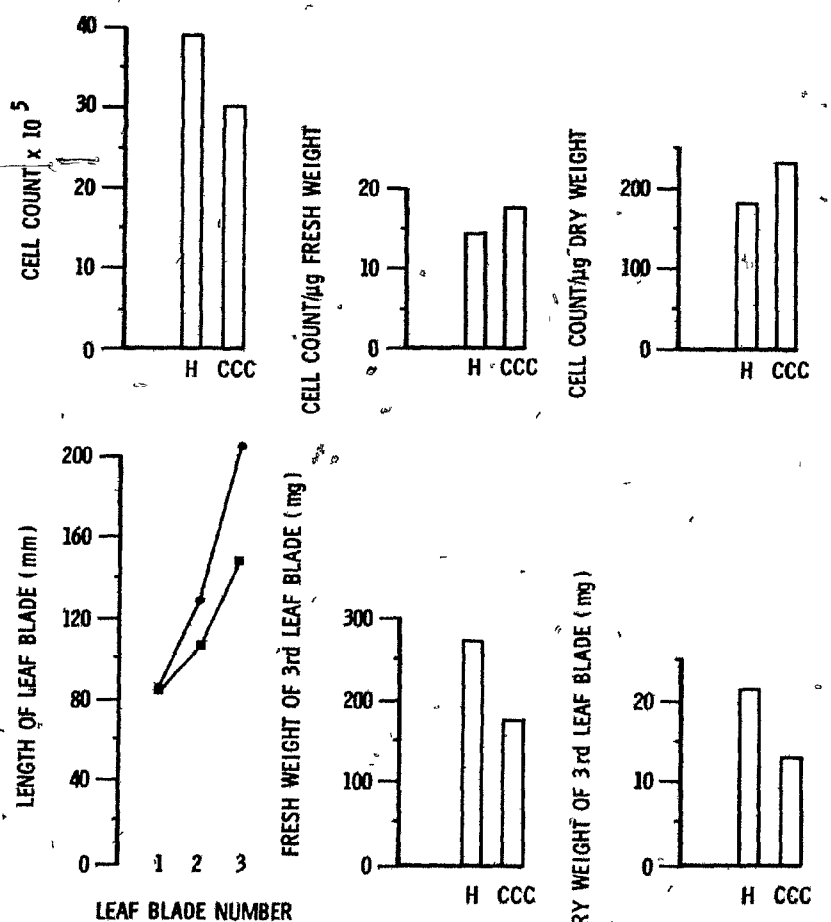


Figure 3.16. Effect of CCC on mean number of cells in third leaf blade of barley.

Each value is the mean obtained using 28 plants.

●——● H, healthy plants

■——■ CCC, plants treated with 3 mg CCC applied as a root drench.

was found in the material obtained from the blank extraction. The chromatographic distribution of gibberellin-like activity was the same in extracts from healthy and CCC-treated plants, Figure 3.17, but there was a significant reduction in the GA_3-activity of CCC-treated plants. The absence of CCC in the extracts of CCC-treated plants was confirmed using Dragendorf's reagent so that the reduced activity could be attributed solely to the presence of less gibberellins in the extract.

No causal relationship can be inferred since it was not found possible to demonstrate diminished levels of GA_3-activity in CCC-treated plants prior to the onset of retardation, but CCC-induced growth effects were clearly associated with a reduction in endogenous gibberellins.

K. EFFECT OF EXOGENOUS GA_3 ON BYDV-INFECTED PLANTS

Plants were sprayed with a 10 ppm GA_3 solution immediately after removal of the aphids. A single application of GA_3 was found to be as effective as repeated doses at three-daily intervals, and promoted increased growth of healthy and infected plants, Figure 3.18.

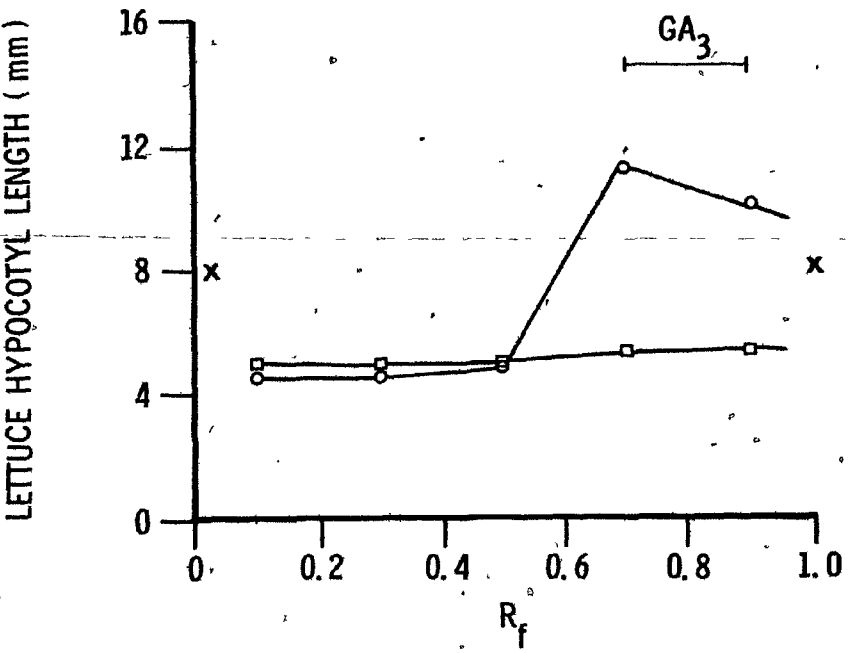


Figure 3.17. The lettuce hypocotyl bioassay of extracts of 100 g fresh weight barley.

○—○ healthy plants

□—□ CCC-treated plants

X X Response of hypocotyls to 10^{-1} mg/L GA_3.

The increase in length of the third leaf blade of both healthy and infected plants was significant at the 1 percent level, but healthy plants treated with GA_3 were considerably larger than infected plants similarly treated, and this difference was also significant at the 1 percent level. In this experiment there was no significant difference in the lengths of third leaf blades of healthy plants and GA_3-treated infected plants, so GA_3 apparently reversed the BYDV-induced dwarfing. In some subsequent experiments, however, a difference significant at the 5 percent level was observed, so that there was not a predictable, total reversal of dwarfing.

The increase in fresh weight of both healthy and BYDV-infected leaves was not significant, so that the apparent reversal of dwarfing was mainly a result of increased expansion of the leaves. Cell counts of the third leaf blades of all four groups of plants showed that exogenous GA_3 had no effect on the meristematic activity of either healthy or BYDV-infected plants, Figure 3.19.

It was concluded that exogenous GA_3 does not reverse BYDV-induced dwarfing of barley but serves only to mask the effect of reduced mitotic activity.

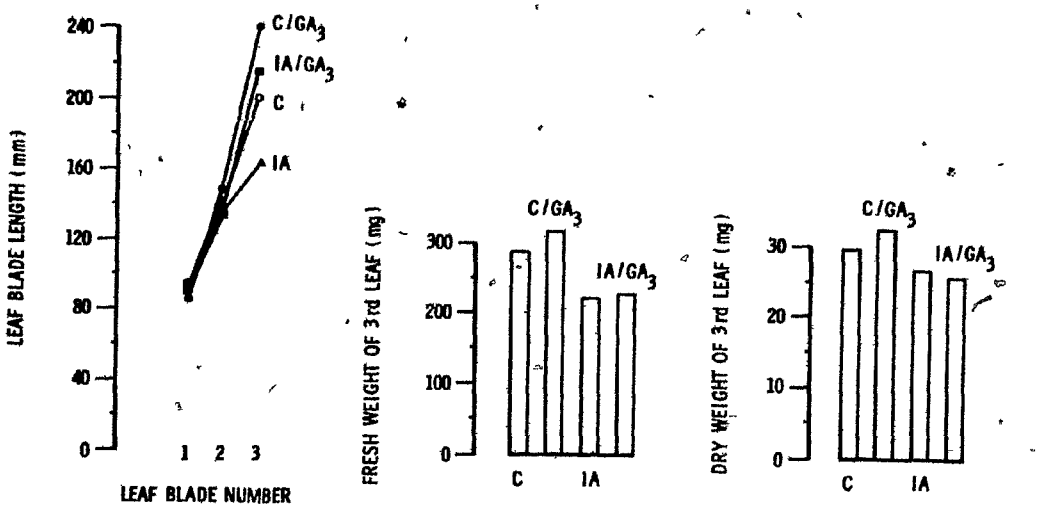


Figure 3.18. Effect of exogenously applied GA_3 on growth of healthy and BYDV-infected barley.

C, ○——○ healthy plants

C/GA_3 ●——● healthy plants sprayed with 10 ppm GA_3

IA ▲——▲ BYDV-infected plants

IA/GA_3 ■——■ BYDV-infected plants sprayed with 10 ppm GA_3

Each value is the mean obtained using 15 plants.

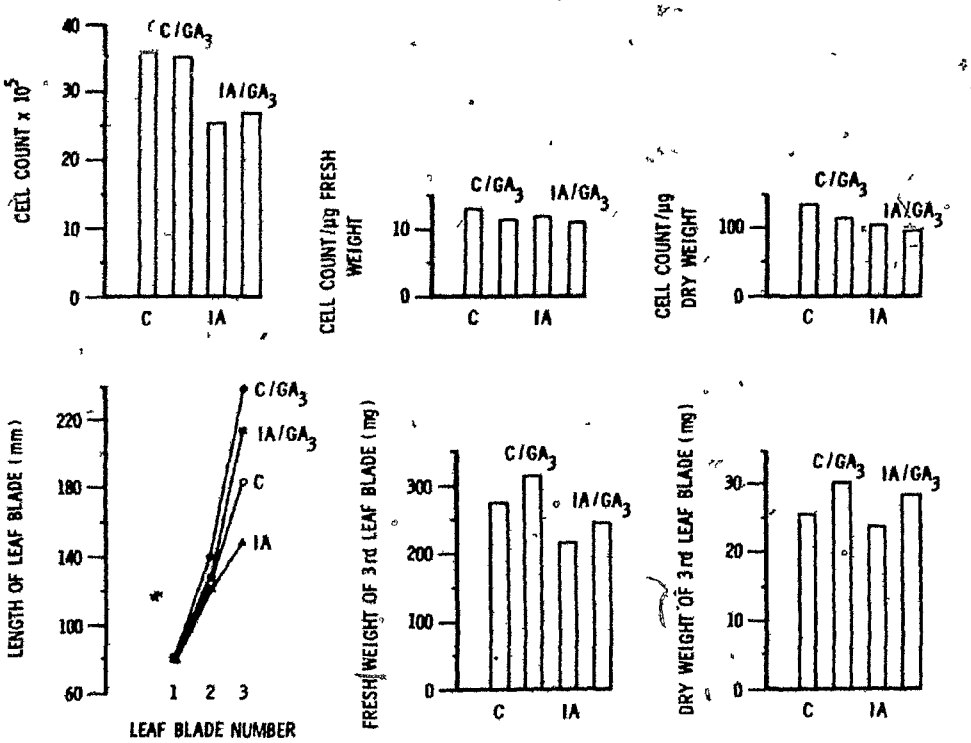


Figure 3.19. Effect of exogenously applied GA_3 on mean cell number of third leaf blade of BYDV-infected and healthy barley.

C ○——○ healthy plants
C/GA_3 ●——● healthy plants sprayed with 10 ppm GA_3
IA ▲——▲ BYDV-infected plants
IA/GA_3 ■——■ BYDV-infected plants sprayed with 10 ppm GA_3

Each value is the mean obtained using 24 plants.

L. EFFECT OF EXOGENOUS GA_3 ON CCC-TREATED BARLEY

Plants were sprayed with a solution containing 10 ppm GA_3 immediately after the application of CCC. This treatment promoted the growth of both healthy and CCC-treated plants, Figure 3.20. CCC-treated plants sprayed with GA_3 were taller than healthy plants, but not as tall healthy plants sprayed with GA_3. Similarly the third leaf blade of GA_3-treated plants was longer and heavier than that of untreated plants, but the CCC-treated leaf blades were still shorter and lighter than those from control plants. Cell counts showed that GA_3 had no effect on the total number of cells in CCC-treated plants, Figure 3.21. However, GA_3-treatment markedly decreased the number of cells per ug fresh weight and per ug dry weight in CCC-treated plants, and it was concluded GA_3 reversed the CCC-inhibited cell expansion, but had no effect on CCC-induced reduction in mitotic activity.

M. EFFECT OF CCC-TREATMENT AND BYDV-INFECTION SIMULTANEOUSLY ON GROWTH OF BARLEY

Eight day old barley seedlings were given a soil drench containing 4.5 mg CCC and then infested with

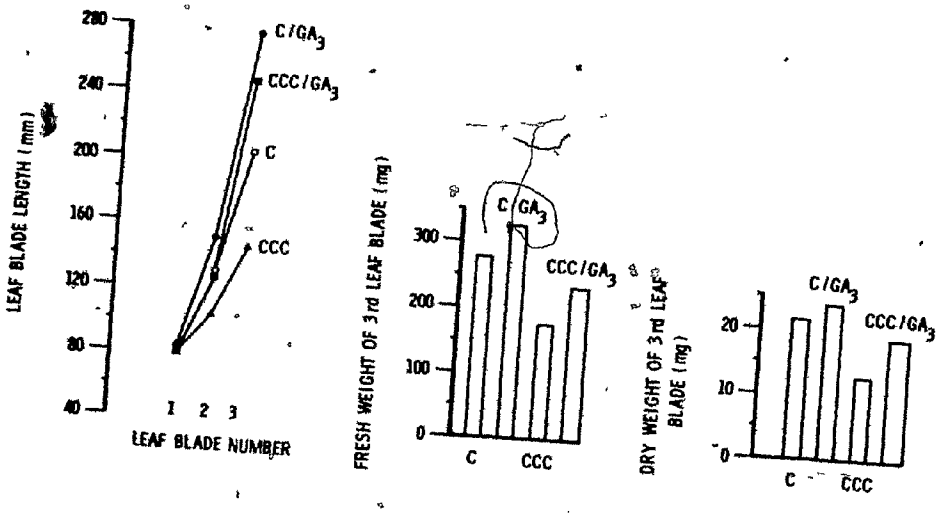


Figure 3.20. Effect of exogenously applied GA_3 on growth of healthy and CCC-treated barley.

C ○——○ control plants

C/GA_3 ●——● healthy plants sprayed with 10 ppm GA_3

CCC ▲——▲ plants treated with 6 mg CCC

CCC/GA_3 ■——■ plants treated with 6 mg CCC and 10 ppm GA_3

Each value is the mean obtained using 20 plants.

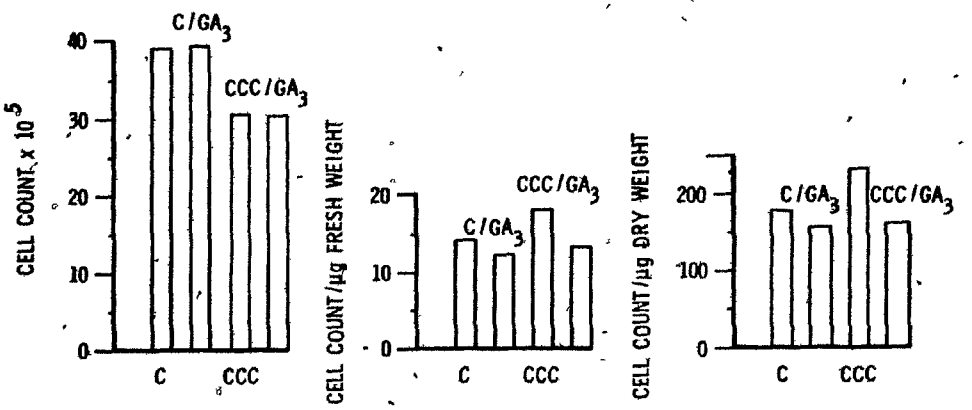


Figure 3.21. Effect of exogenously applied GA_3 on mean cell number of third leaf blade of CCC-treated and healthy barley.

C, control plants

C/GA_3, healthy plants sprayed with 10 ppm GA_3

CCC, plants treated with 6 mg CCC

CCC/GA_3, plants treated with 6 mg CCC and 10 ppm GA_3

Each value is the mean obtained using 20 plants.

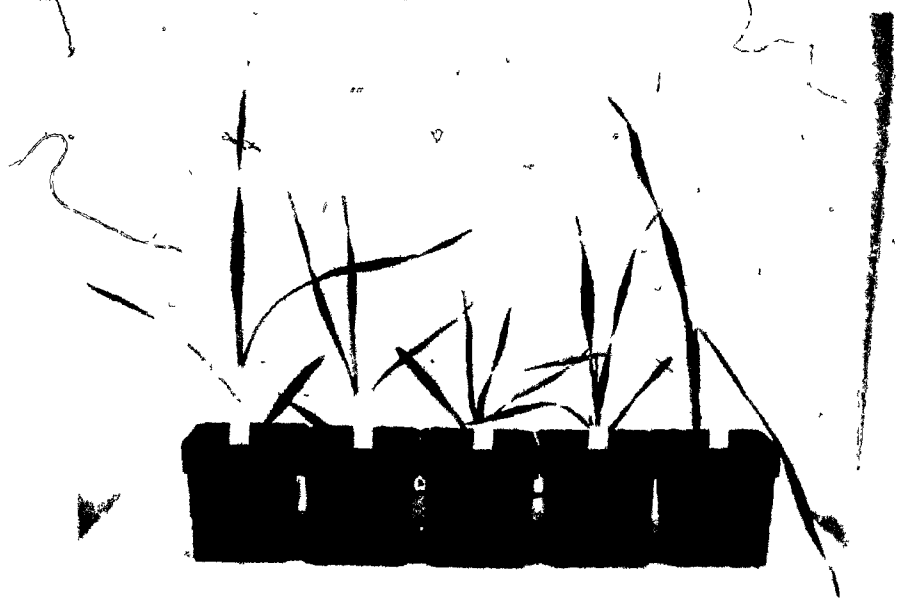


Plate 3.3. The effect of CCC-treatment and BYDV-infection simultaneously on the same plant. (X½)

A healthy plant
B plant infected with BYDV
C plant infected with BYDV and treated with 4 mg CCC
D plant treated with 4 mg CCC
E plant treated with 4 mg CCC and 10 ppm GA_3.

4 BYDV-infected aphids per plant. The growth and development of the treated plants were compared with plants treated with CCC alone, BYDV-infected aphids alone, and with control plants given neither treatment. The double treatment produced considerably greater dwarfing and growth retardation than either treatment alone. The length of the third leaf blade was considerably diminished, Figure 3.22, Plate 3.3. CCC treatment alone reduced the length of third leaf blade by 24.8 per cent, and BYDV-infection alone produced a reduction of 26.8%. The theoretical reduction of CCC-treatment on BYDV-infected plants would be a further 24.8 per cent reduction, giving a theoretical length to the third leaf blade of 110 mm. Similarly the theoretical reduction of BYDV-infection on CCC-treated plants would produce a third leaf blade of 123 mm. The true length of the third leaf blade given the double treatment was 113 mm, which agrees well with the average of the two theoretical values, 116 mm. This implies that there is no synergistic effect between the two treatments, and that the effects are simply additive.

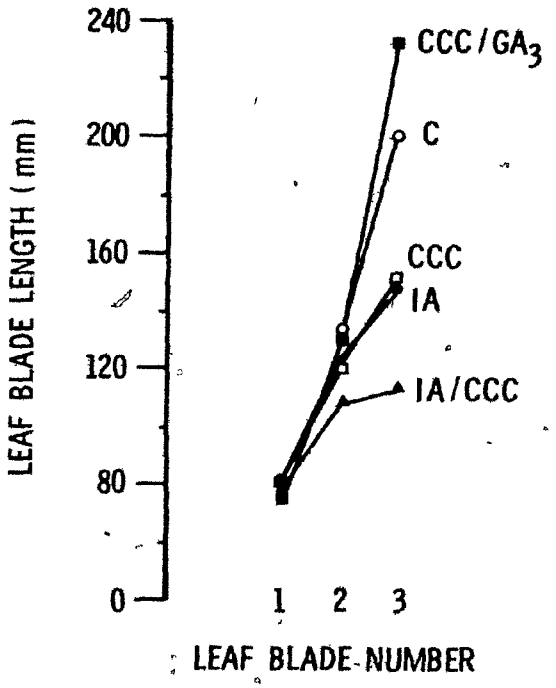


Figure 3.22. Effect of CCC-treatment and BYDV-infection separately and together on the growth of barley.

C ○—○ control plants
IA ●—● BYDV-infected plants
IA/CCC ▲—▲ BYDV-infected plants treated with 4 mg CCC
CCC □—□ plants treated with 4 mg CCC
CCC/GA_3 ■—■ plants treated with 4 mg CCC and 10 ppm GA_3

Each value is the mean obtained using 20 plants.

N. EFFECT OF CCC-TREATMENT AND BYDV-INFECTION SIMULTANEOUSLY ON CELL NUMBER

The number of cells in BYDV-infected plants treated with CCC was grossly reduced, Table 3.4. The number of cells per unit fresh weight was only slightly lowered, implying only a small increase in cell dimensions.

TABLE 3.4. Effect of 4 mg CCC on cell count of BYDV-infected third leaf blade

	C	IA	IA/CCC
mean cell count	39.5×10^5	31.4×10^5	15.5×10^5
cell number per ug fresh weight	13.6	14.7	11.2

It was concluded that CCC-treatment and BYDV-infection act independently, and additively, on meristematic activity when applied together.

0. COMPARISON OF SUSCEPTIBILITY OF OTHER CEREALS TO BYDV-INFECTION AND CCC-TREATMENT

Ethiopian barley C.I. 2376 has been shown to be resistant to BYDV-infection (Oswald and Houston, 1952). Eight day old seedlings of this cultivar, grown and selected as described in the previous section for C.I. 666 seedlings were treated with 6 mg CCC applied as a root drench. A similar group of eight-day old seedlings was infected with 4 BYDV-infected aphids per seedling for 4 days. The plants were all sprayed with 0.25% malathion when twelve days old, and then allowed to develop. BYDV-infection produced a slight dwarfing of the plant and third leaf blade was shorter than that of untreated plants, and CCC-treated plants showed a similar effect, Figure 3.23. There was no significant difference between CCC-treated and BYDV-infected plants. Determination of cell numbers showed no significant difference between the number of cells in the third leaf blade of control plants, CCC-treated plants and BYDV-infected plants, Figure 3.24, and both treatments produced a slight increase in the number of cells per ug fresh weight. It was concluded that C.I. 2376 undergoes some reduction in cell size as a result of both treatments and exhibits a resistance to both CCC-treatment and BYDV-infection induced inhibition of meristematic activity.

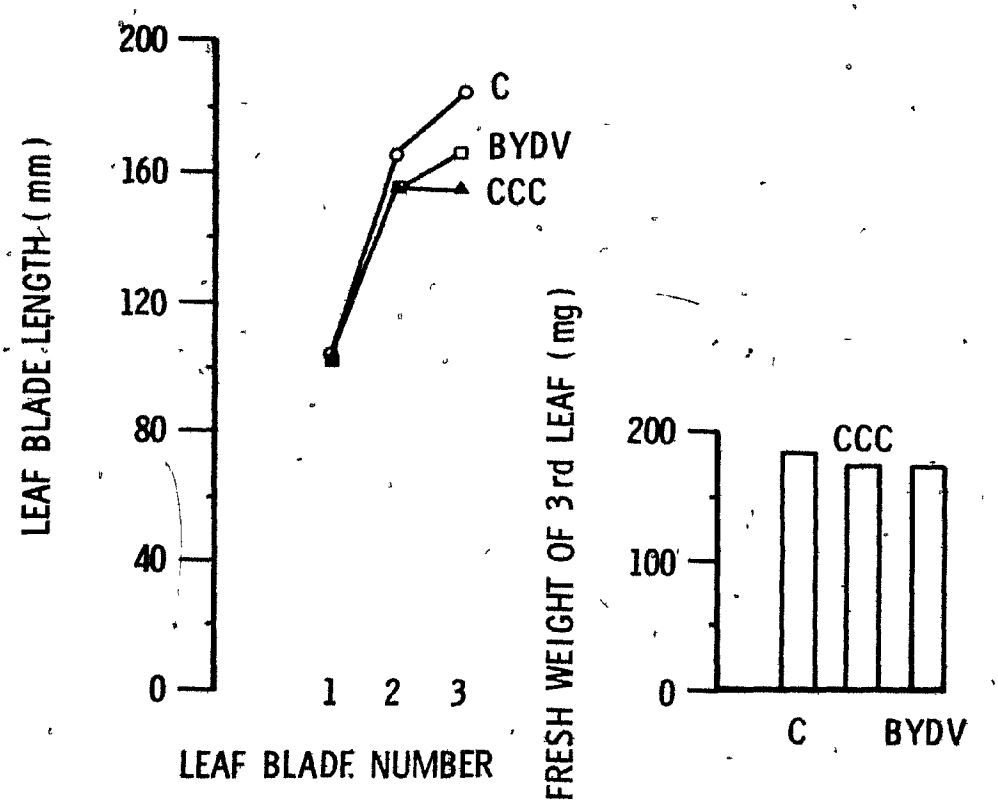


Figure 3.23. The effect of BYDV-infection and CCC-treatment on the growth of C.I. 2376 barley.

C ○——○ control plants

CCC ▲——▲ plants treated with 6 mg CCC

BYDV □——□ plants infested with 4 BYDV-infected aphids.

Each value is the mean obtained using 21 plants.

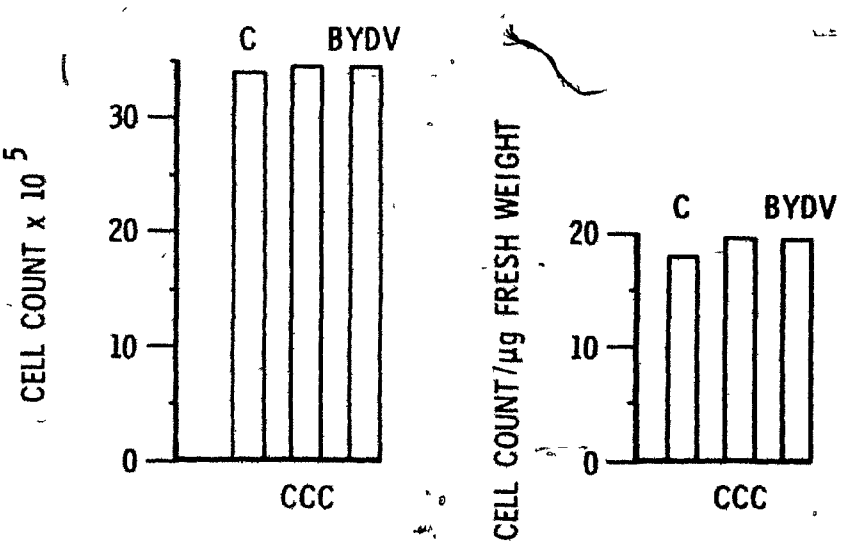


Figure 3.24. The effect of BYDV-infection and CCC treatment on the mean cell count of the third leaf blade of C.I. 2376 barley.

C, control plants

CCC, plants treated with 6 mg CCC

BYDV, plants infested with 4 BYDV-infected aphids.

DISCUSSION

When Black Hulless barley was infected with BYDV there was a significant reduction in the number of cells in the dwarfed leaves. There was no difference in the number of cells per unit fresh weight or unit dry weight. It is concluded that cell size is not involved in the stunting which results from a virus-induced reduction in mitotic activity. This finding is in agreement with that of Esau (1957) who found a suppression of leaf initiation in BYDV-infected barley, and a shorter plastochronic distance between vascular elements and the apex, which she attributed to a reduction in meristematic activity.

A decrease in extractable gibberellins was consistently found in infected plants as compared to healthy plants. The absolute concentration of ethyl acetate soluble gibberellins extracted from healthy plants was nearly six times that extracted from virus-infected plants. Diminished levels of endogenous growth regulators of this order, and less, have been used to explain the reduction of growth in other plant diseases. Jahnel (1939), for example, concluded that the 50% reduction in auxin extractable from potato tubers infected with potato leaf roll virus accounted for the diminished growth of the infected plants.

The concentration of free gibberellins found in healthy barley, was 34.5 ug GA_3 equivalents per kg fresh weight. Krekulé (1971) found the level of gibberellins in young expanded cocoa leaves to be 0.5 to 2.0 ug GA_3 equivalents per kg fresh weight, and Wheeler (1965) found levels of 80 ug GA_3 equivalents per kg fresh weight of 7 day old primary leaves of dwarf french bean.

All these values are very low in comparison to the value of 1 to 10 ug GA_3 equivalents per g fresh weight quoted by Cleland (1969). However, the absolute value depends on the plant under consideration, its age, the part of the plant being extracted, and the extraction procedure, and is probably not a very meaningful figure. The comparison of concentrations in two groups of plants of the same age, extracted under identical conditions, is nonetheless significant.

The gibberellin-like activity was found in all chromatograms of the extracts at the Rf corresponding to authentic GA_3, suggesting that it may have been due to GA_3. This would be in agreement with the work of Nicholls and May (1964) who found GA_2 and GA_3 in developing barley apices, and with Jones, MacMillan and Radley (1963) who found GA_3 in immature barley and other Gramineae. However, further work would be required to confirm this suggestion.

Although no causal relationship can be deduced, a reduction in endogenous gibberellin activity was associated with the virus-induced reduction in mitotic activity. Sachs, Bretz, and Lang (1959) showed that the mitotic activity of the subapical meristem in Hyoscyamus niger is largely regulated by the gibberellin level and Nicholls and May (1964) showed that the highest levels of endogenous gibberellins in barley are found at the period of most rapid cell division. It is possible, therefore, to suggest that the virus-induced reduction in meristematic activity may have resulted from a virus-induced reduction in endogenous gibberellins. However, Bailiss (1968) showed that the dwarfing induced in tomato plants by aspermy virus was the result of reduced subapical mitotic activity which was not associated with diminished levels of endogenous gibberellins, so that other explanations are possible. BYDV-infection may induce, separately, a reduction in endogenous gibberellins and a reduction in mitotic activity.

There was no significant reduction in extractable auxin from infected plants, which implies that the endogenous gibberellin is not acting through diminished levels of endogenous auxin. This agrees with the conclusion of Sachs (1965) that the primary role of gibberellins is not to increase the physiological level of auxin, and also with Cleland (1969) who suggests that

gibberellin acts in some unknown manner in the activation of the subapical meristem.

The level of any substance in a plant system is controlled by the relative rates of biosynthesis, utilisation, and degradation. Considering BYDV-infected barley, the level of available gibberellins would be effectively decreased by a decrease in the rate of synthesis and by an increase in biodegradation. These mechanisms could function separately or together.

A method was devised to examine the activity of gibberellin degradative systems present in barley, and did not demonstrate any significant difference between the activity of extracts of healthy plants and that of severely dwarfed, BYDV-infected plants. It would be presumptuous to conclude, after using one experimental format, that there is absolutely no difference between the ability of healthy and BYDV-infected barley to inactivate gibberellins. It is entirely probable that there is more than one pathway for the inactivation of gibberellins, and it is also possible that not all pathways were active in the extracts prepared as described. However, this latter suggestion seems unlikely. In the method used only the cell debris was discarded and therefore both soluble and membrane bound enzyme systems were present, and while maximum activity of all metabolic process would not be expected, one could expect to

demonstrate some difference between healthy and severely-dwarfed plant extracts, if such a difference existed. It is concluded, therefore, that an increased rate of gibberellin inactivation is not responsible for the diminished levels of endogenous gibberellins found in BYDV-infected barley, although some small increase may occur.

The biosynthesis of gibberellins from acetyl-CoA involves soluble and microsomal enzymes and requires the presence of magnesium ions, ATP, NADPH, and oxygen. The biogenetic pathway is given, in detail, in Appendix 2. If virus particles are present in cells actively synthesising gibberellins it is possible to suggest many ways in which viral activity could repress gibberellin biogenesis. Most simply, a product of viral synthesis could inhibit the activity of any of the enzymes required in the synthesis. Viral activity could repress the synthesis of some, or all, of these enzymes by the repression of the transcription of plant DNA, repression of the translation of plant RNA, or by sequestration of amino acids required for the actual synthesis. Viral activity could limit the availability of magnesium ions for host cell activities, and likewise could utilise the limited supply of ATP for viral replication. Even if virus particles are not present in cells synthesising gibberellins it is possible that a product of viral

activity could be translocated to these cells and once there interfere with the normal biosynthetic processes. Whatever the mechanism involved, the physiological results of an inhibition of gibberellin biosynthesis should be very similar in any given plant.

When Black Hulless barley was treated with the growth retardant, CCC, there was a significant decrease in the level of extractable gibberellins compared to untreated plants. There was also a decrease in cell number and mean cell size. Although it was not possible to demonstrate a significant reduction in extractable gibberellins prior to the onset of dwarfing these results suggest that CCC inhibits the synthesis of gibberellins in barley and that dwarfing results from a decrease in gibberellin-promoted cell division and cell expansion.

There are striking similarities between the response of barley seedlings to BYDV-infection and CCC-treatment. In both cases there is a marked reduction in meristematic activity associated with a decrease in extractable gibberellins. The timing of the response is also similar: plants given either treatment as seven day old seedlings exhibit the same order of retardation and a marked stunting of the third leaf blade. However two points of difference are observable. CCC causes a significant reduction in cell size while BYDV-infection does not and BYDV-infection causes an increase in

percentage dry matter in infected plants while CCC does-not. The increase in percentage dry matter in BYDV-infected barley is probably quite unrelated to its effects on gibberellin biosynthesis since it has been demonstrated to result from carbohydrate accumulation (Watson and Mulligan, 1960), and one would not expect CCC to mimic this effect.

The differing effect on cell expansion is less easy to understand. If both the processes of cell division and cell expansion are controlled by gibberellin, or under the influence of gibberellin, as the results of CCC-treatment suggest, then one would expect that the diminished levels of gibberellins produced by BYDV-infection would influence both processes. It is possible that the cell elongation process is less sensitive to changes in concentration of gibberellins than the process of cell division, so that the reduction in level of gibberellins produced by BYDV is not sufficient to inhibit cell elongation. It is also possible that both processes are not under the same control in barley, and that the inhibitory action of CCC on cell expansion is not mediated by any action on gibberellin biosynthesis. It has been observed (Harada and Lang, 1965) that growth retardants are not solely inhibitors of gibberellin biosynthesis. It is also possible that there is a spatial effect. The regions of cell division and cell

expansion are spatially separate in monocotyledonous plants and it is possible that BYDV-induced inhibition of gibberellin biosynthesis is not occurring in that region which produces gibberellins for cell expansion, while CCC-treatment inhibits gibberellin biosynthesis in all regions. The first explanation appears most likely, and it should be possible to apply varying quantities of CCC to barley plants and observe whether the effect on cell expansion is produced only when there is a great diminution of endogenous gibberellins.

When two treatments produce the same, or very similar, effects on a living system it is possible that the effects may be produced by entirely different mechanisms, or by the same. If both treatments are applied simultaneously to the same test system they may interact, to produce either a marked synergism or an effect differing from that produced by either treatment independently, or there may be no apparent interaction, each treatment producing the expected result, both qualitatively and quantitatively. Two interpretations are possible when there is no interaction between the two treatments. One can conclude that the treatments are functioning quite independently along different pathways. However, it is also possible that the same pathway is used by both treatments, and that there is no limiting factor in the pathway which prevents complete expression

of both treatments. When CCC and BYDV-infected aphids were applied simultaneously to barley seedlings there was no evidence of interaction between the two treatments. Since CCC is recognised as an inhibitor of gibberellin biosynthesis, and would appear to be acting as such in barley, and BYDV-infection has been shown to have little, if any, effect on gibberellin inactivation it would seem reasonable to infer from this lack of interaction that BYDV-infection causes an inhibition of gibberellin biosynthesis in infected plants. While an experiment of this type is not capable of providing any evidence that BYDV-infection causes an inhibition of gibberellin biosynthesis the results are in agreement with this thesis.

Berry and Smith (1970) showed that high concentrations of CCC inhibit the synthesis of chlorophyll and protein in barley leaf segments, and they further showed that these effects were not reversed by application of GA_3. They concluded that CCC was acting as an inhibitor of protein synthesis, in the production of these effects, rather than through a direct effect on gibberellin production. It is interesting, however, to observe that plants which are severely infected with BYDV exhibit a yellowing of the leaves and, according to Orlob and Arny (1961), a decreased content of protein. This great similarity of symptom expression may be purely coincidental, but a closer inspection of both CCC-treated and

BYDV-infected plants might well reveal further information which could be helpful in elucidating the mechanism of action of both CCC and BYDV.

Barley seedlings C.I. 2376, which are resistant to BYDV, were shown not to be susceptible to CCC-treatment, and this similar pattern of resistance is taken as further inference of a similar mode of action for the two treatments. It would be informative to investigate the response of other BYDV-resistant strains by barley to CCC-treatment. If all showed a similar lack of susceptibility to both treatments it would be hard to avoid the conclusion that both treatments were involved in the same mode of action.

Exogenous GA_3 had no effect on cell division in healthy, or BYDV-infected, or CCC-treated barley seedlings, and the reversal of dwarfing was solely the result of increased cell elongation. GA_3 stimulated cell expansion to a lesser extent in healthy than in CCC-treated plants, but there was no difference between the response of BYDV-treated and healthy plants. The fact that exogenous GA_3 reverses the inhibitory action of CCC on cell expansion suggests that CCC inhibits cell expansion by an effect on gibberellin biosynthesis. The failure of exogenous GA_3 to promote mitotic activity in any barley plants could be due to the inaccessibility of the meristematic regions to applied gibberellins. Since the

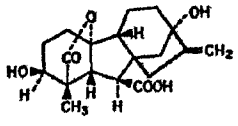
regions of cell division and cell expansion are spatially separate in grass leaves this is entirely possible. The failure of exogenous GA_3 to promote cell division in healthy barley plants does not exclude the possibility of endogenous GA_3 involvement in meristematic tissue. Cleland (1964) showed that although exogenous auxin is ineffective in promoting the growth of oat leaf sections, growth is inhibited by the presence of antiauxins. Alternatively, endogenous GA_3 may not be involved in the control of mitotic activity in barley, in which case neither CCC- nor BYDV-inhibition of meristematic activity would involve endogenous GA_3. One would then be forced to conclude that the diminished levels of GA_3 in treated plants were either a coincidence or the result, not the cause, of diminished growth. In the light of the known ability of GA_3 to stimulate mitotic activity, the presence of GA_3 in barley, and the inhibitory action of CCC on growth and gibberellin biosynthesis, this explanation seems very unlikely. It seems more probable that mitotic activity is under the control of endogenous GA_3, and the exogenous GA_3 fails to promote mitotis due to a failure of penetration.

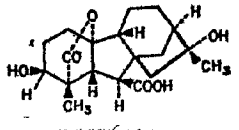
The results of all the experimental work are in agreement with the thesis that BYDV inhibits the synthesis of gibberellins in barley and that the reduction of mitotic activity in infected seedlings is a result of

this. However none of the experimental work constitutes proof of causality, nor, indeed, does this hypothesis explain all the symptoms of BYDV-infection. For reasons of time the possible involvement of other growth regulators was not examined during this piece of work, but there is no suggestion, by this omission, that these may not be involved, or, indeed, important, in the pathology of the disease.

Cytokinins and abscisic acid are growth regulators which are involved in the processes of extension growth and senescence, and it is entirely possible that these could be involved in the BYDV-induced dwarfing of barley. Both have been implicated in the growth deformations produced in plants infected by bacteria (Thimann and Sachs, 1966; Steadman and Sequeira, 1970). Since both cytokinins and abscisic acid are associated with leaf yellowing it is tempting to speculate that one or other is involved in BYDV-infection. However, this is pure speculation, and the yellowing of the BYDV-infected plant may result from nutritional deficiencies without the involvement of any further growth regulators.

APPENDIX 1. THE STRUCTURAL FORMULAE OF KNOWN GIBBERELLINS (LANG, 1970).


GIBBERELLIN A_1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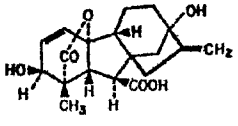
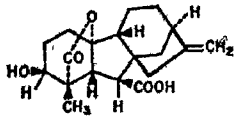
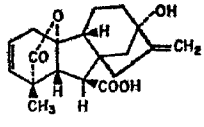

GIBBERELLIN A_3 = GIBBERELLIC ACID


GIBBERELLIN A_4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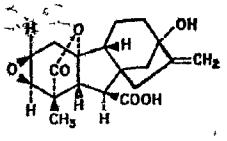
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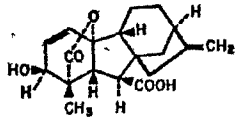
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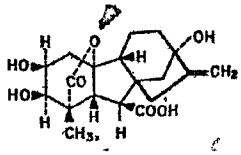
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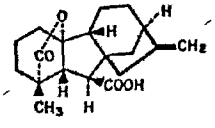
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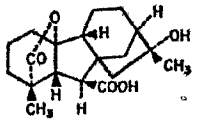
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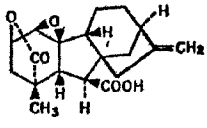
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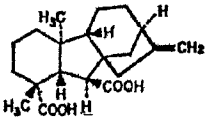
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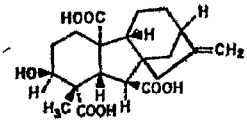
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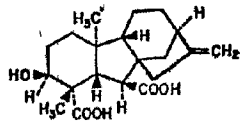
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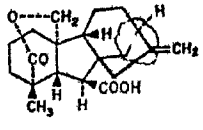
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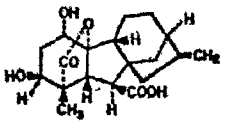

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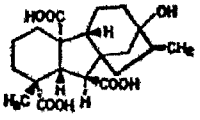
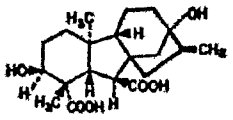
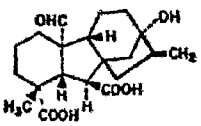


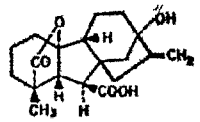
GIBBERELLIN A_{17}



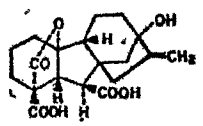
GIBBERELLIN A_{18}
(Lupinus gibberellin I)



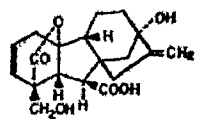
GIBBERELLIN A_{19}
(Bambee gibberellin)



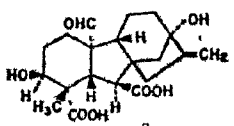
GIBBERELLIN A_{20}
(Phorbitis gibberellin I)



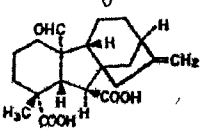
GIBBERELLIN A_{21}
(Canavalia gibberellin I)



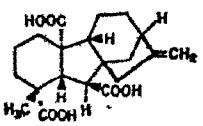
GIBBERELLIN A_{22}
(Canavalia gibberellin II)



GIBBERELLIN A_{23}
(Lupinus gibberellin II)



GIBBERELLIN A_{24}



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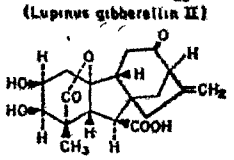
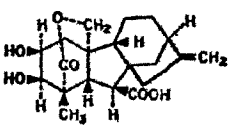
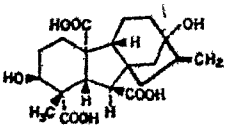


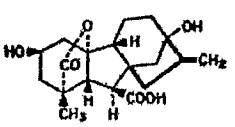
GIBBERELLIN A_{26}



GIBBERELLIN A_{27}



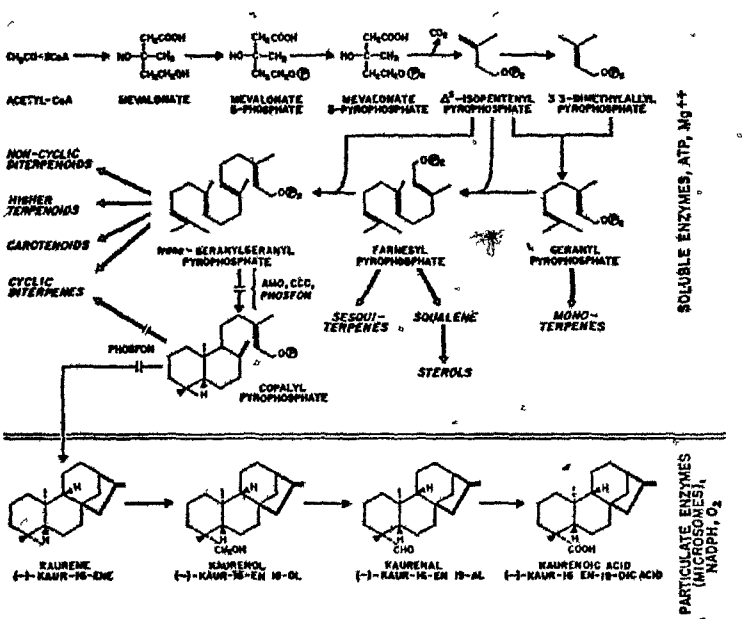
GIBBERELLIN A_{28}



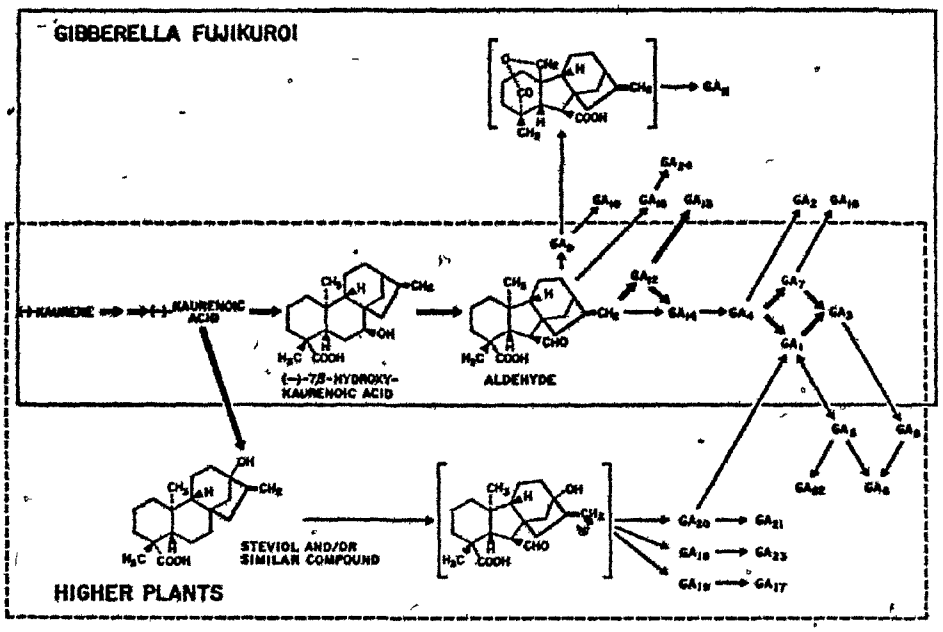
GIBBERELLIN 29

APPENDIX 2. THE BIOSYNTHESIS OF GIBBERELLINS (LANG, 1970)

1. From acetyl-CoA to kaurenoic acid.



2. From kaurene to all known gibberellins.



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