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An investigation of the effects of light, gibberellic acid and growth retardants on growth and lignification of Pisum sativum, L.

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AN INVESTIGATION OF THE EFFECTS OF LIGHT, GIBBERELLIC ACID AND GROWTH RETARDANTS ON GROWTH AND LIGNIFICATION OF *PISUM SATIVUM*, L.

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INTRODUCTION

The gibberellin (GA) family of phytohormones promotes the growth of many dwarf plants. The stems of GA-treated plants in some respects are similar to those of dark grown plants, i.e., they typically have thin and elongated internodes. Leaves and roots, in contrast, seldom respond to GA although the foliage of treated plants is usually of a lighter green color than that of the controls. GA-promoted stem elongation is the result of an enhanced rate of both cell division and elongation, and apparently is dependent on protein, RNA, and DNA synthesis. Other phytohormones (auxins and kinins) stimulate protein synthesis and RNA formation but GA appears to be unique in promoting DNA formation. The auxins have been implicated in stem growth, but the GA phytohormones are unique in that they alone of the phytohormones known to date elicit a several-fold increase in stem elongation.

Brian and Hemming (1958) suggested that the growth of pea internodes is normally regulated by a three-factor system; auxin, inhibitor and a hormone with physiolcgical properties similar to GA. Nevertheless, despite intensive investigation of the gibberellins since their discovery over 30 years ago and the recent discovery that they promote DNA formation, the specific, metabolic responses to GA which

result in the promotion of stem elongation remain obscure.

A number of observations suggest that GA somehow affects phenolic metabolism. The accumulation of lignin, which is an end-product of phenol metabolism and a constituent of the cell wall, has been reported to be promoted by GA. It also has been reported that darkness had an inhibitory effect and light stimulated the lignification of plants. These observations raise some interesting questions. First, how does GA promote lignin accumulation? Second, since lignification is supposed to occur after growth ceases and lignified cells are not considered readily extensible, what is the relationship of GA-promoted lignification to GA-promoted growth, if any. The purpose of the present experiments was to study the possible effects of light and GA on growth and lignification.

LITERATURE REVIEW

It is now generally recognized that GA promotes stem elongation. This enhanced elongation is the result of an increased rate of both cell division and expansion (Sachs, Bretz & Lang, 1959), although GA-promoted cell elongation can occur without concomitant cell division (Haber and Foard, 1963). There is, however, little definitive information regarding the metabolic reactions involved in cell elongation and the role of GA in this process.

Brian and Hemming (1958) suggested that GA might neutralize an inhibitory system in the shoot which prevented the internode from extending to the full extent potentially allowed by the available auxin. Kuraishi and Muir (1964a) reported that GA increased the diffusible auxin in dwarf and normal peas by a factor of three and two-fold, respectively, over the level obtained from untreated plants. Ockerse and Galston (1967) recently reported that GA and 3-indoleacetic acid applied to excised stem sections of green dwarf peas resulted in a markedly synergistic growth response. Further, these authors claimed that the GA action preceded the auxin action in this system.

Kuraishi and Muir (1964b) suggested that the increase in diffisible auxin in dwarf peas following treatment with GA did not involve the inhibition of IAA oxidase, the rate of

auxin transport, a decrease in the amount of a growth inhibitor or the formation of a GA-auxin complex. Instead, these authors claimed that the mechanisms whereby diffusible auxin increased as a result of GA treatment involved the increased production of water soluble auxin by the tryptophan conversion system. Kuraishi and Muir interpreted their observations as evidence that the increase in auxin was not a result of GA-stimulated elongation since no synerigism was found between IAA and gibberellin in the Avena curvature test.

Light inhibits the stem growth of all plants. All varieties of peas reach approximately the same size in darkness, but in light, the growth of dwarf plants is reduced much more than that of tall plants (Gorter, 1961). The height of tall peas is related to the logarithum of light intensity under white light conditions. In contrast, dwarf plants are more sensitive to the light intensity. In yellow light, tall peas show no growth reduction and no response to higher light intensity, but "yellow-etiolation" at lower light intensities while the height of dwarfs is reduced by all light intensities. GA counteracts the light reduction. According to Gorter (1961), intact plants of all varieties of peas treated with saturating quantities of GA grow to the same height. Sale and Vince (1960) found that the light inhibition of pea internode elongation was partly but not completely prevented by the application of large doses of GA. Although elongation following the application of GA was greater in the light than in the dark, internode lengths in the light with GA treatment

were on all cases less than those of plants grown in the dark with GA. Dwarf pea internode elongation was inhibited most by red light while tall peas were inhibited by both red and blue light. Infra-red light largely reversed the inhibitory effects of red light but there was no apparent interaction between GA and infra-red light, although both promoted internode elongation.

That GA promotes enzyme activity was clearly demonstrated by the studies of Paleg (1960) and Varner (1964). The release of reducing sugar from excised barley endosperm was wholly dependent on an exogenous supply of GA which induced the de novo synthesis of an α-amylase in the endosperm (Varner, 1964). The apparently specific dependence on GA for α-amylase production may be unique to barley endosperm. In wheat endosperm GA promoted α-amylase activity, but amino acids were equally as effective (Rowsell and Goad, 1964). The activities of a number of different hydrolytic enzymes have recently been reported to be promoted by GA (Murakami and Hayashi, 1963, Black and Altschul, 1965). The relationship between the activities of these enzymes and cell elongation is not clear, however.

Evidence is accumulating that the three known families of phytohormones: auxins, kinins, and gibberellins, promote nucleic acid synthesis (Key, 1964, and Srivastava & Ware, 1965). Ram and Varner (1965) reported that GA stimulated the synthesis of a salt-soluble RNA fraction in the aleurone cells of barley. Actinomycin-D completely inhibited the GA-stimulated incorporation of labelled precusor into RNA.

Recent work by Lang's group (Nitsan and Lang, 1966) indicated that GA-promoted cell elongation is dependent on a GA-promoted DNA synthesis.

Several different families of chemicals are effective plant growth retardants (Cathey, 1964). Examples of these retardants are: Phosphon-D (2,4-dichlorobenzyltributyl, phosphonium chloride), CCC (2-chloroethyl trimethylammonium chloride), and AMO-1618 (4-hydroxy-5-isopropyl-2-methyl-phenyltrimethyl ammonium chloride, 1-piperdine carboxylate). Morphologically, they cause plants to develop with relatively short internodes, thick stems and dark green leaves (Sachs and Lang, 1961 and Cathey and Stuart, 1961). Cathey and Stuart applied AMO-1618, Phosphon-D, and CCC to many species of plants. They found that CCC reduced stem elongation more than did Phosphon-D or AMO-1618. Phosphon-D produced permanently chlorotic leaves, whereas CCC produced only temporary chlorosis. AMO-1618 had the least effect on leaves. Species differences in responce to the various retardants were also noted. In the case of Phaseolus vulgaris plants treated with AMO-1618, maturity was delayed by approximately a month but the formation of viable seeds was not affected. The inhibitory factor responsible for the reduced stem elongation was translocated into the seeds, but its action was considered non-genetic because this effect did not persist beyond the second generation. (Marth, Preston and Mitchell, 1953).

GA on the other hand, causes most plants to grow in a manner opposite to that induced by these retardants, and, when applied with the retardants to whole plants, completely

overcomes their inhibitory action (Cathey, 1964).

Kuraishi and Muir (1963) reported that CCC and Phosphon-D retarded the growth of Avena coleoptile sections and these retarding effects were not reversed by GA. Knypl (1966) found that IAA and coumarin reversed, and kinetin markedly reduced, the retarding effect of CCC on the growth of sunflower hypocotyl sections. GA was without any effect in this respect. Phosphon-D at 10ppm slightly stimulated growth in the control, but markedly reduced both IAA and coumarin-induced growth (Knypl, 1966). Halevy and Cathey (1960) reported that AMO-1618 at 10^{-3} M inhibited the elongation of dark- and light-grown bean seedlings. AMO-1618 at 3×10^{-4} M drastically inhibited radicle elongation of cucumber seedlings but in this system GA did not reverse the inhibitory action of AMO-1618. The presence of AMO-1618 and Phosphon-D in the incubation medium did not enhance or depress the GA-induced reducing sugar released from excised barley endosperm (Paleg et al, 1965). Sachs and Lang (1961) reported that one of the major functions of GA was to control mitotic activity in the subapical meristems and thus increased the length of the plant stems. In contrast, AMO-1618 decreased mitotic activity. The authors concluded that GA modified the effects of AMO-1618 in the subapical meristems but they did not speculate on the interactions of these chemicals in cell elongation.

It is now well documented (Baldev, Lang and Agatep, 1965 and Harada and Lang, 1965), however, that GA will reverse the inhibition induced by AMO-1618 in intact plants. Convincing evidence has appeared in the past few years which indicate that these growth retardants inhibit gibberellin biosynthesis.

Baldev, Lang and Agatep (1965) reported that AMO-1618 at 5-50ppm inhibited production of all gibberellin-like materials in excised pea seeds (in pods) without appreciably reducing the growth of the seed. They concluded that the developing pea seed is dependent on only a fraction of the gibberellin synthesis at this stage. Kinetic analysis indicated that these growth retardants interacted competitively with gibberellins by partially blocking the biosynthesis of GA required for normal growth. Harada and Lang (1965) reported that application of AMO-1618 to the gibberellin producing fungus Fusarium moniliforme greatly suppressed biosynthesis of GA without affecting growth of the mycellium.

The metabolism of $C_6-C_3-C_6$ compounds (anthocyanins and flavonols) is influenced by light and by GA, (Furuya and Thimann, 1964). In some systems light appears to antagonize the effect of gibberellin on anthocyanin formation. Furuya and Thimann indicated that light at 300 ft-c. stimulated anthocyanin production. GA at 10^{-7} to 10^{-6} M inhibited anthocyanin production about 50% when Spirodela was grown either in darkness or under 300 ft-c. for 20 days. However, at these concentrations, GA slightly stimulated anthocyanin production the first 10 days but had little effect on fresh weight. Under 800 ft-c., GA had little effect on anthocyanin content. The gibberellin was presumed to be affecting anthocyanin production at an early stage of its biosynthesis since GA and red light had similar effects on other $C_6-C_3-C_6$ flavonols.

Troyer (1964) found that light promoted anthocyanin production but the light requirement was not absolute. The age of the seedling, the length of exposure to light and the supply of exogenous sugars were all factors in the amount of anthocyanin accumulated by excised segments of buckwheat. Downs and Siegelman (1965) reported that a high energy photoreaction (HER) is required for anthocyanin synthesis in many plants. The photo receptor for the HER was claimed to be chlorophyll b on the basis of action spectra studies.

Kogl (1960) reported that GA at a concentration of 100µg per ml promoted a 3-fold increase in the content of three polyhydroxy phenolic acids in pea plants. These acids were shown to be ferulic acid, caffeic acid, and chlorogenic acid. Ferulic acid was the most important quantitatively and increased the most in response to GA.

A recent report by Walton (1966) is of interest. This author found that the axis growth of Phaseolus vulgaris plants was stimulated by p-fluoro-DL-phenyalalanine while β-2-thienylalanine had an inhibitory effect. At $5x10^{-4}$M concentration p-fluoro-DL-phenylalanine always increased the fresh weight in comparison with the controls. The stimulation by p-fluoro-DL-phenylalanine was essentially reversed by L-phenylalanine but not by tyrosine. Presumably the effect of p-fluoro-DL-phenylalanine was on cell elongation. Nevertheless, Nooden and Thimann (1966) indicated that fluorophenylalanine had an inhibitory effect in some plant tissues. The inhibitory effect of fluorophenylalanine on growth is generally attributed to reduced enzymatic activities resulting from the replacement

of phenylalanine by fluorophenylalanine in proteins (Richmond, 1962). In higher plants, however, phenylalanine is also a metabolic intermediate for other types of compounds; ie. phenols, coumarins and flavonoids. Therefore, the effects of fluorophenylalanine on plant growth may be complicated by its effects in these metabolic conversions of phenylalanine.

Furuya and Thomas (1964) reported that in etiolated pea plumules low intensity red light stimulated both leaf growth and the production of an IAA oxidase inhibitor which contained Kaempferol-3-triglucoside (KG) and Kaempferol-3-triglucosyl-p-coumarate (KGC). Red light promoted the growth rate of etiolated Alaska pea plumules and increased the concentration of KGC, but had only a slight effect on KG. Far-red reversed the red-promoting effect but the increased growth rate and KGC concentration were not directly correlated to the phytochrome response. The authors argued that red light may stimulate many processes in the cell, two of which are growth and Kaempferol-3-triglucoside-p-coumarate synthesis.

Cell wall biogenesis presumably is associated with cell extension. Lignin, a terminal product of phenol metabolism, is an integral part of some plant cell walls and is considered to confer rigidity upon stems. Lignin is chemically an illdefined polymer of phenylpropanoid compounds. The site of lignin synthesis probably is either in the cell wall per se, or in cytoplasm tightly bound to the wall (Stafford, 1965). As mentioned in the introduction, lignification is thought to occur in mature cells after extension has ceased (Stafford, 1967).

The phenylpropanoids, phenylpyruvic acid and p-hydroxyphenylpyruvic acid are the first aromatic products formed via the shikimic acid pathway to be converted to lignin in plants (Brown and Neish, 1955a). Carbon-labelled phenylalanine, tyrosine and cinnamic acid were efficiently utilized for the synthesis of both coniferyl and sinapyl lignin while ferulic acid was efficiently converted only to coniferyl lignin in heading wheat (Brown and Neish, 1955b). Higuchi (1962) found that L-phenylalanine, p-hydroxycinnamic acid and ferulic acid were good precusors of the coniferyl lignin of Pinus cambial tissue and concluded that the cambium contains the enzyme system necessary for their metabolism to lignin. McCalla and Neish (1959) administered C^{14}-labelled phenylalanine to cuttings of Salvia splendens and isolated radioactive p-coumaric, caffeic, ferulic and sinapic acids from the stems. Measurement of the rate of incorporation of C^{14} into each acid agreed with the sequence of reactions; phenylalanine ⟶ cinnamic acid ⟶ p-coumaric acid ⟶ ferulic acid ⟶ sinapic acid. This sequence was confirmed by feeding cinnamic acid and each of the other acids separately; there was ready conversion to later members of the sequence and a relative slight conversion to earlier members. Higuchi and Brown (1963) recovered labelled coniferyl alcohol from wheat plants to which ferulic acid--C^{14} had been administered. Labelled coniferaldehyde was also recovered in all experiments and its specific activity was invariably higher than of coniferyl alcohol. This finding

suggested the sequence; ferulic acid --→ coniferaldehyde --→ coniferyl-alcohol. The scheme presented in figure 1 summarizes the proposed pathway of lignin biosynthesis in higher plants, as suggested by Brown, Wright and Neish, 1959, and Brown, 1961 and 1964. Many of the compounds shown in figurela are naturally occuring metabolites but none has been established as an obligatory intermediate in lignin biosynthesis.

The presence of ester linkages in lignin was first reported by Smith (1955). Kuc and Nelson (1964) recently carried out kinetic studies on the formation of ester linkages with p-coumaric acid and ferulic acid using maize tissue; they found that the content of p-coumaric acid and ferulic acid rose rapidly from the time of planting and then declined rapidly until maturity. El-Basyouni, Neish and Towers (1964) administered to wheat shoots $C^{14}O_2$ and various C^{14} labelled lignin precussors including phenylalanine, tyrosine, cinnamic acid, p-coumaric acid, ferulic acid and sinapic acid. They found that labelled $C^{14}O_2$, phenylalanine and tyrosine were incorporated more readily into the cinnamic acid bound to the insoluble residue than into ethanol soluble derivatives. In contrast, the reverse was true for labelled percussors such as cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid and sinapic acid. Time course studies with labelled $C^{14}O_2$ suggested the sequence CO_2 --→ aromatic amino acids --→ ethanol insoluble derivatives of cinnamic acid --→ lignin. On the basis of their observations they concluded that the natural route to lignin from CO_2 may involve

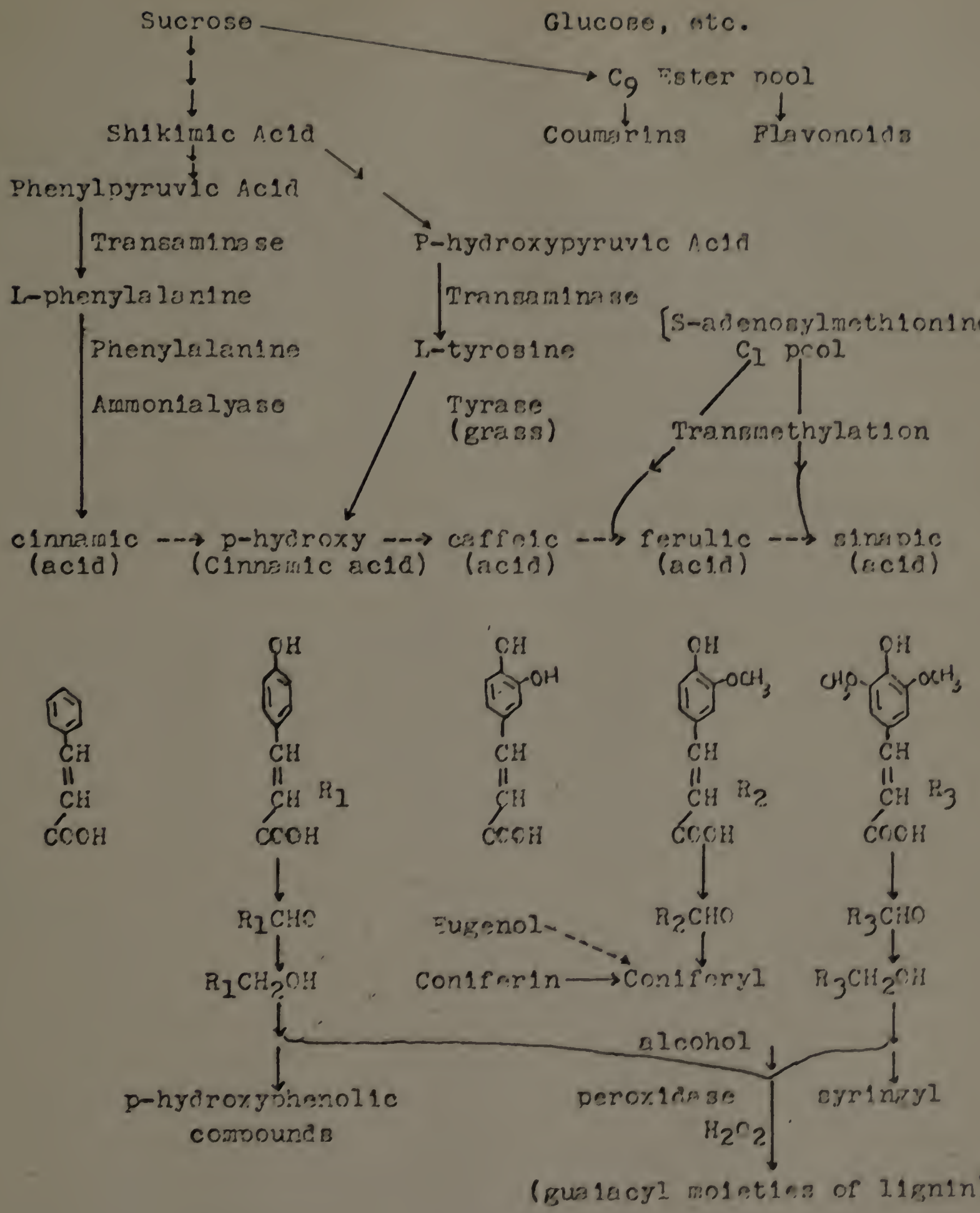
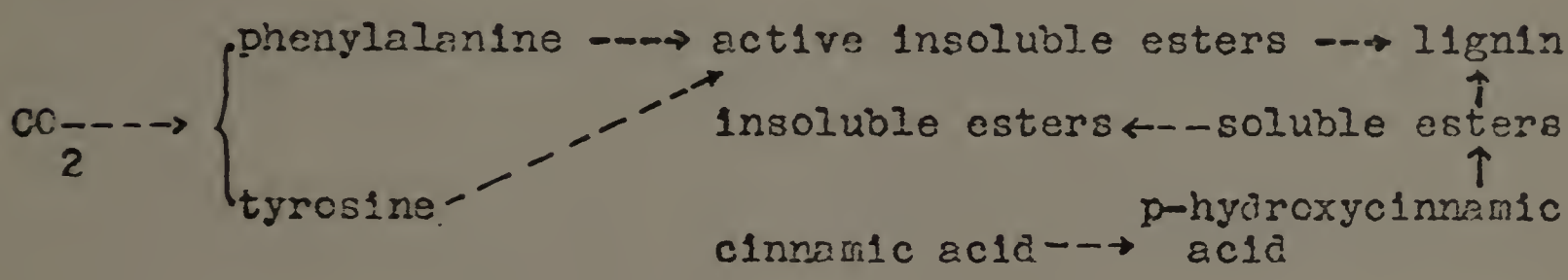


Figure 1a. Phenol metabolism & Lignin formation

the formation of "active insoluble esters" (ethanol insoluble plant residues) from phenylalanine and tyrosine whereas the administration of salts of the free acids may lead to the formation of "soluble esters" also capable of conversion to lignin. These esters were postulated to be sulfurhydryl esters, perhaps of coenzyme A type. The following scheme was proposed as a working hypothesis for the role of esters in lignin formation (El-Basyouni, Neish and Towers, 1964):



Very little is known about the enzymology of lignification or the control mechanisms involved. Koukol and Conn (1961) first demonstrated the existence in plants of phenylalanine ammonia lyase which converted L-phenylalanine directly to cinnamic acid. Grasses possess in addition to phenylalanine ammonia lyase, tyrosine ammonia lyase which converts tyrosine to p-hydroxycinnamic acid (Neish, 1961).

Phenylalanine ammonia lyase is a key enzyme in phenol biogenesis (Zucker, 1965). In the potato tuber the formation and amount of chlorogenic acid was proportional to the activity of the ammonia lyase (Zucker, 1965). Interestingly, in this tissue, the enzyme was light-induced. Examples are accumulating for the importance of the ammonia lyases in phenolic metabolism. There was a pronounced increase in the activities of phenylalanine and tyrosine ammonia lyases during lignification

of bamboo shoots (Higuchi, 1966). The pattern of the activity of the enzymes was observed to be highest in the upper part of the tissue where the most active lignification was taking place. Also, the amount of cinnamic acid derivatives in the tissue was found to be in good accordance with the activity of the enzymes.

Young and Neish (1966) also reported that acetone powders prepared from wheat shoots contained ammonia lyases which catalysed the conversion of a number of rings substituted phenylalanine derivatives to corresponding cinnamic acid compounds. L-phenylalanine was the most effecient substrate deaminated. L-phenylalanine ammonia lyase was relatively stable and had a broad substrate specificty whereas L-tyrosine ammonia lyase was less stable and fairly specific for L-tyrosine, (Young and Neish, 1966).

Only one other enzyme specifically implicated in phenol boisynthesis has been reported. In an unconfirmed observation Nair and Vining (1965) reported a cinnamic acid hydroxylase which converted trans cinnamic acid to p-coumaric acid. The enzyme, which was obtained from spinach chloroplasts, required tetrahydrofolic acid and reduced phosphate dinucleotide as coenzymes.

The control mechanisms in lignification remain obscure. Preliminary experiments by Stafford with C^{14}-labelled compounds indicated that a primary block may be at the level of deamination of phenylalanine and tyrosine. Also, according to Stafford (1967), there appeared to be a regulatory control that prevented the cells of the cortex from becoming lignified

in the excised first internodes of Phleum. Stafford (1967) suggested that flavoprotein enzymes may be responsible for the production of hydrogen peroxide which is believed required for lignification and also for the hydroxylation reactions involved in the interconversions of the various hydroxylated intermediates. Activities of peroxidase, polyphenol oxidase and ascorbic acid were also supposed to control lignin polymerization.

Darkness is reported to have an inhibitory effect on lignification. Evidence has been found that the controlling factor in lignification of the fibre walls is the illumination received by plants. Ishikawa and Takaichi (1957) suggested that light intensity and quality, the concentration of CO_2 and the activity of peroxidase are all limiting factors of lignin biosynthesis in young plants. Bean plants grown in yellowish-light had the highest lignin content, whereas dark grown plants had less than half the lignin content found in plants grown in white light. In addition, yellowish light also increased shikimic acid, phenylalanine and tyrosine content and peroxidase activity. However, the amount of starch, shikimic acid, phenylalanine and tyrosine increased proportional to the concentration of CO_2.

Swain (1960) suggested that light probably promoted the linking of C_2 units or promoted the linking of the C_6 and C_7 moieties to produce the flavonoid compounds. Both of these reactions probably involved Coenzyme-A intermediates. Synthesis of simple phenol derivatives (C_6-C_3, C_6-C_2, C_6-C_1 and C_6) from the shikimic acid pathway apparently did not

have a direct light requirement (Weinstein, Porter & Laurencot, 1961). A light requirement may exist prior to shikimate, but this may be related to the necessity for carbohydrates formed in photosynthesis. Nevertheless, chlorogenic and caffeic acid synthesis are markedly stimulated by light in sliced potato tubers (Zucker, 1963), although Stafford (1967) found that there was no detectable light effect on lignification in excised internodes of Phleum.

Auxin may be involved in the control of lignification. The polymerization of eugenol by Elodea extracts was inhibited by 10^{-4} M IAA, which inhibited completely peroxidase catalyzed oxidation (Stafford, 1965). Siegel (1954) suggested that high levels of auxin (4×10^{-5} M) in rapidly elongating organs would suppress peroxidase activity and hence lignin deposition but with declining auxin concentration accompanying maturity, lignification would be increased (Siegel, 1953 and 1960). Stafford (1965) considered that two possible inter-related mechanisms controlling lignification in intact tissue of Phleum are the level of caffeic acid esters and the level of IAA. Ascorbic acid and glutathione were also postulated to act as anti-oxidants in the lignification of plants.

The enhanced lignification observed in the presence of kinetin may result from a stimulation of carbohydrate metabolism, (Bergman, 1964). Under the influence of the adenine moiety of kinetin, an increased formation of adenine phosphates could lead to conversion of hexoses via sedoheptulose phosphate to aromatic metabolites at the expence of carbohydrate metabolism. The increase in lignin parallelled a general

increase in the metabolism of phenylpropanoids. Bergman (1964) postulated that kinetin stimulated shikimic acid synthesis and thereby promoted phenylpropanoid metabolism. Koblitz (1964) also found that kinetin increased the percentage of lignin in the cell wall of cultured carrot tissue. The proportion of methoxy groups and nitrogen content in lignin was also stimulated by kinetin. GA induced a large lignin formation in this tissue which was suppressed by γ-aminobutyric acid (Koblitz, 1964).

Among synthetic growth substances 1, 4-naphthoquinone and 2, 4-dichlorophenoxyacetic acid (2, 4-D) stimulate lignin formation. GA also promoted lignin accumulation. The stimulation caused by GA was depressed by maleic hydrazide, but the herbicide had no effect on 2, 4-D promoted lignification (Parups, 1964 and Gauthert, 1963).

Some observations by Stafford (1965) suggested that 10^{-4}M IAA inhibited lignin production. Neither kinetin nor GA, at a physiolcgical concentration, singly or in combination, reversed the IAA inhibition. It was also noted (Stafford, 1967) that the accumulation of lignin was parallelled by an increase in alkaline sensitive esters of p-hydroxy cinnamic acid, ferulic acid and sinapic acid, and by flavin coenzymes, especially flavin-adenine dinucleotide. There was, however, no detectable evidence of competition for substrate with other phenols such as anthocyanin.

No investigation has been found about the effects of any of the growth retardants on lignification of higher plants.

Materials and Methods

Seeds of dwarf green peas (Pisum sativm L., cult. Improved Laxton's Progress) and tall green peas (cult. Tall Telephone) were obtained from Stokes Seeds, Limited, St. Catharines Cntario, Canada. Gibberellic acid, 84.1% K salt, was supplied by Eastman Kodak Company. 4-Hydroxyl-5-isopropyl-2-methylphenyltrimethylammonium chloride 1-piperidine carboxylate (AMO-1618) and (2,4-dichlorobenzyltributyl, phosphonium chloride) (Phosphon-D) were obtained from Rainbow Color and Chemical Company, Northridge, California and Virginia-Carolina Chemical Corporation, Richmond, Va., respectively. L-phenylalanine and glutathione was purchased from Sigma Chemical Co., and ammonium sulfate, enzymological grade, and Bovine albumin, from Mann Research Lab Inc. Trypsin was purchased from Worthington Biochemical Co.

Growth rate studies:

Green house studies: These experiments were done in the green house during the summer, prior to obtaining growth chamber facilities. Pea seeds were germinated in Vermiculite in the dark. After 8 to 10 days germination, 10 seedlings were transplanted into soil in 8 inch plastic pots which were then placed in the greenhouse. The transplanted seedlings were treated with different concentrations of GA, AMO-1618

and Phosphon-D by pouring 200ml of each solution directly into each pot one time. Three pots were used for each treatment. Following treatment daily measurements were made of the height of the shoots from the basal part of the first node to the stem tip. The average height of the 30 seedlings per treatment was taken as an estimate of growth rate of the plants.

Growth chamber studies:

Seeds were germinated and seedlings were grown in Vermiculite (200 seeds per flat) in the dark, red light or white light. The temperature was maintained at 26°-27° C for seed germination and 20°-21° C for the seedling growth. White-light grown peas were irradiated by two 25 watt incandescent bulbs and 8 white fluorescent lamps. The light intensity was 1,000 ft- c. Red-light grown peas were irradiated by one fluorescent lamp wrapped in red cellophane. The light intensity was 8 ft- c. Radiation was for 14 hours per day in both cases. The seedlings were treated by spraying all the plants in each flat with 50ml of a solution of GA or AMO-1618.

The heights of 10 shoots in replicate flats were measured daily and the average height estimated from these measurements. The plants thus grown were used subsequently for determinations of both lignin content and the activity of phenylalanine ammonia lyase.

Lignin determination:

The young plants harvested from the growth chamber were at a stage of constant growth rate (10 to 20 days from

germination). Ten to 30 pea stems of each treatment were used for lignin determination. There were three and four replications for each treatment. The following two methods were used for lignin determination: 1) The method of Siegel (1955): Fresh tissue was macerated and extracted once with 25ml of boiling water.. The residue then was extracted three times with boiling ethanol and once with chloroform. The plant residue was soaked with 72% H_2SO_4 at 2° C for 15 hours and then the acid was diluted to 3% and heated in an autoclave at 20° C and 15 lbs pressure for three hours. The residue was collected by filtration, dried and weighed. The dried material constituted the "lignin" fraction according to the method of Siegel. 2) The method of Armitage, Ashworth, and Ferguson (1948): One gram of finely ground dried material was extracted by boiling in 40ml of ethanol-benzene (1:2 w/w) for 2.5 hours. The residue was refluxed with 100ml of 5% HCL for one hour and then filtered. The residue was incubated at 38° C for 18 hours in 100ml of 0.25% Na_2CO_3 containing 100mg of trypsin. After the tryptic digestion, the residue was again collected by filtration and dissolved in 10 ml of 72% H_2SO_4. The acid solution was held for 2 hours at 15°-20° C and then refluxed with 240ml of water for two hours (the acid was thus diluted from 72% to 3%) and filtered through a tared Gooch crucible. The "lignin" thus purified and collected was washed with hot water, dried and weighed.

Preparation and Assay of L-phenylalanine ammonia lyase:

Phenylalanine ammonia lyase was assayed by a modification of the method of Zucker (1965). Unless otherwise noted, all procedures were performed at 0° to 4° C. Twenty to 40 stems were dehydrated by grinding two times in cold acetone in a Virtis homgenizer. One gram of the acetone powder was extracted with 20ml of 0.1M Borate buffer, pH 8.7, containing 10mg of reduced glutathione. The slurry was centrifuged at 20,000 x g for 10 minutes to sediment the residue. The supernant fluid was brought to 70% saturation with solid ammonium sulfate. After a 10 minute equilibration period, the solution was centrifuged at 20,000 x g for 10 minutes. The precipitated protein was suspended in 3ml of Borate buffer, pH 8.7 and the final volume of solution measured with a pipette. This crude preparation was used for enzyme assays. The standard reaction mixture for assay of the ammonia lyase contained 100 μmoles of Borate buffer, pH 8.7, 20 μmoles of L-phenylalanine, 0.5ml of extract and water to a final volume of 3.0ml. The reaction was started by the addition of substrate and followed by recording the increase in absorption at 290mμ on a Beckman DU-2 spectrophotometer for a period of 12 minutes. The temperature was maintained at 30°C. One unit of enzyme activity was defined as that amount of protein which formed one μmole of cinnamic acid per hour as calculated from the extinction coefficient for cinnamic acid at 290mμ, (Zucker, 1965).

Protein determinations:

Protein was estimated by the standard Biuret assay

using a standard curve prepared from bovine albumin, (Gornall, 1949 and Robinson and Hogden, 1940).

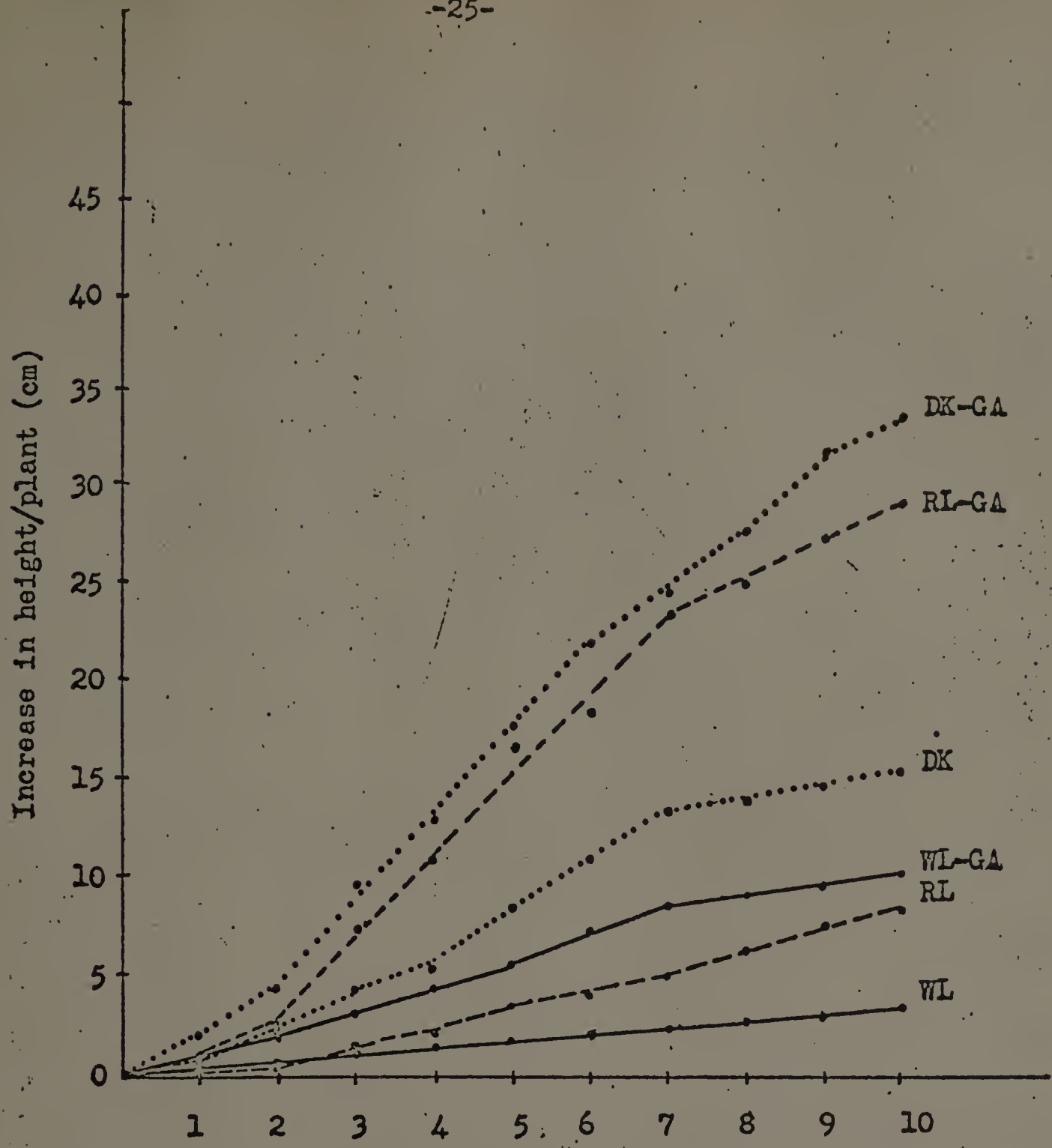
Results

Growth rate studies:

The inhibitory effects of light and growth retardants on stem elongation, or conversely, the promotive effects of GA and darkness on stem elongation, are well known (Sale and Vince,1960, Sachs and Lang, 1961 and Kuraishi and Muir, 1964b). A series of experiments were conducted to establish the responses of the two pea cultivars selected for study in the present investigation to various light regimes and to growth regulator treatments.

The data presented in figure 1b is a summary of the effects of various light regimes and GA treatment on the growth of dwarf peas. The white light, at 1,000 ft-c, caused the most inhibition but 8 ft-c of red light was almost as effective as much higher intensity white light. GA (100 µg/ml) promoted elongation of the stems in the dark as well as in the light. It is noteworthy that the enhanced growth rate in response to the GA treatment was detectable the first day after treatment under both light and dark conditions.

The effect of AMO-1618 on the growth of dwarf peas under different light conditions is shown in figure 2. There was a detectable inhibition of growth caused by 50 ppm of AMO-1618 in the dark, but the growth retardant had no detectable



Days after treatment

Fig.1b. The effect of GA on the growth of dwarf peas grown under different light regimes. Pea seeds were germinated in the growth chamber at 27° for 6 days (red light & darkness) and 8 days (white light), then the seedlings were sprayed with 100µg/ml of GA (50ml/200 plants) and grown at 21°. Each point was the average of duplicate samples, each of which contained 10 plants.

DK----dark, control.

DK-GA----dark, treated with GA.

RL----red light, control.

RL-GA----red light, treated with GA.

WL----white light, control.

WL-GA----white light, treated with GA.

effect on the growth of the dwarf plants under white light and red light conditions. Again, as in the case of GA, the plants responded to AMO-1618 in the dark the first day after treatment.

Although AMC-1618 had no detectable effect on growth of dwarf peas in light, 50 ppm of AMC-1618 did inhibit growth of tall peas under these conditions (Fig 3). GA at 10^{-4} M promoted the growth of both dwarf and tall peas, and in fact, caused the dwarf peas to grow at a faster rate than the tall peas.

Lignin studies:

Two different methods were employed for lignin determination. No method is available which yields a homogenous preparation of lignin and different methods all give slightly different results. Because of this technical limitation the chemical composition of lignin is not precisely known. In fact, the structure of lignin probably varies with the source. Nevertheless, it is possible to obtain reproducible "lignin" fractions which presumably give an estimate of the lignin content of the tissue.

Siegel's method for lignin determination was originally employed to study the peroxidase catalyzed polymerization of eugenol in the presence of hydrogen peroxide to a product with properties similar to lignin. It is a relatively simple method, but used on natural tissue yields more "lignin" than other methods. Armitage, Ashworth & Ferguson's method for lignin determination was used for the latter part of the present investigation. The method of Armitage et al is a modified

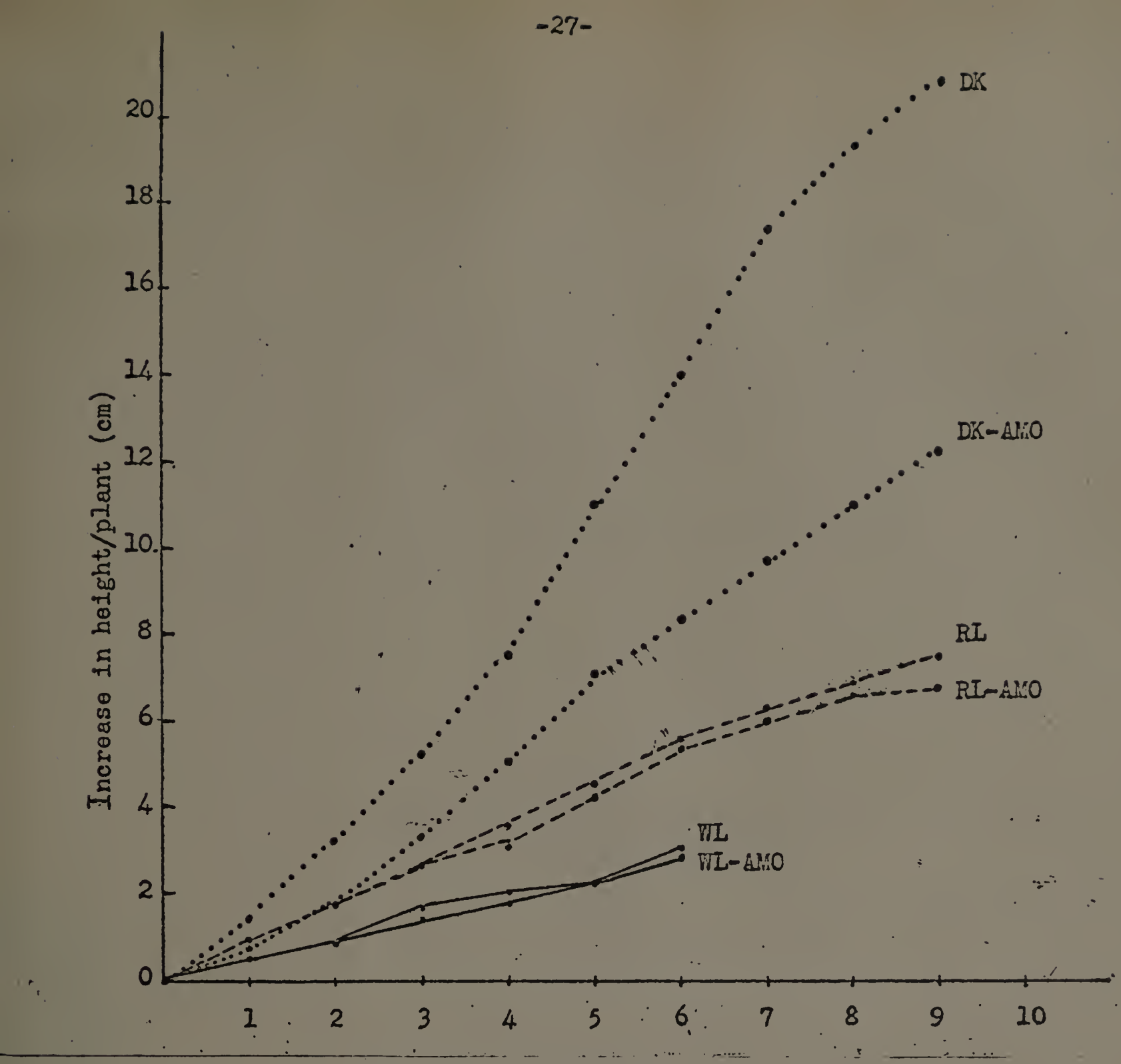


Fig. 2. The effect of AMO-1618 on the growth of dwarf peas grown under different light regimes. Pea seeds were germinated in the growth chamber for 8 days at 27°, the seedlings were sprayed with 50ppm Of AMO-1618 one time (50ml/200 plants) and grown at 21°. Each point was the average of duplicate samples each of which contained 10 plants.

DK----dark, control.

DK-AMO----dark, treated with AMO.

RL----red light, control.

RL-AMO----red light, treated with AMO.

WL----white light, control.

WL-AMO----white light, treated with AMO.

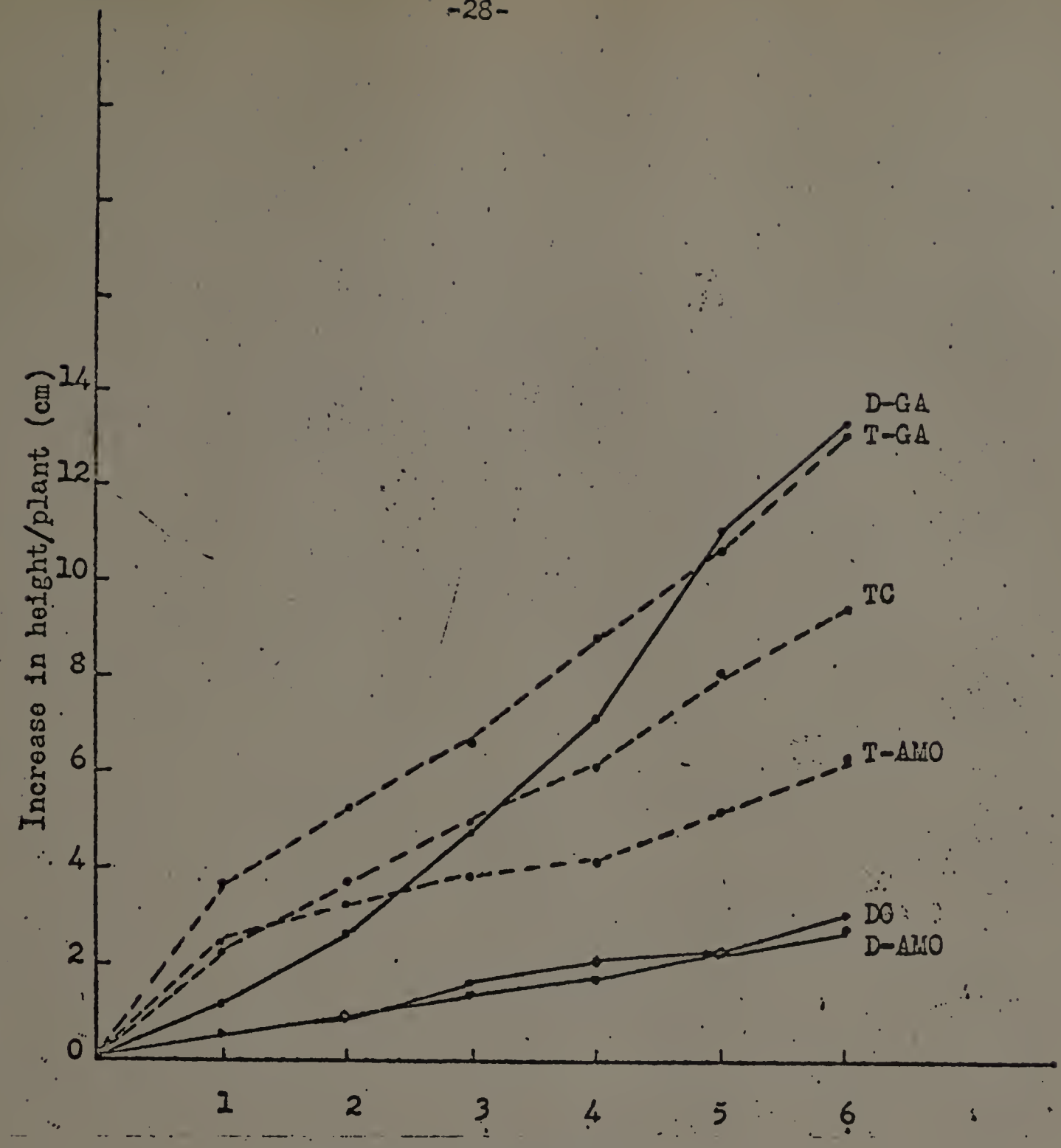


Fig. 3. The growth of dwarf peas and tall peas treated with AMO-1618 or GA Pea seeds were germinated in the green house for 10 days during summer, then the seedlings were treated with 50 ppm of AMO-1618 or 10 M^{-4} of GA. Each point was the average of triplicate samples each of which contained 10 plants.

DC----dwarf, control.

D-GA----dwarf, treated with GA.

D-AMO----dwarf, treated with AMO.

TC----tall, control.

T-GA----tall, treated with GA.

T-AMO----tall, treated with AMO.

method for the determination of total lignin in high protein plant material (Norman, 1937). The main modifications are the use of a simple digestion with trypsin to remove protein contamination in the lignin complex and the use of Gooch crucibles which give more accurate quantitative results.

In a preliminary experiment, the stems of dwarf peas were assayed for lignin content by Siegel's method. Dwarf peas were grown in the green house during the summer: 8 day old seedlings were treated with 10^{-3} M, 10^{-4} M, and 10^{-5} M GA and 1, 5 and 50 ppm AMO-1618 and Phosphon-D, separately. Six days after treatment the plants were harvested for lignin determination. The results (Table 1) suggested that GA stimulated lignin accumulation. The effects of the two growth retardants on lignification were also examined in this experiment. AMO-1618 and Phosphon-D in a concentration range of 1-50 ppm had no consistant effect on lignification of dwarf peas.

Because GA appeared to promote lignification in the dwarf peas, the effects of GA on lignification in tall peas also was investigated. Seedlings were grown for 8 days in the green house and then treated with 10^{-4} M GA. Four days after treatment, the stems were harvested for lignin determination by the method of Siegel. The GA treatment almost doubled the growth of the tall peas but had no effect on the lignin content of the stems on either a fresh weight or dry weight basis, (Table 2). It will be noted, however, that the lignin content of the GA treated and control tall peas was appreciably greater than that of dwarf control peas (Table 1). The results therefore appeared to suggest that GA promoted lignin accumulation

in dwarf peas but had no effect on the lignin content of tall peas.

The growth rate studies (Fig.1b, 2, & 3) indicated that plants treated with GA or growth retardants responded to treatment within one day. In order to determine the relationship between GA-promoted growth and the apparent GA-promoted lignification, a time course study was conducted (Fig. 4). White light-grown seedlings, 8-days old, were sprayed with 100 µg/ml of GA. The young plants were used for lignin determination by the method of Armitage et al. The average lignin content of the GA-treated plants was higher than that of the controls at all sampling dates, and the apparent GA-promoted lignification was detectable the 2nd day after treatment. The % lignin of both the controls and GA treated plants increased on the 3rd and 4th day after treatment (11 and 12 days after germination) then slightly decreased. On the 10th day after treatment (18 days from germination) there was a very pronounced increase in the lignin content of both the treated and control plants.

The preliminary investigations of the effects of GA on lignification (Table 1 & 2) were repeated using the method of Armitage et al. Seedlings of tall and dwarf peas were grown for 10 days in the growth chamber and then treated with 10^{-4}, 10^{-5}, and 10^{-6} M GA. After a further 9 days growth under white light, the stems were harvested for lignin determination.

The tall plants were approximately twice as tall as the dwarf plants and contained twice as much total lignin as the dwarfs (Table 3). On a fresh or dry weight basis, untreated

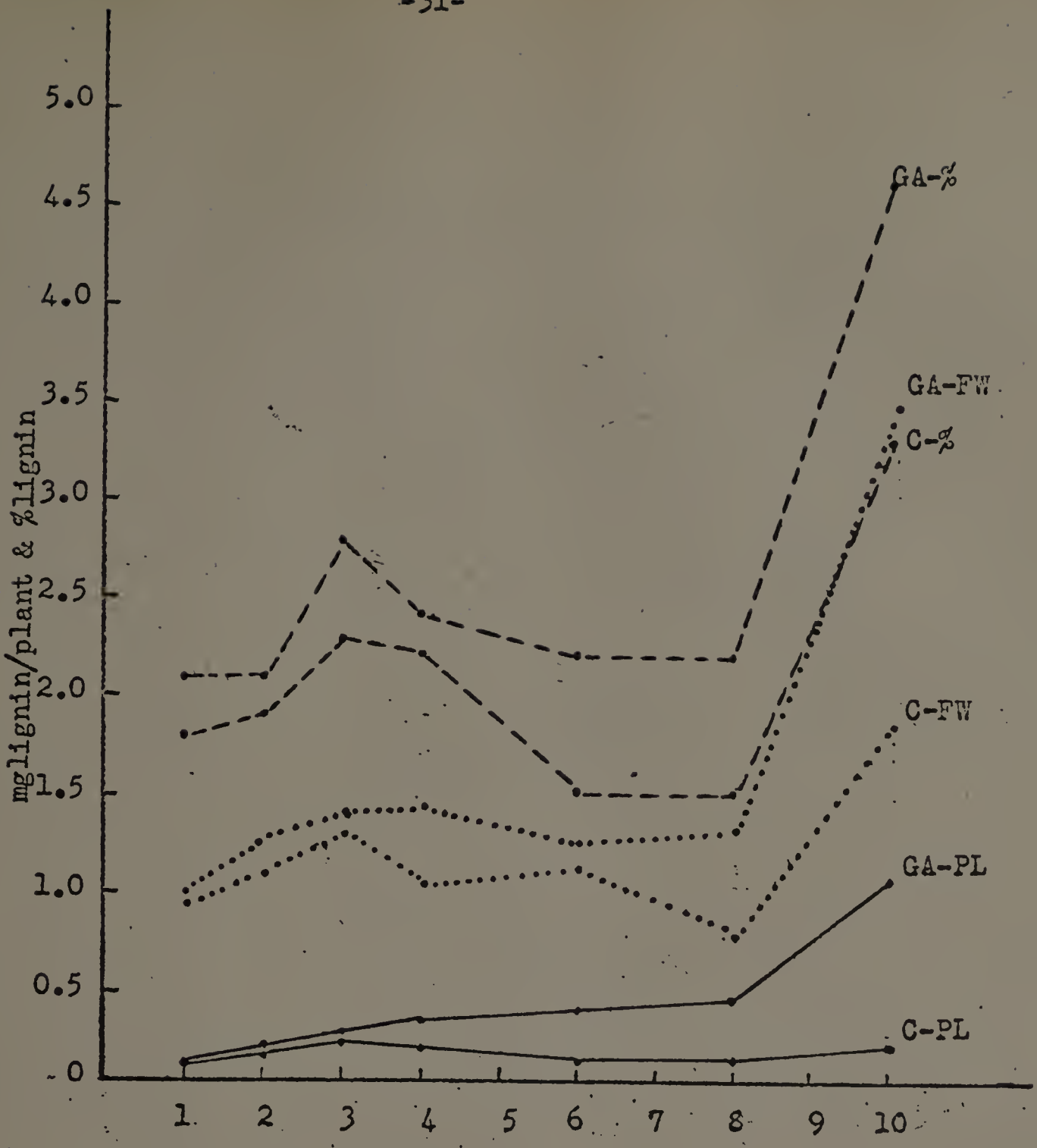


Fig. 4. Time course of lignin accumulation in dwarf pea stems. Pea seeds were germinated in the growth chamber for 8 days at 27°, then the seedlings were sprayed with 100µg/ml of GA (50ml/200 plants) one time and grown at 21°. Each point was the average of duplicate samples each of which contained 20-30 stems. The percentage of lignin is based on dry weight.

C-%----% lignin, control.

C-PL----mg lignin/plant, control.

GA-%---%lignin, treated with GA.

GA-PL----mg lignin/plant, treated with GA.

C-FW----mg lignin/g fresh wt.

GA-FW----mg lignin/g fresh wt. treated with GA.

Table 1. The lignin content of dwarf peas treated with AMO-1618, phosphon-D and GA (Siegel's method of lignin determination).

Treatments	Increase of height (cm)	mg of lignin per plant	mg lignin per g fresh wt.	% lignin (based on dry wt.)
Control	3.7	3.3	2.1	1.9
AMO-1618				
1 ppm	3.8	3.7	1.5	1.4
5 ppm	2.6	4.3	2.0	1.8
50 ppm	2.7	4.2	2.4	2.1
Phosphon-D				
1 ppm	2.9	3.7	2.6	2.4
5 ppm	3.3	3.3	1.9	2.0
50 ppm	3.3	3.4	1.7	1.7
GA_3				
10^{-5} M	6.2	5.0	2.9	2.8
10^{-4} M	13.3	5.8	3.1	2.9
10^{-3} M	16.0	5.9	2.9	2.6

Seeds were germinated in the green house for 8 days, then treated with 10^{-5} M, 10^{-4} M and 10^{-3} M GA, or 1, 5, and 50 ppm of both AMO-1618 and phosphon-D, for another 6 days. The data are the means of duplicate samples each of which contained 10 plants.

Table 2. The lignin content of tall peas treated with GA (Siegel's method of lignin determination).

Treatment	Height (cm)	mg lignin per plant	mg lignin/g of fresh wt.	% lignin (based on dry wt.)
Control	11.7	4.0	3.1	3.2
GA 10^{-4} M	20.2	4.6	3.1	3.4

Seeds were germinated in the green house for 8 days then treated with 10^{-4} M of GA for another 4 days. 200 ml of 10^{-4} M GA solution was poured directly into each pot (10 plants/pot). All data are the means of duplicate samples.

tall plants had more lignin than the dwarfs. Treating with GA greatly promoted lignification in the dwarf peas, i.e., GA at concentrations of 10^{-4} M, 10^{-5} M, and 10^{-6} M promoted lignin accumulation by factors of 6.5, 6 and 2, respectively and growth by factors of 4.5, 2.7 and 1.3. In the tall pea, in contrast, GA had no effect on lignin, although 10^{-4} M GA did promote growth slightly.

Light, GA and AMO-1618 all affected plant growth (Fig. 1b, 2 and 3) and GA was found to stimulate lignification of dwarf peas grown under white light (Table 3). Further experiments were conducted to establish the effects of light, darkness, GA and the growth retardant, AMO-1618, on lignification of pea plants. Dark and red light grown dwarf peas (8-days old) were sprayed with 100 µg/ml of GA or 50 ppm of AMO-1618.

Table 3. The lignin content of dwarf and tall peas treated with different concentrations of GA, (the method of Armitage, Ashworth & Ferguson for lignin determination).

Treatment	Height (cm)	mg lignin per stem	mg lignin/g fresh wt.	% lignin (based on dry wt.)
Dwarf Peas				
Control	9.8± .07	.9±.30	2.7± .78	3.1±.07
GA 10^{-6} M	12.6± .04	1.9±.28	4.8± .11	4.5±.25
10^{-5} M	26.9± .20	6.1±.84	9.6±1.30	7.7±.44
10^{-4} M	44.3±1.07	6.4±.78	6.4± .76	6.8±.60
Tall Peas				
Control	24.8± .36	1.8±.06	3.2± .26	4.8±.37
GA 10^{-6} M	24.2±1.50	1.6±.12	3.2± .23	4.2±.24
10^{-5} M	26.8±1.26	1.7±.29	2.7± .40	4.2±.74
10^{-4} M	36.0± .62	2.4±.13	3.2± .49	4.2±.55

Seeds were germinated in growth chambers for 10 days, then sprayed with 50 ml of 10^{-6} M, 10^{-5} M, and 10^{-4} M GA and grown for another 9 days under 1,000 ft-c white light, (50 ml of GA solution per pot and 10 plants per pot). All data are the means of duplicated samples each of which contained 10 plants. The standard deviation is given.

Ten days later the stems were harvested for lignin determination by the method of Armitage et al. The results of this experiment are presented in Table 4. Data from the time course experiment (Fig. 4) are included for comparison of white light grown tissue to the dark and red-light grown tissues. Relative to the dark grown tissue, red light slightly stimulated lignin formation on a fresh weight but not a dry weight basis in the dwarf peas. The lignin content of the dwarf plants grown in the darkness or red light on a fresh or dry weight basis was essentially unaffected by either GA or AMO-1618, although these two growth regulators promoted and inhibited, respectively, the growth of the plants. The total amount of lignin per plant was promoted in both the dark and red light by GA. In the dark, AMO-1618 inhibited growth but had little effect on the total lignin content of the plants. Examination of the data for the % lignin on a fresh or dry weight basis would suggest light promoted lignification in the controls. Actually, however, control plants grown in the dark had 2 to 3 times more total lignin per stem than the red and white light grown plants.

The effects of GA and AMO-1618 on lignification of tall peas grown under darkness were also investigated (Table 5). Six-day old seedlings were sprayed with 10^{-4} M GA and 50 ppm AMO-1618 separately. Ten days after treatment the stems were harvested for lignin determination by the method of Armitage et al. Neither GA nor AMO-1618 had a marked effect on either the growth or lignification of tall peas in the dark,

Table 4. The lignin content of the stems of dwarf peas treated with GA and AMO-1618 under red light, white light, and darkness.

	Expt. No.	Treatments	Height (cm)	mg lignin per stem	mg lignin/ g fresh wt	%lignin
Dark	1	Control	15.7	.9±.03	.57± .06	1.5±.07
		GA	33.9	1.4±.10	.71± .06	1.7±.13
	2	Control	20.8	.7±.04	.61±1.09	1.7±.14
		AMO-1618	12.3	.6±.03	.72± .03	1.8±.49
Red Light	3	Control	7.4	.4±.01	.90± .02	1.7±.03
		GA	31.3	1.0±.01	.91± .01	2.0±.07
		AMO-1618	6.7	.3±.01	.90± .03	1.6±.04
White Light	4	Control	3.7	.3±.05	1.90± .61	3.3±.26
		GA	10.4	1.1±.01	3.50± .05	4.6±.13

Seeds were germinated in growth chambers for 8 days, then sprayed with 50 ml of 100 µg/ml GA and 50 ml of 50 ppm AMO-1618 per 100 plants and harvested 10 days later. All data are the means of four samples ± the standard deviations. Each sample contained 30 plants.

although 50 ppm AMO appeared to slightly increase the total lignin per stem. It is also noteworthy that in this experiment the growth regulator had no effect on the growth of tall peas in the dark, in contrast to their effects in the light (fig. 3).

A number of observations (Minaniskawa & Uritani, 1965, Young & Neish, 1966, and Zucker, 1965) suggested that phenylalanine ammonia lyase was a key enzyme in phenolic metabolism and the lignification of higher plants. Experiments were conducted, therefore, to determine the relation, if any, between the activity of phenylalanine ammonia lyase and lignification in pea plants, and the effects of GA, light and AMO-1618 on the activity of the enzyme.

GA has been shown to promote elongation and lignification of dwarf pea stems. In contrast, GA typically has no effect on root growth. A preliminary experiment was conducted to determine the effects of GA on phenylalanine ammonia lyase activity in the roots and shoots of white light grown dwarf peas. Nine day old seedlings were sprayed with 200 µg/ml of GA and after a further 9 days growth the plants were harvested for enzyme assay. It appeared that GA did stimulate the activity of phenylalanine ammonia lyase in the shoots but not in the roots of dwarf peas (Table 6). Furthermore, the activity of this enzyme on both a per plant and specific activity basis in GA treated stems approached the level of activity found in the roots.

It was of interest to determine how soon after GA treatment the enhanced phenylalanine ammonia lyase activity could be

Table 5. The lignin content of tall peas treated with 10^{-4} M GA or 50 ppm AMO-1618, (the method of Armitage, Ashworth & Ferguson for lignin determination).

Treatments	Height (cm)	mg lignin per plant	mg lignin/ g fresh wt	% lignin (based on dry weight)
Control	12.4	.26±.01	.53±.01	1.2±.03
AMO-1618 50 ppm	12.7	.32±.01	.61±.06	1.3±.09
GA 10^{-4} M	13.1	.28±.01	.60±.02	1.2±.02

Seeds were germinated in growth chambers for 6 days under total darkness, then sprayed with 50 ml of 10^{-4} M GA and 50 ml of 50 ppm AMO-1618 and harvested 10 days later. All data are the means of 3 samples. Each sample contained 30 plants.

Table 6. The activity of phenylalanine ammonia lyase in the shoots and roots of dwarf peas.

Part of Plants	Treatments	Enzyme Activities Unit/g fresh wt.	Unit per Plant	Specific Activity
Shoot	Control	.05	.009	.023
	GA	.06	.030	.056
Root	Control	.05	.038	.053
	GA	.05	.034	.048

Seedlings were grown in the growth chamber under 1000 ft-c white light for 9 days, then sprayed with 200 µg/ml GA (50 ml/200 plants) and harvested 9 days after treatment. The data are the averages of duplicate samples each of which contained 20 plants.

detected and therefore a time course study was conducted. Dwarf pea plants were grown under the same conditions as those of the experiment reported in figure 4. GA (100 µg/ml) slightly but consistently stimulated the activity of the enzyme throughout the course of this experiment (Table 7). This enhanced activity was evident on both a fresh weight and specific activity basis. The activity per stem increased most markedly in response to GA treatment.

In this and subsequent experiments the amount of protein per stem was estimated as based on the protein extracted from the acetone powder by the borate buffer used in preparing the enzyme preparation. The data reported in Table 7 shows that in the control tissue the amount of this borate soluble fraction of protein was relatively constant throughout the duration of the time course experiment. The amount of the borate soluble protein was greater in the GA-treated tissue the first day after treatment and in 3 days rose to a level twice that of the control tissue.

The time course study of the effects of GA on phenylalanine ammonia lyase activity was repeated using a higher concentration of GA (fig. 5). Ten-day old seedlings were sprayed with 200 µg/ml of GA and 2, 4, and 6 days later the plants were harvested for assay of phenylalanine ammonia lyase. GA markedly stimulated enzyme activity the 2nd day after treatment on both a per plant and specific activity basis. The specific activity of the ammonia lyase in the GA-treated tissue was slightly higher in this experiment using 200 µg/ml GA than in the preceding

Table 7. The time course of the activity of phenylalanine ammonia lyase in dwarf pea stems.

Days after Treatment	Height (cm)	Enzyme Activity: Unit per g fresh wt.	Unit/ stem	mg protein per stem	Specific Activity
Control					
1	.3	.08	.008	.58	.014
2	.6	.07	.007	.71	.010
3	1.0	.06	.007	.51	.014
4	1.2	.07	.009	.51	.017
6	1.6	.05	.006	.57	.012
8	2.0	.07	.010	.66	.015
10	4.5	.05	.006	.59	.011
GA					
1	.7	.11	.013	.73	.017
2	2.0	.10	.015	.89	.017
3	3.1	.07	.016	1.20	.014
4	4.3	.11	.026	1.20	.022
6	6.7	.13	.039	1.47	.027
8	8.3	.08	.024	1.35	.018
10	10.0	.08	.026	1.22	.021

Seeds were germinated in growth chambers for 8 days, then the seedlings were sprayed with 100 µg ml of GA (50 ml/200 plants). The plants were grown under 1000 ft-c white light and harvested at the indicated days. The data are the means of duplicate samples each of which contained 20 plants.

experiment (table 7) where 100 ug/ml of GA was used. However, the increase in enzyme activity was not proportional to the concentration of GA.

In order to understand more about the effects of light, GA and AMO-1618 on lignification and plant growth, the activity of phenylalanine ammonia lyase in dwarf peas grown under different light regimes and subjected to different growth regulators was also investigated (table 8). The plants were grown under the same conditions as those of the experiment reported in table 4. Data from the 10th day of the time course study (table 7) are included for comparison of white light conditions. Relative to the dark grown tissues, red light slightly stimulated phenylalanine ammonia lyase activity on both a fresh weight and specific activity basis, although the plants grew to a greater height in the dark. GA stimulated enzyme activity only in light and was most effective on the white light grown plants.

Interestingly, the protein content of the stems was highest in the dark grown tissue and was not affected by either growth regulator, although GA promoted and AMO-1618 inhibited stem elongation in this experiment (table 8). In the red light, however, as was observed in the white light, there was approximately twice as much protein per stem in the GA treated tissue as in the controls. AMO-1618 had no appreciable effect on the growth or amount of protein per stem in the red light.

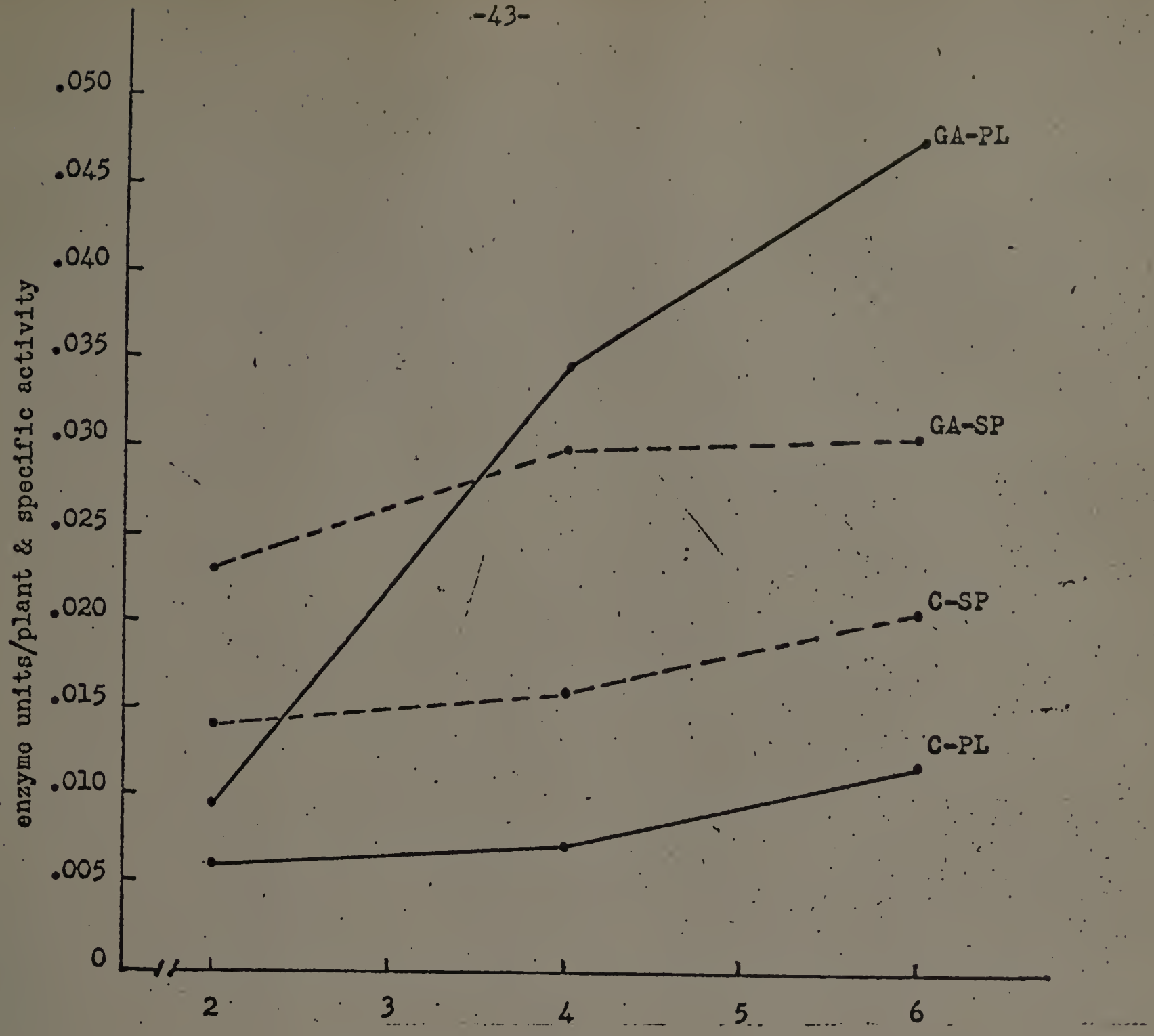


Fig. 5. Time course of phenylalanine ammonialyase activity in dwarf pea stems. Pea seeds were germinated in the growth chamber in white light for 10 days at 27°, then the seedlings were sprayed with 200µg/ml of GA one time (50ml/200 plants). Each point was the average of duplicate samples each of which contained 30 stems.

C-PL----control, enzyme units/plant.

C-SP----control, specific activity.

GA-PL----GA treatment, enzyme units/plant.

GA-SP----GA treatment, specific activity.

Table 8. The activity of phenylalanine ammonia lyase in the stems of dwarf peas treated with GA or AMO-1618 and grown under red light, white light or in darkness.

	Treatments	Height (cm)	Unit/ plant	Unit/g frs. wt.	mg protein per plant	Specific Activity
	Control	15.7	.008	.008	1.7	.005
	GA	33.9	.008	.007	1.8	.005
DARK						
	Control	20.8	.007	.006	1.8	.004
	AMO-1618	12.3	.009	.011	1.7	.005
RED LIGHT	Control	7.4	.005	.012	0.6	.009
	GA	31.3	.017	.015	1.2	.015
	AMO-1618	6.7	.004	.010	0.7	.006
WHITE LIGHT	Control	3.7	.006	.050	0.6	.011
	GA	10.4	.026	.080	1.2	.021

Plants were germinated and grown under the same conditions as those of the experiment of Table 7. The data are the means of duplicate samples each of which contained 20 plants.

Discussion

It has long been known that light inhibits while GA promotes the growth of many dwarf plants. The experiments reported here confirm that both red and white light inhibited while GA promoted the growth of pea plants.

AMO-1618 has been reported to suppress gibberellin production in cultures of the fungus Fusarium moniliforme without affecting their growth (Harada and Lang, 1965). Direct evidence that the production of gibberellin in higher plants is inhibited by AMO-1618 has been shown by Baldev, Lang and Agatep (1965). Therefore, the interpretations of the present experiments are based on the assumption that AMO-1618 inhibits growth by inhibiting GA biosynthesis. It is also logical to assume that the growth retardant would be equally effective in inhibiting GA biosynthesis in the light and dark.

Under the experimental conditions of this study, AMO-1618 inhibited the growth of dwarf peas only in the dark and of tall peas only in the light. That AMO-1618 had no effect on the growth of dwarf peas in the light can be interpreted as evidence that while GA promotes plant growth it is not absolutely necessary for growth. If the growth of the dwarf plants in the light was due to the presence of small amounts of GA, AMO should have retarded their growth, just as it did

the tall plants. But it did not do so and therefore it seems reasonable to conclude that a certain amount of growth can occur in the absence of GA.

A definitive explanation of the light inhibition and GA promotion of plant growth cannot be given at this point. However, as a working hypothesis it is postulated that light stimulates the formation of a compound, or compounds, which inhibits growth. GA is proposed to promote growth by either blocking the formation of this compound(s) or by nullifying its action. The present experiments can be interpreted in terms of this postulation. In the light, plants require more GA than they do in the dark to overcome the effects of the light-promoted inhibitor. In the dwarf peas, the level of GA was not sufficient to overcome this inhibitor either because the dwarf pea contained less GA or more inhibitor. In the dark, where a lesser amount of GA is required, the dwarf plants grew much taller than in the light. AMO reduced the level of GA and thus reduced growth in the dark. In the tall plants, in contrast, there was sufficient GA to partially overcome the effects of the light-promoted inhibitor. The observation that exogenous GA stimulated the growth of the tall plants in the light indicates that the endogenous GA was not present in saturating quantities. AMO reduced the amount of GA and thus reduced growth of the tall peas in the light. In the dark, the tall plants had an excess of GA and thus, although AMO reduced the level of GA, it had no effect on growth.

The identity of the light-promoted inhibitor of stem elongation is not known. However, a number of observations suggest

That it may be a flavonol compound. It is well established that the formation of some flavonols is enhanced by light (Furuya and Thimann, 1964, Downs and Siegilman, 1965, Furuya and Thomas, 1964) and, in certain cases (Furuya and Thimann, 1964) inhibited by GA. Parups (1967) has recently reported inhibition of protein synthesis by coumarin and by the flavonols, kaempferol and quercetin. Growth is now known to be dependent on protein synthesis (Key, 1964). In the present studies, dark grown stems contained 3-fold more protein than the stems of plants grown in white light. GA doubled the amount of protein in the stems of plants grown in white light but had no effect in the dark. These observations support in part the premise that a factor is produced in the light which inhibits protein synthesis and thus growth. However, GA promoted and AMO inhibited growth in the dark although these growth regulators did not affect protein content in the dark. This observation argues against the possibility that the amount of protein per stem is strictly a function of the amount of stem. On the other hand, it also suggests that the role of GA in plant growth is more complex than a simple non-specific stimulation of protein synthesis. The controlling mechanisms of lignification may involve the activity of phenylalanine ammonia lyase, light and GA. Stafford (1967) suggested that the activity of phenylalanine ammonia lyase might be the controlling factor in lignification in Sorghum plants. The observations here that there was slightly more enzyme activity and 2- to 3- fold more total lignin per stem in the dark-grown tissue than in the light-grown tissue might suggest that phenylalanine ammonia lyase was a

controlling factor of lignification. Also, enzyme activity and the percentage of lignin were stimulated by GA in the light which might be interpreted as evidence that the enzyme controls lignification in the dwarf plants. However, the time course studies of the changes in lignin content and enzyme activity suggest that the activity of phenylalanine ammonia lyase was not a primary limiting factor in lignification in the dwarf plants. In the time course studies, there was no obvious correlation between enzyme activity and lignin content. Thus, it would appear that phenylalanine ammonia lyase is probably only one of the controlling factors of lignification in the dwarf peas.

The synthesis of many phenolic compounds (some of which are precusors of lignin) and lignin formation are reported to be stimulated by light and GA. (Zucker, 1963, Kogl, 1964 & Parups, 1964). In the present experiments, light and GA did stimulate lignification in the dwarf plants when the results are expressed on a percentage basis. In contrast, the total lignin content per plant was higher in dark-grown plants than in light-grown plants. Therefore, the light effect on lignification still remains obscure. At least, the observation that light and GA had no effect on lignification of tall plants suggests different control mechanisms for lignification between these two cultivars.

The observations that GA promoted growth the first day after treatment indicate a relatively rapid response to the phytohormone. Any metabolic change which can only be detected a few days after treatment must be a secondary response.

Therefore, the enhanced lignification observed in the GA-treated dwarf plants can not be a direct cause of the GA-promoted elongation. The question remains, however, why GA promoted lignification in the dwarf but not the tall peas, although both cultivars responded to GA in the light.

The observation that darkness and GA promoted stem elongation and lignin accumulation on a per plant basis at the same time can be explained in terms of the working hypothesis proposed above, assuming that some flavonoid compounds are the light-promoted inhibitors of growth. In the dark or in GA-treated plants, the carbon of cinnamic acid may be diverted from the flavonoid compounds into lignin. Perhaps the formation of these flavonoid compounds is promoted by light and inhibited by GA. It is thus possible that light and GA may control the growth and lignification in plants by regulating the metabolism of cinnamic acid derivatives.

Summary

A series of experiments were conducted to establish the growth and lignification responses of two pea cultivars (Pisum Sativm L., cult. Improved Laxtons Progress and cult. Tall Telephone) to various light regimes and to growth regulator treatments.

1) GA at 100 µg/ml promoted stem elongation of dwarf peas in the dark as well as in the light. The enhanced growth rate in response to the GA treatment was detectable the first day after treatment under both light and dark conditions. AMO-1618 at 50 ppm inhibited the growth of dwarf peas in the dark but not in the light. The inhibition caused by AMO-1618 in the dark was also detectable the first day after treatment.

2) GA at 10^{-4} M promoted the growth of both dwarf and tall peas in the light and in fact caused the dwarf peas to grow at a faster rate than the tall peas. GA at 10^{-4} M also increased the growth of dwarf peas in the dark but had no effect on the growth of tall peas. AMO-1618 at 50 ppm inhibited the growth of tall peas in the light but not in the dark.

3) Light promoted lignification in the controls of dwarf peas on a dry wt basis. Actually, however, control plants grown in the dark had 2 to 3 times more total lignin per stem than the light-grown plants. GA stimulated lignin accumulation of dwarf plants only in the light. The lignin content of the

dwarf plants in the dark on a fresh or dry wt basis was essentially unaffected by either GA or AMO-1618 although these two growth regulators promoted and inhibited, respectively, the growth of the dwarf plants.

4) Neither GA nor AMO-1618 had a detectable effect on lignification of tall peas under light or dark conditions although 10^{-4} M GA and 50 ppm AMO promoted and inhibited, respectively, the growth of plants in the light.

5) GA slightly but consistantly stimulated the activity of phenylalanine ammonia lyase in the stems of dwarf peas in the light. The enzyme activity per stem increased most markedly in response to GA treatment.

6) The protein content (based on the protein extracted from an acetone powder by borate buffer) of the stems of dwarf peas was 3-fold higher in the dark-grown tissue than the light-grown tissue. GA promoted protein synthesis only under light conditions.

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