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Shoot apex senescence is defined as the collective progressive and deteriorative processes which ultimately terminate the functional life of the shoot apex. Senescence of shoot apices of Pisum sativum L. 'Alaska' as measured by cessation of stem elongation was delayed by removal of flowers and by treatment with gibberellin A_3 and was hastened by treatment with AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride). Ontogenetic changes in relative endogenous gibberellin levels and in capability of gibberellin biosynthesis in deflowered and control (flower- and fruit-bearing) plants were determined indirectly by studying time-course changes in the sensitivity, as indicated by the growth response, of these plants to applied gibberellin and AMO-1618. The results of these experiments suggest that the endogenous gibberellin level varies directly with the growth rate.

Analyses of total RNA, nitrogen, protein and inorganic

phosphorus in shoot tips of deflowered and control plants throughout ontogeny revealed that the levels of all these metabolites declined during senescence. Also throughout ontogeny shoot tips of control and deflowered plants were compared with respect to their ability to enzymically degrade RNA and to take up and incorporate ^{32}P-orthophosphate into RNA. The specific activity of ribonuclease increased as senescence progressed while the absolute activity appeared to decrease in correlation with the decrease in total nitrogen content. When compared with nonsenescing shoot tips, senescing shoot tips accumulated less ^{32}P, but exhibited an apparent enhancement of ^{32}P incorporation into RNA, which was attributed to a reduction in the endogenous phosphorus pool causing a smaller dilution of the accumulated ^{32}P.

It is concluded that decreases in the levels of RNA, protein, gibberellin and inorganic phosphorus and in the transpirational uptake of nutrients are factors correlated with senescence of the shoot apex. Thus, while shoot apex senescence is correlated with numerous interrelated processes, senescence apparently results most directly and basically from deterioration of RNA and protein synthesis and concomitant enzymic degradation of these macromolecular species.

Physiology of Senescence of the Shoot Apex in *Pisum sativum* L.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
Concepts of Senescence	1
Quantitative Nature of Senescence in Plants	2
Mobilization Hypothesis	2
Other Aspects of Plant Senescence	5
Physiological, Cytological and Biochemical Changes Associated with Senescence	7
PURPOSE OF THE STUDY	17
MATERIALS AND METHODS	20
Source and Purity of Reagents	20
General Procedures	20
Culture of Plants	20
Preparation of Growth Regulator Solutions and Treatment of Plants	21
Measurement of Plants and Statistical Analyses	22
Preparation of Plant Shoot Tips for Biochemical Studies	22
Determination of Dry Weight	23
Definitions	23
Repetition of Experiments	23
Extraction and Determination of RNA and Protein	24
Assay of Ribonuclease Activity, Nitrogen and Inorganic Phosphorus	25
Preparation of Enzyme Extracts	25
Estimation of Ribonuclease Activity	26
Estimation of Nitrogen	26
Estimation of Inorganic Phosphorus	27
Incorporation of ^{32}P into RNA	28
Preparation of Plant Material	28
Incubation of Explants	28
Extraction and Estimation of RNA	29
Determination of Radioactivity	29
Determination of Incorporation of ^{32}P into RNA	30

Page

RESULTS 31

Effect of Deflowering on Growth and Senescence 31
Effect of Gibberellin Treatments on Control and Deflowered Plants 40
Effect of AMO-1618 Treatments on Control and Deflowered Plants 41
Ontogenetic Changes in Sensitivity of Plants to Applied Gibberellin or AMO-1618 42
Ontogenetic Changes in the Levels of Total RNA and Protein in Plant Shoot Tips 50
Ontogenetic Changes in the Levels of Nitrogen and Inorganic Phosphorus in Plant Shoot Tips 50
Ontogenetic Changes in Soluble Ribonuclease Activity in Shoot Tips 53
Ontogenetic Changes in the Ability of Shoot Tips to Incorporate ^{32}P into RNA 65

DISCUSSION 69

Delay of Senescence by Deflowering 69
Correlation of Plant Growth Rate and Senescence with the Level of Endogenous Gibberellin 70
Gibberellin Involvement in Apical Dominance 72
Physiological Manifestations of Senescence Associated with Development and Removal of Reproductive Structures 72
Correlation of Biochemical Changes with Senescence 73

BIBLIOGRAPHY 78

LIST OF FIGURES

Figure		Page
1	Stem elongation of 'Alaska' peas in response to flower removal and to weekly shoot-tip applications of 1 μg of KGA_3 per plant.	33
2	Effect of deflowering and shoot-tip applications of GA on the growth rate of 'Alaska' peas.	35
3	Effect of deflowering and shoot-tip applications of GA on the average length of internodes of 'Alaska' peas.	37
4	Typical senescent shoot tip of a flower- and fruit-bearing plant.	39
5	Typical shoot tip of a deflowered plant prior to the occurrence of determinate growth.	39
6	Terminal flower and leaf at the final node of a deflowered plant displaying determinate growth.	39
7	Terminal leaf with fused, degenerate stipules at the final node of a deflowered plant exhibiting determinate growth.	39
8	Stem elongation of 'Alaska' peas in response to deflowering and to weekly shoot-tip applications of 50 μg of AMO-1618 per plant.	44
9-12	Ontogenetic changes in sensitivity of fruit-bearing and deflowered 'Alaska' peas to a single shoot-tip application of 1 μg of KGA_3 per plant or 50 μg of AMO-1618 per plant.	47
13	Ontogenetic changes in growth rate and in the levels of total RNA and protein in the shoot tips of fruit-bearing (control) and deflowered plants.	52
14	Ontogenetic changes in the levels of total and soluble nitrogen in shoot tips of plants bearing reproductive structures (controls) and deflowered plants.	55
15	Ontogenetic changes in the level of inorganic phosphorus in shoot tips of plants bearing flowers and fruits (controls) and deflowered plants.	57

Figure		Page
16	Ontogenetic changes in the activity of soluble RNase in shoot tips of plants bearing reproductive structures (controls) and deflowered plants.	60
17	Time-course change in the ratio of protein nitrogen in the soluble RNase extract to shoot tip dry weight from shoot tips of deflowered plants and plants bearing reproductive structures (controls).	63

LIST OF TABLES

Table		Page
1	Comparisons between concentrations of total N in shoot tips and protein N in R Nase extracts prepared from shoot tips of control and deflowered plants.	64
2	Summary data on the incorporation of ^{32}P-orthophosphate into RNA.	66

PHYSIOLOGY OF SENESCENCE OF THE SHOOT APEX IN *PISUM SATIVUM* L.

INTRODUCTION

Concepts of Senescence

Senescence is one of the most poorly defined and least understood phenomena in biology. The animal physiologist Alex Comfort (1965) suggested that senescence involves a decrease in viability and an increase in vulnerability which is exemplified in higher plants by a reduction in growth rate and vigor and an increase in susceptibility to adverse environmental factors. Aging has been described by Lansing (1947, p. 327) as "a problem of cellular change--of unfavorable change in the ability of protoplasm to maintain itself by self-synthesis." Varner (1965) regarded senescence in plants as the terminal phase of development and differentiation. Leopold (1964, p. 194) attempted to distinguish between aging and senescence by defining senescence as the collective "deteriorative processes which naturally terminate the functional life of an organ or organism," and by considering aging to involve changes which occur in time but which do not ultimately result in death. However, at best Leopold's definitions serve only to connote that senescence is the terminal phase of the aging process, and generally the terms "aging" and "senescence" are used synonymously in the literature. In this paper the term

"senescence" is used and is defined as the collective progressive and deteriorative processes which ultimately terminate the functional life of the shoot apex.

Quantitative Nature of Senescence in Plants

Leopold (1961, 1964) characterized the types of senescence which occur in higher plants. "Plant senescence" is displayed by all annual plants in which the life cycle is terminated at the end of one growing season by the death of the entire organism. "Organ senescence," which is the senescence of particular parts of the entire plant organism, has a variety of manifestations. Perennial herbaceous plants exhibit "top senescence" in which the above ground portions die at the end of the growing season, but the underground system remains viable. "Deciduous senescence," involving the annual senescence of leaves, is exemplified by many woody perennials, and "progressive senescence" is characterized by the progressive death of leaves from the base of the plant up the stem. Fruit ripening and deterioration are also considered to be forms of "organ senescence."

Mobilization Hypothesis

Apparently Reichart's publication in 1821 (as cited by Molisch, 1938) was the first report on the antagonism between the development

of reproductive structures and vegetative growth. He found that senescence in the Vienna wall-flower, an annual plant, could be postponed by the removal of flower buds. Reichart's results have been confirmed and extended by several investigators using other species (e. g. Leopold, Niedergang-Kamien and Janick, 1959; Lockhart and Gottschall, 1961; Murneek, 1926; Molisch, 1938).

Murneek (1926) concluded that the developing fruits of tomato plants were able, in some manner, to monopolize most of the available nitrogen in the plant, but Molisch (1938) apparently was the first to attempt to provide a general explanation for plant senescence and its relationship to the development of reproductive structures. He attributed senescence to the mobilization and transport of metabolites from the vegetative plant parts to the developing reproductive structures, thus causing the vegetative parts to die of starvation. The removal of flowers or developing fruits eliminated this mobilizing and assimilating force and vegetative growth was able to continue. Molisch's proposal found considerable support from more recent investigators such as Linck and Swanson (1960), McCollum and Skok (1960), Seth and Wareing (1967), Watson and Petrie (1940) and Williams (1938), all of whom have demonstrated that developing reproductive structures accumulate a large amount of the metabolites which were formerly present in the vegetative plant organs. Studies by Seth and Wareing (1967) have further indicated that the mobilizing

effect provided by developing flowers and fruits may be hormonally controlled.

Reports by Leopold and Kawase (1964) and Mothes and Engelbrecht (1961) indicated that the cytokinin group of plant hormones might be implicated with the mobilization phenomenon. In view of these reports, Herner, Morris and Rappaport (1967) hypothesized that cytokinins present in developing fruits might be responsible for the mobilization and transport of materials into the fruit, thus hastening senescence of the parent plant. Their tests of this hypothesis apparently were not highly conclusive, but the results did suggest that a cytokinin applied to deseeded pea pods could partially substitute for the intact developing seeds in promoting senescence.

The mobilization phenomenon has not been restricted to the development of reproductive structures at the expense of the vegetative plant (Leopold, 1961; Mothes, 1960; Ota, 1964; Srivastava and Atkin, 1968). It has been demonstrated that any actively growing plant structure, whether vegetative or reproductive, is an effective metabolite assimilation center, and such structures grow at the expense of mature tissues and organs. However, it appears that reproductive structures, particularly developing fruits, are the strongest assimilation centers (Leopold, 1961; Linck and Swanson, 1960). Sitton, Itai and Kende (1967) have suggested that a reduction

of cytokinin supply from the roots to the leaves is one of the factors leading to plant senescence in sunflowers. They have also pointed out that when leaves become deficient in cytokinins due to the declining supply from the roots, a concomitant event is the formation of a new center of cytokinin synthesis in the developing fruits, resulting in a relocation of mobilization and assimilation centers.

A mobilization process across the zone of cellular separation and the consequent development of cellular senescence on the distal side of the zone has been demonstrated by Scott and Leopold (1966) to be a component process in bean leaf abscission.

Other Aspects of Plant Senescence

Although the mobilization hypothesis provides a valid partial explanation of senescence in some cases, there are some reports which indicate that the cause of plant senescence involves more than a depletion of food reserves. Leopold, Niedergang-Kamien and Janick (1959) demonstrated that plant senescence was delayed by the removal of filled, unripened fruits from soybean plants and by the removal of flowers from staminate as well as pistillate spinach plants. It seems reasonable to assume that the presence of filled fruits or developing male flowers would have little, if any, effect on the depletion of metabolites from vegetative plant structures. Leopold *et al.* (1959) attempted to explain their results by suggesting that

senescence is imposed upon a plant by an increasing "signal" of unknown character which is intensified with successive reproductive developmental stages of the plant. Krizek, McIlrath and Vergara (1966) reported that inductive photoperiods induced senescence in Xanthium plants which had been debudded and were, therefore, unable to form flowers. Thus, it appears that in some species senescence is triggered by photoinduction even under conditions where the formation of reproductive structures does not ensue.

Lockhart and Gottschall (1961) using peas were able to distinguish two types of senescence: "fruit-induced senescence," which resulted from the development of fruit and might be explained by the mobilization hypothesis of Molisch (1938); and "apical senescence" which occurred even if plants were deflowered or kept vegetative by supplementing the normal photoperiod with far-red radiation. Additional experiments by Lockhart and Gottschall indicated that "apical senescence" resulted from the degeneration of the apex itself and was not caused by the translocation of some factor in the plant. They concluded that since "apical senescence" occurred in peas even when flowering did not occur, it was not directly or causally related to the induction of flowering.

Physiological, Cytological and Biochemical Changes Associated with Senescence

Although the gross morphological and physiological changes associated with senescence in plants have been studied for several years, the majority of the contributions to the understanding of senescence at the cellular and subcellular levels has been made within the past two decades. Both intact and excised tissues and organs have been utilized in such investigations. Apparently due to convenience, a considerable number of studies of senescence at the cellular and subcellular levels have been performed with plant structures in which senescence has been induced or enhanced by excising the structure from the whole organism. In some cases there may be little difference in the senescence-associated processes occurring in excised and intact tissues; however, studies by Küster and Stahl (as cited by Molisch, 1938); Varner, Balce, and Huang (1963) and Young et al. (1960) suggested that other parts of the whole plant imparted an effect on the senescence of cotyledons and leaves. In the following studies mentioned, only a very few reports stated that both intact and detached senescing tissues were compared.

Senescence in leaves and cotyledons has been characterized by a decline in chlorophyll content and reduced photosynthetic and respiratory activity (e. g. Das and Leopold (cited in Leopold, 1964); McDonald and DeKock, 1958; Singh and Lal, 1935; Smillie, 1962;

Yemm, 1956). Smillie (1962) found that the activities of several specific enzymes functioning in photosynthesis and respiration declined during senescence. Cytological studies of progressively senescing leaf and cotyledon cells have shown that the chloroplasts and mitochondria become structurally disorganized and eventually disintegrate, the endoplasmic reticulum and cytoplasmic ribosomes disappear, the nuclei disintegrate and the tonoplast and ultimately the plasmalemma are ruptured and destroyed (Barton, 1966; Butler, 1967; Lund, Vatter and Hanson, 1958; Shaw and Manocha, 1965). With biochemical techniques, Srivastava and Arglebe (1967) have further characterized the destruction of polyribosomes in senescing barley leaves. The expected result of membrane destruction is an alteration in cellular permeability, which has been demonstrated by Glasziou, Sacher and McCalla (1960) and Sacher (1957) using bean endocarp tissue, by Sacher (1959) using leaf tissue from Mesembryanthemum sp. and Rhoeo discolor and by Das and Leopold (cited in Leopold, 1964) using the primary leaves of bean. Lansing (1947) suggested that the increased deposition of calcium which is found in senescing cells of both plants and animals might be partially responsible for senescence by modifying membrane permeability.

Yemm (1956) noted that in some species during the yellowing of mature leaves, the respiratory rate increased in a manner similar to that in the respiratory climacteric of some fruits.

He suggested that the high respiratory activity and the accompanying loss of synthetic activity in such senescing leaves were due to an impairment of the coupling between phosphorylation reactions and oxidation reactions. More recently Huang (1960), using cotyledons of germinating peas, isolated and partially purified and characterized a protein the accumulation of which paralleled the inactivation of mitochondria, and which uncoupled oxidative phosphorylation from the electron transport pathway. Hanson and Swanson (1962) and Hanson et al. (1965) suggested from in vitro studies that an increase in soluble ribonuclease is at least partially responsible for the reduced phosphorylation and respiratory activity of mitochondria in senescing maize scutella.

A decline in the levels of protein and nucleic acids, particularly ribonucleic acid (RNA), appears to be a common characteristic of all senescing plant cells, as exemplified by biochemical studies of senescing leaf tissue (e. g. Beevers, 1966; Osborne, 1962; Scott and Leopold, 1966; Shaw, Bhattacharya and Quick, 1965; Srivastava and Atkin, 1968), cotyledons (e. g. Cherry et al., 1965; Oota, 1964), and bean endocarp (Sacher, 1965). Cytological studies by Johnson and Jones (1967) of tobacco cells growing in microculture also indicated that the RNA content of senescing cells declined. It has been well established that nucleoli are involved in RNA metabolism (Birnstiel, 1967; Brown and Gurdon, 1964; Ritossa and Spiegelman, 1965), and

Johnson and Jones found that nucleolar vacuoles occurred less frequently and were less active in senescing cells than in growing cells. A reduction in synthetic activity, an increase in degradation reactions or a combination of both occurrences could explain the decline in nucleic acid and protein levels.

Varner (1961) cited evidence which suggests that in senescing fruit and cotyledon cells anabolic reactions were impaired by a lack of ATP (adenosine triphosphate), presumably due to the inability of the mitochondria to efficiently carry out oxidative phosphorylation. With in vitro preparations, Hanson (1959, 1960) demonstrated that pancreatic ribonuclease could inhibit respiratory activity and uncouple phosphorylation reactions in mitochondria isolated from both plant and animal sources. Extrapolation of Hanson's results to in vivo conditions has credulity in view of the report by Srivastava and Ware (1965) that excised senescing barley leaf tissue had greater ribonuclease and deoxyribonuclease activity than non-senescing tissue, and the demonstration by Hanson et al. (1965) that the specific activity of soluble ribonuclease (RNase) increased in senescing maize scutella and that this soluble RNase impaired both oxidation and phosphorylation in the same tissue. The marked decline in total phosphorus in senescing cotyledons observed by Cherry et al. (1965), and the same trend in both inorganic phosphorus and cold 5% trichloroacetic acid-soluble bound phosphorus levels noted by

Srivastava and Atkin (1968) in intact senescing leaf tissue could provide an additional explanation for the decline in nucleic acid levels and in phosphorylative activity in senescing cells.

Anderson and Rowan (1966) found that the absolute and specific activities of peptidase increased with age in detached, senescing tobacco leaf tissue. The studies of Anderson and Rowan (1966) and Srivastava and Ware (1965) suggest that the decline in nucleic acid and protein levels in senescing leaf tissue could be the result of increased activities of degradative (hydrolytic) enzymes. However, reports by Sigiura (as cited in Oota, 1964) and Goldthwaite and Laetsch (1967) indicated that the loss of protein and chlorophyll observed in senescing leaf discs was due more to a decline in synthetic activity than to degradative processes.

Unfortunately, most of the knowledge of the biochemistry of leaf senescence has been obtained from studies using excised leaf tissues which are frequently exposed to highly unnatural environmental conditions. Obviously, such studies have eliminated any effects the rest of the plant may have in controlling the processes involved with senescence of its organs, and inferences drawn from investigations using excised tissues should be considered with reservation. Biochemical studies concerning changes in protein and nucleic acid levels during cotyledon senescence may be more meaningful because intact cotyledons usually have been used.

Cotyledons generally have been considered as storage organs containing reserve materials, including nucleic acids, for the growing seedling. During the later stages of germination the cotyledons have been regarded as senescing organs (e. g. Cherry, 1962; Oota, 1964), and their senescence is controlled by the other growing and developing parts of the plant (Leopold, 1961; Varner, Balce and Huang, 1963; Young et al., 1960). Oota and associates (Oota, 1964) using seedling of the bean Vigna sesquipedalis found that during the period of heterotrophic growth of the seedling the RNA content in the cotyledons was reduced and its decline was balanced by an increase in RNA content in the growing seedling axis. Associated with the decline in the RNA level in the cotyledons was the degradation of ribosomal RNA to small, metabolically inert "transportable RNA" molecules, suggesting ribonuclease activity. Cherry (1963) reported that senescing peanut cotyledons displayed a decline in the levels of protein and RNA and a concomitant increase in ribonuclease activity. Later it was demonstrated (Cherry et al. 1965) that the amount of ribosomal RNA, and what was considered to be messenger RNA, declined as the soluble RNA content increased in senescing cotyledons, which further indicated the involvement of increasing ribonuclease activity with cotyledon senescence.

With the increasing amount of knowledge concerning the ability of plant hormones and other growth regulators to control

plant growth and development has come a renewed interest in senescence among plant physiologists. Within the last decade several publications have appeared dealing with the effects of auxins, gibberellins, cytokinins and related compounds on the senescence of intact and excised plant tissues and organs, as well as whole plants. Sacher (1957, 1965) showed that exogenous auxin delayed senescence in excised bean endocarp tissue. Senescence in leaf tissue has been retarded by auxins (Osborne, 1959; Osborne and Hallaway, 1964; Sacher, 1959), and gibberellin A_3 (Beevers, 1966; Brian, Petty and Richmond, 1959; Fletcher and Osborne, 1966; Whyte and Luckwill, 1966). Applied gibberellin A_3 also has been reported to delay whole plant senescence (Lockhart and Gottschall, 1961; Stein, 1962). Numerous reports on the ability of cytokinins to postpone senescence in both intact and excised tissues have appeared (e. g. Engelbrecht, 1964; Leopold and Kawase, 1964; Mothes, 1960; Osborne, 1962; Richmond and Lang, 1957; Shaw, Bhattacharya and Quick, 1965; Srivastava and Ware, 1965). Benzimidazole (Person, Somborski and Forsyth, 1957) and certain urea and thiourea derivatives (Bruce, Zwar and Kefford, 1965) also have been demonstrated to retard senescence in leaf tissue.

Synthetic d, l abscisin II, whose d(+) enantiomorph reportedly is identical in structure to that of a natural growth inhibitor (Cornforth, Milborrow and Ryback, 1965, 1966), has been shown to

promote senescence in leaf tissues from several species (El-Antably, Wareing and Hillman, 1967), perhaps by its reported antagonism to the action of auxin (Milborrow, 1966), cytokinins (Khan, 1967) and gibberellin (Chrispeels and Varner, 1966; Thomas, Wareing and Robinson, 1965).

There apparently is a high degree of specificity of particular hormones or growth regulators for different plants, and in some instances gibberellin A_3 (Brian, Petty and Richmond; 1959) and benzyladenine, a synthetic cytokinin, (Halevy, Dilley and Wittwer; 1966) promoted senescence in material from particular plant species while retarding senescence in comparable material from other species. The phenomenon of retarding senescence with plant hormones and related compounds has been further complicated by reports that the senescence-delaying effect of kinetin was reduced by the concurrent application of an auxin in two plant species (Von Abrams and Pratt, 1966), and that the application of certain growth retardants, which interfere with hormone metabolism or are antagonistic to hormonal action, retarded senescence in a few plant species (Harada, 1966; Halevy, Dilley and Wittwer, 1966; Kessler, Spiegel and Zolotov, 1967). Three of the growth retardants used in these studies, 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride (AMO-1618), β-chloroethyl-trimethylammonium chloride (CCC), and tributyl-2,

4-dichlorobenzylphosphonium chloride (Phosphon D), have been shown to inhibit gibberellin biosynthesis (Baldev, Lang and Agatep, 1965; Dennis, Upper and West, 1965; Kende, Ninnemann and Lang, 1963; Ninnemann et al. 1964). The other growth retardant employed, N, N-dimethylaminosuccinamic acid (B-995), and CCC reportedly might affect auxin metabolism (e. g. Halevy, 1963; Norris, 1966; Reed, Moore and Anderson, 1965). The results of the aforementioned studies suggest that a certain balance among the endogenous hormones is required to delay senescence. In all cases the postponement of senescence by growth regulators appeared to be associated with maintenance of RNA and protein levels, but the methods by which the growth regulators accomplish this have not been clearly ascertained. Auxins (Sacher, 1965), gibberellins (Fletcher and Osborne, 1966), and cytokinins (Osborne, 1962; Wollgiehn and Parthier, 1964) reportedly might function by promoting or, at least, maintaining RNA and protein synthesis. However, Anderson and Rowan (1965) have shown that kinetin applied to harvested tobacco leaves retards the increase in peptidase activity which occurs in senescing leaves, and McHale and Dove (1968) and Srivastava and Ware (1965) found that cytokinins applied to detached tomato leaves and barley leaves, respectively, suppressed the activities of nucleases, thus retarding nucleic acid degradation.

Many of the aspects of fruit ripening and deterioration are not

directly pertinent to the research described in this thesis; therefore, studies considering this form of organ senescence have not been extensively reviewed here. Ulrich (1958) and Hansen (1966) have written excellent reviews on this subject.

PURPOSE OF THE STUDY

From the studies considered in the Introduction, it appears that senescence of cotyledons and mature leaves is partially, if not completely, due to the mobilization and transport of metabolites from these structures to more active metabolic sites. Upon reaching maturity, an intact, vegetative plant organ seemingly becomes less efficient in performing anabolic processes and ultimately it is used as a source of metabolites for the growing portions of the plant. Several investigators have shown that developing reproductive structures, particularly fruits, are very active assimilation centers for metabolites, and in annual and some perennial species these developing reproductive structures are capable of inducing or promoting the senescence of the entire vegetative plant. One of the most extensive investigations of plant senescence in annual plants was conducted by Lockhart and Gottschall (1961) using peas (Pisum sativum L.). They found that fruit development resulted in senescence of the shoot apex, as exemplified by a cessation of shoot elongation. However, although senescence in the shoot apex was delayed by the removal of reproductive structures, the shoot apex eventually experienced determinate growth even in the absence of developing fruits. They also found that applied gibberellin delayed, but did not prevent, shoot apex senescence. The shoot apex is a true meristem (Esau, 1965); therefore,

it and the developing shoot tip are physiologically different from a mature plant structure. This fact, plus the observation by Lockhart and Gottschall (1961) that apex senescence was apparently independent of leaf senescence, suggest that senescence of the shoot apex may be unique, as compared to plant organ senescence. It appears that developing fruits are able to outcompete the shoot apex for available metabolites; but this has not been adequately demonstrated and cannot account for apex senescence in deflowered pea plants.

The primary purposes of this investigation were to: (1) confirm and extend the reported observations that flower removal and gibberellin treatments will delay senescence of the apex of the pea plant; (2) determine whether changes in growth rate and occurrence of senescence in the shoot apex are correlated with changes in the level of endogenous gibberellin in the shoot tip; and (3) attempt to ascertain some of the fundamental biochemical changes which are correlated with senescence of the intact shoot tip, and to compare these changes with those reported for senescing leaves, fruits and excised tissues and organs, all of which are clearly different physiologically from intact shoot tips.

The first objective was accomplished by comparing deflowered plants with plants bearing flowers and fruits in regard to their growth rate, longevity and responses to applied gibberellin and a known inhibitor of gibberellin biosynthesis.

Comparisons of flower- and fruit-bearing plants with deflowered plants throughout ontogeny with respect to their growth responses to a single treatment with gibberellin or a known inhibitor of gibberellin biosynthesis resulted in fulfillment of the second objective.

The shoot tips of deflowered plants were compared with those of plants bearing reproductive structures with respect to the levels of certain metabolites, the ability to enzymically degrade ribonucleic acid and the ability to take up ^{32}P-orthophosphate and incorporate it into ribonucleic acid, as a means of accomplishing the third objective.

MATERIALS AND METHODS

Source and Purity of Reagents

The gibberellin used was Gibrel, of Merck and Company, 81% of which was the potassium salt of gibberellin A_3 (KGA_3). The plant growth retardant 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride (AMO-1618) was obtained from Rainbow Color and Chemical Company, Sepulveda, California. Reagent grade yeast RNA from Nutritional Biochemicals Corporation was used to determine a standard curve for optical density versus RNA concentration and for the ribonuclease assays. The ^{32}P was obtained from International Chemical and Nuclear Corporation as carrier free phosphoric-32 acid in 1N HCl solution. Constituents of the scintillation fluid, cellosolve (2-ethoxyethanol) and scintillation grade 2, 5-diphenyloxazole (PPO), were products of Eastman Kodak Company and Packard Corporation, respectively. All other chemicals were of reagent grade.

General Procedures

Culture of Plants

All experiments were performed with Pisum sativum L. 'Alaska' obtained from W. Atlee Burpee Company, Riverside,

California. Seeds were planted without presoaking in plastic pots filled with vermiculite, and the plants were irrigated alternately with complete mineral nutrient solution and water as needed. All plants were grown in a greenhouse where the light and temperature conditions consisted of a 16-hour photoperiod at 20-29° C and an eight-hour dark period at 16-20° C. The natural light intensity was supplemented with and the natural photoperiod extended by Gro-Lux fluorescent lamps; the light intensity at plant level varied between 800 and 1,000 foot-candles.

Preparation of Growth Regulator Solutions and Treatment of Plants

All GA concentrations were based on 100% KGA_3. Ten mg quantities of KGA_3 were each dissolved in 0.5 ml of 95% ethanol, and subsequent dilutions were prepared with an aqueous 0.05% Tween 20 (polyoxyethylene sorbitan monolaurate) solution. AMO-1618 concentrations were based on the assumption that the compound was 100 % pure and dilutions were made with 0.05% Tween 20 solutions. Aliquots of 10 µl of GA, AMO-1618 or Tween 20 solutions were applied to the shoot tips with a micrometer buret. Fresh solutions were made immediately before each treatment.

Measurement of Plants and Statistical Analyses

Shoot height was measured as the distance from the cotyledonary node to the highest visible node, and internodes were numbered from the cotyledonary node to the shoot tip. At least ten, and in most experiments 15 or more, plants were utilized for each treatment concerning the effects of deflowering and growth regulators. Where plotted on the graphs, variations are expressed as the standard errors of the means or least significant differences between sample means.

Preparation of Plant Shoot Tips for Biochemical Studies

Plants were grown as previously described with some plants being deflowered as flowers appeared and others being allowed to bear flowers and fruits (controls). At two-week intervals samples of plants were randomly selected from each group of deflowered and control plants. All visible developing flowers were removed from the shoot tips so that, practically speaking, only vegetative plant material was used. Shoot tips were then removed and those from each experimental group (deflowered and control) were sorted into three or four sample replicates of 25 shoot tips each, which were refrigerated and used for biochemical studies. Variations of this procedure are mentioned under appropriate headings.

Determination of Dry Weight

The fresh weight of each sample (25 shoot tips) of deflowered or control plants was determined. One sample was taken from each experimental group and dried at 70-80°C for 24 hours or longer, and its dry weight was determined. The ratio of dry weight to fresh weight for each dried sample was then used to calculate the dry weight of corresponding samples used for biochemical studies.

Definitions

The criterion for senescence was the cessation of shoot elongation. The time of anthesis was arbitrarily selected as the time when 50% of the plants had one fully open flower. For use of a standard morphological structure for the biochemical studies, the shoot tip was arbitrarily defined as the upper portion of the stem above and including the uppermost node having expanded stipules.

Repetition of Experiments

Each experiment concerning the effects of deflowering and growth regulators on plant development and senescence was repeated at least once. Complete experiments concerning ontogenetic biochemical changes in shoot tips were not repeated because of various prohibitive circumstances. However, critical parts of these

experiments were repeated at least once.

Extraction and Determination of RNA and Protein

RNA was extracted by the procedure of Smillie and Krotkov (1960) with a modification employed by Osborne (1962) to determine the protein. Shoot tips were ground in methanol in a Thomas teflon-to-glass tissue homogenizer and then extracted twice with methanol. The residue was then extracted twice with cold 5% trichloroacetic acid and once with cold 95% ethanol. This was followed by two extractions with boiling ethanol, one extraction with boiling ethanol-ether (2:1) and one extraction with boiling ether. The residue was then dried to a powder and suspended in 0.3 N KOH with which it was extracted for 16 hours at 37° C. Following centrifugation the supernatant was collected and the residue was twice washed with cold water and centrifuged. The KOH hydrolysate and washing supernatants were combined and made to volume and an aliquot of this solution was used for protein determinations. A second aliquot was adjusted to pH 2 with perchloric acid, centrifuged and the supernatant adjusted to pH 8 with KOH. Insoluble $KClO_4$ was removed by centrifugation and total ribonucleotides were determined by comparing the optical density of the supernatant at 260 nm in a Beckman DB spectrophotometer with that on a standard curve obtained from KOH-hydrolyzed reagent grade yeast RNA.

The insoluble residue from the KOH extraction was extracted with 1 N NaOH at 100° C for five minutes to solubilize previously insoluble protein. Protein was determined by the Biuret method (Dawson et al. 1959) and the total protein value was the sum of the protein in the KOH extraction supernatant and the NaOH extraction supernatant. Interfering pigments in a few of the Biuret assay samples necessitated the employment of micro-Kjeldahl nitrogen determinations of dried shoot tip samples as an additional means of determining total protein. Experimental results all are expressed in terms of protein measured by the micro-Kjeldahl procedure. Micro-Kjeldahl values were consistently higher than those obtained using the Biuret method; accordingly, Biuret values were corrected by using an experimentally determined conversion factor.

Assay of Ribonuclease Activity, Nitrogen and Inorganic Phosphorus

Preparation of Enzyme Extracts

Each shoot tip sample was homogenized in cold 0.1 M potassium acetate buffer (1 g fr wt/6 ml buffer), pH 5.0, with a Thomas teflon-to-glass homogenizer contained in an ice bath. The homogenate was centrifuged at 20,000 x g for 20 minutes at 0° C. The volume of the resulting supernatant was determined and the supernatant was used as the enzyme source.

Estimation of Ribonuclease Activity

Each reaction mixture contained 1.5 ml of the enzyme extract and 1.0 ml of 4% yeast RNA in 0.1 M acetate buffer, pH 5.0. Zero time blanks were prepared by adding 5.0 ml of cold 5% perchloric acid in absolute ethanol to reaction mixtures immediately after they were made; the other reaction mixtures were incubated for one hour at 30° C, and the reaction was stopped by the addition of 5.0 ml of 5% perchloric acid in absolute ethanol. Following refrigeration for several hours, each mixture was centrifuged at 20,000 x g for 15 minutes at 0° C. The precipitate was washed once with 3 ml of cold 5% perchloric acid in absolute ethanol and centrifuged. The supernatant and washing from each mixture were combined and diluted to 200 ml and optical density (OD) at 260 nm was determined on a Beckman DB spectrophotometer. Ribonuclease activity was estimated by the difference in OD between incubated reaction mixtures and corresponding zero time blanks. One unit of activity was defined as the amount of RNase required to produce a difference in OD of 0.10 between a diluted incubated mixture and a corresponding diluted zero time blank.

Estimation of Nitrogen

A micro-Kjeldahl procedure (A. O. A. C., 1965) was used for

all nitrogen determinations. Total nitrogen content of shoot tip samples was estimated by using 1. 0 ml aliquots of thoroughly mixed homogenates.

Trichloroacetic acid (TCA) was added to a final concentration of 5% to aliquots of enzyme extracts. These mixtures were refrigerated for several hours and then centrifuged at 0° C. One-milliliter aliquots of the supernatant were used to estimate soluble nitrogen, which was then corrected for the dilution with TCA.

Protein nitrogen in the enzyme extract was estimated by the difference between the total nitrogen content of the enzyme extract and the soluble nitrogen content of the extract.

Estimation of Inorganic Phosphorus

The method of Fiske and Subbarow (1925) was employed to estimate inorganic phosphorus. Aliquots of the previously described enzyme extracts were prepared for the colorimetric assay. The optial density of each extract preparation at 680 nm in a Beckman DB spectrophotometer was compared with the OD on a standard curve obtained with reagent grade monobasic potassium phosphate.

Incorporation of ^{32}P into RNA

Preparation of Plant Material

Plants were grown, treated and randomly selected as previously described. Visible developing flowers were removed from each intact shoot tip and all leaves and reproductive structures (on control plants) were removed from approximately a 20 cm section of the stem immediately below the defined shoot tip. Plant stems were then cut approximately 30 cm below the shoot tip and the cut bases of the explants were immediately placed in water. Each explant stem was cut under water 8 cm below the defined shoot tip and the cut base of the explant was immersed immediately in a phosphoric-32 acid solution.

Incubation of Explants

One-pint plastic containers with perforated lids were used to hold the explants. Each container held 25 explants, which were supported by holes in the lid and a small piece of 1/4-inch mesh screen elevated slightly from the container bottom. The bases of the explants in each container were immersed in approximately 70 μc of phosphoric-32 acid diluted to 60 ml with distilled water. Explants were illuminated with two 100 watt incandescent lamps mounted in reflector shades 70 cm above the shoot tips. The light intensity at

the shoot tips was approximately 200 foot-candles. A small blower mounted 40 cm from the explants provided a gentle air current to facilitate the transpirational uptake of the ^{32}P solution. The incubation period was 15 hours, during which the containers were rotated occasionally.

Extraction and Estimation of RNA

RNA was extracted and estimated as previously described except that all supernatants obtained prior to the 0.3 N KOH extraction were retained and combined, as were the precipitates.

Determination of Radioactivity

One-milliliter aliquots of the ribonucleotide solution were combined with 15 ml of scintillation solution in liquid scintillation vials. The scintillation solution consisted of seven volumes of cellosolve mixed with ten volumes of 0.6% PPO in toluene. Pigmented supernatants and precipitates were prepared by the procedure of Mahin and Lofberg (1966). Each 0.2 ml aliquot of combined supernatants or suspended precipitates was thoroughly mixed with an equal volume of 60% perchloric acid in a scintillation vial to which subsequently was added 0.4 ml of 30% hydrogen peroxide. Vial caps were screwed on tightly and the vials were placed in a water bath at 75°C until the mixtures were colorless (approximately 30 minutes). After the vials

had cooled, 15 ml of the aforementioned scintillation solution were mixed with each preparation. Radioactivity was measured in a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3375. Liquid scintillation data are expressed in counts per minute (cpm). Radioactivity was corrected for decay and the data are expressed in terms of the radioactivity present in the shoot tips at the termination of the incubation period.

Determination of Incorporation of ^{32}P into RNA

The fraction of radioactivity incorporated into RNA was calculated as the ratio of cpm in the ribonucleotide solution to total cpm uptake (cpm in ribonucleotide solution plus cpm in combined supernatants). It was determined that less than 2% of the total radioactivity taken up was present in the combined precipitates from the RNA extraction. Due to considerable difficulty in accurately measuring and preparing aliquots of the combined precipitates for radioassay, the cpm in this fraction was not included in the total cpm uptake.

RESULTS

Effect of Deflowering on Growth and Senescence

Removal of flowers as they formed delayed senescence of the shoot apex resulting in a prolonged period of vegetative growth and flower production (Figures 1, 2A, 8). All plants experienced a measurable decline in growth rate with the production of the first flower and a rapid decline in growth rate following anthesis. Plants from which flowers were not removed normally produced four or five flowers (the last one of which often aborted) and ceased to grow after seven or eight weeks, with the shoots terminating in dried and shriveled tips (Figure 4). Deflowering partially prevented the rapid decline in growth rate (Figure 2A) and resulted in the production of more nodes (Figure 3A) and also more flowers, since each node above the first flowering node produced a flower.

At 10 to 12 weeks of age, most plants which had been continually deflowered exhibited determinate growth with the shoot terminating in a final flower and leaf (Figure 6) or a final leaf with a pair of degenerate stipules (Figure 7). However, about 20 to 30% of the deflowered plants did not exhibit this determinate growth and continued to produce leaves and flowers (Figure 5); such plants were grown to an age of 32 weeks.

Figure 1. Stem elongation of 'Alaska' peas in response to flower removal and to weekly shoot-tip applications of 1 μg of KGA_3 per plant. Control plants were allowed to bear flowers and fruit. Vertical arrow indicates the time of anthesis. Vertical bars indicate the standard errors of the means.

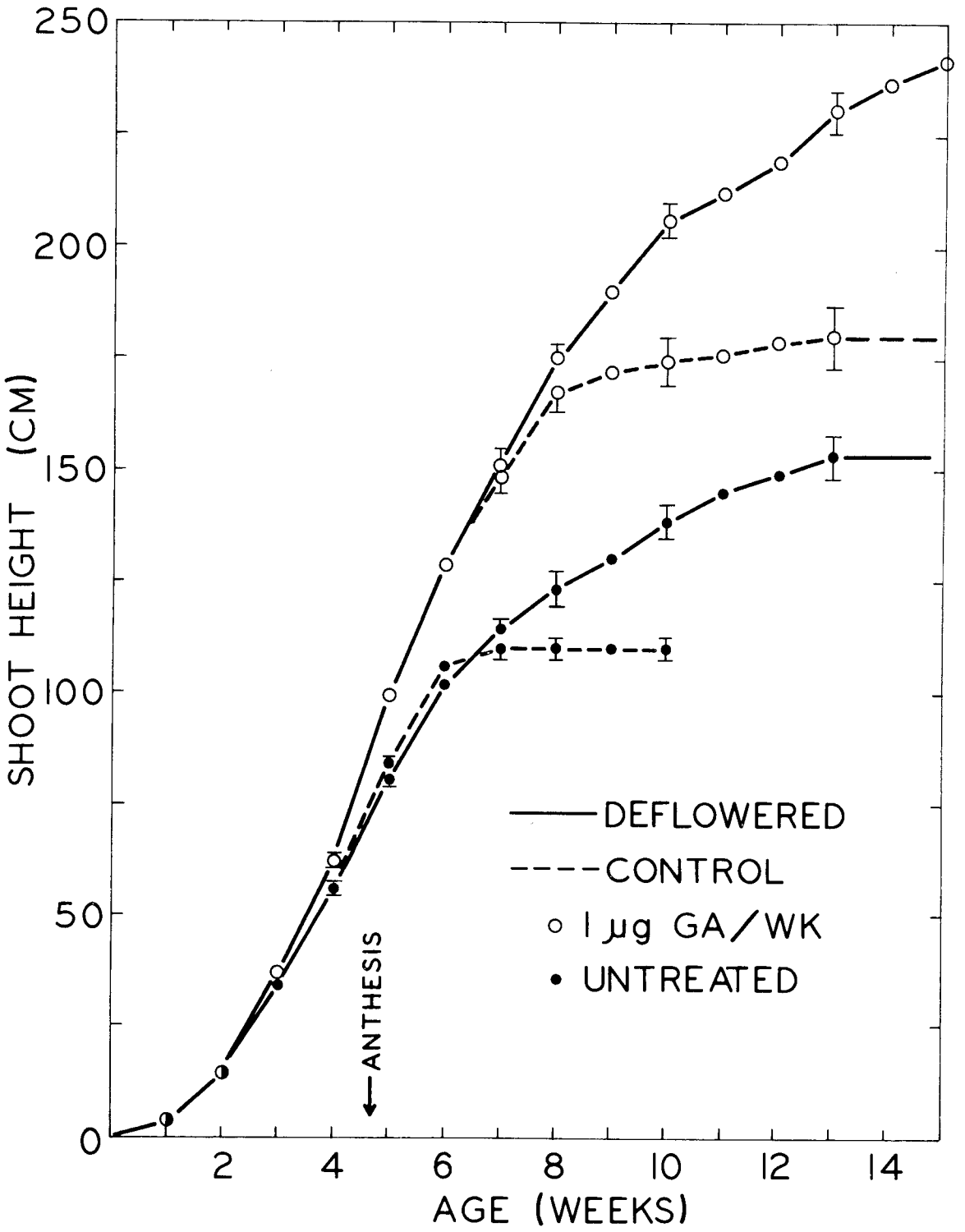

250
200
150
100
50
0
SHOOT HEIGHT (CM)
2
4
6
8
10
12
14
AGE (WEEKS)
ANTHESIS
DEFLOWERED
CONTROL
1 µg GA/WK
UNTREATED

Figure 2. Effect of deflowering and shoot-tip applications of GA on the growth rate of 'Alaska' peas. A, effect of deflowering. B, effect of deflowering plus GA treatments. Control plants were allowed to bear flowers and fruit. Vertical arrows indicate the time of anthesis.

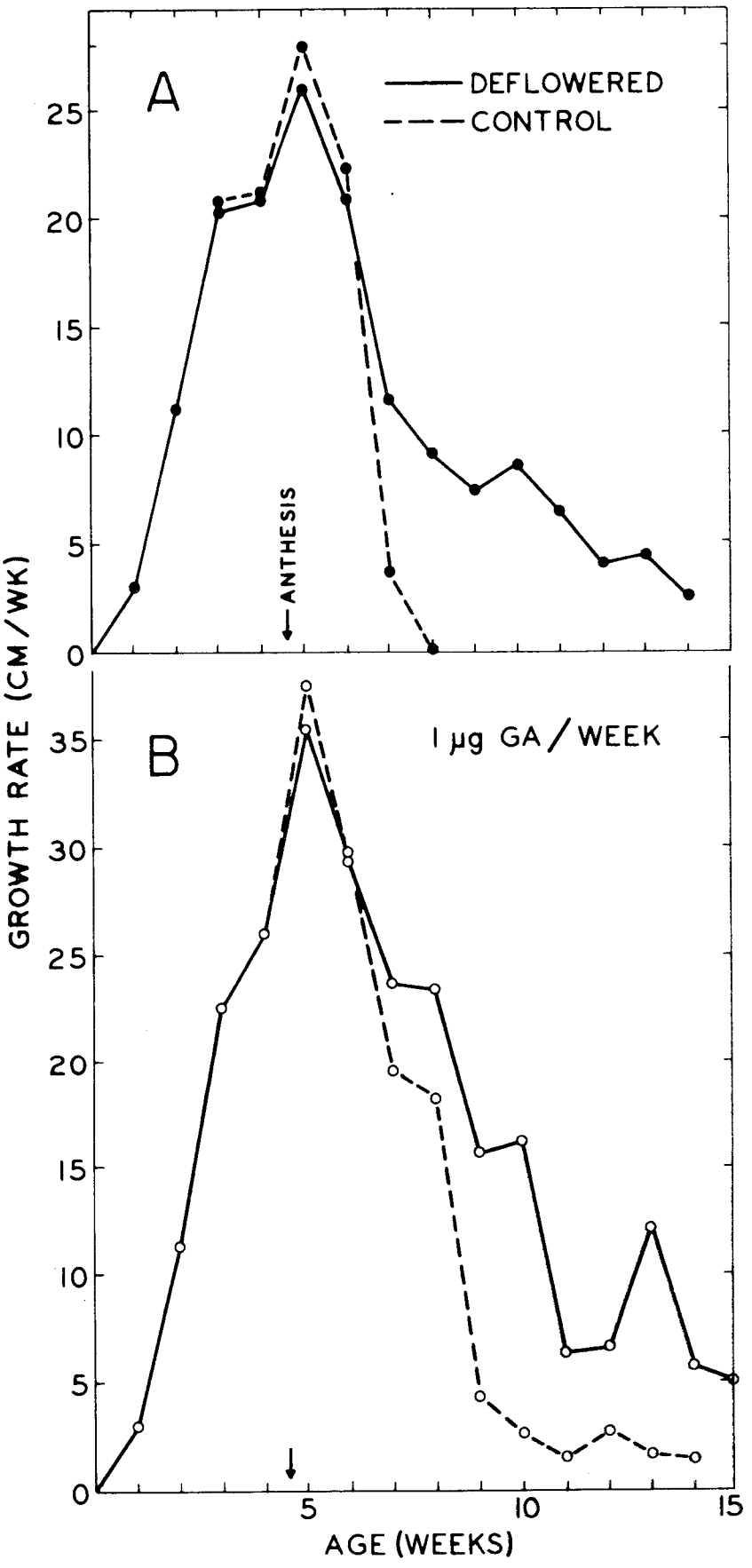
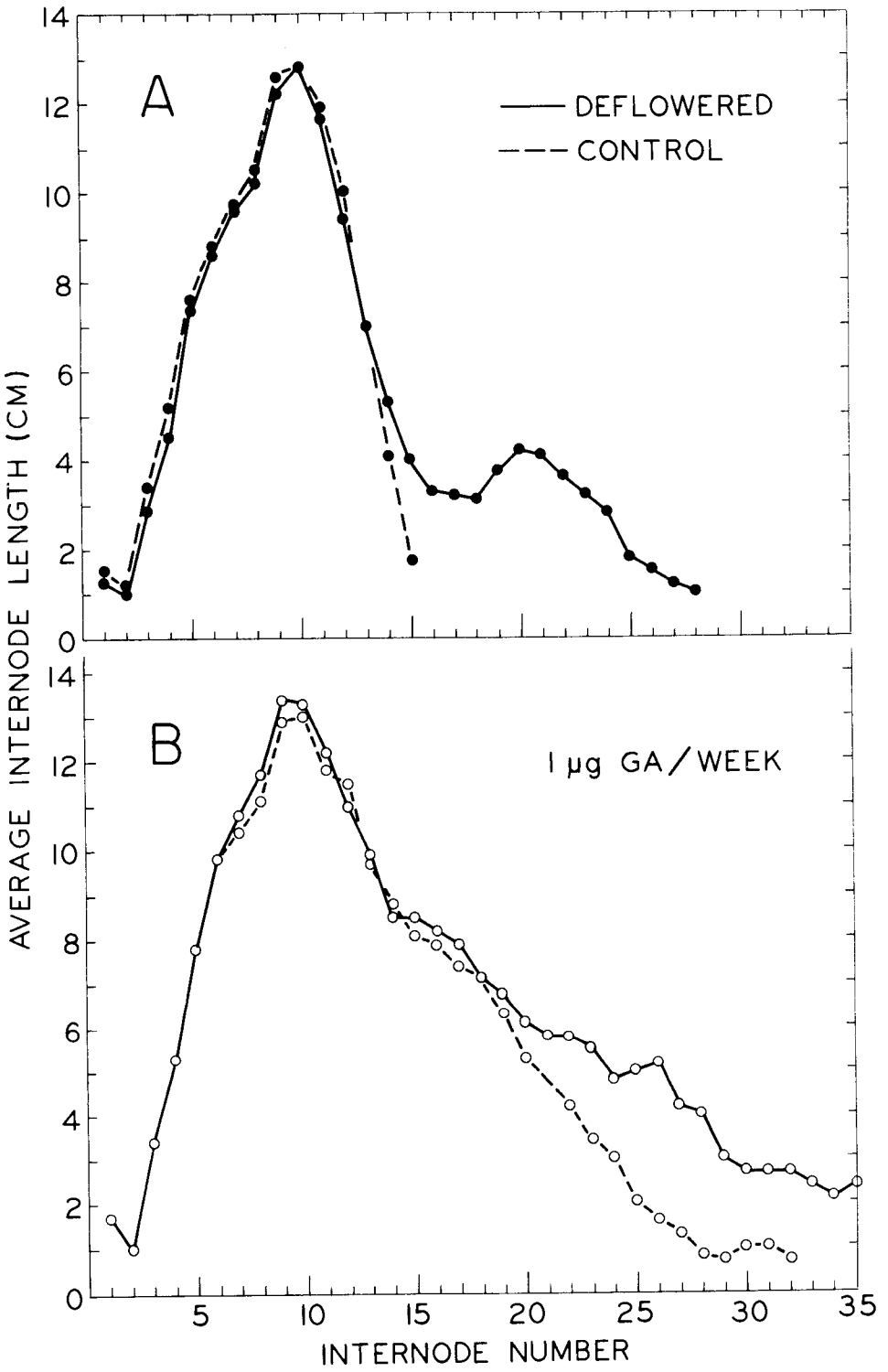

A
DEFLOWERED
CONTROL
ANTHESIS
B
1 µg GA / WEEK
GROWTH RATE (CM / WK)
AGE (WEEKS)
0
5
10
15
20
25
30
35

Figure 3. Effect of deflowering and shoot-tip applications of GA on the average length of internodes of 'Alaska' peas. The average number of internodes per plant for each plant group was determined; then the average lengths of the mean number of internodes for each plant group were determined. Internodes were numbered from the cotyledonary node to the shoot tip. A, effect of deflowering. B, effect of deflowering plus GA treatments. Control plants were allowed to bear reproductive structures.


A
DEFLOWERED
CONTROL
B
1 µg GA/WEEK
AVERAGE INTERNODE LENGTH (CM)
INTERNODE NUMBER
0
2
4
6
8
10
12
14
5
10
15
20
25
30
35

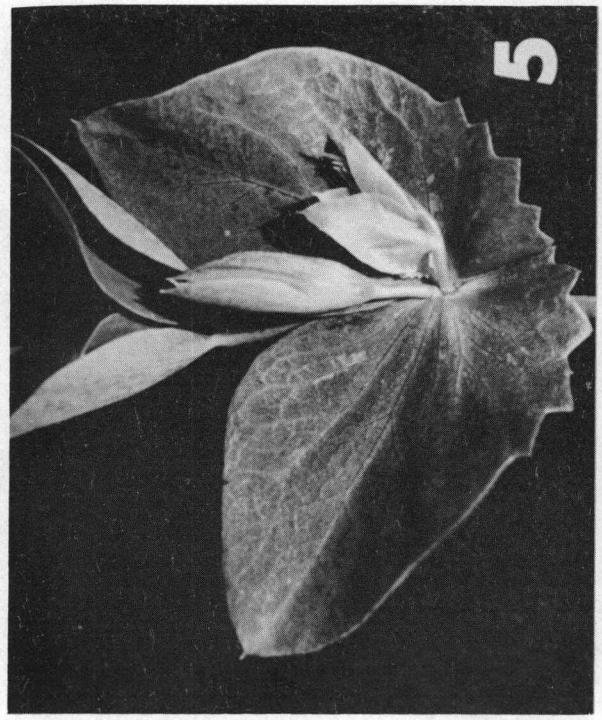
Figures 4-7. Shoot tips of 'Alaska' peas.

Figure 4. Typical senescent shoot tip of a flower- and fruit-bearing plant.

Figure 5. Typical shoot tip of a deflowered plant prior to the occurrence of determinate growth.

Figure 6. Terminal flower and leaf at the final node of a deflowered plant displaying determinate growth.

Figure 7. Terminal leaf with fused, degenerate stipules at the final node of a deflowered plant exhibiting determinate growth.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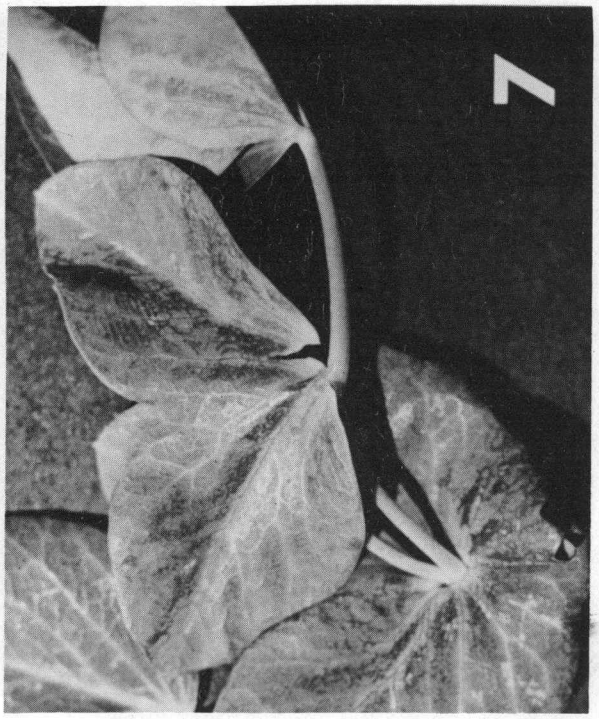
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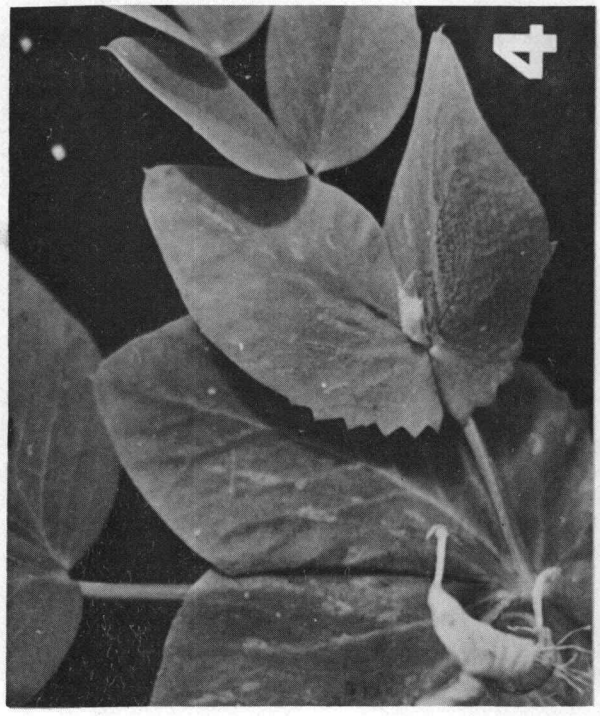
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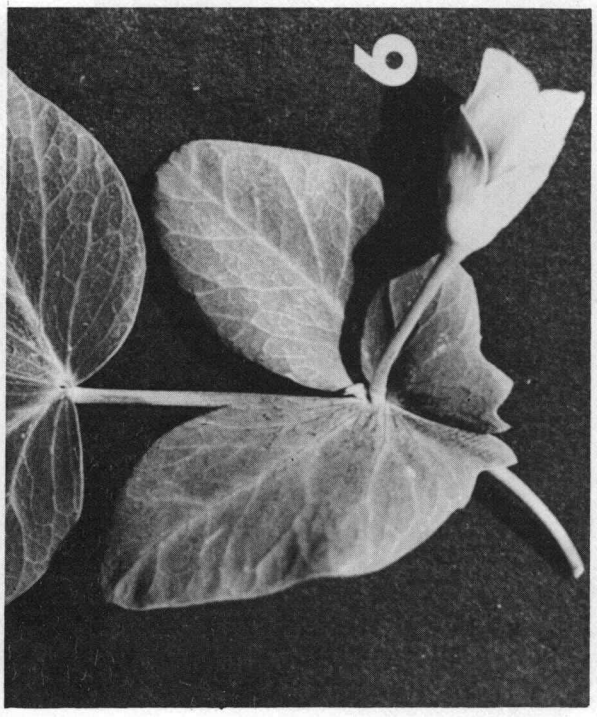

6

Effect of Gibberellin Treatments on Control and Deflowered Plants

Deflowered plants and those with flowers and fruits intact (controls) were treated weekly with different dosages of GA. One μg of KGA_3 per plant applied weekly was growth-saturating, and only the results of this treatment are here presented. GA was first applied when the plants were two weeks old, an age at which they are essentially unresponsive to exogenous GA (Moore, 1967); therefore, there was no immediate growth response by the GA-treated plants. Shortly after anthesis the growth promoting effect was evident in treated plants (Figure 1). Exogenous GA caused an increased growth rate, prevented somewhat the rapid decline in growth rate subsequent to anthesis (cf. Figures 2A, B) and resulted in the formation of more nodes (cf. Figures 3A, B). GA treatment had no apparent effect on the time of flower appearance, but two or three of the first few flowers formed on GA-treated plants aborted, while corresponding flowers on untreated control plants set normal fruit.

Although applied GA delayed senescence in plants with flowers and fruits intact (Figure 1), the appearance of these plants was quite different from that of GA-treated, deflowered plants. The effect of GA applications on fruit-bearing plants was primarily on internode elongation (cf. Figures 3A, B). In addition, the stems of treated plants were slender and the leaves and fruits were abnormally small.

Several seed-bearing pods were formed on each GA-treated plant; however, the uppermost fruits were parthenocarpic and the apex of each of these plants ultimately displayed senescence by drying and shriveling as on untreated control plants (Figure 4). Deflowered plants treated with GA had thick stems and large leaves, and most of them ultimately terminated shoot elongation with the formation of a final flower or leaf as on untreated deflowered plants (Figures 6, 7).

Continual deflowering of plants resulted first in the development of axillary branches below the flowering nodes and then in the formation and development of axillary branches at flowering nodes. GA treatment had no effect in delaying the appearance and development of these branches. In all experiments the axillary branches were removed as they developed.

Effect of AMO-1618 Treatments on Control and Deflowered Plants

Preliminary experiments showed that weekly shoot-tip applications of 50 μg of AMO-1618 caused a marked reduction in growth without evoking apparent toxicity symptoms on plants; therefore, this amount of the growth retardant was used in the experiments reported. Weekly applications of AMO-1618 were started when the plants were nine days old and an immediate growth-retarding effect was noted (Figure 8). In addition to slowing the growth rate, AMO-1618

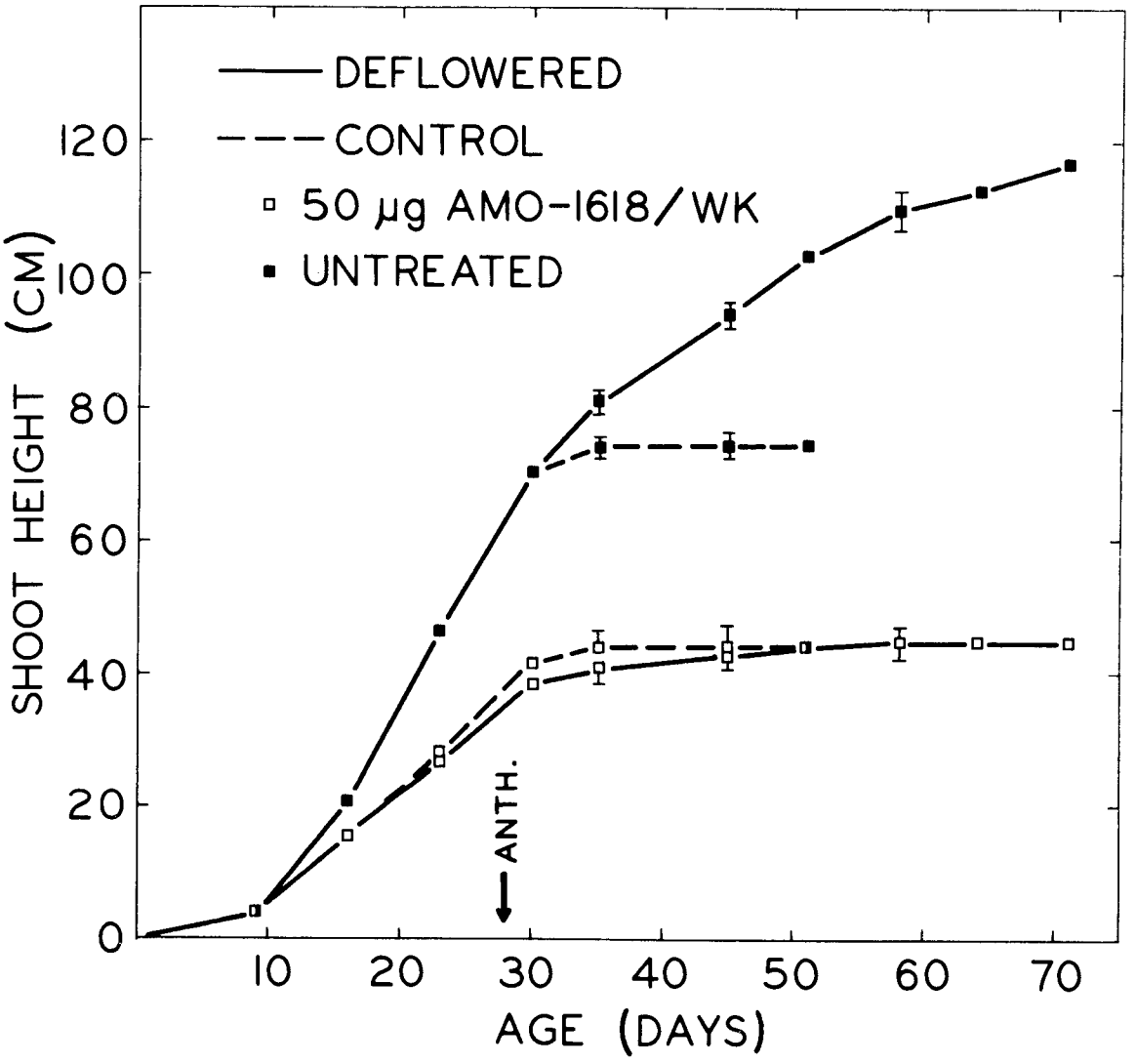
interfered with apical dominance, as was manifested by the development of axillary branches after the first application.

There was no significant difference in the growth of deflowered plants and controls (flowers and fruits intact) treated with AMO-1618, and the growth retardant had no effect on the time at which senescence occurred in the control plants or on the time of appearance and development of flowers and fruit. However, weekly AMO-1618 treatments promoted senescence in deflowered plants. AMO-1618-treated deflowered plants ceased growing at an age of 60 days while untreated, deflowered plants were still growing (Figure 8). The AMO-1618-promoted senescence in deflowered plants resulted in small, shriveled shoot tips characteristic of senescence in plants with flowers and fruits intact (Figure 4).

Ontogenetic Changes in Sensitivity of Plants to Applied Gibberellin or AMO-1618

It is conceivable that the rate of gibberellin biosynthesis and the level of active, endogenous gibberellin in the shoot tip could be estimated indirectly by relating the growth rate with the growth response of the plant to a standard dosage of exogenous GA or AMO-1618, an inhibitor of gibberellin biosynthesis. In view of this hypothesis, time-course changes in the growth response (sensitivity) of plants to a single shoot tip application of a standard dosage of GA

Figure 8. Stem elongation of 'Alaska' peas in response to deflowering and to weekly shoot-tip applications of 50 μg of AMO-1618 per plant. Control plants were allowed to bear reproductive structures. Vertical arrow indicates the time of anthesis. Vertical bars designate the standard errors of the means.


DEFLOWERED
CONTROL
50 µg AMO-1618/WK
UNTREATED
SHOOT HEIGHT (CM)
0
20
40
60
80
100
120
ANTH.
10
20
30
40
50
60
70
AGE (DAYS)

or AMO-1618 were studied throughout ontogeny. It was assumed that the growth response of a plant to exogenous GA would be inversely related to the level of active, endogenous gibberellin in the shoot tip during the time the response is measured and that a specific amount of AMO-1618 would inhibit the biosynthesis of a specific amount of endogenous gibberellin; therefore, the magnitude of growth inhibition by AMO-1618 would depend on the rate of gibberellin biosynthesis throughout the time interval that inhibition is measured.

Sensitivity to exogenous GA was measured as the percentage increase in stem elongation over that of untreated plants one week after the application. Sensitivity to AMO-1618 was determined as the percentage inhibition in stem elongation compared with untreated plants one week after application. In Figures 9A-12B sensitivity to a growth regulator is plotted at the age of the plants when the regulator was applied.

The results of two experiments using 1.0 μg KGA_3 per plant (Figures 9A-10B) and two experiments using 50.0 μg AMO-1618 per plant (Figures 11A-12B) are shown. The first experiments with GA (Figures 9A, B) and AMO-1618 (Figures 11A, B) were performed simultaneously, as were the second experiments with each growth regulator (Figures 10A, B, 12A, B). Uncontrollable differences in temperature and light intensity in the first and second sets of experiments resulted in different rates of plant development which

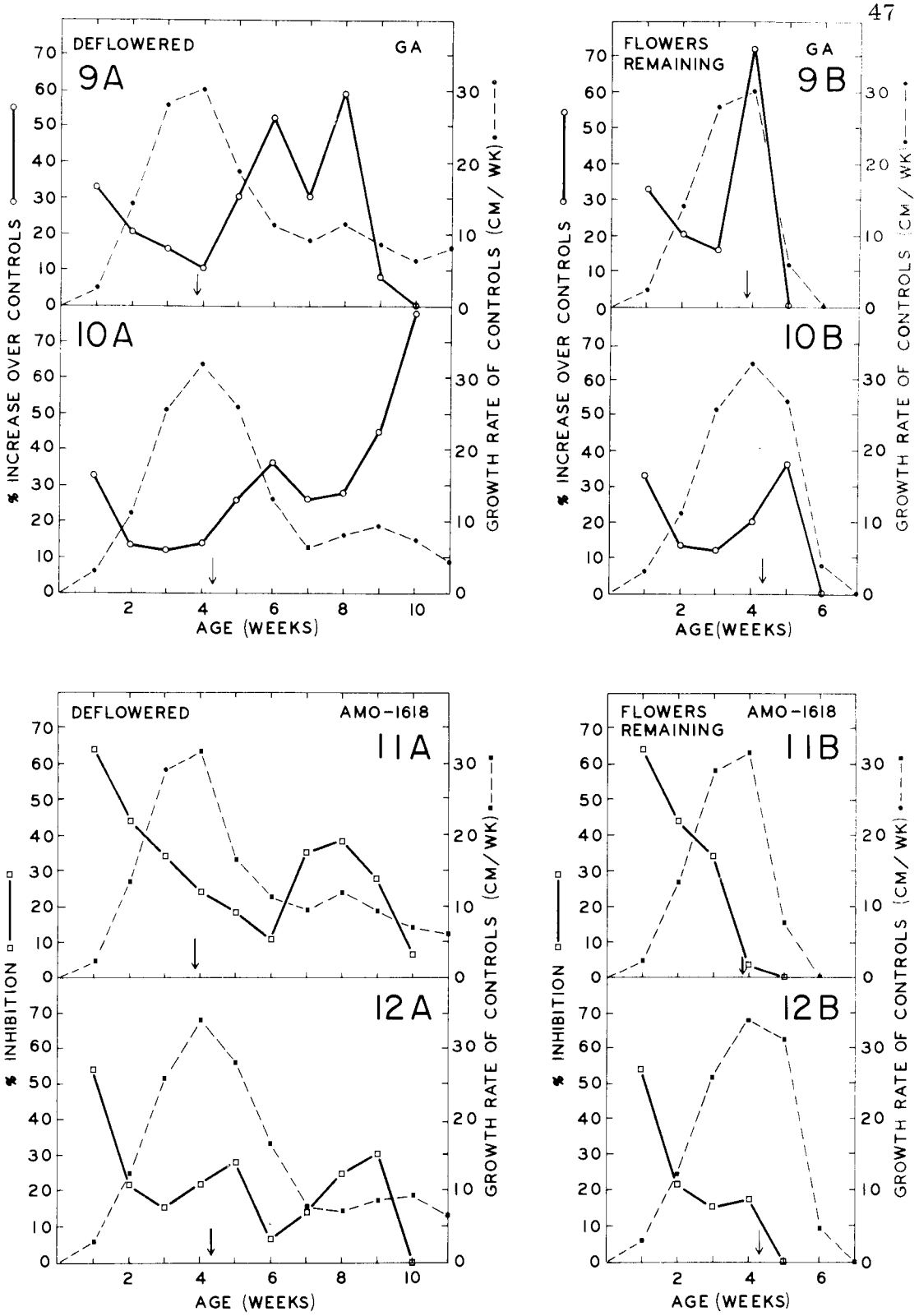
Figures 9-12. Ontogenetic changes in sensitivity of fruit-bearing and deflowered 'Alaska' peas to a single shoot-tip application of 1 μg of KGA_3 per plant or 50 μg of AMO-1618 per plant. Experiments for which results are shown in Figures 9A, B and 11A, B were performed simultaneously, as were those for which results are shown in Figures 10A, B and 12A, B. Vertical arrows indicate the time of anthesis. Deflowered control plants were those from which flowers were removed as they formed and which received no growth regulator. Controls for experiments using plants with flowers remaining were flower- and fruit-bearing plants which received no growth regulator. Sensitivity to a growth regulator is plotted at the plant age at which the regulator was applied.

Figures 9A, 10A. Time-course change in sensitivity of deflowered plants to exogenous GA.

Figures 9B, 10B. Time-course change in sensitivity to exogenous GA by plants with flowers and fruits intact. Sensitivity to exogenous GA was measured as the percentage increase in stem elongation over that of untreated plants one week after the application.

Figures 11A, 12A. Time-course change in sensitivity of deflowered plants to AMO-1618.

Figures 11B, 12B. Time-course change in sensitivity of flower- and fruit-bearing plants to AMO-1618. Sensitivity to AMO-1618 was measured as the percentage inhibition in stem elongation compared with untreated plants one week after the application.


DEFLOWERED
GA
9A
10A
FLOWERS REMAINING
9B
10B
% INCREASE OVER CONTROLS
GROWTH RATE OF CONTROLS (CM/WK)
AGE (WEEKS)
DEFLOWERED
AMO-1618
11A
12A
FLOWERS REMAINING
11B
12B
% INHIBITION

account for variations in the curves for either of the growth regulators. The results of two separate experiments using each regulator are given to show the fundamental similarities.

The plants displayed high sensitivity to both exogenous GA and AMO-1618 during the exponential phase of growth, suggesting that a relatively low level of endogenous gibberellin was present in the shoot tip, but a fairly high rate of gibberellin biosynthesis was occurring. During the linear phase of growth, sensitivity to both exogenous GA and AMO-1618 declined, presumably because the rate of gibberellin biosynthesis was rapidly increasing, thereby increasing the endogenous gibberellin to a level that was practically growth-saturating.

In the case of plants with flowers and fruits intact, a second period of high sensitivity to exogenous GA appeared near the time of anthesis (Figures 9B, 10B), while sensitivity to AMO-1618 continued to decline, or remained quite low (Figures 11B, 12B). These results suggest that subsequent to the linear growth phase the level of endogenous gibberellin was decreasing because the rate of gibberellin biosynthesis was rapidly declining. A lack of response to exogenous GA or AMO-1618 (Figures 9B, 10B, 11B, 12B) was associated with the rapid decline in growth rate, presumably because as apex senescence progressed, irreversible catabolic processes in the apex resulted in a loss of growth capability.

The decline in growth rate following anthesis was not so

precipitous, the second period of high sensitivity to exogenous GA was delayed (e. g., compare Figures 9A, B), and inhibition by AMO-1618 did not decrease as rapidly (e. g., compare Figures 11A, B) with deflowered plants as with flower- and fruit-bearing plants. These differences are assumed to result from a slower decline in the rate of gibberellin biosynthesis following anthesis in deflowered plants. Throughout the period of prolonged growth due to flower removal, the sensitivity to exogenous GA was inversely related to the growth rate of the plants (Figures 9A, 10A), while inhibition by AMO-1618 was directly related to the growth rate (Figures 11A, 12A). The troughs in the GA-sensitivity curves correspond with the peaks in the AMO-1618-sensitivity curves for deflowered plants, suggesting that a second period of increasing gibberellin biosynthesis occurred in the shoot tips of plants in which apex senescence was delayed. As deflowered plants approached the stage of determinate growth with the apparent disappearance of the shoot apex, the growth potential of the shoots was lost and the sensitivity to exogenous GA or AMO-1618 disappeared. The striking difference in the sensitivity of ten-week-old deflowered plants to GA in the two experiments (Figures 9A, 10A) was due to different rates of plant development. With respect to the occurrence of determinate growth, plants in the first experiment (Figure 9A) were ontogenetically older than those in the second experiment (Figure 10A).

Ontogenetic Changes in the Levels of Total RNA and Protein in Plant Shoot Tips

At eight weeks of age, plants with flowers and fruits intact were senescent as was evident by their appearance and lack of growth (Figure 13C). The total RNA and protein levels of these senescent plants were significantly lower than those of plants of the same age in which senescence was delayed by flower removal (Figures 13A, B, D). Differences in values at eight weeks were also significant at the 1% probability level. Changes in the RNA: protein ratio were directly related to changes in growth rate on a two-week basis throughout ontogeny (cf. Figures 13A, C), and a decline in total protein appeared to occur before a decline in total RNA in both deflowered and control plants (Figures 13B, D).

Ontogenetic Changes in the Levels of Nitrogen and Inorganic Phosphorus in Plant Shoot Tips

The results of one representative study of the time-course changes in the levels of nitrogen and inorganic phosphorus in shoot tips are presented in Figures 14 and 15, respectively. Due to differences in plant developmental rates with each repetition of the experiment, the results are not expressed as the averages of more than one experiment. In the experiment represented here developing flowers were apparent when the plants were four weeks old.

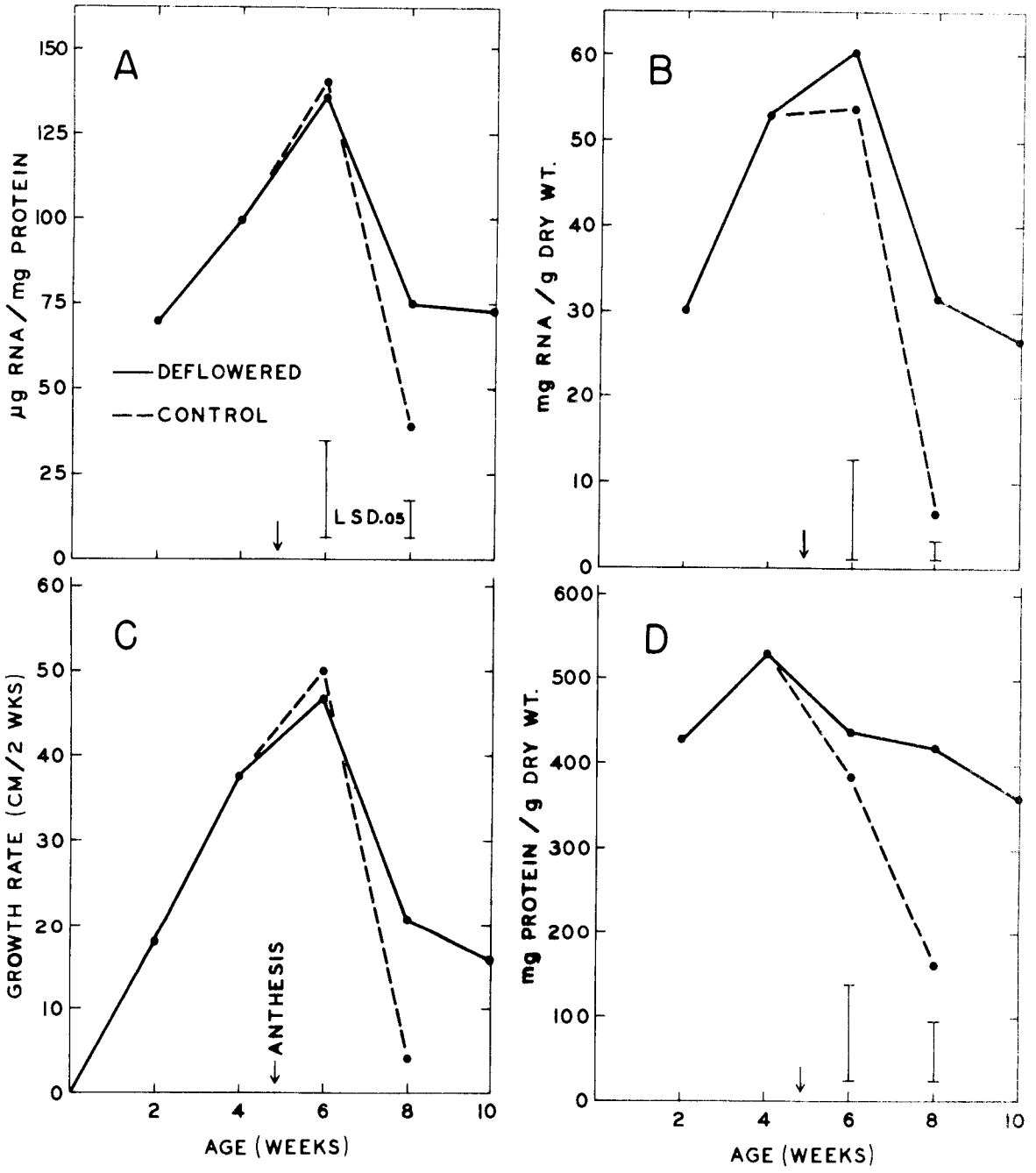
Figure 13. Ontogenetic changes in growth rate and in the levels of total RNA and protein in the shoot tips of fruit-bearing (control) and deflowered plants. Vertical arrows indicate the time of anthesis. Vertical bars at six and eight weeks show the least significant difference between mean values for control and deflowered plants at those ages; in all cases the differences between mean values were also significant at the 1% probability level for eight-week-old plants.

A. Time-course change in the ratio of RNA to protein.

B. Time-course change in the ratio of total RNA to shoot tip dry weight.

C. Time-course change in plant growth rate on a two-week basis.

D. Time-course change in the ratio of total protein to shoot tip dry weight.


A
B
C
D
µg RNA / mg PROTEIN
mg RNA / g DRY WT.
GROWTH RATE (CM/2 WKS)
mg PROTEIN / g DRY WT.
DEFLOWERED
CONTROL
LSD.05
ANTHESIS
AGE (WEEKS)
AGE (WEEKS)

The results in Figure 14 show a significant decline in the levels of both total and soluble nitrogen in shoot tips of control plants from four to eight weeks of age. Shoot tips of deflowered plants between the ages of four and six weeks experienced a decline in total nitrogen content but the soluble nitrogen content remained practically constant. An increase in the levels of both total and soluble nitrogen was observed in shoot tips of deflowered plants from six to eight weeks of age.

The results in Figure 15 indicate that the level of inorganic phosphorus declined significantly in the shoot tips of control plants from four to eight weeks of age. Shoot tips of deflowered plants displayed no decline in inorganic phosphorus content during this period, and an increase in the level of inorganic phosphorus was found between the ages of six and eight weeks.

Repetition of this study demonstrated that the earlier the time of anthesis occurred, the greater was the difference between shoot tips of six-week-old control and deflowered plants with respect to their levels of total nitrogen, soluble nitrogen and inorganic phosphorus.

Ontogenetic Changes in Soluble Ribonuclease Activity in Shoot Tips

The results of one experiment concerning the time-course changes in soluble RNase activity in shoot tips are shown in Figures

Figure 14. Ontogenetic changes in the levels of total and soluble nitrogen in shoot tips of plants bearing reproductive structures (controls) and deflowered plants. Vertical arrow indicates the time of anthesis. Vertical bars designate the least significant difference between mean values for control and deflowered plants at the 5% probability level.

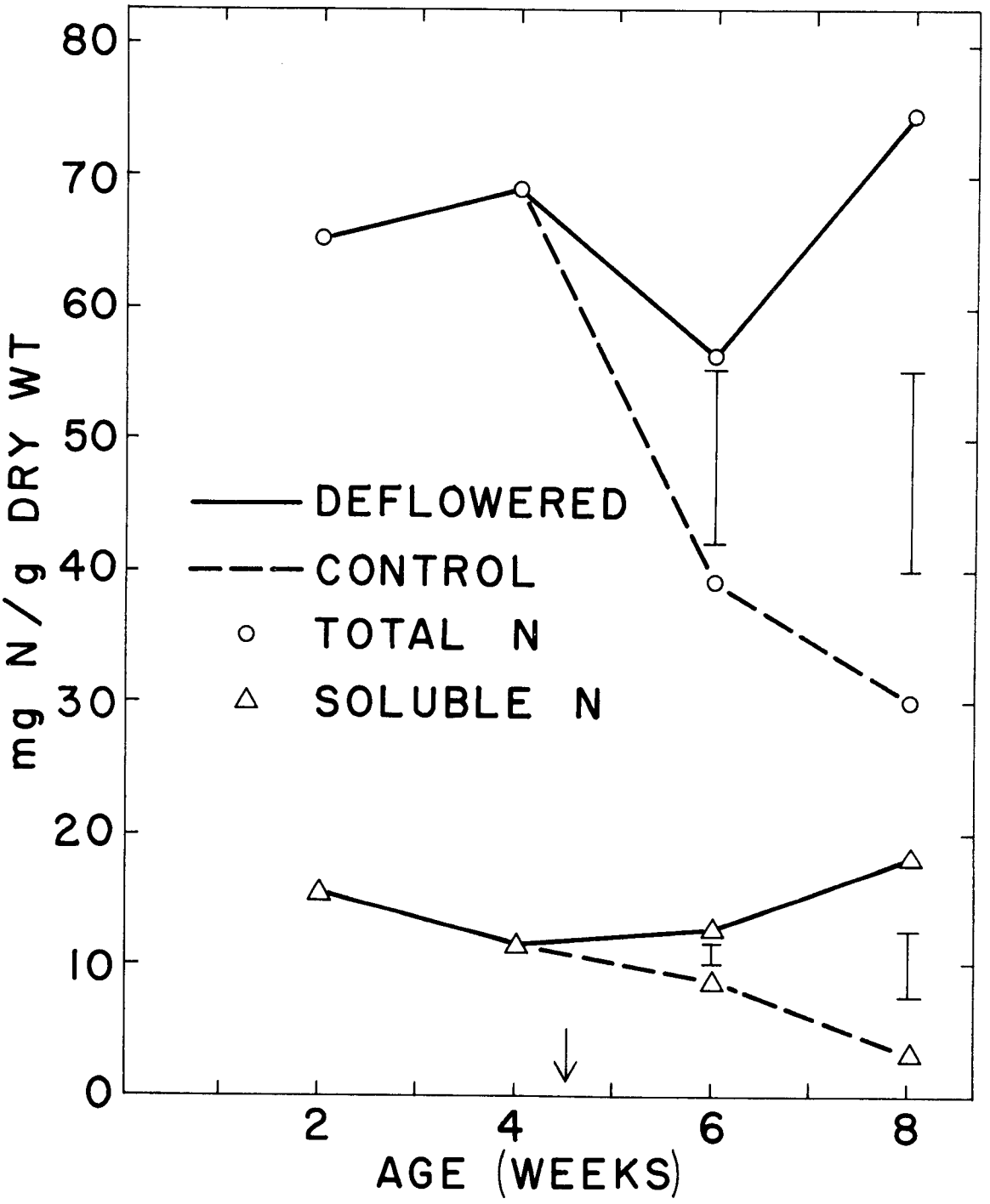
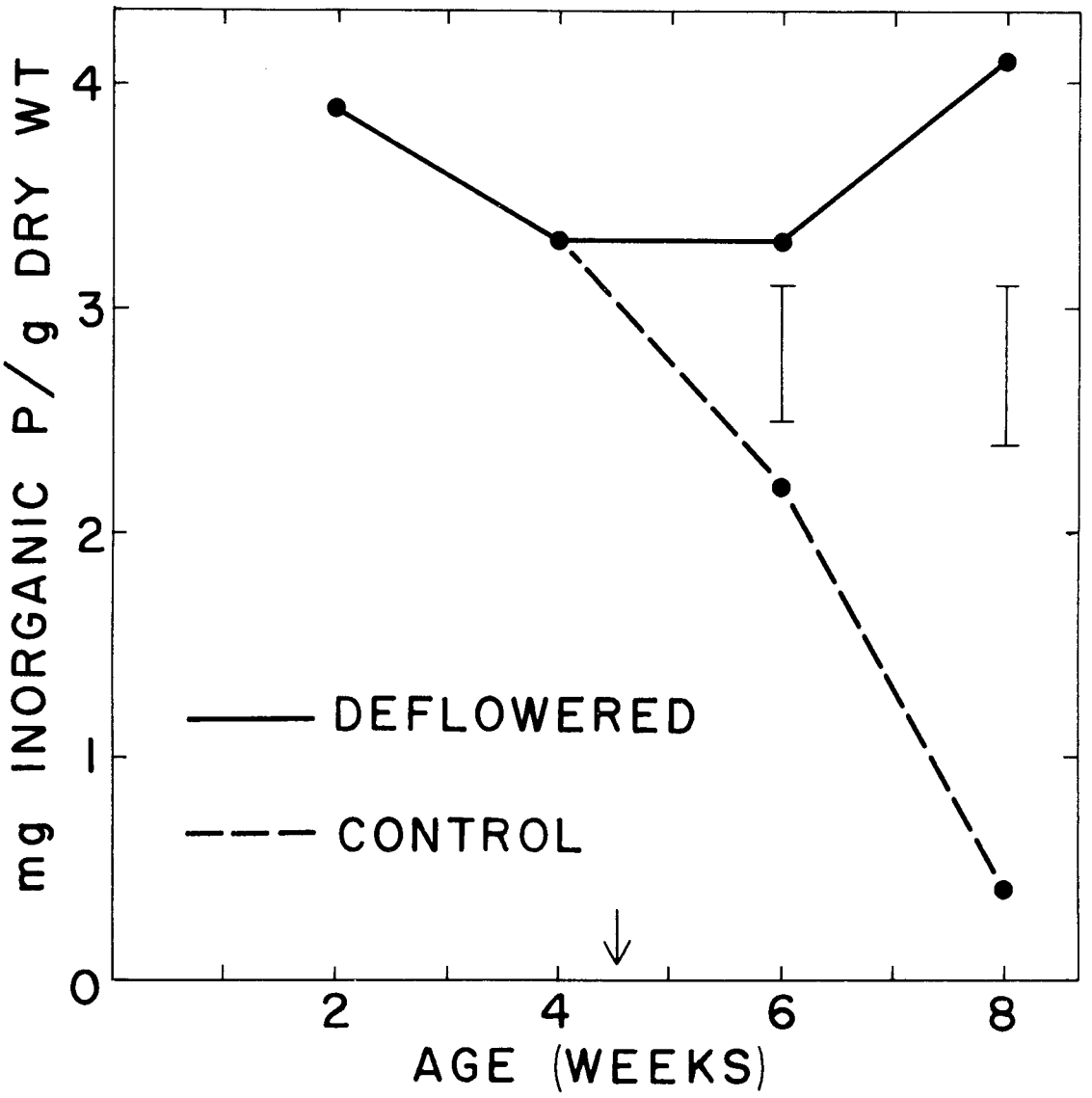

80
70
60
50
40
30
20
10
0
mg N/g DRY WT
DEFLOWERED
CONTROL
TOTAL N
SOLUBLE N
2
4
6
8
AGE (WEEKS)

Figure 15. Ontogenetic changes in the level of inorganic phosphorus in shoot tips of plants bearing flowers and fruits (controls) and deflowered plants. Vertical arrow indicates the time of anthesis. Vertical bars designate the least significant difference between mean values for control and deflowered plants at the 5% probability level.


mg INORGANIC P/g DRY WT
0
1
2
3
4
2
4
6
8
AGE (WEEKS)
DEFLOWERED
CONTROL

16A and B. The plant material used for this study was the same as that used to study the ontogenetic changes in nitrogen and inorganic phosphorus levels. For the previously mentioned reason, the results in Figures 16A and B do not represent averages of data for more than one experiment.

When RNase activity was expressed in terms of the total nitrogen content of the shoot tips, as in Figure 16B, the enzyme activity showed little variation throughout the two-to eight-week age interval, and there was no significant difference between control and deflowered plants with respect to RNase activity in their shoot tips.

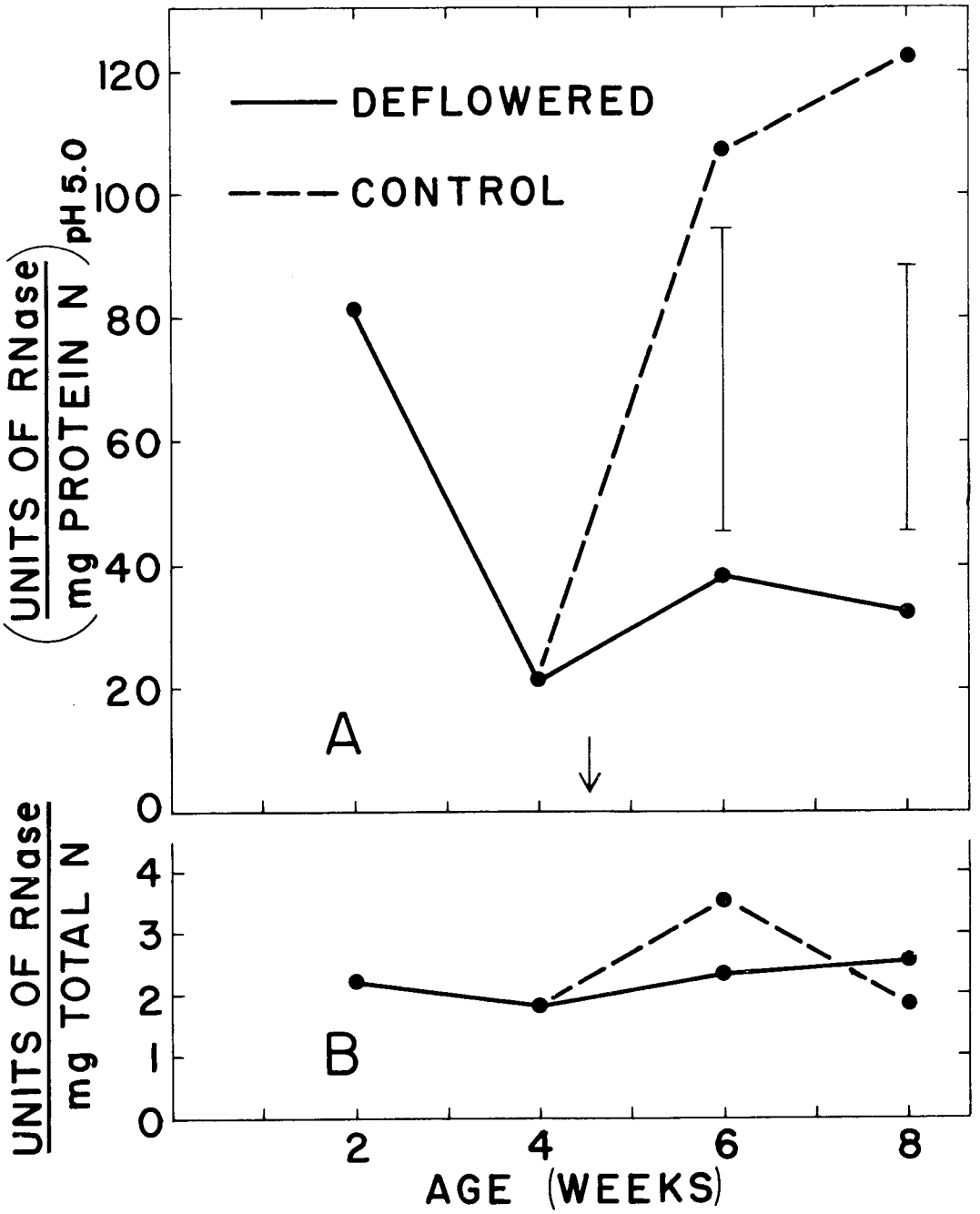
The results in Figure 16A show that when RNase activity was expressed in terms of the protein nitrogen present in the enzyme extract, the activity varied throughout the ontogeny of the plants. RNase activity appeared to be relatively high in the shoot tips of young plants, then decline to a minimum near the time of anthesis and increase in control plants as flowers and fruits developed. When plants were deflowered the soluble RNase activity in the shoot tips appeared to increase only slightly as the plants aged.

When parts of this experiment were repeated, considerable variance in the absolute values for the specific activity (units RNase/mg protein N) was observed; but with each repetition the specific activity of RNase was significantly higher in shoot tips from control plants than in those from deflowered plants at six weeks of age, and

Figure 16. Ontogenetic changes in the activity of soluble RNase in shoot tips of plants bearing reproductive structures (controls) and deflowered plants. RNase activity was assayed at pH 5.0. Vertical arrow indicates the time of anthesis. Vertical bars designate the least significant difference between mean values for control and deflowered plants at the 5% probability level.

A. Time-course change in soluble RNase activity per unit protein nitrogen in the RNase extract from shoot tips.

B. Time-course change in soluble RNase activity per unit total nitrogen in the shoot tips.


DEFLOWERED
CONTROL
(UNITS OF RNase / mg PROTEIN N) pH 5.0
120
100
80
60
40
20
0
A
UNITS OF RNase / mg TOTAL N
4
3
2
1
0
B
2
4
6
8
AGE (WEEKS)

the ratio of RNase specific activity in tips of control plants to that in tips of deflowered plants was nearly constant for all repetitions.

The fact that soluble RNase activity displayed no notable change during senescence in shoot tips of control plants when it was expressed on a total nitrogen basis, but it increased significantly when expressed in terms of the protein nitrogen in the enzyme extract suggested that the apparent increase in RNase activity might actually be due to a preferential decrease in the level of acid-soluble (pH 5. 0) protein with progressive senescence. A comparison of Figure 16 with Figure 17 shows the inverse relationship between RNase activity per unit protein nitrogen at pH 5. 0 and the concentration of protein nitrogen in the RNase extract from shoot tips throughout eight weeks of the plants' ontogeny. Table 1 indicates that the amount of protein in the RNase extract diminished at a greater rate than the amount of total nitrogenous substances in senescing shoot tips.

During progressive senescence the absolute activity of soluble RNase seemed to decrease in a manner similar to that of total nitrogenous substances (cf. Figures 14, 16B), but the specific activity (units RNase/mg protein N)$_{\text{pH 5. 0}}$ appeared to increase, presumably because soluble RNase was more stable than some of the other proteins which could be extracted at pH 5. 0.

Figure 17. Time-course change in the ratio of protein nitrogen in the soluble RNase extract to shoot tip dry weight from shoot tips of deflowered plants and plants bearing reproductive structures (controls). Vertical arrow indicates the time of anthesis.

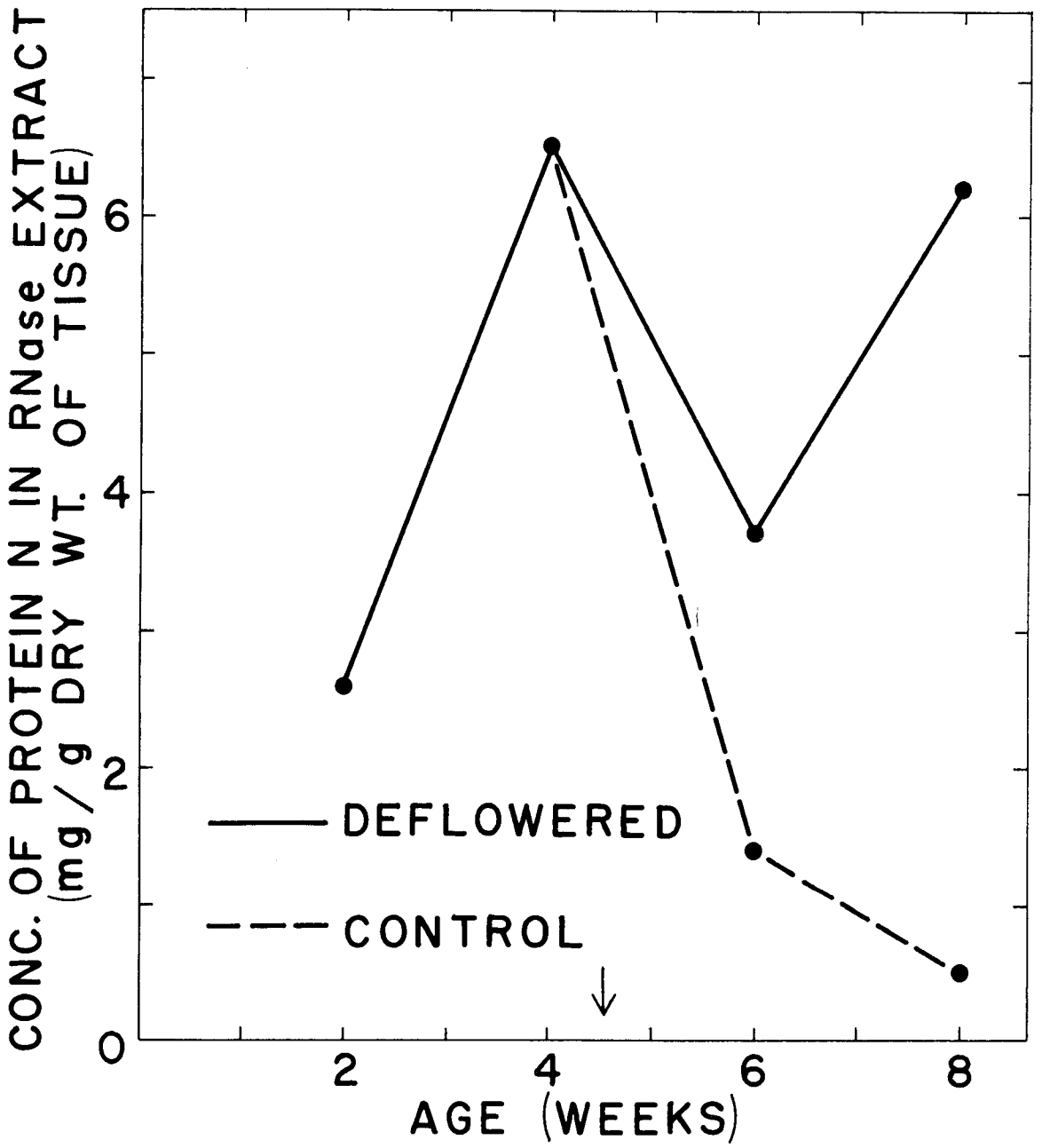

CONC. OF PROTEIN N IN RNase EXTRACT
(mg / g DRY WT. OF TISSUE)
0
2
4
6
2
4
6
8
AGE (WEEKS)
DEFLOWERED
CONTROL

Table 1. Comparisons between concentrations of total N in shoot tips and protein N in RNase extracts prepared from shoot tips of control and deflowered plants. *

Plant Age (week)	Ratio: mg N/g dry wt for control plants / mg N/g dry wt for deflowered plants	
	Total N	Protein N in RNase extract (pH 5.0)
6	0.69	0.38
8	0.40	0.08

*Samples of shoot tips excised from six- and eight-week-old control and deflowered plants were assayed for total N content. Concentrations of protein N were measured in RNase extracts prepared from the same tissue samples. Actual values for total N and protein N concentrations which were used in computing ratios are plotted in Figures 16A and 17, respectively. The data are expressed as ratios of control:deflowered plants for the purpose of illustrating the disproportionate decrease in protein N, as compared to total N, in senescing shoot tips.

The enzyme extracts used for the RNase assays were quite crude. No attempt was made to purify the enzyme and an optimal pH for soluble RNase activity was not determined; however, preliminary experiments indicated greater activity at a pH of 5.0 than at 5.6.

The notable variability in the absolute values for soluble RNase specific activity with repeated experiments might be due to a less than adequate reaction time. The one-hour incubation period seemed adequate in preliminary experiments and in the first of the reported experiments. To provide for standardization in the RNase assay procedure, the one-hour reaction time was used in all

subsequent assays. It is felt that the absolute differences are not as important as the relative differences between senescing (control) and nonsenescing (deflowered) plants with respect to the specific activity of soluble RNase in the shoot tips.

Ontogenetic Changes in the Ability of Shoot Tips to Incorporate ^{32}P into RNA

One experiment using four-, six- and eight-week-old plants was performed to determine if the ability of plant shoot tips to take up ^{32}P and incorporate it into RNA changed throughout the ontogeny of the plants. No part of this experiment was repeated. Two replicates of each sample (25 shoot tips) were used; however, in some cases a sample replicate was lost in the RNA extraction procedure.

The experimental results are presented in Table 2. Flowers had not been removed from four-week-old plants prior to sampling, so there were no separate control and deflowered groups for these plants. The amount of ^{32}P taken up by the explants during each incubation period varied considerably; shoot tips of six-week-old explants accumulated much more radioactivity than did those of four- or eight-week-old explants. Shoot tips from six- and eight-week-old control plants took up considerably less ^{32}P, but incorporated a greater percentage of the accumulated ^{32}P into RNA than did shoot tips from corresponding deflowered plants. In shoot tips from control

Table 2. Summary data on the incorporation of ^{32}P-orthophosphate into RNA.

Parameter	Replicate no.	4-wk-old plants	6-wk-old plants		8-wk-old plants	
			Control	Deflowered	Control	Deflowered
total cpm in sample	1	9, 344, 900	15, 649, 100	44, 346, 600	792, 900	5, 348, 000
	2	9, 317, 300				2, 800, 900
cpm incorp. into RNA	1	502, 200	1, 135, 100	2, 552, 400	96, 100	331, 800
	2	488, 300				187, 100
mg RNA/sample	1	35. 8	15. 1	34. 0	7. 1	42. 8
	2	38. 1				38. 1
mg RNA/mg N	1	0. 36	0. 36	0. 32	0. 11	0. 30
		0. 38				0. 29
cpm in RNA/mg RNA (specific activity)	1	14, 050	75, 050	75, 000	13, 550	7, 750
	2	12, 800				4, 900
% cpm incorp. into RNA	1	5. 37	7. 25	5. 75	12. 12	6. 20
	2	5. 24				6. 68
$\frac{\%\text{cpm incorp. into RNA}}{\text{mg RNA}}$	1	0. 150	0. 479	0. 168	1. 707	0. 144
	2	0. 137				0. 175
mg RNA/g dry wt	1	24. 4	13. 9	17. 9	3. 2	22. 4
	2	26. 0				21. 8
µg RNA/mg protein*	1	57. 6	57. 6	51. 2	17. 6	48. 0
	2	60. 8				46. 4
mg inorg P/g dry wt prior to ^{32}P uptake**		3. 3	2. 2	3. 3	0. 4	4. 1

*Protein determined from total nitrogen content. **An average value determined from similar samples.

plants, the percentage of the total radioactivity taken up which was incorporated into RNA increased as senescence progressed and was inversely related to the endogenous inorganic phosphorus level.

In regards to nonsenescing shoot tips (four-week-old and deflowered six- and eight-week-old plants) the amount of radioactivity found in the RNA appeared to be proportional to the total amount of radioactivity accumulated by the shoot tips. Since there were great differences in the amount of radioactivity taken up by the explants during each incubation period, the specific activity of ^{32}P in terms of RNA (cpm in RNA/mg RNA) or in terms of inorganic phosphorus (cpm/mg inorganic P) did not provide adequate means of comparing samples. It was rationalized that the data could be expressed and compared on a common basis as "percentage of total accumulated radioactivity incorporated into RNA per unit RNA." When the data were expressed in this manner, nearly constant values were obtained for nonsenescing shoot tips of deflowered plants from four to eight weeks of age. However, such values for shoot tips from control plants increased considerably as senescence progressed, suggesting a much greater incorporation of ^{32}P into RNA with respect to the levels of RNA present.

The results in Table 2 show that the amount of RNA diminished in senescing shoot tips, but in this experiment the level of RNA, expressed in terms of either total nitrogen or sample dry weight,

remained relatively constant in shoot tips from deflowered plants as compared with the results of previous experiments (Figures 13A, B).

DISCUSSION

Delay of Senescence by Deflowering

The results of experiments performed to determine the ability of flower removal and GA treatments to delay senescence in 'Alaska' peas were essentially the same as those obtained by Lockhart and Gottschall (1961). The removal of flowers or developing fruit postponed senescence in the shoot apex and in the entire vegetative plant. As mentioned earlier, Leopold et al. (1959) and Krizek et al. (1966) reported that in some annual species senescence cannot be completely explained by the depletion of metabolites in the vegetative plant structures by the developing fruits. However, Linck and Swanson (1960) have shown that while young pea flowers are ineffective in promoting the movement of solutes, fertilized flowers and developing pods provide a strong mobilizing force for the transport of solutes from the leaves and that the fruits are a metabolically controlling factor in the distribution of solutes through the phloem. Seth and Wareing (1967) have demonstrated that developing bean fruits strongly promote the mobilization of metabolites from the leaves to the developing fruits and that this mobilization process may be hormonally controlled.

An interesting aspect of senescence in 'Alaska' peas is the determinate growth and seeming disappearance of a shoot apex in plants

which have been continually deflowered. This phenomenon was also reported by Lockhart and Gottschall (1961) and was considered by them to be a type of "apical senescence," as was the determinate growth which occurred in plants kept vegetative by supplementing the normal photoperiod with far-red radiation. Although these two forms of "apical senescence" both were apparently unrelated to the formation of flowers (Lockhart and Gottschall, 1961), they obviously were not equivalent. A shoot tip terminating with a final flower is morphologically and physiologically different from one on a nonflowering plant displaying determinate growth. Contrary to the results reported by Lockhart and Gottschall (1961), 20-30% of the continually deflowered plants exhibited apparent indeterminate growth and continued to grow and produce flowers for several months.

Correlation of Plant Growth Rate and Senescence with the Level of Endogenous Gibberellin

Periodic GA treatments delayed but did not prevent senescence in fruit-bearing plants. Lockhart and Gottschall (1961) concluded that the GA effect was indirect inasmuch as these treatments substituted for the effect of deflowering by delaying fruit set. In the experiments described here, weekly GA treatments resulted in the abortion of some flowers and in the production of some abnormally small filled pods and parthenocarpic fruit, which would support the assumption that the GA effect is indirect. On the other hand, results obtained in

experiments employing periodic shoot-tip applications of AMO-1618, an inhibitor of gibberellin biosynthesis (Baldev, Lang and Agatep, 1965; Dennis, Upper and West, 1965) cast some doubt on the validity of that assumption. AMO-1618 had no effect on flower formation, fruit development, nor the time of senescence in fruit-bearing plants, but in deflowered plants the growth retardant promoted apex senescence which appeared morphologically similar to that in fruit-bearing plants. These results suggest that a decrease in active gibberellin in the shoot tip may be directly correlated with the occurrence of senescence in the shoot apex. The implication of a declining level of gibberellin with senescence has been previously suggested from similar studies by Ruddat and Pharis (1966a) who have shown that treatment with AMO-1618 enhances the senescence of intact, fully expanded leaves of soybean (Glycine max (L.) Merr.) and Stevia rebaudiana and that simultaneous treatments with GA can reverse the AMO-1618 effect.

Studies of the ontogenetic changes in the sensitivity of both deflowered and fruit-bearing plants to applied GA and AMO-1618 further support the suggestion that a decrease in endogenous gibberellin is a causal factor in the senescence of the shoot apex. The results support the observation by Moore (1967) that 'Alaska' peas which are not deflowered exhibit two periods of sensitivity to exogenous GA in their ontogeny; the first occurs during the growth of the young seedlings,

prior to the linear phase of growth, and the second occurs during the period of declining growth rate and the onset of apex senescence, when endogenous gibberellin is presumably rate-limiting for stem elongation. The lack of responsiveness to exogenous GA by plants in the linear growth phase is assumed to be correlated with the presence of a growth-saturating level of endogenous gibberellin.

Gibberellin Involvement in Apical Dominance

Although not directly pertinent to the objectives of the research problem, the ability of shoot-tip applications of AMO-1618 to interfere with apical dominance in peas as observed in this study further corroborates the conclusions of other investigators (Jacobs and Case, 1965; Pharis et al., 1965; Ruddat and Pharis, 1966b; Scott, Case and Jacobs, 1967) that endogenous gibberellin participates in the control of apical dominance.

Physiological Manifestations of Senescence Associated with Development and Removal of Reproductive Structures

Leopold et al. (1959) suggested that senescence is imposed on a plant by an increasing "signal" which is intensified with successive reproductive development stages of the plant. Lockhart and Gottshall (1961) and the author have found with 'Alaska' peas that the intensity of such a proposed "signal" varies little from the time of flower

production through the early stages of fruit development and then greatly increases during seed development. The formation and development of reproductive structures apparently imposes new physiological conditions in the shoot tip which are partially manifested by a rapidly declining plant growth rate and a decreasing level of gibberellin. Flower removal seemingly results in a temporary reversal of these conditions as suggested by the increase in growth rate and gibberellin biosynthesis a few weeks after anthesis (Figures 2A, 9A, 10A, 11A, 12A) and by the corresponding increase in internode lengths (Figure 3A). A second, smaller peak following the time of anthesis was characteristic of the growth rate curves of untreated, deflowered plants obtained in all similar experiments performed, and in many cases the magnitude of the second peak was larger than that in Figure 2A.

Correlation of Biochemical Changes with Senescence

The results of experiments concerning biochemical changes in shoot tips during the ontogeny of pea plants indicate that among the events causally associated with senescence are a decrease in the levels of total RNA, total and soluble nitrogen and inorganic phosphorus. Thus it appears that senescence in intact pea shoot tips involves many of the biochemical changes reported for other senescing structures.

A reduction in the levels of metabolites could be explained by one or a combination of the following possibilities: (1) there is an increase in the activity of catabolic enzymes; (2) there is a decline in the activity of anabolic enzymes; and (3) during the turnover of metabolites, mobilization and net transport of catabolism products from the shoot tip occurs resulting in a diminution of anabolic activity.

It has been reported that increasing activities of catabolic enzymes such as RNase (Cherry, 1963; Hanson et al., 1965; Srivastava and Ware, 1965) and peptidase (Anderson and Rowan, 1966) are associated with senescence in cotyledons and leaves. The destruction of polyribosomes and ribosomes during the senescence of detached barley leaves described by Srivastava and Arglebe (1967) suggested net catabolic activity with respect to RNase and proteinases. An increase in the specific activity of soluble RNase also seems to be a factor contributing to the decline of the total RNA level in intact senescing pea shoot tips. Although the absolute activity of soluble RNase diminished during progressive senescence, the enzyme appeared to be more stable than the bulk of the protein in the RNase extract.

A dwindling of the total RNA level might also be attributed to a decline in RNA polymerase activity which was not measured as such. However, experiments which measured the uptake of ^{32}P and its incorporation into RNA were employed in an attempt to determine

the capability of senescing shoot tips to synthesize RNA. The results of these experiments were similar to results reported by Cherry et al. (1965) and Srivastava and Atkin (1968), who found an apparent enhanced incorporation of ^{32}P into RNA in senescing peanut cotyledons and barley leaf tissue, respectively. Cherry et al. and Srivastava and Atkin attributed their results mainly to a diminution in the endogenous phosphorus pool which resulted in a smaller dilution of the absorbed ^{32}P, and Cherry et al. were able to correlate a declining phosphate content with an increasing specific activity (cpm $^{32}P/\mu g$ phosphate). Considerable variation in the transpirational uptake of ^{32}P by pea explants at different incubation times rendered specific activity determinations meaningless in interpreting the results of the present investigation. When the amount of ^{32}P incorporated into RNA was expressed in terms of the total amount of ^{32}P accumulated by the shoot tips, an apparent enhancement of ^{32}P incorporation into RNA was noted in senescing shoot tips. However, since the total RNA level declined during senescence (Table 2) the increased ^{32}P incorporation into RNA could not represent net synthesis of RNA. Furthermore, the enhancement of ^{32}P incorporation into RNA can be correlated with the decrease in the inorganic phosphorus pool in senescing shoot tips (Table 2).

Cherry et al. (1965) also proposed that the seeming augmentation of ^{32}P incorporation into RNA could be the result of an increased

rate of RNA degradation during senescence while the rate of RNA synthesis remains practically unchanged. The increased incorporation of ^{32}P into RNA in senescing pea shoot tips suggests that the metabolic pathways involved in RNA synthesis remain active during progressive senescence; however, it is unknown if their activity remains constant. Pertinent to this discussion is the report by Huang (as cited by Varner, 1961) that during the senescence of pea cotyledons there was no change in the ability of the nuclei to incorporate tritiated thymidine. It was concluded that a reduction in nuclear activity is not the primary event in pea cotyledon senescence.

A diminishing endogenous phosphorus pool seems to be one of the principal factors contributing to the senescence of intact plant structures. One can only speculate on whether a decline in the phosphorus level is an initial cause of senescence or a consequence of primary events and then contributes to senescence. Several investigators have demonstrated that phosphorus compounds and other metabolites are mobilized and transported from mature organs to developing vegetative or reproductive structures, and that senescence of the mature organs is associated with the loss of metabolites. In the case of apex senescence, anabolic cells and tissues of the apex and shoot tip seemingly become catabolic in nature before reaching morphological maturity. Developing reproductive structures might be capable of mobilizing and assimilating metabolites from the

growing shoot tip, or perhaps they merely outcompete the shoot tip for available metabolites. The results of experiments to study the uptake and incorporation of ^{32}P (Table 2) indicate that during progressive senescence, the ability of shoot tips to take up nutrients by transpirational activity declines, thereby intensifying the decrease in the endogenous phosphorus pool. A decline in the phosphorus level obviously would have a considerable effect on the anabolic activities of cells, in view of the fact that phosphorus is a constituent of nucleic acids and of lipids which are components of cellular membranes, and it is involved in the conservation and transfer of energy in biochemical reactions.

What effect endogenous gibberellin has in regulating the senescence of the shoot apex is unknown. Neither extractable nor diffusible gibberellin in the shoot tips was measured. However, the indirect methods employed to estimate the relative amounts of active endogenous gibberellin present in the shoot tips yielded results which indicate that senescence of the shoot tip is associated with a reduction in the level of active gibberellin. It remains as a subject for future investigation to determine whether the decline in the rate of biosynthesis of endogenous gibberellin is the cause or the consequence of the decline in RNA and protein levels in the senescing shoot tip.

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