PHYSIOLOGY OF FLOWERING IN RED RASPBERRY
RUBUS IDAEUS L.

BY

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TO MY WIFE

DIMITRA

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ABSTRACT

PHYSIOLOGY OF FLOWERING IN RED RASPBERRY

RUBUS IDAEUS L.

BY

MILTIADIS D. VASILAKAKIS

Under the supervision of Professor Malcolm N. Dana

The effect of low temperature in flowering of red raspberry (*Rubus idaeus* L.) and also the possible involvement of endogenous gibberellins and cytokinins in flower induction were studied.

For studying the effect of low temperature in flower induction and flower bud development in mature or overwintering canes of red raspberry, 'Heritage', fall-bearing, and 'Latham', June-bearing, cultivars were used. Flower induction occurred in warm temperatures but low temperatures hastened not only flower induction but also flower bud development. In non-cold treated plants no development beyond the stage of initiation was observed. In cold treated plants a gradual degree of flower bud development, basipetally expressed, occurred.

For studying the effect of low temperature on the time of flowering in fall-bearing red raspberries, 'Heritage' plants grown under controlled environmental conditions

were used. Plants at any stage of growth, even at the stage of preformed buds on the roots, responded to cold, the response being expressed as shortening of the vegetative plant-life. The older the shoots were at time of cold treatment, the earlier the flower induction occurred. Shoots never flowered at less than 20 visible nodes of growth. The flowering response to cold was not transmissible by grafting. 'Heritage' plants responded to vernalization in a way similar to that of most annuals and biennials.

Removal of the developing inflorescences did not alter the total number of inflorescences produced. The more the number of inflorescences removed, the greater the number of suckers produced.

The possible involvement of plant hormones in flower induction was studied by comparing changes (qualitative and quantitative) that occurred in endogenous gibberellin-like substances and cytokinins when plants were shifting from the vegetative to the reproductive phase. Cold-treated and non-cold treated fall-bearing cultivars, and cold treated June-bearing cultivars as well, were involved in the analyses. Samples were taken at two stages of growth, 10 and 20 visible nodes. At 10 node stage of growth all plants were vegetative, at 20 nodes only

cold-treated fall-bearing cultivars were at or before the beginning of flower induction.

Qualitative differences in endogenous gibberellin-like substances were observed between 10 and 20 node stage of growth in cold treated and flower induced plants of fall-bearing cultivars and between cold treated and non-cold treated 'Heritage' at the 20 node stage of growth.

Major quantitative changes from 10 to 20 node stage of growth occurred only in cold treated fall-bearing cultivars. Sixty percent greater activity of gibberellin-like substances was observed at 20 node stage of growth than at 10 nodes and 100% greater activity at 20 node stage of growth in cold-treated and flower-induced than in non-cold treated and non-flower-induced fall-bearing cultivars.

In all cultivars and treatments cytokinin activity was always greater at the 20 than at the 10 node stage of growth. However, greater cytokinin activity was observed at both stages of growth in cold treated than non-cold treated fall-bearing cultivars.

It was concluded that both gibberellins and cytokinins may be involved in the production of the floral stimulus in this species.

Approved Malcolm N. Dana
Professor of Horticulture

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INTRODUCTION

Red raspberry (Rubus idaeus L.) bears woody, short-lived shoots on a long-lived perennial root. The biennial shoots usually arise in spring from adventitious buds on the roots or from basal axillary buds on old canes. Shoots that do not start growing until late summer or fall continue apical growth in the following spring. Shoots of fall-bearing cultivars continue to elongate until the terminal inflorescence appears and vegetative growth ceases. Inflorescences continue to develop basipetally to a certain node, the number developing varies among cultivars. Fruit matures into the first year's crop, the canes overwinter, and in the spring the rest of the axillary buds elongate and give mixed shoots where the second year's crop appears. June-bearing cultivars grow later in the season than fall-bearing cultivars and normally do not form a terminal inflorescence the first year of growth. In the spring of the second year, axillary buds give mixed shoots similar to those of fall-bearing cultivars and yield the full crop.

The first year's crop on fall-bearing cultivars is not usually important in states having a climate similar to Wisconsin because only a small amount of fruit ripens before frost. If the flowering process could be advanced by

two or three weeks, then the first year's crop of fall-bearing cultivars would be of great value.

Therefore, an understanding of the flowering process in red raspberries and especially of the fall-bearing type was necessary as a basis for developing cultural practices to advance the fruiting period.

Besides producing seed, flowering in annuals and biennials usually means the termination of vegetative growth and later death. Each plant species has a genetically controlled program for growth and development, the course of which may be shortened or prolonged by environmental manipulation (i.e., photoperiod, cold). The endogenous changes that occur under different environmental conditions are main concerns of plant physiologists and biochemists.

Growth regulating substances are involved in many phases of plant growth and development. These substances play a role in flowering either directly or indirectly. Gibberellins are a group of growth regulating substances which have been widely studied in relation to flowering because they have been shown capable of modifying long photoperiod or cold requirements of many plant species, mostly annuals and biennials. The external application of gibberellins to such plants growing under non-flower-inductive conditions may bring flowering in most but not all of them. In addition there is good correlation

between flowering and internal changes of gibberellins both qualitative and quantitative in many plant species.

Some people believe that the balance of growth regulating substances might be the real factor controlling the flowering process. The existence of some unknown flowering hormone cannot be excluded and still remains a speculation.

The purpose of the present study was to better understand the flowering process in red raspberries and to find possible ways of changing the endogenously controlled flowering program.

LITERATURE REVIEW

A. General Review on Flowering Related to Cold and Growth Regulating Substances.

Systematic research on vernalization was undertaken as early as 1857 by Klippart (52). He showed that among the various climatic factors of winter, the determining factor was the cold temperature to which young plants were subjected for a few weeks; this stimulates winter cereals to flower soon after the return of warmer temperature. This observation was extended to many plant species by Gassner in 1918 (31) who showed the great variety of "cold requirements" (measured by minimal chilling time) displayed by numerous species known as "biennials" or "winter annuals" which have no significant chilling requirement for flowering. Gassner also showed that in winter cereals the early swollen germinating seed is already sensitive to the specific cold effect. Finally, in 1928, Lysenko (66) established that slight imbibition (e.g., 50 parts water to 100 parts dry matter) makes the cereal seed susceptible to this action of cold without inducing the excessive germination that could prevent the use of a sowing machine. It was Lysenko who gave a name to the phenomenon. As spring cereals are called Jarovoe in Russian (from "Jar" formerly fire, or the god of spring),

he called the process which makes a winter cereal behave like a spring cereal "Jarovization", and translated the word in his own English, French, and German translation into"vernalization" (Latin *vernum* meaning spring).

From their study of vernalization, Lysenko and his followers built their famous phasic theory of the stadial development of plants and felt they had continued Mitchourin's theory of the transmissibility of certain characteristics acquired by adaptation (19).

Two main groups have studied the physiology of vernalization on an accurate experimental basis: Gregory and Purvis (37, 93) in London using winter rye 'Petkus'; and Melchers and Lang (72) at Tubingen, working with the biennial strain of henbane (*Hyoscyamus niger* L.)

Chouard (19) in his review uses the original restricted definition of vernalization. Vernalization induces or hastens the development of the capacity for flowering and although its action is not visible at first, it appears as an after-effect, chilling is the normal agent. Vernalization is a preparatory process to flowering but not the flower initiation process itself.

In the present review, low temperature and flowering will be discussed generally because although Chouard's' view is correct in most cases, there are some complicating exceptions, especially those plants that are not seed

vernalizable. In such plants, the response to cold depends on the age at which the plant receives the cold. They they receive the cold during their juvenile phase, then a preparation by the plant is necessary for flowering to occur. When mature plants receive the cold, then flower induction can rapidly occur.

<u>Example of plants responding to vernalization</u>

<u>Rye 'Petkus</u>' (monocotyledonae)

Petkus, a cultivar of <u>Secale</u> <u>cereale</u> L. consists of two races. The spring race is a typical annual rosette plant and can be considered as a quantitative long day (LD) plant. When seeds are planted during spring under LD, the primary axis produces seven leaf primordia and then forms the young ear, recognizable by double ridges on the sides of the apex. In short day (SD), it produces about 25 leaves before earing. The winter race is a typical biennial rosette plant. When seeds are planted during spring, under LD conditions, the primary axis produces 25 leaf primordia before earing, and 16 under SD conditions, but if the seed has been previously chilled, it grows exactly like the spring cultivar. Ear primordia have been formed under LD conditions after the 7th leaf, even sometimes after the 6th, when chilling has been extended up to 90 days (36). Thus, in both strains the first six or seven lateral primordia formed by the apex are predetermined as leaves, and "ripeness to flowering" is reached at the 6th or 7th leaf.

Short days can replace part of the vernalization as if the plant was a "facultative SD" plant in relation to flower initiation (93). In all winter cereals after vernalization the plant is a quantitative LD plant (36).

Chilling can effectively induce vernalization on very young plants. The immature embryo within the milky caryopsis can be vernalized, either because of the cold nights occurring during early summer maturation in subarctic countries or local chilling of the ear on the stalk (38). In immature embryos, as in mature seeds or seedlings, the time required for complete vernalization is about the same (40 to 45 days). When the treatment is applied to an older plant, the minimal exposure required to vernalize decreases with age (94).

The vernalized state is not transmissible by grafting but is transferred without reduction through cell division to all meristematic tissues originating at the apex of the embryo, e.g., to tillers even though they are formed after the vernalizing treatment (44).

In cereals the organs already formed (young leaves, young culms) are most responsive to the elongating effect of gibberellins but this substance cannot cause them to flower and cannot replace vernalization by chilling (94).

Henbane (dicotyledonae)

The classical information concerning vernalization was based on henbane as much as on 'Petkus' rye, because

of Melchers' and Lang's remarkable work. Chouard (19) summarizes their work as follows: The species Hyoscyamus niger L. consists of two physiological races, the biennial and annual strains. The important characteristics of henbane that differ from winter cereals are as follows:

a) Neither the seed nor the immature embryo is vernalizable; chilling is only effective on plants at least ten days old and in the rosette stage. The capacity for vernalization increases until the thirtieth day and then stays constant for a long time. That is to say, in the terms used by Wellensiek, "this plant presents a juvenile phase for the first ten days of life after germination since the plant cannot respond to the vernalizing effect of cold before the rosette is ten days old;" or in Chouard's terms, "the plant reaches its ripeness for vernalization at that age."

b) The leafy plant is a strictly LD plant.

c) The winter strain has an obligate requirement for vernalization.

d) Partial replacement of vernalization by previous exposure to SD is not possible.

e) This plant shows a remarkable ability for grafting and the transmission of vernalization from donor to receptor through grafts.

Graft transmission of the vernalized state is one of the most extraordinary properties displayed by henbane (72). A donor scion can be side-grafted onto the rosette, the scion either is a branch or a separate leaf from one of the numerous solanaceous species among which all possible types of vernalization or photoperiodic requirements are found. By these means, Melchers (70) succeeded in making a nonvernalized, biennial black henbane flower by grafting with a donor. The donor could be a leaf or shoot from an individual of the same strain previously vernalized and exposed to LD, or from the annual strain, or from the Hyoscyamus albus L. species which is indifferent to vernalization and photoperiodism, or from annual species that are photoperiodically insensitive (such as Petunia hybrida Vilm., Nicotiana sylvestris L.), or even Nicotiana tabacum L. 'Maryland Mammoth' the famous SD annual. Since a leaf of this latter plant exposed to LD cannot be induced to flower, it is not a donor of the so-called flowering hormone or "florigen', however, because it induces flowering in non-vernalized black henbane, it must contain another hypothetical flowering hormone called "vernaline" by Melchers (71). Except for the tobacco 'Maryland Mammoth', the donor must always be at the flowering stage. Reciprocally, a non-vernalized, LD black henbane cannot be used as an effective donor to make a defoliated 'Maryland

Mammoth' branch flower. The black henbane does act as a donor if previously vernalized (71).

Lang successfully substituted treatment with gibberellin (GA) for chilling using the biennial black henbane. The formation of flower primordia preceded stem elongation in vernalization by chilling, the reverse occurred when gibberellin was used (57). However, if the GA concentration was kept to a minimum, both processes occurred simultaneously (6).

Other plants that have been shown to require cold for flowering are presented in Table 1, adapted from Chouard, 1960 (19).

Flowering and plant growth regulating substances

The influence of environmental factors on flowering has been established and extensively studied by many investigators. It was proposed as early as 1880 by Julius Sachs (101) that the environment may ultimately lead the plant to flowering through the formation of a hypothetical hormone. This idea was revived and adopted by Chailakhyan (12) who proposed the florigen theory for flowering. Several attempts have been made to isolate and characterize florigen but without success (62, 63, 98).

In the present review, only known growth regulating substances proven to play some role in flower induction will be discussed.

Table 1. Plants requiring cold for flowering (cereals not included). Adapted from Chouard, 1960

Biennial plants in rosette species	Degree of cold requirement	Photoperiod after cold	Response to external GA	Vernalizable stage	
				Seed	Plant
Beta vulgaris L. sugar beets	Absolute	LD flowering SD shooting	No flowering	X	X
Beta maritima L.	Less cold		Yes		
Arabidopsis thaliana (L.) Heyn.	Absolute or facultative	--	No Yes	X	
Lactuca sativa L.	Different degrees varietal response	Facultative LD	Yes	X	X
Hyoscyamus niger L.	Obligate	LD	Yes		X
Cichorium endivia L.	Quantitative	Quantitative LD	Yes	X	
Petroselinum crispus Hoffm.	Obligate	LD	Yes		X
Campanula medium L.	Absolute or facultative	LD	No Yes	 X	X X
Oenothera biennis L.	Obligate	LD	Yes	X	X
Oenothera lamarkiana L.	Less obligate	LD	Yes		
Daucus carota L.	Varietal differences	LD	Yes		X
Digitalis purpurea L.	Obligate	SD or LD	Yes		X
Scrofularia vernalis L.	Obligate	Day neutral	-		X
Lunaria annua or *biennis* L.	Absolute	LD or SD	No		X

Table 1. (continued)

Caulescent biennial of monocarpic plant species	Degree of cold requirement	Photoperiod after cold	Response to external GA	Vernalizable stage	
				Seed	Plant
Euphorbia lathyrus L.	Genotype differences	SD or LD	No		X
Iberis sp.	Obligate	SD or LD	No		X
Brassica oleracea capitata L.	Strain difference	LD	Some strains Yes		X
Brassica oleracea gemnifera L.	Cold induces flowering				X
Lycopersicon esculentum Mill.	Cold promotes flowering			X	X
Pisum sativum L.	In dwarf late cultivars, cold promotes flowering GA delays flowering			X	X
Perennial or polycarpic plants					
Geum urbanum L.	Obligate to less cold required	Day neutral	No Yes		X
Scabiosa succisa L.	Obligate	Quantitative LD	No		X
Meadow *Graminaceae*	Species dependent	LD or SD	No		X
Saxifraga rotundifolia L.	Obligate	Day neutral	No		X
Scrofularia alata L.	Obligate to less cold requirement	LD or SD	No Yes		X
Scabiosa canescens L.	Obligate or not genotype dependent	LD or SD	No		X

Table 1. (continued)

Caulescent perennial plants	Degree of cold requirement	Photoperiod after cold	Response to external GA
Dianthus sp.	Species dependent	LD or SD	No
Leucanthus sp.	Obligate	Day neutral	No
Teucrium scorodinia L.	Obligate	LD but not always	No
Chrysanthemum morifolium Ramat.	Cultivar differences	SD	In some cultivars SD and GA flower
Woody plants			
Olea europea L.	Obligate or not (cultivar differences)	LD	No

Plants possess great genetical variation as concerns requirement of vernalization for flowering and response to GA. GA can replace cold in most of the rosette biennial plants requiring cold, especially in those which do not have an absolute requirement of cold for flowering. GA has no effect on caulescent biennials and perennials and on woody species.

1. Auxins

Chailakhyan (18) summarizing the results obtained from studies with auxins and flowering noted that auxins are produced in terminal apices regardless of the nature of the photoperiodic response and are not connected with flowering; they are connected with daylength being more intensive in LD species. There are no experiments where treatment with auxin has resulted in flowering of annual plants under unfavorable daylength conditions. Flowering of LD species was slightly enhanced while flowering of SD species was slightly inhibited by auxin treatment under inductive conditions. At the same time such growth inhibitors as TIBA (Tri-iodo-benzoic acid) and MH (1,2-dihydro-3,6-pyridazine-dione) proved able to stimulate flowering and to sharply inhibit growth of SD species like cocklebur, soybean and _Perilla_. Thus, he concluded, auxins and synthetic growth-promoting preparations which function as auxins and as auxin antagonists have indirect influence on flowering, but do not play a decisive role.

2. Cytokinins and nucleic acids

Experiments have shown that cytokinin application can promote flowering in SD plants. In _Perilla_, spraying with kinetin solutions under inductive conditions greatly accelerated flowering (65). In _Pharbitis_ seedlings the stimulating effect was observed only when applications were made to the cotyledons and not to the apical

bud (84). Isolated terminal buds of Perilla grown on nutrient media induced flower primordia by kinetin even under LD (13). Similarly, flowers have been induced on stem segments of the obligate SD plant Plumbago indica L. growing in culture in LD with a combination of a cytokinin and adenine (81, 82).

In the LD plant Rudbeckia, kinetin applications greatly suppressed flowering both in the entire plants and in buds in culture (16), but induced flowering of the LD plants Arabidopsis thaliana (L.) Heyn. and Calendula officialis L. grown under SD (7, 76). Similar flower initiation was claimed for the cold requiring Cichorium intybus L. and 'Petkus' rye grown under warm non-inductive conditions (11, 75), which suggested a possible action through the control of GA level. Maheshwari and Venkataraman (68) have shown that zeatin application induced flowering in duckweed [Wolfia microscopica (Griff) Kurtz.] a SD plant growing under LD conditions.

In one cultivar of Chrysanthemum benzyladenine had little effect on flower formation, but a combination of GA_5 and benzyladenine induced formation of inflorescences (89).

Beever and Woolhouse (8) reported that photoperiodic induction of SD Perilla was associated with a striking increase in cytokinin levels in the xylem sap from the roots. Analytical tests showed that leaves of SD plants species

soybeans, Streptocarpus wendlandii Dam. and Chenopodium album L.--under SD conditions contained more nucleic acids than under LD conditions; the content of nucleic acids in leaves of LD species varied depending on the day-length (32).

Nevertheless, flowering induction and promotion are not confined to the cytokinins but are shared by the closely related bases adenine, guanine and uracil, and also the derived nucleosides adenosine and guanosine (18). There is an extensive literature (18, 60) which shows clearly that certain analogues of nucleic acid bases, which act as antimetabolites in nucleic acid synthesis (2-thiouracil,5-fluorouracil, 5-flourodisoxyuridine, 2,6-diaminopurine, 8-azaguanine) effectively prevent flower formation. Chailakhyan (18) commenting on the results obtained by using antimetabolites of nucleic acids believed that plants can flower only if meristems of stem buds synthesize a significant quantity of deoxyribonucleic acid (DNA) and that flowering is preceded by the synthesis of a specific ribonucleic acid (RNA), which is called "reproductive" in contrast to "vegetative". Audus (2), discussing cytokinins and flowering, stated that flowering must involve a switch in gene expression and that this involves changes in the type of RNA synthesized. Cytokinins and the related bases may likely affect flowering by influencing such nucleic acid synthesis.

There appears to be a gradient in flowering capacity along a stem axis. Aghion-prat (1) established the existence of an apex-to-base gradient in the capacity of stem segments to produce flower buds *in vitro* (day neutral tobacco cultivar). The axillary buds of an orchid (35) and of *Hieracium floribundum* Wim. and Grab. (125) showed a similar gradient in the potentiality to flower. The flowering gradient in tobacco was paralleled with DNA content by Wardell and Skoog (116). The uppermost stem tissue of flowering tobacco plants contained ten times more DNA per gram fresh weight than tissue further down the stem. Additional work led to the finding that DNA isolated from the inflorescence region caused flower formation when applied to defoliated axillary buds of decapitated plants, whereas DNA prepared from vegetative plants had no flower inducing activity. Thermal denaturation of DNA increased the flower inducing activity, while treatment with DNase completely eliminated it (117). Zeevaart (131) in a pre-review comment, stated that the presence of DNase in tobacco could be expected to lead to rapid degradation of external added DNA molecules. The best approach, he suggested, to demonstrate conclusively the involvement of intact DNA in flowering would seem to lie in application of radioactive and density-labelled DNA, and to see if this DNA could be subsequently recovered intact from axillary buds. Without such evidence, the claim

that DNA, as opposed to a breakdown product or contaminant of the preparation, has flower inducing activity must be viewed with some caution.

3. Abscisic acid (ABA)

Zeevaart (131) summarized the results obtained from studies with ABA and flowering and noted that ABA could not induce flowering in SD plants under strictly non-inductive conditions, but it could enhance the flowering response of slightly induced plants of *Pharbitis* and *Chenopodium*. In the SD plant *Kalanchoe* ABA as well as xanthoxanthin inhibited flower formation. Applied ABA was not inhibitory to flower formation in the LD plant *Rudbeckia*. With regard to endogenous ABA, the levels were higher under LD than under SD conditions in some cases, the level remained virtually unchanged after transferring LD plants from SD to LD. Thus, he concluded, that these observations were not in agreement with the postulated role of ABA as an inhibitor of flower formation in LD plants held under SD conditions.

4. Gibberellins (GA)

Among the endogenous plant hormones, only gibberellins consistently replace environmental requirements for flowering in a large number of plants belonging to different categories (60).

There are many outstanding reviews on GA (50, 61, 91) and flowering (60, 103, 105, 131). A most recent review is by Krishnamoorthy (54) in the book edited by him "Gibberellins and Plant Growth".

a. External application of gibberellins and flowering.

Rosette LD plants and cold requiring plants.

Lang (56, 57) was the first to demonstrate that a rosette LD plant, the cold requiring biennial henbane (*Hyoscyamus niger* L.) could be flower-induced by application of GA_3 to the apices of plants grown under non-inductive conditions. Since then, a number of plants belonging to these two categories have proven to respond similarly to GA_3 applications. There is considerable evidence to show that gibberellins differ in their effects on stem elongation and flowering in individual plant species (58, 73, 90, 123, 124).

It has been argued frequently that the failure of a particular gibberellin to cause flowering in a plant can not be generalized as there is always the possibility that an appropriate concentration of one of the known gibberellins applied by a proper method at the right stage, may cause flowering (60). This can be tested only when sufficient quantities of these gibberellins become available.

Gibberellins can substitute for the cold requirement but not for long days in plants requiring both cold and LD for flowering. An exception is henbane (57).

Long-short day and short-long day plants

GA in these plants successfully replaces the LD requirement but not the SD requirement (47, 51, 86, 130).

Short day plants

Generally, gibberellins fail to cause flowering of SD plants under non-inductive conditions, although stem elongation may occur (60). Gibberellins were able to cause flowering under strictly non-inductive conditions in *Impatiens balsamina* L. (79), *Chrysanthemum morifolium* Ram. (89), *Zinnia elegans* Jacq. (102), *Panicum miliaceum* L. and *P. miliare* Lank. (55). Gibberellins promoted flowering of some SD plants maintained under suboptimal photoinduction (39, 55, 83, 100). In contrast, GA inhibited flowering of some SD plants maintained under optimal inductive conditions (22, 41, 47, 81).

b. Role of endogenous GA in flowering

There are two approaches to the study of this problem 1) treating plants with growth retardants which presumably inhibit GA-biosynthesis and thus reduce the endogenous level, 2) direct determination of endogenous GA-content of plants exposed to varying degrees of inductive conditions and to correlate the change in the quantity and quality of GA with the progress of the plant towards flowering (54).

Treatment with growth retardants under otherwise favorable environmental conditions inhibits flowering in many plants. This inhibition is overcome by applying GA to growth retardant treated plants. There is, however, strong evidence to indicate that the action of growth retardants is not exclusively on the biosynthesis of gibberellins. More often the growth retardants failed to inhibit flowering in *Hieracium* spp. (88) and *Brassica oleracea* L. var. *capitata* (29) under inductive conditions and increased the gibberellin content rather than decreased it (42, 97). Sometimes the retardants are synergistic rather than being antagonistic to endogenous GA-level as in the flowering of *Lolium temulentum* L. (27), *Clerodendrum thomsonae* Balf. (53) and internode elongation of strawberry (40).

Krishnamoorthy (54) stated that conclusions drawn from growth retardant studies must be tentative until the precise mode of action is fully understood.

Long day plants

In most LD plants, quantitative and qualitative changes in endogenous GA have been shown in induced plants (45, 46, 59, 95). Results from studies conducted with growth retardants on LD plants appear to be more consistent than those from similar studies on SD plants in relation to participation of GA in flowering. *Samolus parviflorus* Raf.

is a rosette LD plant requiring at least 8 LD for flowering (6). Growth retardants, Amo 1618 (4-hydroxyl-5-isopropyl-2-methylphenyl trimethyl ammonium chloride, 1-piperidine carboxylate) and CCC (2-chloroethyl trimethyl-ammonium chloride) inhibited flowering and the inhibition was overcome by supplying GA_3 or by exposing the plants to additional LD . Therefore, LD and exogenous GA_3 appeared to be similar in action and one could replace the other. The higher the concentration of the retardant used, the more the number of LD or the higher the concentration of GA_3 required to overcome inhibition. The authors explained that in Samolus exposure to LD probably resulted in a gradual increase in endogenous GA level. At least eight LD were required to build up an optimum GA level required for flowering. Lolium temulentum L. can be induced to flower by a single LD. This requirement could be met by an exogenous supply of GA_3, GA_4 or GA_7 (26). The effect of gibberellins and LD were additive. However, a large dose of CCC or Amo 1618 applied for prolonged periods before or after a single LD induction did not inhibit flowering, although stem elongation was inhibited. In fact, the effect of CCC in flowering was synergistic with GA rather than being antagonistic (27).

Long-short day plants

Flowering and gibberellin level are closely related in these plants. Zeevaart (129), working with Bryophyllum

days at 3-4°C, endogenous GA level was similar to that of summer cultivars. Cycocel (CCC) applied before or during vernalization of winter wheat seeds inhibited flowering and reduced the level of endogenous GA (74). In addition Phosphon D, B-nine, and Amo 1618 also had similar effects on this plant (111). In radish, which requires both low temperature and LDs for flowering (112), endogenous GA increased with vernalization reaching a peak four days after transferring to a greenhouse. However, neither GA_3 nor GA_7 could replace the vernalization required for flowering, although these caused bolting. Cycocel (CCC) reduced endogenous GA content and inhibited bolting but not flowering. Therefore, they concluded that the mechanisms of bolting and flowering were different and endogenous GA did not appear to play a role in flowering, in this plant. Harada's data (46) with vernalization of Shuakan cultivar of *Chrysanthemum morifolium* Ram.,which requires cold treatment for flowering,showed a large increase in gibberellin-like substance (substance E) during and just after vernalization. A similar situation was observed in *Brassica oleracera* L. var. Italica in which new zones of activity also appeared on the chromatogram of vernalized plants indicating both a quantitative and qualitative change in endogenous gibberellin content during vernalization (29). Levels of endogenous gibberellin also increased

diagremontianum Hamet and Pert. concluded that the level of gibberellins appeared to be the limiting factor for flowering in this plant under SD conditions. This deficiency could be made by either exposing the plant to LD which built up endogenous gibberellin level or by supplying exogenous gibberellins.

Short day plants

Gibberellins are not the limiting factor for flowering in these plants. Vegetative plants under LD had more gibberellin-like substances than flowering plants under SD (14, 15, 25, 126). Indirect evidence indicates that gibberellin may play some role in the flowering of SD plants. In *Pharbitis nil* L. all the four retardants, Amo 1618, Phosphon D (2,4-dichlorobenzyl-tributylphosphonium chloride), CCC, and B-nine (N,N-dimethylamino succinamic acid) inhibited vegetative growth and flowering which was overcome by GA_3 treatment (127). In *Impatiens balsamina* L. Phosphon D increased the critical dark period requirement for flowering by one hour, while GA_3 completely removed this requirement (80).

Cold requiring plants

Chailakhyan and Lozhnikova (17) found that summer cultivars of wheat, rye and rape (*Brassica napus* L.) had higher gibberellin content than corresponding winter cultivars. However, when the latter were vernalized for 45

In some conifers of the families Cupressaceae and Taxodiaceae GA promoted flowering markedly and consistently (90).

5. Gibberellins and "florigen"

The weight of evidence is against the hypothesis that gibberellin is identical to florigen. Chailakhyan (18) postulated that florigen is really a system of two hormones, one a gibberellin and the other a hypothetical "anthesin". Both are needed in adequate quantities to induce flower formation. In LD plants anthesins are always adequate under any daylength. Gibberellins are at sub-optimal threshold levels in SD and have to be increased by synthesis during LD before an effective florigen system is constituted. In SD plants, gibberellins are adequate under any daylength but the anthesins are lacking under LD. Anthesin synthesis would be increased to effective levels by SD. Of course, anthesins never have been isolated. Lang (60) concluded that gibberellins could be a factor causing, under appropriate conditions, the synthesis of "florigen". Such synthesis probably takes place in the leaves since a leaf from a plant grown under SD but treated with gibberellin could induce flowering in a LD stock after grafting (129).

In genotypically non-flowering segregates of red clover (Trifolium pratense L.) flowering could not be induced under any photoperiod. Endogenous GA was found to

be low in these plants. An application of exogenous GA_3 caused stem elongation under SD and in addition flowering when given before LD (108, 109, 110). Therefore, it was concluded that in red clover both an adequate level of endogenous GA and subsequent exposure to LD were required for the formation of the floral stimulus. Similarly, the clone produced by Hilman and Smith (48) by crossing *Nicotiana tabacum* L. with *N. rustica* L. flowered only when both exogenous GA_3 and low temperature were present. Either of them alone was ineffective. Thus, it was concluded that gibberellin was required for the formation of the floral stimulus.

Evans in his review (28) concluded that gibberellins are a prerequisite for evocation in all plants and that GA level is often limiting in LD plants, while in some, such as *Samolus*, it may be the primary limitation. Zeevaart (131) tends to support the idea that GA is not important in flowering. His argument is that selection of different strains of *Silene armeria* L. and genetic analysis have demonstrated that GA-induced stem elongation and flower formation are determined by two separate genes. Growth retardants partly or fully suppressed stem growth while flower formation took place normally. Suppression of stem growth in *Silene* with a growth retardant was associated with reduction in the GA level below the limits of

detection. Thus he concluded, at least in this plant, flower formation proceeded in the absence of GA.

In cold requiring plants the effects of gibberellins vary, sometimes inducing bolting and flowering and sometimes inducing bolting only. This would suggest that "vernaline" in these latter plants is not gibberellin but another hormone independently induced by cold treatment while gibberellins are concerned only with the phenomena of bolting, a conclusion reached by Suge and Rappaport (112). Evans (28) seems to support the view that gibberellins may be needed only for the expression of the vernalization response. These two last views seem to oppose Chailakhyan's view (18) that "vernaline" is a precursor of gibberellins which then induces flowering, but under SD no conversion takes place and the plant remains vegetative under SD.

Zeevaart (131), commenting on flowering hormones, said that negative and non-reproducible results with extracts led several workers to question or abandon the concept of a single substance being the limiting factor for flower formation. On the other hand, there is no evidence today that precludes the existence of such a chemical. Any worker attempting to extract and assay a flower-inducing substance faces many technical problems.

Contrary to the idea of a single substance controlling flowering, several authors have proposed that flower

formation is the result of a specific balance of hormones acting in the shoot apex. This hypothesis, according to Zeevaart, is readily applicable to woody perennials, but is less attractive to explain flower formation in herbaceous plants.

Srinivasan and Mullins (107), working with grapes, reported that inflorescences did not form on tendrils if cytokinins were applied in the agar. Tendrils cultured in agitated liquid medium containing BA (benzyladenine), BAP (6-[benzylamino]-a-[2-tetrahydro-pyranyl]-9H purine), or zeatin riboside showed profuse branching and were transformed into inflorescences. Calyptra (calyx and corolla), stamens and pistils developed normally in the presence of both zeatin riboside and BAP but micro- and macrosporogenesis were absent. These results support Zeevaart's view (131) that in woody species a specific balance of hormones is necessary for flower induction to occur. Bernier et al. (9) proposed that the mitotic component of the floral stimulus in the herbaceous *Sinapis alba* L. is a cytokinin but since cytokinin application could not induce flowering, they concluded that there is a multicomponent floral stimulus in this species. This last report suggests that the floral stimulus was not a single chemical even in non-woody species.

B. Literature Review on Red Raspberries

1. Time of flower initiation in red raspberries.

The time of flower bud differentiation in red raspberry, Rubus idaeus L. var. strigosus (Mich.) was first determined by Goff (34) who reported that flower primordia were present in primocanes of 'Bonanza' and 'Wallace' in Wisconsin in the fall of the season previous to fruiting. MacDaniels (67) reported that growing points in the buds of 'Cuthbert', 'Marlboro' and 'Herbert' in New York State were partially differentiated early in January. He stated that all axillary buds in red raspberry are potentially fruit buds. Waldo (115) stated that in red raspberries the fall fruiting cultivars, such as 'Ranere' and 'Lloyd George' form fruit buds as soon as shoot elongation ceases in the summer. The 'Lloyd George' in Oregon ceased growing about July 15 in 1933, and fruit bud formation developed rapidly, starting first in buds at the terminals and progressively in buds downward gradually during September. Mathers (69) in Scotland found that 'Malling Promise', 'Malling Landmark' and 'Lloyd George' buds were entirely vegetative in July and floral development was initiated in the middle of September. Similarly, Robertson (99) found flower primordia in 'Lloyd George' by late September. Williams (121) reported that in 'Malling Promise' flower initiation was first evident in terminal buds

in the second week in September. In 'Lloyd George' flower initiation was found in the terminal bud in the last week of July. In both cultivars flower initiation in axillary buds occurred in early autumn. He also suggested that once flower initiation had occurred, the rate of development of the inflorescences was largely governed by prevailing temperature, with periods of low temperature arresting development and retarding what otherwise would have been a continuous process. Haltvick and Struckmeyer (43) reported that in Wisconsin the earliest flower bud initiation in axillary buds occurred by mid-September for 'June' and 'Sunrise', the end of September in 'Chief', in October for 'Taylor', and early November for 'Latham'. Waldo (115) found that fall fruiting cultivars such as 'Ranere' showed no evidence of fruit bud differentiation below the fall-fruiting region until November 15 and little development was noted between December 15 and March 8. He also found that in Oregon, fruit bud differentiation proceeded generally from terminals of the canes downward in all red raspberries. Mathers (69) found that the buds in the region of 5-10 nodes below the terminal bud were always the most advanced.

2. Low temperature and flower induction in red raspberries.

Williams and Hudson (118) found that by exposing plants with elongated canes to a period of low temperature

between 2.5 - 4.5°C for periods up to 6 weeks, flowering and fruiting could be initiated ('Malling Promise' and 'Lloyd George'). They found that the stage of growth of the cane as measured by the number of nodes produced, was an important factor in flower initiation and development. Young canes with fewer than 12 nodes failed to flower in response to any treatment applied. Williams (121) reported that 'Lloyd George' developed a terminal inflorescence in autumn of the same year in which the vegetative shoot was formed. He suggested that flower initiation in the meristem of the apical bud of this cultivar occurred when the meristem reached a certain physiological stage, a stage of "ripeness to flower", and was not a direct response to change in specific environmental factors. Williams (12) reported that short days and cool temperatures could bring about the dormancy of young shoots but flower initiation did not occur. Williams (122) reported that shoots with 20 nodes were physiologically mature to flower in 'Malling Promise'. The vegetative phase of 'Malling Promise' could be reduced to a few weeks or prolonged for at least 19 months. With 'Lloyd George' a reduction was possible but flowering on terminal meristems was initiated after about seven months of shoot growth.

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CHAPTER I

GROWTH, FLOWER INDUCTION AND FLOWER BUD DEVELOPMENT OF RED RASPBERRIES (*RUBUS IDAEUS* L.) IN RELATION TO TEMPERATURE AND PRUNING MANAGEMENT

A. Flower Bud Induction, Initiation and Differentiation in Mature Overwintering Canes of Red Raspberry.

Introduction

Flower bud initiation of the terminals and further development of the inflorescences, giving the first year's crop, in fall bearing cultivars, such as 'Lloyd George' and 'Ranere' occurred in summer (115, 119). Flower bud initiation and differentiation in the rest of the buds of the cane in fall-bearing and in the whole cane of June-bearing cultivars took place from fall to spring during the coldest period of the year. The cultivar and the geographic location affected the time of initiation (43, 67, 69, 115, 119).

The present investigation was undertaken to determine whether temperature had any effect on flower bud induction, initiation and differentiation of the overwintering cane of fall-bearing and June-bearing red raspberries.

Materials and Methods

'Heritage' a fall-bearing cultivar and 'Latham' a June-bearing cultivar were selected to represent these two growth types.

Sixty plants of 'Heritage' were grown in 20 cm diameter clay pots in cold frames. In late August, 30 plants were placed in the greenhouse to grow under natural daylength and a temperature of 22-24°C (Treatment 1-warm).

Thirty plants remained outside exposed to natural cold and daylength (Treatment 2-cool). Bud samples were taken at monthly intervals at every 6th node below the last developed inflorescence from both sets of plants. Five plants from each treatment were involved in each sampling from October 4 to March 27. Buds were fixed in FAA (formalin-aceto-alcohol), dehydrated with a tertiary butyl alcohol series, embedded, sectioned, and stained in safranin and fast green. The bud stage of development was compared with standards produced by taking photomicrographs at different stages of bud development (vegetative, early initiation, initiation, differentiation) (Fig. 1). Camera lucida was used for making the drawings.

In 'Latham' only plants grown under the same non-cold treatment conditions as those of 'Heritage' were studied because Haltvick and Struckmeyer (43) had reported that flower induction and differentiation were completed in November when plants were grown under field conditions and received the winter cold. Samples were taken in February and March only and sampling began with the terminal bud.

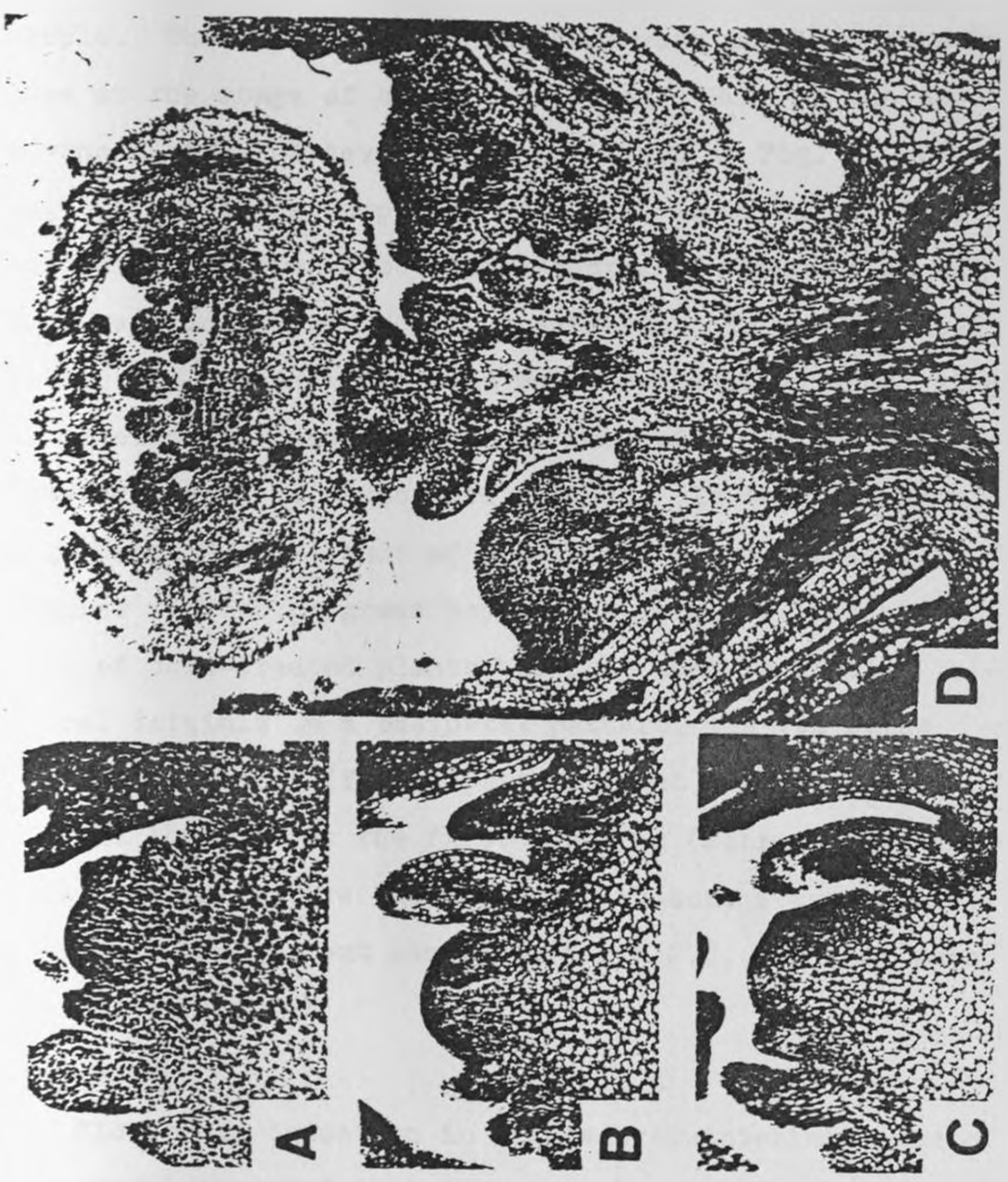
Results

Because there was little difference in flower bud development from month to month, only three out of the six samplings are described. In 'Heritage' the first bud

Fig. 1. Longitudinal sections of axillary buds of red raspberry 'Heritage' at the vegetative, early initiation, initiation, and differentiation stage.

A. vegetative, B. early initiation,

C. initiation, D. differentiation. X105.


A
B
C
D

below the last inflorescence was a flower bud in both treatments 1 and 2 (Fig 1, Plate D), in the October 4 sample. The buds lower on the cane were mostly vegetative, some at the stage of early initiation. Buds in the most advanced stage of development are shown in Fig. 2. During the following samplings more buds were induced in a basipetal pattern in both treatments. On January 3, all buds were initiated in both treatments. In cold treated plants the 7th and 13th buds were in a more advanced stage of development than the rest of the buds. In non-cold treated plants all buds were similar. In the last sampling (March 27) all buds of non-cold treated plants were similar with no progress beyond the stage of initiation. Buds of cold-treated plants had continued to develop floral initials in a basipetal pattern, the 7th being the most advanced (floral parts evident).

In 'Latham' at the first sampling (February 27) some buds were vegetative but most were at some stage of initiation. In the next sampling (March 27), all buds were initiated (Fig. 3).

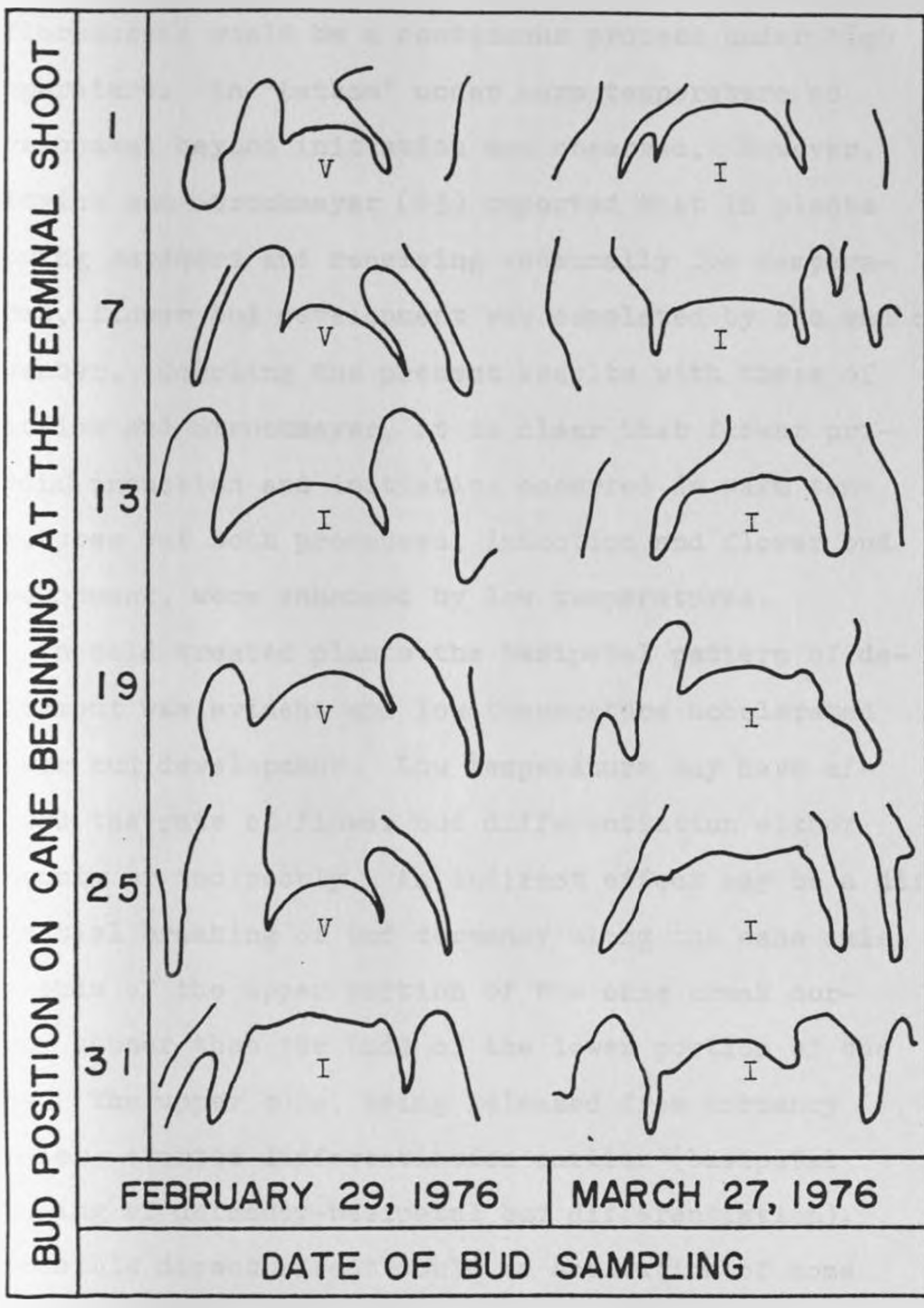
Discussion

Flower bud induction in mature overwintering canes of 'Heritage' occurred in a warm greenhouse. Further, flower bud development was stimulated by low temperature. These results disagree with the statement made by Williams

Fig. 2. Camera lucida drawings showing stages of flower bud development at different nodes on mature canes of 'Heritage' fall-bearing red raspberry. Axillary buds from plants grown in greenhouse with temperature 22-24°C (warm) and natural daylength and from plants grown outdoors (cool). V. Vegetative, I. Initiation, D. Differentiation. X66.

BUD POSITION ON CANE BEGINNING AT 7th NODE BELOW LAST INFLORESCENCE	OCTOBER 4, 1975		JANUARY 3, 1976		MARCH 27, 1976	
	WARM	COOL	WARM	COOL	WARM	COOL
7	V	I	I	D	I	D
13	V	I	I	I	I	I
19	V	I	I	I	V	I
25	I	I	V	V	I	I
31	V	V	V	I	I	I

Fig. 3. Camera lucida drawings showing stages of flower bud development at different nodes on mature canes of 'Latham', June-bearing red raspberry. Terminal and axillary buds from plants grown in the greenhouse at temperature 22-24°C and natural daylength. V. Vegetative, I. Initiation. X150.



BUD POSITION ON CANE BEGINNING AT THE TERMINAL SHOOT
1
7
13
19
25
31
V
I
FEBRUARY 29, 1976
MARCH 27, 1976
DATE OF BUD SAMPLING

(119) that after induction, the rate of development of the inflorescence would be a continuous process under high temperature. In 'Latham' under warm temperature no development beyond initiation was observed. However, Haltvick and Struckmeyer (43) reported that in plants growing outdoors and receiving seasonally low temperatures, flower bud development was completed by the end of November. Coupling the present results with those of Haltvick and Struckmeyer, it is clear that flower primordial induction and initiation occurred in warm temperatures but both processes, induction and flower bud development, were enhanced by low temperatures.

In cold treated plants the basipetal pattern of development was evident and low temperature accelerated flower bud development. Low temperature may have affected the rate of flower bud differentiation either directly or indirectly. An indirect effect may be a differential breaking of bud dormancy along the cane axis. The buds of the upper portion of the cane break dormancy sooner than the buds of the lower portion of the cane. The upper buds, being released from dormancy earlier, started differentiation earlier (basipetal breaking of dormancy-basipetal bud differentiation). A possible direct effect could be activation of some floral stimulus expressed basipetally. The presence of a basipetal floral gradient was demonstrated in the

flowering of 'Wisc. 38' tobacco by Aghion-prat (1). Another possible direct effect could be a change in internal balance of growth regulating substances (promoters vs. inhibitors) in favor of the promoters. Badr et al. (5) working with flower induction in the olive, which requires low temperature, found an increase in GA-like substances and a drop in inhibitory substances before flower induction and also an increase in GA-like substances during the early development of inflorescences.

B. Flower Induction in Primocanes of 'Heritage' Red Raspberry.

1. <u>Introduction</u>

In fall-bearing raspberry cultivars, flowering occurs at the top of the primocane during summer, giving the first year's crop. This crop is usually not important in areas where fall frosts prevent ripening of the full crop; an advance of 2-3 weeks in flowering would increase the volume of the fall crop. An understanding of the flowering process in fall-bearing red raspberries is needed before such improvement can be developed.

All buds on the primocane may, but not all do, become flower buds in the first year (67). Buds below the fruiting portion of the cane become flower buds during fall or winter. The time of development depends on the cultivar and the area where the plants are grown. The data

presented earlier showed that flower induction in overwintering mature canes of 'Heritage' occurred in warm temperatures, but flower bud development was greatly enhanced by low temperatures. However, data presented by Williams and Hudson (118) showed that elongated canes of 'Lloyd George', a fall-bearing cultivar, could be flower-initiated when they received a cold treatment of 2.5 - 4.5°C for a period up to six weeks. Also they reported that flowering could not be induced by low temperatures on primocanes with fewer than 12 nodes.

'Heritage' (85) was chosen for this study because of its potential economic value. Flowering of 'Heritage' occurs in Wisconsin about the last week of July and fruit ripening starts in late August.

Since low temperature appears to be a key factor in the flowering of red raspberries, experiments were conducted to determine the influence of and essentiality of cold temperature for flowering of fall-bearing red raspberry primocanes.

2. Effect of Temperature on Flowering of the Primocane of Fall-Bearing Red Raspberry 'Heritage'.

a. Experiment 1

Materials and Methods

Plants for this study came from root cuttings that had received no previous low temperature exposure. They were grown in 20 cm diameter clay pots in a greenhouse with temperature of 22-24°C and with sunlight supplemented with fluorescent and incandescent lamps to produce a 16-hour daylength. From late May to late August the plants were outside with no supplemental lighting.

Differential treatments in a growth chamber consisted of exposing shoots at 14-16 node stage of growth to a time series at 7°C and 16 hours of light for periods of 0, 25, 45 and 60 days. After treatment the plants were returned to the greenhouse.

Results

All treated plants resumed vegetative apical growth and flowered terminally and basipetally. Control plants (no cold) flowered in the same pattern but much later than cold treated plants. There were no differences among cold treatments in the total number of nodes produced but a great difference between cold treated and non-cold treated plants. There were no differences in the number of nodes with inflorescences among all treatments (Table 1).

Table 1. Total number of nodes and inflorescences produced by 'Heritage' primocanes after plants were treated at 7°C and 16 hr daylength at 14-16 node stage of growth for a period of 0, 25, 45 or 60 days.

Days at 7°C	Average total no. of nodes	Average no. of nodes with inflorescences
0	78 ± 6	12 ± 1
25	30 ± 3	10 ± 1
45	28 ± 4	11 ± 1
60	28 ± 3	10 ± 2

After cold treated plants and controls had ripened their fruits, buds taken every third node starting below the last inflorescence were fixed in FAA (formalin-aceto-alcohol), dehydrated in a tertiary butyl alcohol series, embedded, sectioned and stained with safranin and fast green for microscopic examination.

The first bud below the last developed inflorescence was in all treatments a flower bud. All the rest of the buds in the control treatment were vegetative. In cold treated plants, the number of buds initiated was directly related to the length of the low temperature treatment period. Buds close to the soil level were vegetative in all treatments (Table 2).

b. Experiment 2

<u>Introduction</u>

'Heritage' plants cold treated at 14-16 node stage of growth for at least 25 days at 7°C flowered much earlier than non-cold treated plants. Growing in the open, plants are at this stage of growth in the middle of the summer. The application of a cold treatment at that time would be impractical as a field practice for advancing flowering.

It is known that buds which form adventitiously on the roots and are a source of primocanes may remain dormant or may elongate for a few nodes in the first season.

Table 2. Microscopic examination of buds below the last inflorescence after the plants had ripened their fruits.

Days at 7°C	Node below last inflorescence						
	1st	4th	7th	10th	13th	16th	Below 16th
0	F	V	V	V	V	V	V
25	F	I	3I,1V	2I,2V	V	V	V
45	F	I	I	I	I	V	V
60	F	I	I	I	I	2I,2V	V

F = Flower bud, well differentiated.

I = Flower initiation.

V = Vegetative.

In nature, these shoot buds are exposed to winter cold. In order to measure the extent of low temperature needed to bring about the flowering response, such buds were treated with shorter periods of cold than would be expected in nature.

Materials and Methods

Plants for this study came from root cuttings which were outside until December 15. It was calculated that the buds had received an exposure of more than 700 hours below 7°C at this time. Plants were moved to the greenhouse on December 15 and grown in 20 cm pots with temperatures of 22-24°C under 16 hours daylength. Plants were allowed to flower normally. After all the inflorescences were expanded, the flowering portion of the cane was removed (pruned). On treatment plants, an additional 2, 4, 6, 8 or 10 nodes were removed from the primocane. The stage of development of the axillary bud was derived from observing the form of emerging axillary shoots.

Results

All plants flowered terminally at the same stage of growth as those which grow outside under natural environmental conditions (about 40 nodes). The number of nodes with inflorescences (about 13) was also similar. Axillary buds below the last inflorescence were quiescent. One or two buds below the pruning cut grew when the terminal portion of the cane was removed. All axillary

shoots were vegetative but most flowered later in a manner similar to the primocanes.

Those that flowered produced the same number of inflorescences but the total number of nodes produced was dependent on the position of the axillary bud on the primocane (Table 3). The time between pruning and bud breaking increased to a maximum of 15 days as the bud location progressed down the primocane. Two laterals out of five in Treatment 6 became dormant shoots after a period of growth.

Discussion of Experiments 1 and 2.

'Heritage' plants that received cold treatment for 25 days or more at 7°C at 14-16 visible nodes (actually about 20 nodes total) were flower induced. The existence of a basipetal floral gradient was noticed, similar to that found in mature overwintering canes. The degree of floral expression was dependent on the quantity of cold treatment received. These results agree with those reported by Williams and Hudson (124) working with another fall-bearing cultivar 'Lloyd George'. When comparing plants grown from root cuttings taken from the field in mid-December with non-cold treated plants, a great difference in time of flower expression was evident. A cold treatment even at the stage of preformed buds on the root hastened flowering. The influence of cold

Table 3. Response of primocanes and axillary buds below the last inflorescence following removal of flowering head. Plants received cold treatment until December 15 in the open.

Treatment #	Nodes removed plus the flowering head	Total number of nodes of primocanes	Number of inflorescences produced	1st axillary		Plants naturally grown	
				Total no. of nodes	No. of inflorescences	Total no. of nodes	No. of inflorescences
1	0	40±3	13±1	25±6	13±1	41±4	14±2
2	2	40±3	13±1	32±6	12±1		
3	4	42±5	12±0	33±4	13±1		
4	[illegible]	43±4	13±1	39±7	14±1		
5	[illegible]	43±4	13±1	42±3	13±1		
6	10	39±6	13±1	39±2	14±1		

treatment on preformed buds as well as on shoots at the 14-16 node stage of growth suggests that plants at any stage of growth respond to cold treatment.

The results lead to the conclusion that flowering could be advanced by forcing plants to start growth earlier in the spring. This might be done by covering the plants in such a way as to protect them from cool nights and to raise the daytime temperature.

3. Influence of Removal of Inflorescences on the Total Number Produced.

Introduction

Time of flowering and plant height were influenced by cold applied at any stage of growth but the number of nodes with inflorescences was not. Williams (121) stated that other factors than environment controlled the number of inflorescences produced. One of these factors might be the developing inflorescences themselves; developing inflorescences are recognized as strong sinks and developing seeds produce quantities of growth regulating substances. Such an influence could be dual 1) photosynthates shortage, and 2) production of inhibitors. An experimen[illegible]s conducted [illegible]tudy t[illegible]elationship among inflor[illegible]nce and suck[illegible]oduction, fruit size and further bud initiation.

<u>Materials and Methods</u>

Forty plants grown from root cutting buds previously cold treated were divided into four groups. As the inflorescences developed, they were removed beginning from the top at regular intervals to make a total removal of 100, 75, 50 and 0 percent. After the plant stopped producing any additional inflorescences, the final number was recorded. Fruits were weighed as they ripened. The number of suckers produced was counted after 75% of the fruit was ripened. Following fruit harvest, the fruiting head was removed and the emergence of axillary buds was observed.

<u>Results</u>

All plants reached about the same stage of growth when they flowered. The number of inflorescences produced was the same in all treatments. The higher the percentage of inflorescences removed, the more the suckers produced. The fruit weight for Treatments 2 and 3 was the same but larger than that of the control plants (Table 4).

Axillary buds below the last inflorescence were quiescent and did not grow until removal of the flowering head occurred, even in Treatment 1 in which all inflorescences had been removed as they appeared. Axillary shoots produced a number of inflorescences comparable to that of primocanes (Table 5).

Table 4. Effect of reduction of the developing inflorescences in the total number of inflorescences produced by the plant, number of suckers and fruit size. 10 plants per treatment.

Treatment #	% of infl. removed	Total no. of nodes	No. of infl. produced	No. of suckers produced	Fruit wt. in gr.
1	100	39±6 a[z]	13±1 a	4±1a	--
2	75	37±5a	13±1 a	3±1ab	2.2a
3	50	39±5a	13±1 a	2±1cb	2.2a
4	0	40±3a	13±1 a	1±1c	1.9b

[z] Mean separation, within column, in Duncan's new multiple range test, level 5%.

Table 5. Response of axillary buds below the last inflorescence to removal of the top stem with the inflorescences after plants had ripened most of their fruits.

Treat-ment #	% of infl. removed	1st axillary		2nd axillary	
		Total no. of nodes	No. of infl.	Total No. of nodes	No. of infl.
1	100	23±6	11±2	27±5	12±2
2	75	25±4	12±2	28±5	11±2
3	50	28±4	12±1	29±2	12±1
4	0	25±6	13±1	24±0	12±0

Discussion

The number of suckers produced per plant was positively related to the number of inflorescences removed which suggests that sucker production was food dependent. Fruit size was affected by the same treatment and it is known that fruit size depends, to a certain degree, on photosynthate availability. The number of inflorescences produced by primocanes and laterals was not influenced by the same treatment. It is possible that there was no increase in number of inflorescences because no increase in photosynthates occurred after removal of the inflorescences. There are many reports confirming that photosynthesis drops dramatically when the sink (developing fruit) is removed from the plant (78, 96). However, since sucker production and fruit size were affected by the treatment but not the number of inflorescences, it is concluded that other factors might determine the total number of inflorescences produced.

The discovery that the number of suckers was increased after removal of the developing inflorescences may be an important lead for modifying nursery practices. Nurserymen could increase the number of plants per acre, at least in fall-bearing cultivars, by removing the flowering portion of the primocane either mechanically or chemically before the fruit crop is fully developed.

4. Role of the Apical Portion of the Primocane in Flowering.

Introduction

Flowering on the primocane of fall-bearing red raspberries always occurs first at the terminal and progresses basipetally, irrespective of the conditions under which plants are growing. The apical meristem is the first to either receive or to respond to the flowering stimulus. Since the number of inflorescences produced per cane is the same each year for each cultivar and since all buds are potentially fruit buds, it was hypothesized that the apical stem portion controls the whole flowering process.

For understanding the possible role of the apex in the flowering process of the primocane, the following two experiments were conducted.

a. Experiment 1

Materials and Methods

Plants used were grown in clay pots 20 cm diameter, in a greenhouse under 22-24°C temperature and 16 hours daylight. Plants received a cold treatment of 7°C for a month as root cuttings. When the inflorescences appeared on the distal 3 to 5 nodes, primocanes were pinched at the 6th, 9th and 12th node. The flowering behavior of the laterals emerging from the axillary buds after pinching was examined.

Results

Primocanes pinched at the 6th node produced laterals with inflorescences, those at the 9th node produced inflorescences or mixed shoots and those at the 12th node produced mixed shoots or vegetative shoots. Non-pinched control plants produced only inflorescences (12 ± 1) (Table 6). The floral expression of the axillary bud at the top 12 nodes was similar whether or not the pinching procedure was used. The removal of the apical stem region with the terminal inflorescences already formed did not have any influence on the total number of nodes with floral expression. However, there were differences in the type of growth of laterals. In controls, 12 axillaries produced inflorescences but shoots pinched below the ninth node produced some inflorescences and some mixed shoots. Any interaction between the apical meristem and the number of nodes with inflorescences occurred before the terminal inflorescences were visible.

b. Experiment 2

Materials and Methods

Twenty-eight plants were grown to the 10-12 node stage of growth under 22-24°C temperature and 16 hours daylength in 20 cm pots. Of these 24 were treated at 7°C for one month and four served as controls. This stage of growth was chosen because Williams and Hudson (118) reported that plants at this stage could not be

Table 6. Flowering behavior of laterals after pinching the 3-5 terminal inflorescences plus 6, 9, and 12 nodes without inflorescences.

Pinching at node[a]	Response of laterals
6	100% infl.
9	70% infl. 30% mixed shoots
12	80% veg. shoots 20% mixed shoots
Control	100% infl.

[a] All remaining nodes developed inflorescences, mixed shoots or vegetative shoots.

flower induced by any treatment (cv. Lloyd George). Following the cold treatment, plants were returned to the warm greenhouse.

Four plants were pinched at the 10th node (counting from the soil level) the day they were taken out of the growth chambers. Following the resumption of apical growth and as the plants were growing, four plants were pinched at the 14th node, four at the 19th, four at the 23rd and four at the 27th node. Four cold treated and the four non-cold treated plants were left to grow as controls. No plants were pinched beyond the 27th node, for at that stage of growth the plants had started flowering. In each pinched plant, only the two uppermost laterals were left to grow. The laterals which developed after pinching were pinched at eight, 10 or 12 nodes if they had not formed terminal inflorescences.

Results

Non-pinched, cold-treated primocanes produced 32 ± 4 total number of nodes and 13 ± 1 inflorescences. Non-pinched and non-cold treated primocanes produced 81 ± 3 total number of nodes and 13 ± 1 inflorescences. The flower expression in the primary laterals changed from vegetative, to vegetative and mixed (beginning at the 23rd node, and to mixed shoots. Similar responses were observed in secondary laterals (Table 7).

Table 7. Growth response of axillary buds when primocanes were pinched at 10, 14, 19, 23 and 27 node stage of growth and vegetative laterals at 8, 10 and 12 node stage of growth. Plants received cold treatment (7°C) for 30 days at 10-12 node stage of growth (4 plants/treatment).

Primocane pinched at node	Type of growth of axillary shoot after pinching of the primocane	Total No. of nodes (inflorescences included), produced by laterals	Type of growth of axillary shoot after pinching of the vegetative laterals at node		
			8	10	12
10	Vegetative[a]	16	Veg.	Mixed	Infl.
14	Vegetative	15	Veg.	Mixed	Infl.
19	Vegetative	14	Infl.		
23	Mixed[b]	13	--	--	--
27	Mixed	11	--	--	--

[a] Vegetative apex turned into flowering apex after shoot produced a certain number of nodes.

[b] Elongating shoot with developing inflorescences from the axillary buds.

Discussion

The results show that plants with only 10-12 nodes of growth that received cold treatment were not flower induced. These results agree with those reported by Williams and Hudson (118). However, plants responded to cold treatment and the response was carried over and was expressed as flower induction much earlier [(32 ± 4) nodes] than in non-cold treated plants [(81 ± 3) nodes].

In cold treated plants, flower induction did not occur until plants had reached at least 20 nodes of growth. At this stage the actively growing apical meristem was able to change from vegetative to reproductive. From this stage of growth on, all buds formed by the apical meristem became flower buds, but failed to grow unless forced by pinching, probably because of apical dominance.

That all plants produced almost the same number of inflorescences can be explained only if it is accepted that the number of inflorescences produced was predetermined, and by being predetermined, could not be influenced after the appearance of the terminal inflorescence. This agrees with the results of Experiments 1 and 2.

In the attempt to determine whether the plant response was translocatable or not, cold treated plants were approach-grafted to non-cold treated plants. Grafted plants did not show any kind of interaction, which probably means that the

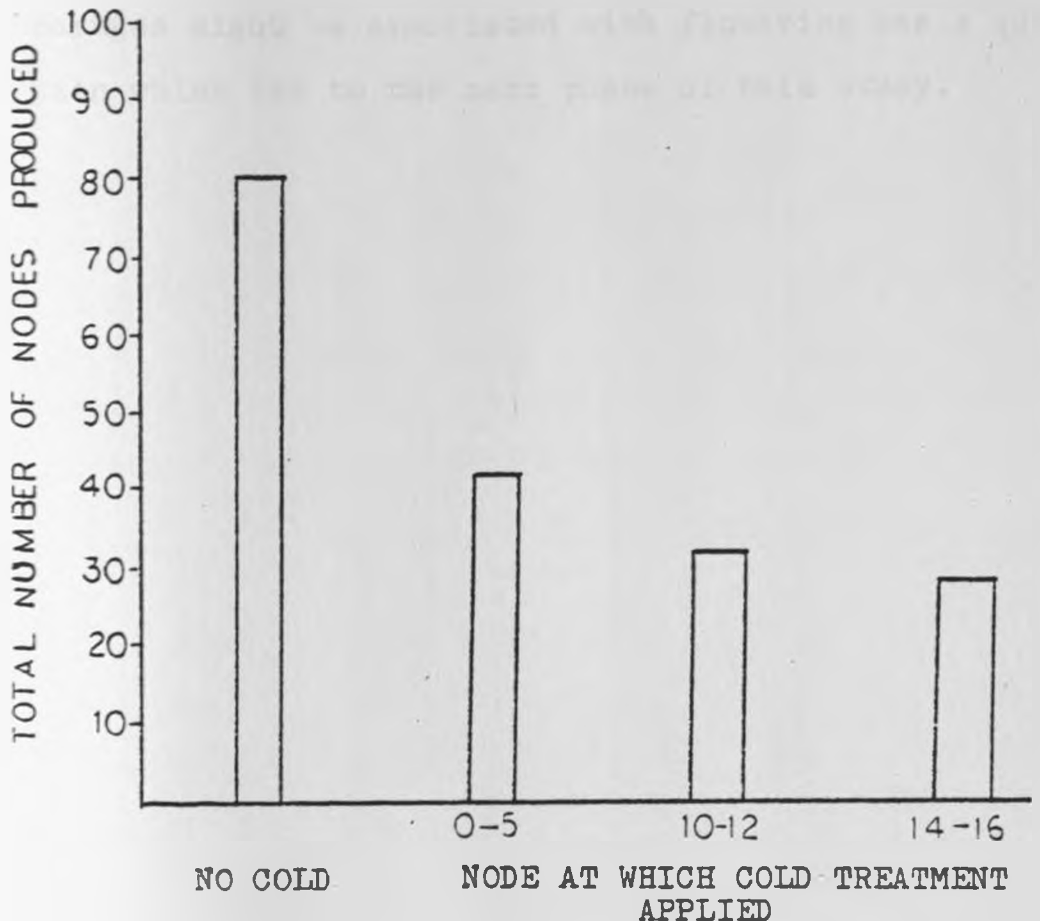
Fig. 4. Total number of nodes produced by raspberry plants exposed to cold at different stages of growth.

response was carried over through cell division in the apical meristem of the main axis.

C. Conclusions and Remarks on Chapter I.

'Heritage' plants grown continuously under long phoroperiod (16 hours daylength) and temperature 22-24°C will flower. The time period between shoot emergence and flowering may be manipulated by environmental control. Plants do not respond to an external stimulus unless they have reached a certain stage of growth. It appears that a cold treatment enables plants to respond to an external flowering stimulus at a younger age or stage of growth. In fact, the stage of growth at which flowering can be expressed depends on the stage of growth at which plants receive a cold treatment (aging of plants((Fig. 4).

It is clear that raspberry plants have two separate phases of growth and development. The first is the vegetative phase in which the plants reach a certain size (age). The second is a reproductive phase. In non-cold treated plants, the vegetative phase lasts until 80 nodes have been produced but can be shortened to a minimum of 20 nodes if cold treatment is applied. The older the plants, the more responsive they are to the flower-promoting effect of a cold treatment; this effect lasts until an age of 20 nodes, after which plants flower rapidly after a cold treatment and greater age has no further effect.



TOTAL NUMBER OF NODES PRODUCED
100
90
80
70
60
50
40
30
20
10
0-5
10-12
14-16
NO COLD
NODE AT WHICH COLD TREATMENT APPLIED

Thus 'Heritage' and probably all fall-bearing red raspberries respond to the vernalization in a similar way as most annual and biennial herbaceous plants.

What endogenous hormonal changes might occur before and after the 20 node stage of growth and how such changes might be associated with flowering was a question which led to the next phase of this study.

CHAPTER II

CHANGES IN ENDOGENOUS GIBBERELLIN-LIKE SUBSTANCES AND CYTOKININS AND THEIR ASSOCIATION WITH FLOWER INDUCTION IN RED RASPBERRIES

Introduction

A century ago (1880) Sachs suggested that flowering is not a process dependent on trophic factors but is a hormonal response. After the discovery of photoperiodism and the identification of the leaf as the receptor organ that produced a stimulus for transmission to the shoot apex, the search for the "florigen" was begun in earnest.

Vernalization was known before photoperiodism but intensive studies started about the same time on both concepts. Years later another hypothetical flowering hormone "vernaline" was proposed. This one also remains to be isolated.

Later, with the discovery of gibberellins and their influence on flower induction in some plant species, another hypothetical flowering hormone "anthesin" was named. Florigen, vernaline, and anthesin all are still hypothetical, just concepts.

In 1956 Lang reported that gibberellins, known for quite a few years, could induce flowering in some species under non-inductive conditions. Since that time much work has been published dealing with flowering, "florigen" and gibberellins. These publications all agree that gibberellins are not florigen, but play a very important role in flowering, especially in those plant species

(annuals and biennials) that require either long photoperiods or long photoperiods and low temperatures for flower induction.

One aspect which was realized very early was the specificity of species response to specific gibberellins (qualitative response) and the quantity of gibberellins required to bring flower induction in different species (quantitative response). Some species responded only to certain gibberellins or some gibberellins were more effective at lower concentration than others. Some investigators have suggested that the negative results obtained were due to the lack of the right gibberellin for the right plant. Because they are generally available, GA_3, GA_4, GA_7 and GA_9 have been used in most of the research.

Although most of the information available concerning gibberellins and flowering has come from external application of gibberellins, plant analysis has provided a great deal of evidence that changes in endogenous gibberellins occur during or before flower induction in many species. Such evidence suggests that gibberellins play a role in flower induction.

It is currently accepted that gibberellins are involved in flower induction and are either necessary for flowering or are part of the florigen complex. Also, gibberellins may play a role in flower induction by affecting

the balance of growth hormones and thus bringing flowering in many plant species, especially woody species.

Raspberry plants appeared to be a good material for comparative flowering studies because; 1) plants of 'Heritage' (fall-bearing cultivar) will flower if they grow under 22-24°C temperature and 16 hours daylength after they have formed about 80 nodes of growth; if they receive a cold treatment, flowering occurs at 30-40 node stage of growth (data presented in Chapter I). 2) June-bearing cultivars exposed to cold at the same stage of growth as fall-bearing cultivars and growing under the same environmental conditions do not flower in the first year. However, preliminary experiments showed that these cultivars do flower if they are kept growing under greenhouse conditions (22-24°C and 16 hours daylength). Thus, June-bearing cultivars behave in the same way as non-cold treated fall-bearing 'Heritage'. Therefore, a study was initiated to detail the changes in endogenous gibberellins in raspberry plants changing from vegetative to reproductive growth.

Analysis for cytokinins was included in the same study because some reports suggest that cytokinins might play a role in flower induction, either alone or in combination with gibberellins and also because cell division and flower induction are very closely related.

Materials and Methods

Five cultivars were used for the analysis, two fall-bearing cultivars 'Heritage' and 'Fallred', and three June-bearing cultivars, 'Latham','Hilton' and 'Sentry'. All five cultivars were grown in the field. In addition, plants of 'Heritage' that had not received any cold treatment were grown in a greenhouse under 22-24°C and 16 hours daylength.

Samples were taken when plants had produced 10 or 20 visible nodes. Stem tips and the unfolded leaves were the only plant parts sampled. It was decided to take samples at these two stages of growth because; 1) at the 10 node stage of growth all plants were vegetative, 2) at the 20 node stage of growth all June-bearing cultivars and also non-cold treated 'Heritage' were vegetative, and 3) 'Heritage' and 'Fallred' plants with 20 nodes (26-27 nodes including those microscopically visible) were not flower induced or were at the very beginning of flower induction. Field grown 'Heritage' plants produce 41 ± 4 total nodes and 14 ± 2 nodes with inflorescences. 'Fallred' plants produce 38 ± 3 total nodes with 12 ± 2 nodes with inflorecences.

Samples were harvested, weighed (F.W.) and were put in cold 80% MeOH and chloroform (2:1, V:V ratio). Then they were stored at -60°C for later analysis.

Sixty grams (F.W.) of tissue were used for each analysis. Samples were ground in a Sorvall-Omni mixer at 0°C in approximately 100 ml 80% MeOH-chloroform (2:1, V/V). Ground samples were stored at -20°C for a minimum period of 6 hours, were brought to room temperature (20°C) for 2 hours, and were vacuum filtered at 20°C. Residue was washed three times with 10 ml MeOH/chloroform and the combined filtrates were stored at -20°C. Residue was re-suspended in MeOH/chloroform (100 ml) and was stored at -20°C for a period of 6 hours (minimum). The same extraction procedure was repeated twice. The total extract collected in MeOH/chloroform was approximately 400 ml . All filtrates were combined and stored at -20°C.

The extracted hormones were separated by ethylacetate partitioning using a method adapted from Shindy and Smith (106). Two main modifications were introduced; 1) a high salt concentration was maintained during solvent partitioning because of reports by Durley and Pharis (23) and Miller (77). The former suggested that the greater the molarity of the buffer, the greater the tendency of the gibberellins to enter the acidic ethylacetate phase"salt effect" and the latter suggested the same importance of the "salt effect" for zeatin separation at basic pH. 2) Cytokinins were taken with ethylacetate at pH 8.2 and not with water saturated n-butanol at pH 5.5, because it was reported

by Upper et al. (114) that at pH 7.7 and above, cytokinins could be taken very effectively with ethylacetate.

The adapted procedure was as follows: the combined filtrates were evaporated in a flask evaporator at 40°C and the water phase (100 mls) was adjusted to pH 8.2 with dibasic potassium phosphate. Then it was partitioned, first with ethylacetate three times and second, with purified hexane three times (1:1 volume). The water phase was adjusted to pH 2.8 with phosphoric acid and partitioned with ethylacetate three times (1:1 volume). The organic solvent containing the acidic fraction was evaporated to dryness in a flask evaporator at 40°C. The dried acidic fraction was stored at -20°C for further use [Fraction I (F_I), auxins, gibberellins, abscisic acid (ABA)].

The organic solvents (ethylacetate and hexane) containing the basic fraction were combined and evaporated in a flask evaporator at 40°C. The dried basic fraction was taken with water (40 mls) and then with ethylacetate to assure that nothing remained in the flask. The water phase adjusted to pH 2.8 was partitioned three times, first with ethylacetate and then three times with hexane (1:1 volume). The organic solvents were discarded. The remaining water phase was adjusted to pH 8.2 with dibasic potassium phosphate and was partitioned three times with ethylacetate (1:1 volume). The organic phase was evaporated in a flask

evaporator at 40°C. The dried basic fraction was stored at -20°C for further use [Fraction II (F_{II}) cytokinins].

Purification of the Extract

For further purification of the extracts, column chromatography was employed. Polyclar AT, an insoluble form of the polymer poly-N-vinylpyrrolidone (PVP) was used as the stationary phase and buffers pH 8.0 and pH 4.0 as the moving phase. The procedure was the same as that reported by Glenn et al. (33) with a few modifications:

1) Purification of the acidic fraction (F_I).

A column with dimensions 1.9x30 cm was used. Phosphate buffer (pH 8.0) was used as the moving phase. The eluate between 40 and 180 mls was collected. In this range all gibberellins and ABA were collected, but no IAA. The eluate was adjusted to pH 2.8 with concentrated phosphoric acid and was partitioned three times with ethylacetate. The organic solvent was evaporated in a flask evaporator at 40°C and the dried F_I was stored under nitrogen at -20°C for further use.

2) Purification of the basic fraction (F_{II}).

The same column and PVP which were used for F_I were used for F_{II}. Sodium acetate-acetic acid buffer (pH 4.0) was used as the moving phase. This pH was chosen because minimal inhibition was shown in the tobacco callus bioassay (as compared to higher pH) and in addition, at pH 4.0 all

cytokinins (known) were collected with less volume of buffer than at pH 5.0. Elution profiles of known cytokinins are shown in Table 1. Zeatin was tested at pH 4.0 separately because of the OH^- group on the side chain. Between 70 and 170 mls of elute were collected, adjusted to pH 8.2 with dibasic potassium phosphate and partitioned three times with ethylacetate. The organic solvent was evaporated in a flask evaporator at 40°C and the dried basic fraction (F_{II}) was stored under nitrogen at -20°C for further use. The whole procedure for extraction and partitioning of F_I and F_{II} is shown in Fig. 1 and the purification procedure is shown in Fig. 2.

Paper Chromatography

For further separation of active compounds contained in F_I, paper chromatography was employed. The procedure which was followed was the same as that reported by Browning and Saunders (10). Whatman No. 1 paper, 10 cm wide, was used in the descending method of development with a solvent system of 88% isopropyl-alcohol, ammonia, water (10:1:1, V/V ratio). Chromatograms were developed for a distance of 40 cm, were dried and then were cut into ten pieces, each piece corresponding to 1 Rf value. Each chromatogram contained the extract from 15 g (F.W.) of tissue. For standardization and checking of procedure, the chromatograms with GA_3, GA_4 and GA_7 were run

Table 1. Fraction number and UV absorbance of elutes containing four known cytokinins (2iP,[z] 2iPA,[y] Zeatin,[x] Zeatin Riboside[w]) from a 1.9 x 30 cm column containing PVP as the stationary phase and buffer at different pHs as the moving phase. Absorption measured at 270 nm with a Beckman DB-GT grating spectrophotometer. Fractions consisted of 10 ml, the total amount of each cytokinin being 100 ug. The flow rate was 3 ml/min. and a total of 400 ml was collected.

pH 5.0						pH 4.0			
1.	0.06	11.	0.19	21.	0.14	1.	0.00	11.	0.45
2.	0.06	12.	0.18	22	0.18	2.	0.00	12.	0.42
3.	0.04	13.	0.18	23.	0.16	3.	0.00	13.	0.44
4.	0.02	14.	0.25	24.	0.14	4.	0.00	14.	0.40
5.	0.02	15.	0.30	25.	0.12	5.	0.00	15.	0.26
6.	0.02	16.	0.26	26.	0.08	6.	0.00	16.	0.14
7.	0.01	17.	0.20	27.	0.04	7.	0.00	17.	0.06
8.	0.02	18.	0.12	28.	0.04	8.	0.08	18.	0.03
9.	0.08	19.	0.10	29.	0.02	9.	0.03	19.	0.00
10.	0.16	20.	0.12	30.	0.00	10.	0.50	20.	0.00

Zeatin pH 4.0					
1.	0.00	6.	0.00	11.	0.28
2.	0.00	7.	0.00	12.	0.26
3.	0.00	8.	0.00	13.	0.06
4.	0.00	9.	0.04	14.	0.02
5.	0.00	10.	0.10	15.	0.00

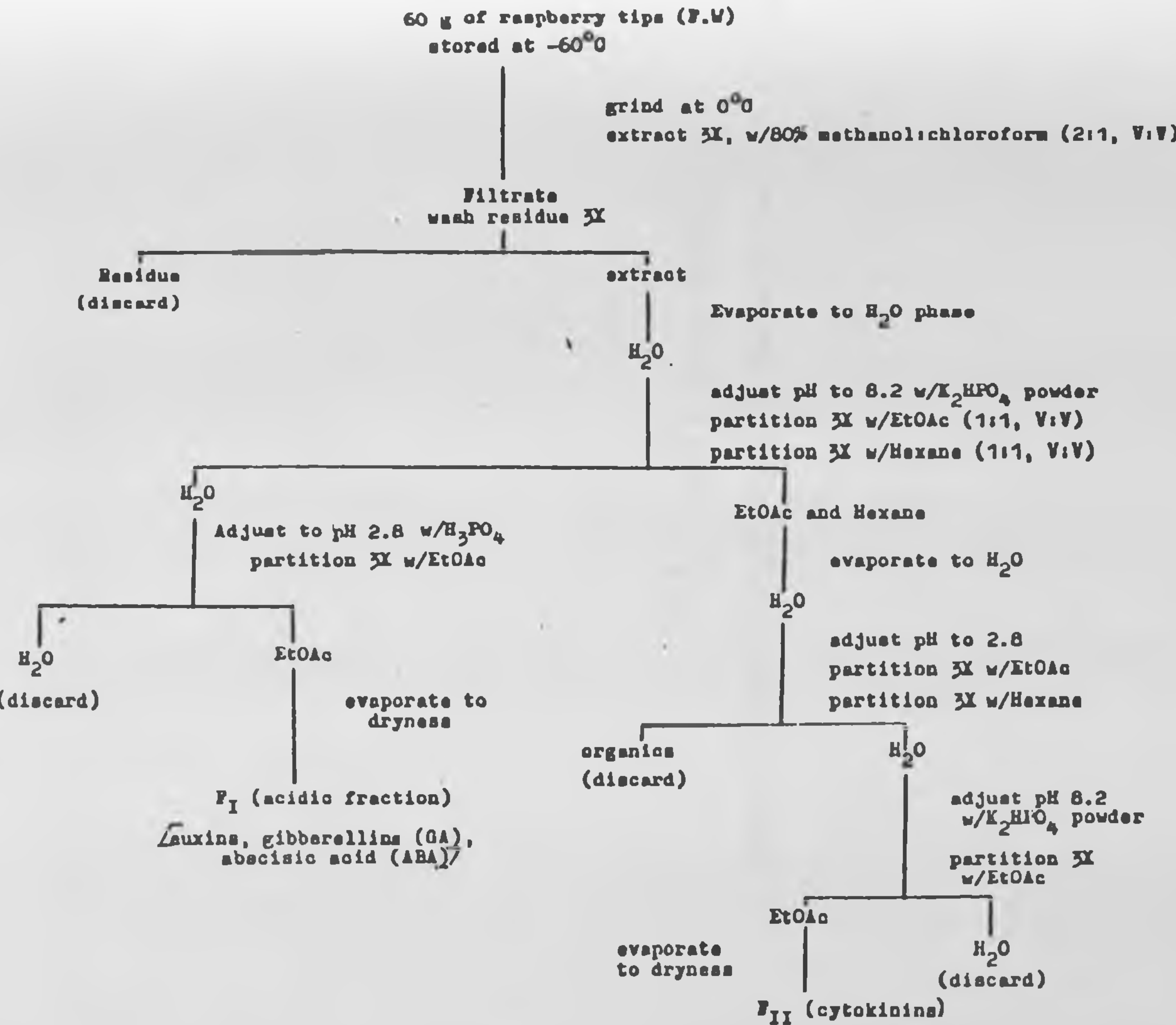
[z]2iP = 6-(γ-γ-dimethylallylamino) purine

[y]2iPA = 6-(γ-γ-dimethylallylamino)-9-β-D-ribofuranosyl-purine

[x]Zeatin = 6-(4-hydroxy-3-methylbut-trans-2-enyl)amino-purine

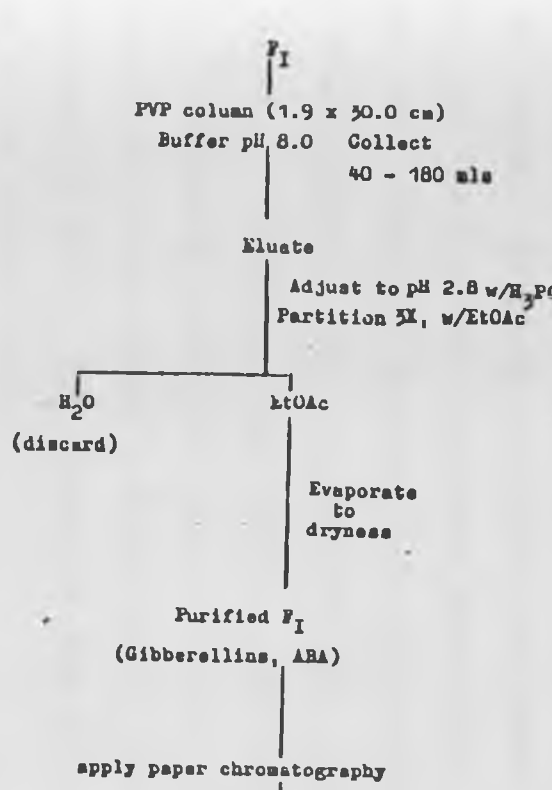
[w]Zeatin Riboside = 9-β-D-ribofuranosyl-Zeatin

Fig. 1. Flow diagram for extraction and partition of plant hormones from stem tips of red raspberries.

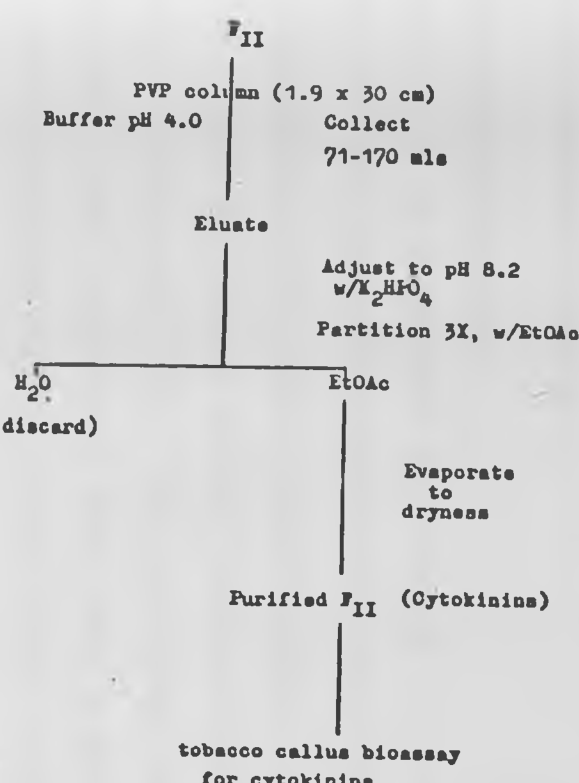


60 g of raspberry tips (F.W)
stored at -60°C
grind at 0°C
extract 3X, w/80% methanol:chloroform (2:1, V:V)
Filtrate
wash residue 3X
Residue
(discard)
extract
Evaporate to H_2O phase
H_2O
adjust pH to 8.2 w/K_2HPO_4 powder
partition 3X w/EtOAc (1:1, V:V)
partition 3X w/Hexane (1:1, V:V)
H_2O
Adjust to pH 2.8 w/H_3PO_4
partition 3X w/EtOAc
EtOAc and Hexane
evaporate to H_2O
H_2O
H_2O
(discard)
EtOAc
evaporate to dryness
adjust pH to 2.8
partition 3X w/EtOAc
partition 3X w/Hexane
organics
(discard)
H_2O
F_I (acidic fraction)
[auxins, gibberellins (GA), abscisic acid (ABA)]
adjust pH 8.2 w/K_2HPO_4 powder
partition 3X w/EtOAc
EtOAc
evaporate to dryness
H_2O
(discard)
F_{II} (cytokinins)

Fig. 2. Flow diagram for purification of plant hormones from F_I and F_{II}.



F_I
PVP column (1.9 x 30.0 cm)
Buffer pH 8.0
Collect
40 - 180 mls
Eluate
Adjust to pH 2.8 w/H_3PO_4
Partition 3X, w/EtOAc
H_2O
(discard)
EtOAc
Evaporate
to
dryness
Purified F_I
(Gibberellins, ABA)
apply paper chromatography
Lettuce Hypocotyl bioassay
for gibberellins



F_{II}
PVP column (1.9 x 30 cm)
Buffer pH 4.0
Collect
71-170 mls
Eluate
Adjust to pH 8.2
w/K_2HPO_4
Partition 3X, w/EtOAc
H_2O
(discard)
EtOAc
Evaporate
to
dryness
Purified F_{II} (Cytokinins)
tobacco callus bioassay
for cytokinins

simultaneously every 10 runs. In addition, co-chromatography of the extracts with GA_3 and GA_7 were conducted. Then the pieces of chromatograms were applied directly in the lettuce hypocotyl bioassay.

Lettuce Hypocotyl Bioassay

The method of Frankland and Wareing (30) with some modifications was chosen because of its simplicity and ease of combination with paper chromatography. Seeds of the lettuce cultivar 'Grand Rapids' were germinated in darkness for 36 hours at 25°C. Ten seedlings selected for uniformity (same root size) were used in each 5 cm diameter petri dish previously filled with a single section (10 x 4 cm) of the chromatogram, which represented a single Rf value. Three ml of distilled water containing 100 ng GA_3/ml were poured into each petri dish. The 100 ng GA_3/ml were employed because under the conditions of running the bioassay, poor sensitivity was noticed below 100 ng GA_3/ml. Seedlines were left to grow under continuous fluorescent light (two bulbs of cool white, 40 W per fixture, 20 cm distance from the petri dishes) and at a temperature of 30°C. The length of the hypocotyl was measured in mm after 3 days.

Each time the test was run, standard GA_3 was used for comparison. Concentrations used were 100, 1,000 and 10,000

ng GA_3/ml. In all tests applied with standard GA_3, there was a linear relationship between the hypocotyl length in mm and the $\log_{10}$ of GA_3 concentration. GA_3 equivalents were calculated according to the formula:

$$\frac{\text{Growth in mm (extract} + 100 \text{ ng } GA_3/\text{ml)} - \text{Growth in mm } (100 \text{ ng } GA_3/\text{ml}}{\text{Growth in mm}(1{,}000 \text{ ng } GA_3/\text{ml}) - \text{Growth in mm}(100 \text{ ng } GA_3/\text{ml})} = \frac{GA_3 \text{ equivalent}}{\text{ml}}$$

The GA_3 equivalent/ml was adjusted to ug of GA_3 equivalents/ 5g tissue (F.W.).

Tobacco Callus Bioassay for Cytokinins

The method of Linsmaier and Skoog (64) and Schmitz and Skoog (104) was used. Extracts were dissolved in DMSO (dimethyl-sulfoxide) and then diluted with sterilized sodium acetate-acetic acid buffer at pH 4.0. Total DMSO used was 25 µl/50 ml of media. Three serial dilutions were made X, 0.X, 0.0X [X = unknown amount of cytokinins contained in extract coming from 15 g of tissue (F.W.)].

RM-1965 medium was used. For the production of the standard curve, kinetin was used in tenth dilutions of 1 to 0.001 uM. Three pieces of callus were put in a flask containing 50 mls of media. After a period of 35 days of growth in darkness and a temperature of 25°C, the callus was harvested and the fresh weight was measured.

Kinetin equivalents were calculated according to the formula:

1 kinetin equivalent = growth (g) on a medium containing 0.01 µM kinetin (2.1 µg kinetin/liter medium)

Results

1) Gibberellin-like substances

The main activity of gibberellin-like substances was always noticed in the first 5 or 6 Rf values with little activity in the rest of the chromatogram (Figs. 3-8). Specific comparisons between the 10 and 20 node stage of growth in cold treated fall-bearing cultivars (Fig. 3, 5) showed that at 10 nodes the greatest activity was at Rf 0.1 and 0.4 and at 20 nodes the greatest activity was at Rf 0.1, 0.3 and 0.5. In non-cold treated 'Heritage' (Fig. 4) at the 10 node stage of growth the greatest activity was at Rf 0.2 and 0.4, at the 20 node stage the greatest activity was at Rf 0.2. At 10 nodes in cold-treated 'Heritage' the greatest activity was at Rf 0.1 and 0.4 (Fig. 3B) but in non-cold treated 'Heritage' (Fig. 4B) the greatest activity was noticed at Rf 0.2 and 0.4. At 20 nodes in cold treated 'Heritage' the greatest activity was noticed at Rf 0.3 and 0.5 (Fig. 3A), but in non-cold treated 'Heritage' the greatest activity was noticed at Rf 0.2 (Fig. 4A).

Fig. 3. GA activity in cold treated 'Heritage' red raspberry canes at the 20 node (A) and 10 node (B) stage of growth. GA activity measured by the Lettuce Hypocotyl Bioassay using an extract of 15 g of tissue. Average of three chromatograms.

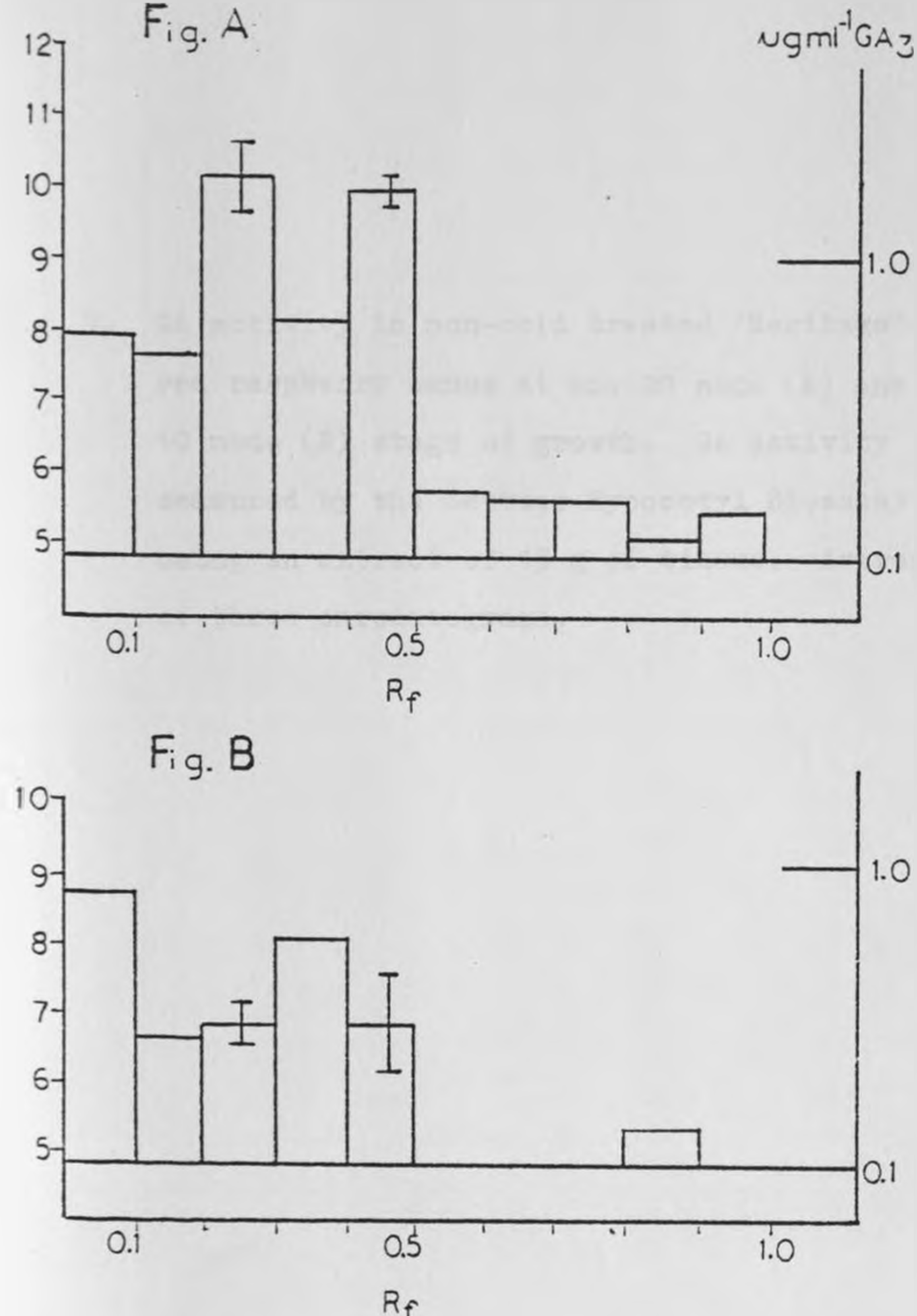


Fig. A
Fig. B
Hypocotyl length (mm)
µgml-1 GA3
Rf
0.1
0.5
1.0
12
11
10
9
8
7
6
5

Fig. 4. GA activity in non-cold treated 'Heritage' red raspberry canes at the 20 node (A) and 10 node (B) stage of growth. GA activity measured by the Lettuce Hypocotyl Bioassay using an extract of 15 g of tissue. Average of three chromatograms.

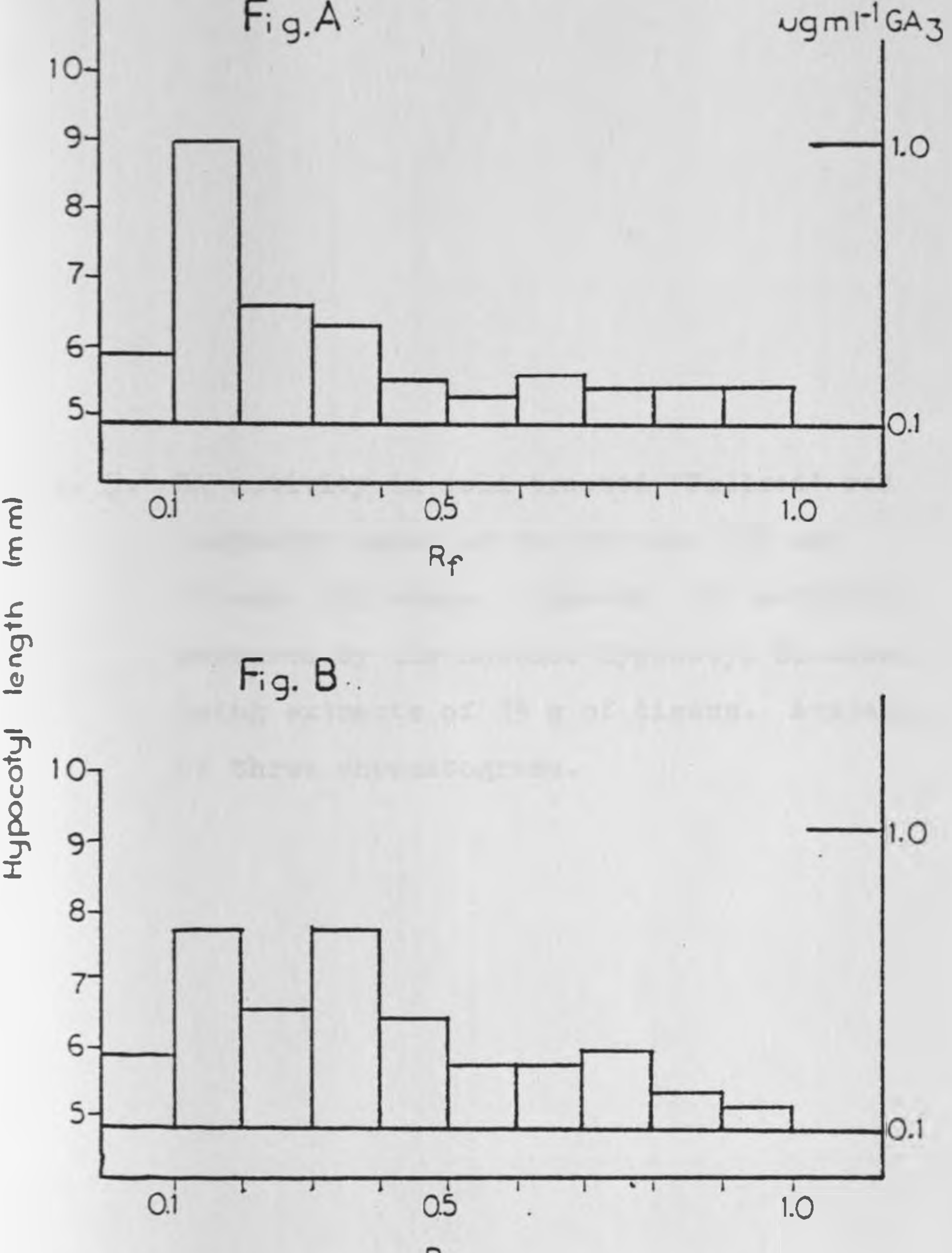


Fig.A
ugml-1 GA3
10
9
8
7
6
5
1.0
0.1
0.1
0.5
1.0
Rf
Hypocotyl length (mm)
Fig. B
10
9
8
7
6
5
1.0
0.1
0.1
0.5
1.0
Rf

Fig. 5. GA activity in cold treated 'Fallred' red raspberry canes at the 20 node (A) and 10 node (B) stage of growth. GA activity measured by the Lettuce Hypocotyl Bioassay using extracts of 15 g of tissue. Average of three chromatograms.

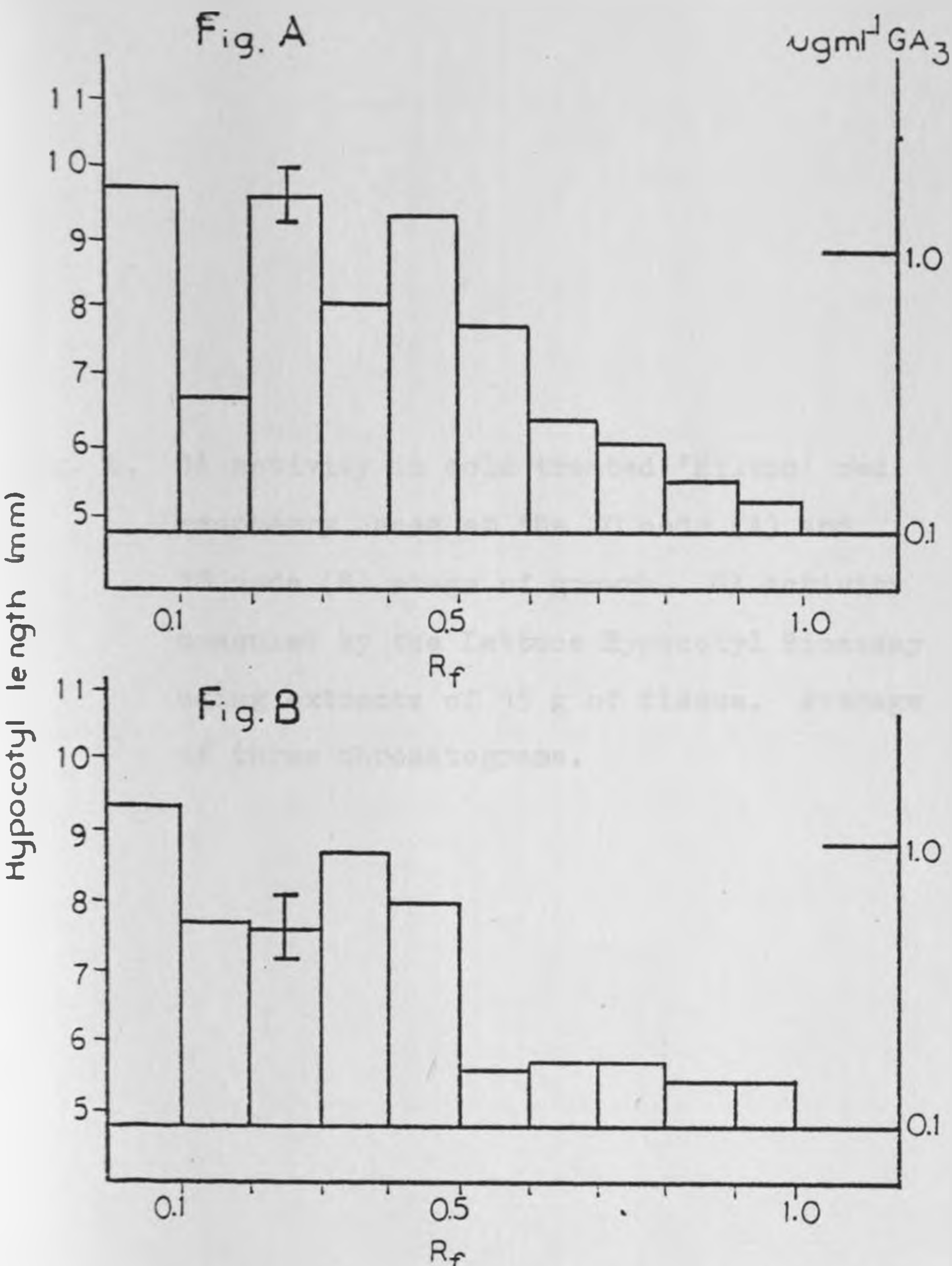


Fig. A
ugml^{-1} GA_3
11
10
9
8
7
6
5
1.0
0.1
0.1
0.5
1.0
R_f
Hypocotyl length (mm)
Fig. B
11
10
9
8
7
6
5
1.0
0.1
0.1
0.5
1.0
R_f

Fig. 6. GA activity in cold treated 'Hilton' red raspberry canes at the 20 node (A) and 10 node (B) stage of growth. GA activity measured by the Lettuce Hypocotyl Bioassay using extracts of 15 g of tissue. Average of three chromatograms.

Hypocotyl
10
9
8
7
6
5
1.0
0.1
0.1
0.5
1.0
R_f

Fig. A

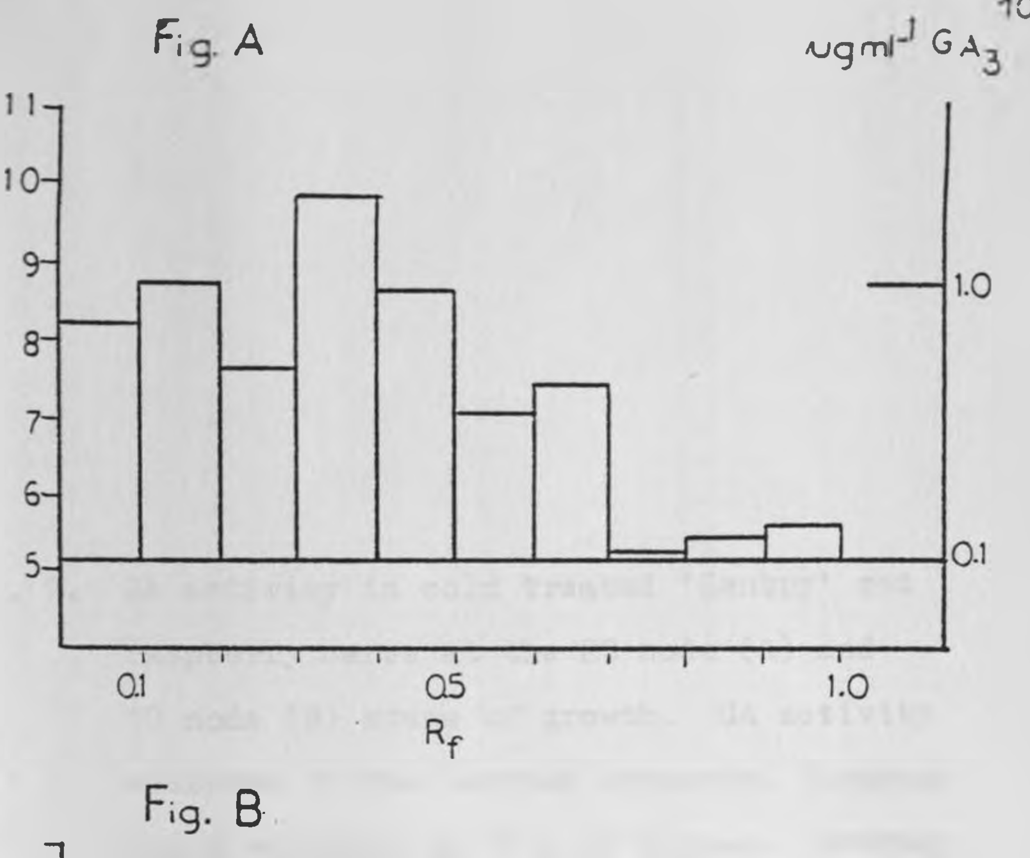


Fig. B

Fig. 7. GA activity in cold treated 'Sentry' red raspberry canes at the 20 node (A) and 10 node (B) stage of growth. GA activity measured by the Lettuce Hypocotyl Bioassay using extracts of 15 g of tissue. Average of three chromatograms.

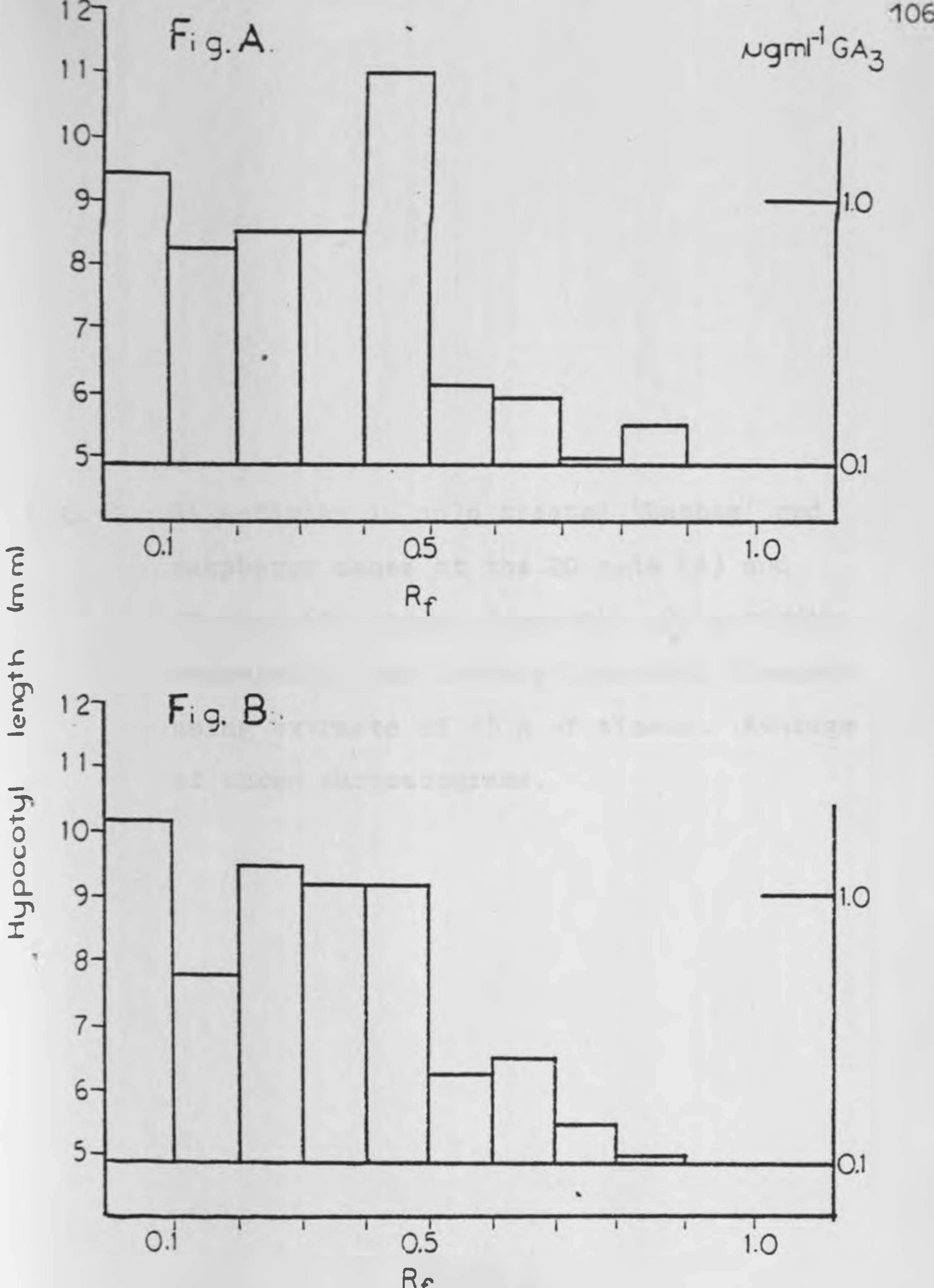
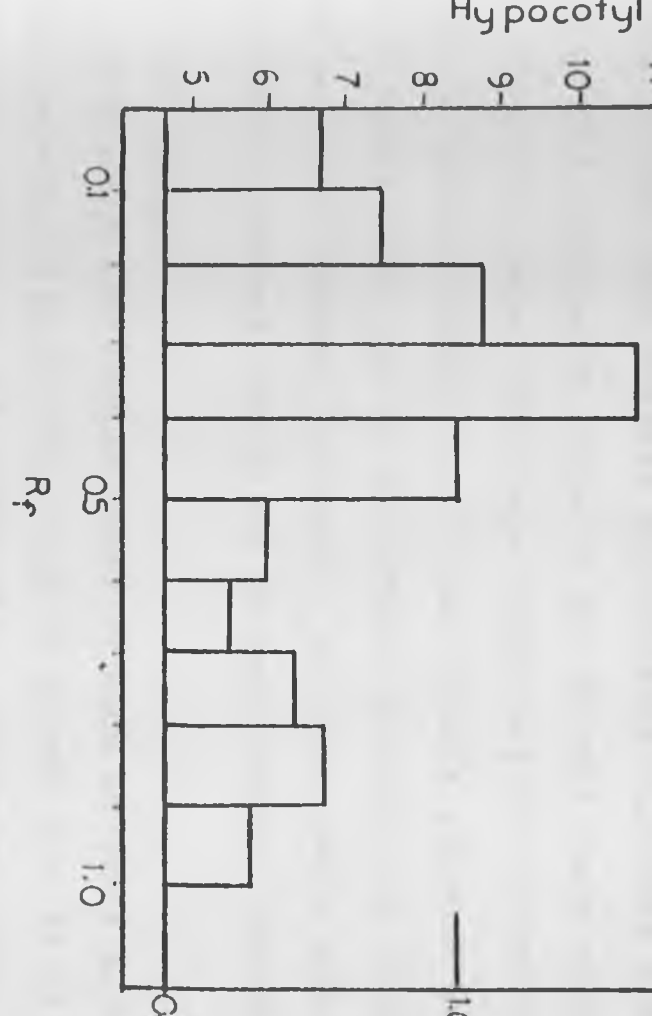


Fig. A.
Fig. B.
$\mu g ml^{-1}$ GA_3
Hypocotyl length (mm)
12
11
10
9
8
7
6
5
1.0
0.1
0.1
0.5
1.0
R_f

Fig. 8. GA activity in cold treated 'Latham' red raspberry canes at the 20 node (A) and 10 node (B) stage of growth. GA activity measured by the Lettuce Hypocotyl Bioassay using extracts of 15 g of tissue. Average of three chromatograms.


Hypocotyl
5
6
7
8
9
10
0.1
0.5
1.0
R_f
1.0

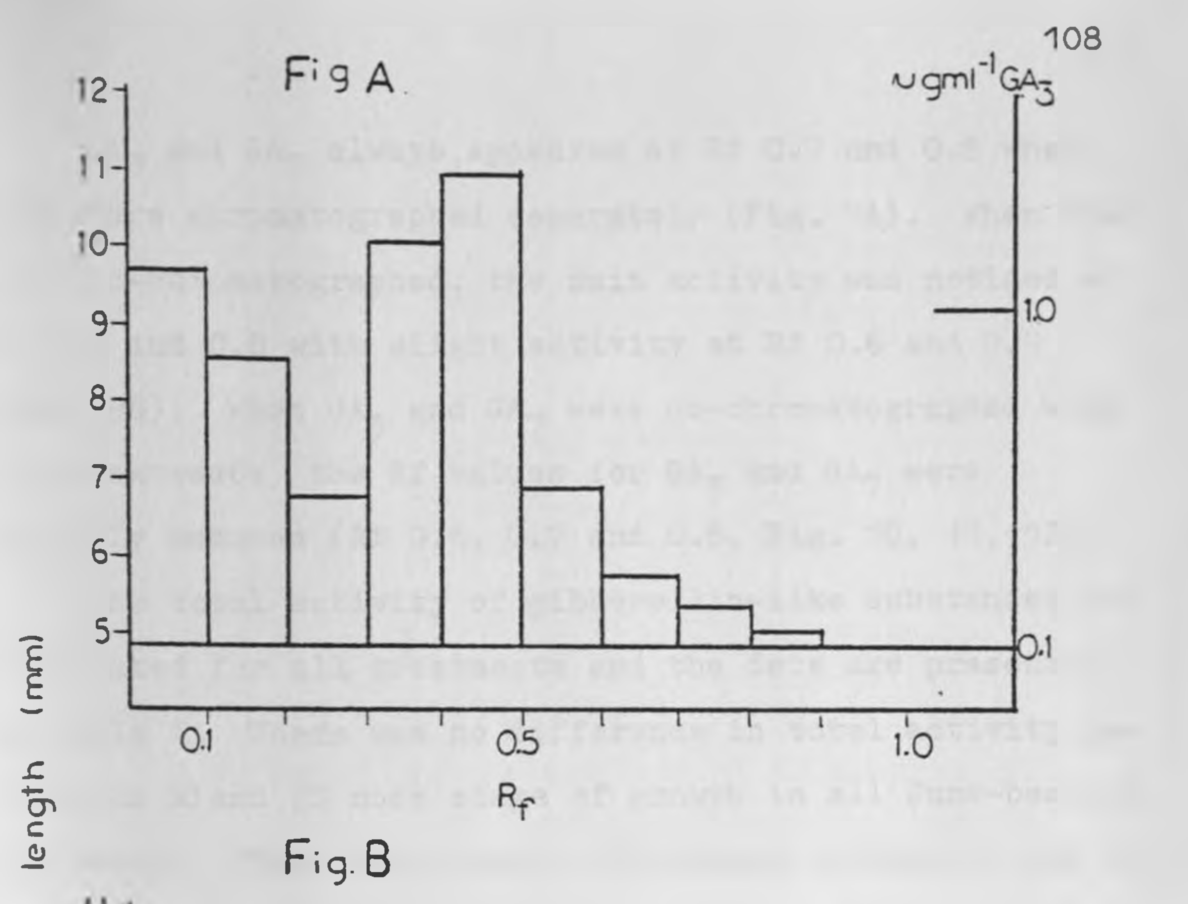


Fig. B

GA_3 and GA_7 always appeared at Rf 0.7 and 0.8 when they were chromatographed separately (Fig. 9A). When they were co-chromatographed, the main activity was noticed at Rf 0.7 and 0.8 with slight activity at Rf 0.6 and 0.9 (Fig. 9B). When GA_3 and GA_7 were co-chromatographed with plant extracts, the Rf values for GA_3 and GA_7 were slightly reduced (Rf 0.6, 0.7 and 0.8, Fig. 10, 11, 12).

The total activity of gibberellin-like substances was calculated for all treatments and the data are presented in Table 2. There was no difference in total activity between the 10 and 20 node stage of growth in all June-bearing cultivars. There was a major difference between 10 and 20 nodes in both cold treated fall-bearing cultivars with greater activity at the 20 than at the 10 node stage of growth. In non-cold treated 'Heritage' there was no difference in total activity between the 10 and 20 node stage of growth. At 10 nodes there was no difference between cold treated and non-cold treated 'Heritage', but there was a great difference between them at the 20 node stage of growth.

2) <u>Cytokinins</u>

Cytokinin activity, as shown by data in Table 3, was always greater at the 20 node stage of growth than at 10 nodes with all cultivars and treatments. 'Heritage' cold treated plants had greater activity at both node stage of growth than non-cold treated plants.

Fig. 9. Activity of GA_3 and GA_7 (1 µg/ml) in the Lettuce Hypocotyl Bioassay when chromatographed separately (A) and in combination (B).

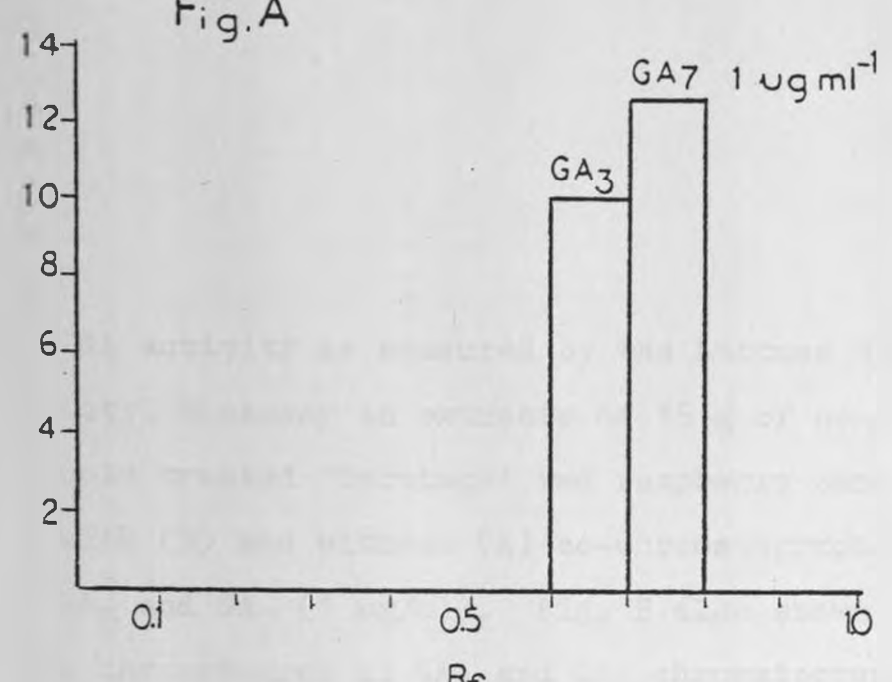


Fig. A
GA7 1 ug ml-1
GA3
14
12
10
8
6
4
2
0.1
0.5
1.0
Rf
Hypocotyl length (mm)

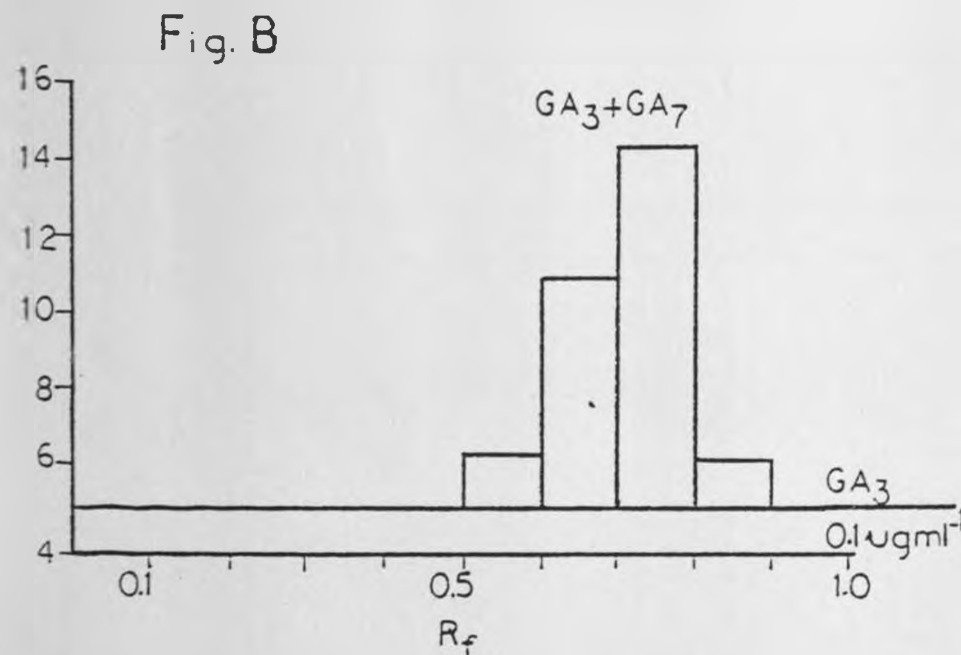


Fig. B
GA3+GA7
GA3
0.1 ugml-1
16
14
12
10
8
6
4
0.1
0.5
1.0
Rf

Fig. 10. GA activity as measured by the Lettuce Hypocotyl Bioassay in extracts of 15 g of non-cold treated 'Heritage' red raspberry canes with (B) and without (A) co-chromatographed GA_3 and GA_7 (1 µg/ml). Fig. B also shows a chromatogram of GA_3 and GA_7 chromatographed separately from the extract.

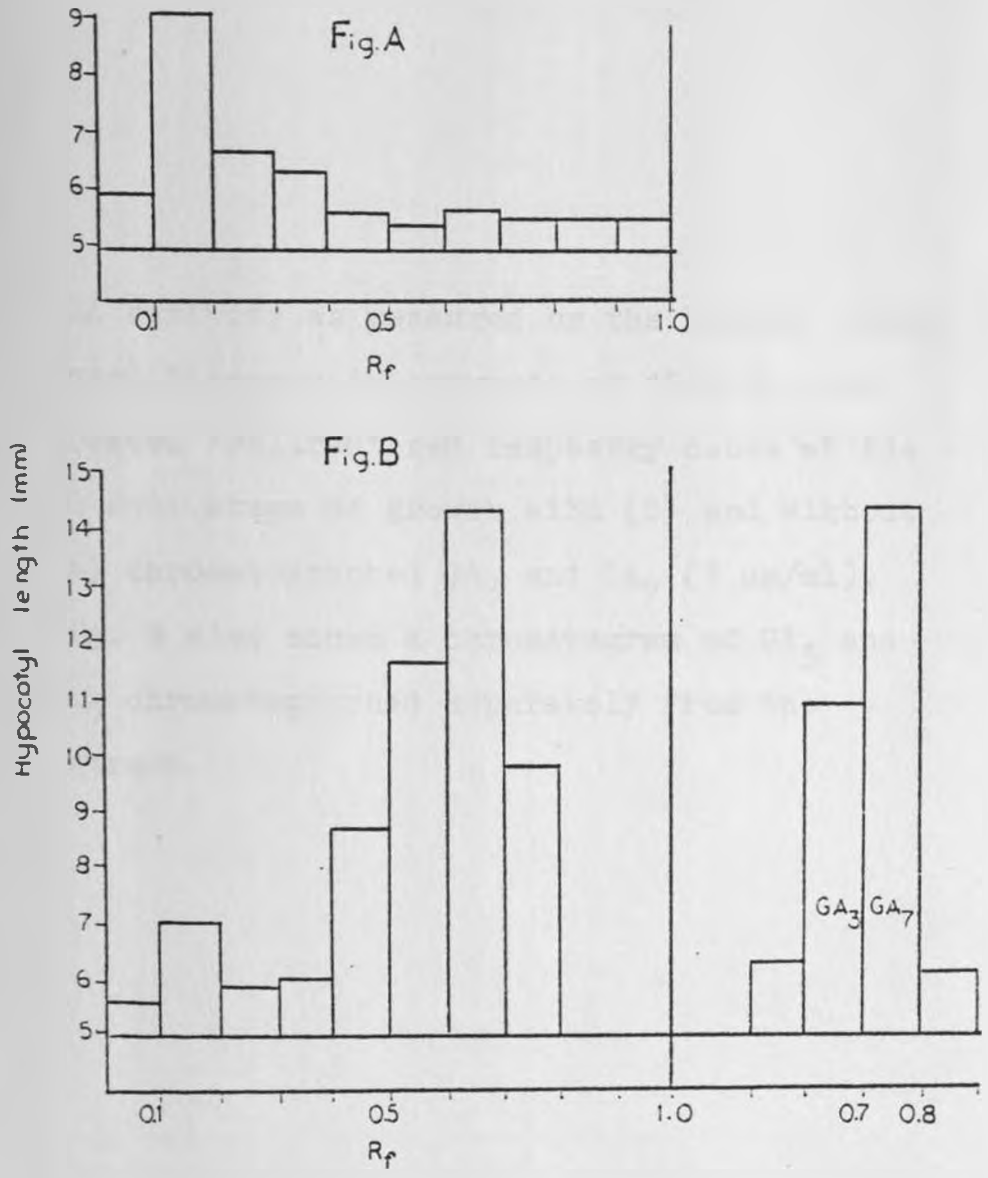


Fig.A
Fig.B
Hypocotyl length (mm)
9
8
7
6
5
15
14
13
12
11
10
0.1
0.5
1.0
0.7
0.8
R_f
GA_3
GA_7

Fig. 11. GA activity as measured by the Lettuce Hypocotyl Bioassay in extracts of 15 g of cold treated 'Fallred' red raspberry canes at the 20 node stage of growth with (B) and without (A) chromatographed GA_3 and GA_7 (1 µg/ml). Fig. B also shows a chromatogram of GA_3 and GA_7 chromatographed separately from the extract.

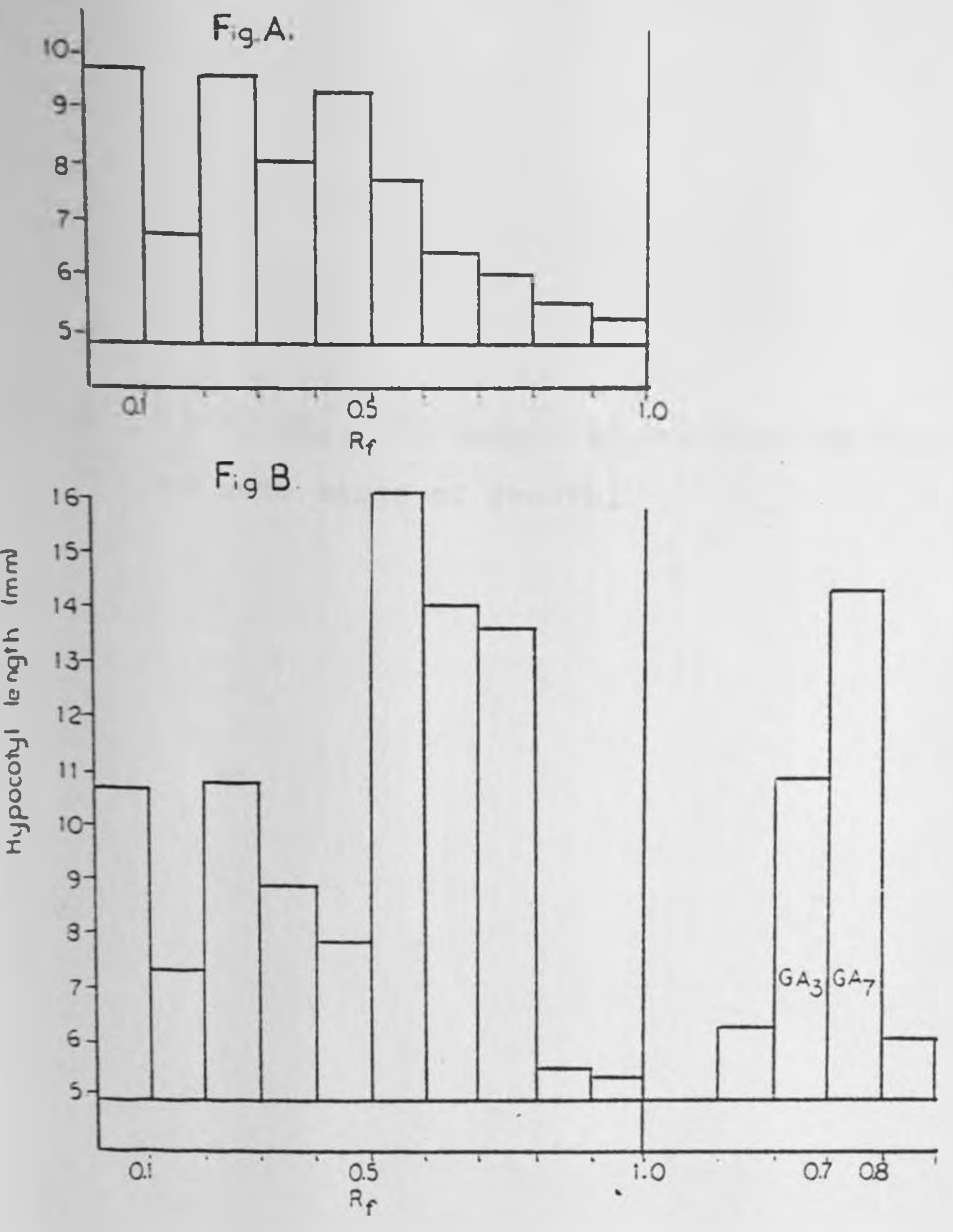


Fig. A.
10
9
8
7
6
5
0.1
0.5
1.0
Rf
Fig B.
Hypocotyl length (mm)
16
15
14
13
12
11
10
9
8
7
6
5
0.1
0.5
1.0
0.7
0.8
Rf
GA3
GA7

Fig. 12. As in Fig. 11, except plants were at the 10 node stage of growth.

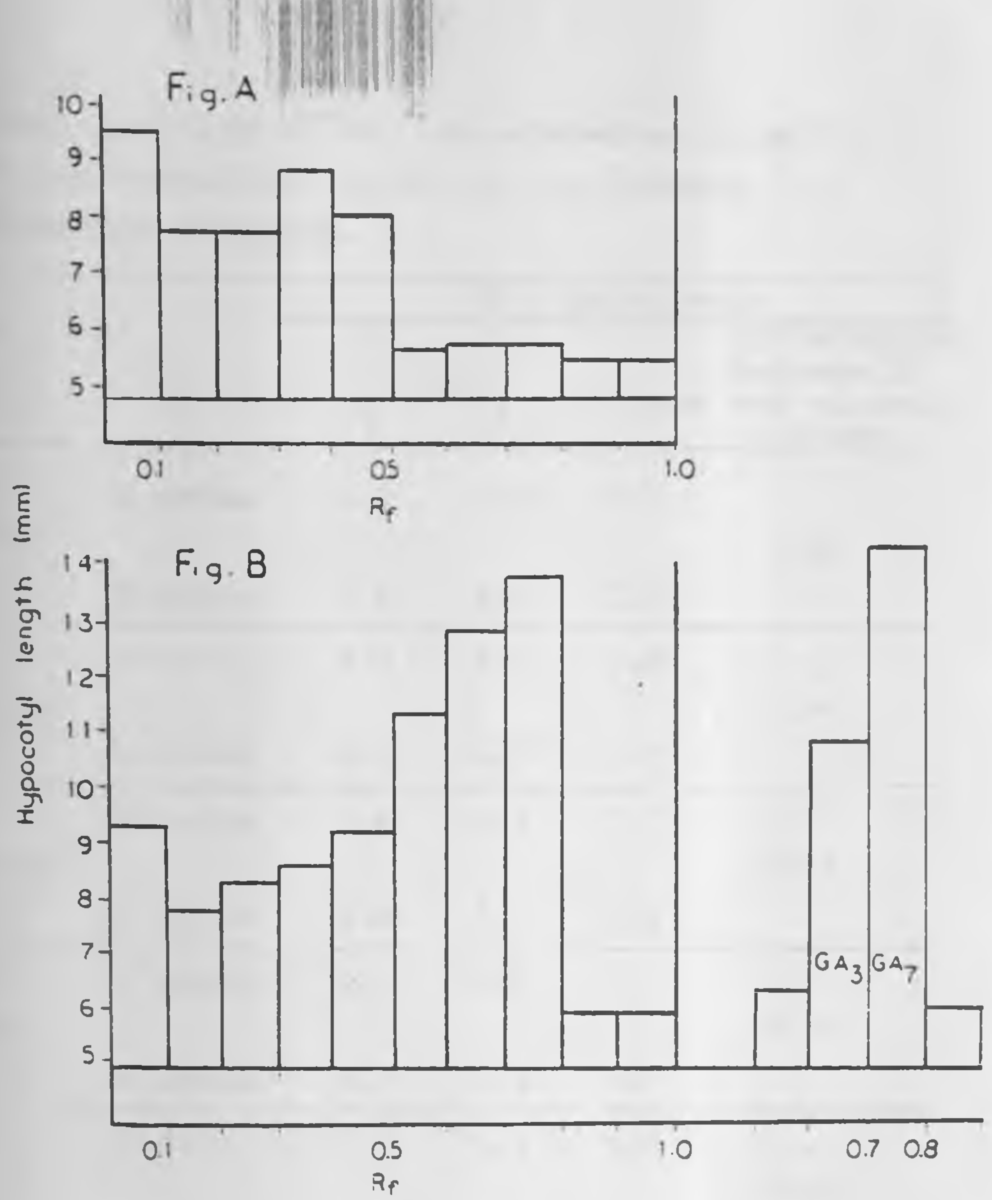


Fig. A
Fig. B
Hypocotyl length (mm)
10
9
8
7
6
5
14
13
12
11
0.1
0.5
1.0
0.7
0.8
Rf
GA3
GA7

Table 2. Total activity of GA-like substances in µg GA_3 equivalents/5 gr. tissue in the Lettuce Hypocotyl Bioassay.

Treatments			GA_3 equivalents			
			Reps[a/]		Average	Differences between 20 and 10 node stage
			1	2		
1	Fallred	10 nodes	4.0	4.3	4.2	2.8
2		20 nodes	7.6	6.4	7.0	
3	Heritage	10 nodes	3.5	4.0	3.8	2.4
4		20 nodes	6.4	6.0	6.2	
5	Heritage[b/]	10 nodes	3.4	2.9	3.2	-0.3
6		20 nodes	2.8	3.0	2.9	
7	Hilton	10 nodes	4.4	5.6	5.0	+0.1
8		20 nodes	4.7	5.5	5.1	
9	Sentry	10 nodes	5.9	6.1	6.0	-0.1
10		20 nodes	5.8	5.9	5.9	
11	Latham	10 nodes	6.5	-	6.5	+0.3
12		20 nodes	7.4	6.2	6.8	

[a/] Three chromatograms per rep.

[b/] Heritage without cold treatment.

Table 3. Total cytokinin activity contained in extracts of 15 g (F.W) tissue of red raspberry plants at the 10 and 20 node stage of growth, expressed in kinetin equivalents.

Treatments			Kinetin equivalents[a]		
			Rep 1 [c]	Rep 2	Average
1	Fallred	10 nodes	0.84	1.69	1.26
2	Fallred	20 nodes	1.81	3.01	2.41
3	Heritage	10 nodes	1.73	1.98	1.85
4	Heritage	20 nodes	3.33	4.04	3.75
5	Heritage[b]	10 nodes	0.75	1.47	1.08
6	Heritage[b]	20 nodes	1.48	2.47	1.97
7	Hilton	10 nodes	1.24	1.53	1.38
8	Hilton	20 nodes	3.24	3.52	3.38
9	Latham	10 nodes	0.80	-	0.80
10	Latham	20 nodes	2.78	-	2.78
11	Sentry	10 nodes	0.93	1.60	1.27
12	Sentry	20 nodes	1.75	1.83	1.79

[a] 1 kinetin equivalent = growth obtained in media containing 0.01 µM kinetin in tobacco callus bioassay.

[b] Heritage without cold treatment.

[c] Three or four flasks containing three pieces of callus each.

Discussion

1. Gibberellin-like substances and flowering

Qualitative differences were observed between the 10 and 20 node stage of growth and between cold and non-cold treated 'Heritage' plants. In fall-bearing cold-treated plants, the main changes between the 10 and 20 node stage of growth occurred at Rf 3 and 5 or 6. In 'Heritage' this change accounted for 54% of the total increase in GA activity from 10 to 20 nodes and in 'Fallred' for 42% of the total increase. In induced fall-bearing plants at the 20 node stage of growth, the greatest activity was observed at Rf 1, 3 and 5, although in non-cold treated 'Heritage' activity was noticed mainly at Rf 2. Since changes in GA-like substances were constant only in flower-induced plants, one interpretation is that these GA are involved in flower induction.

The co-chromatography results (Fig. 10, 11, 12) show that raspberry plants had gibberellin-like substances of generally high polarity. Little activity coincident with GA_3 and other less polar gibberellins was evident. Coupling these results with those reported by Browning and Saunders (10) and by Durley et al. (24), the most likely gibberellins would be $GA_{2,\ 8,\ 13,\ 17,\ 18,\ 21,\ 23,\ 26\ \text{or}\ 28}$. However, all these gibberellins have low activity in the Lettuce Hypocotyl Bioassay (Crozier et al., 21) and since

very good activity was noticed using this bioassay with the raspberry extracts, either these gibberellins were present in very large quantities or some unknown gibberellins which are very active in the Lettuce Hypocotyl Bioassay were present.

The results support the idea that raspberry plants are specific to producing other gibberellins less polar than GA_3 and demonstrate the specificity of plant species in producing and probably responding to specific gibberellins exogenously applied. In a way the results support the statement made by Lang (58) and supported by other studies that a specific gibberellin must be used for a specific plant for flower induction to occur.

Differences were evident in the total quantity of gibberellin-like substances between the two stages of growth in flower induced (cold treated) fall-bearing cultivars. Cold treated June-bearing cultivars and the non-cold treated 'Heritage' had same total activity of gibberellin-like substances at both growth stages and were not flower-induced. The increased gibberellin-like activity at the 20 node stage of growth when flower induction had occurred or was imminent suggests that a relatively high level of gibberellin-like substances was necessary for flower induction. Similar conclusions were reached by many investigators working with other plant species, particularly

with cold-induced flower induction (3, 17, 29, 46, 74, 92, 103, 111, 113).

Evans in his review (28) concluded that the presence of gibberellin may be a prerequisite for flower evocation in all plants and that the level of GA is most often limiting in LD plants. However, because some reports suggest that in rosette plant species, stem elongation and flowering are two separate processes and that the prime effect of gibberellins is to cause stem elongation, some investigators (Zeevaart, 131) have tended to minimize the importance of gibberellins in flowering. In the raspberry plant, stem elongation proceeds without a cold treatment and thus the increased level of gibberellins observed before or at the beginning of flower induction is more closely linked to flowering in this species. Zeevaart (131) based his argument on results obtained mostly with the use of growth retardants particularly with the plant *Silene armeria* L. In this plant a 100% increase in endogenous levels of gibberellins under flower inductive conditions was recorded but when plants were treated with Amo 1618, a growth retardant, an 80% decrease in endogenous extractable gibberellins and non-detectable diffusible gibberellins was observed. Since stem elongation was affected but not flowering, it was concluded that flowering could occur in the absence of gibberellins. Application of GA_3 could

overcome the retardant's effect in stem elongation but could not bring flowering (Cleland and Zeevaart, 20). Although the same above investigators reported that both GA_3 and GA_7 could bring flower induction under non-inductive conditions, they applied only GA_3 after the application of the retardant and one wonders what would have happened with GA_7 application. There are reports showing a positive interaction between growth retardants, GA's, and flowering. Koranski (66), working with Clerodendrum, reported that although GA_3 had a strong interaction with ancymidol (a-cyclopropyl-a-(4-methoxyphenyl)-5-pyrimidine-methanol), a growth retardant, GA_7 treated plants did not show such a response. Treatment with GA_7 did not produce a marked stimulation of vegetative growth nor did it overcome the effects of ancymidol. However, GA_7 did stimulate flowering under inductive conditions, a response opposite to that obtained with GA_3. In addition, ancymidol treatment produced increases in endogenous GA-like activity which was associated with flower induction. Other reports also show such a synergistic effect between gibberellins and growth retardants as in the flowering of Lolium temulentum L. (27) and internode elongation in stems of strawberry (40).

Krishnamoorthy (54), commenting on the results obtained by using growth retardants, stated that "the

conclusions drawn with these chemicals have only to be tentative till their precise mode of action is fully understood."

2. Cytokinins and flowering.

The results show that in all treatments there was higher cytokinin activity at the 20 node stage of growth than at 10 nodes. This probably means that there was no relation between flowering and cytokinin content. But by taking into consideration that cold treated 'Heritage' plants had greater cytokinin activity than non-cold treated plants at both stages of growth, it is suggested that cytokinins may be involved in the production of the floral stimulus. A similar conclusion was reached by Bernier et al. (9). They suggested that in _Sinapsis alba_ L. cytokinin was one of the possible components necessary for the production of the floral stimulus.

Since cold treated plants had greater gibberellin and cytokinin activity than non-cold treated plants and the increase in activity was closely related to flower induction, it is further suggested that probably a certain balance of gibberellins and cytokinins might be necessary for the production of the floral stimulus in fall-bearing red raspberries.

Why June-bearing cultivars were not induced to flower at the 20 node stage of growth even when cold treated could

be explained by the two following hypotheses: 1) fall-bearing and June-bearing red raspberries have the same flowering mechanism but June-bearing plants were not at an appropriate stage of growth to respond to cold treatment, or 2) June-bearing red raspberries have a different flowering mechanism than fall-bearing cultivars and do not respond to cold as do the fall-bearing cultivars.

CONCLUSIONS AND PROJECTIONS

The purpose of the present study was to achieve a better understanding of the flowering process in fall-bearing red raspberries and to use this information as a background for suggesting possible ways of improvement of the red raspberry industry.

The results show that 'Heritage' plants responded to cold treatment at any stage of growth and as a consequence, they flowered earlier than non-cold treated plants. Since the amount of cold required for bringing earlier flowering or shortening the plant's vegetative life had been met in Wisconsin by the middle of December, it is suggested that earlier flowering might be achieved if early growth was encouraged by protection during early spring against cold nights and cold days.

The number of suckers produced per plant could be increased by the removal of the flowering head after the appearance of the terminal inflorescences, but before full development of the fruits. This may be done very easily by mechanical or chemical pruning applied at the right time. An increased production of nursery stock should result from such a management program.

The results also show that at or before flower induction in cold treated fall-bearing cultivars an increase in endogenous gibberellins was noticed. No increase was noticed in either non-cold treated 'Heritage' or in June-bearing cultivars which at this stage were vegetative. Thus, it is suggested that the level of gibberellins might play a role in flower induction in these plants.

Cytokinin levels were higher in older than younger plants in all cultivars. However, cold treated 'Heritage' plants had greater cytokinin activity than non-cold treated plants. Thus, it is further suggested that a certain balance between gibberellins and cytokinins might be important for flower induction in fall-bearing red raspberries.

The results of Chapters I and II demonstrate the environmental regulations on the expression of a plant's program of growth and development. Such environmental controls are at least mediated by hormonal changes.