PHYSIOLOGY OF THE TERMINAL BUD OF *VACCINIUM MACROCARPON* AIT. CULTIVAR McFARLIN IN RELATION TO WINTER DORMANCY

by

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ABSTRACT

A study of the relationship between the winter dormant period and subsequent growth and development in Vaccinium macrocarpon Ait. cultivar McFarlin was carried out. The main objectives of the study were to obtain data on this relationship, to investigate the possible role of gibberellins in the induction of flowering, to correlate the data with development of the terminal bud under field conditions, and to provide basic information relating to the problem of frost injury.

Controlled environment facilities were used to investigate the effect of chilling on the subsequent growth and development of the terminal bud. Exposure to an accumulation of chilling temperatures below 7°C was required to break dormancy of the terminal bud. Longer periods of chilling were needed to induce flower development. The faster rate of vegetative response was apparently due to development of the plant during the warmer portion of the daily temperature cycle. The best responses were obtained when the chilling conditions included a period of approximately 10°C during the day.

The floral primordia of terminal buds in the field recommenced development in late February or early March, and were well differentiated by the end of April under British Columbia conditions.

Application of gibberellic acid to dormant, unchilled terminal buds stimulated only vegetative growth. Gibberellin is probably not involved in the induction of floral development.

Cold methanol extraction of gibberellin-like substances and subsequent purification was carried out on the leaves and terminal buds of V. macrocarpon grown in the field. Fractions of R_f 0.1 were scraped from thin-layer chromatography sheets and bioassayed for gibberellin-like activity using a lettuce hypocotyl bioassay. An increase in such activity on March 9, 1970 was observed for both buds and leaves. On April 6, the activity

in the leaves was considerably reduced but the bud level was increased, thus suggesting a translocation of gibberellin-like substances from the leaves to the terminal buds.

Some possible implications of the study in relation to frost injury were discussed. The chilling requirement may be a factor in determining both the southern and northern limits of the geographic range of the species.

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INTRODUCTION

The commercial cranberry, Vaccinium macrocarpon Ait., is a low growing perennial woody vine with persistent leaves. The stems, or runners, range from 0.3 to greater than 6 m in length. Short vertical branches, commonly called uprights, are produced from the runners. Most of the fruit is borne on the uprights. A mixed bud forms at the apex of the upright during the summer. These terminal buds undergo some differentiation before the onset of winter dormancy. Under normal conditions, they complete their development the following spring, producing new stems, leaves and flowers.

The anatomy of the terminal bud of V. macrocarpon has been studied by several authors. Flower bud primordia have been distinguished as early as July 29 under field conditions in Wisconsin (Roberts and Struckmeyer, 1948). Development of these primordia continued until the onset of winter dormancy. Dormant cranberry flower buds collected from Nova Scotia (Bell and Burchill, 1955), Massachusetts (Lacroix, 1926), and Wisconsin (Goff, 1901) were reported as having discernible calyx and petals with recognizable, but rudimentary, stamen and carpel primordia. In most of the major cranberry growing areas, it is apparent that the flower buds overwinter in a somewhat undifferentiated stage.

A general review of experimental studies of the shoot apex and shoot morphogenesis, including the effect of gibberellins, has been done by Cutter (1965). Growth and development of meristems in woody plants have been reviewed by Romberger (1963). The floral morphology of V. macrocarpon in relation to other members of the Ericales has been discussed by Palser (1961). Flower numbers and fruit set of McFarlin cranberries have been recorded for British Columbia (Garlick, 1966), Massachusetts (Bergman, 1950),

New Jersey (Bergman, 1954), and Wisconsin (Bain, 1946).

There has been considerable interest in the relationship between winter conditions and fruit production and quality. Bergman (1947, 1949, 1950) studied the problem of winter injury and concluded that much of the injury was a result of oxygen deficiency caused by the cultural practice of flooding the bogs. Cross (1969) reported that the terminal buds had their best growth during the months of September and October, but did not discuss bud anatomy. He also reported a strong correlation between January sunshine and berry size, which he suggested is related to the oxygen deficiency problem. Doughty[1] approached the problem of winter injury on a degree-hour basis. He reported that new growth may be initiated after 400 hr below 4.5°C under Washington State conditions.

Growth and development of V. macrocarpon in direct relation to the winter rest requirement were investigated by Chandler and Demoranville (1964). They reported that below 7°C 2500 hr of chilling were necessary to fulfil the rest period requirement of McFarlin cranberry plants, while less chilling (1500 hr) resulted in abnormal vegetative growth of the terminal shoot. The present author[2] found that at 5-6°C under nine hour photoperiods, 650 hr were sufficient for the induction of normal growth of the terminal shoot. Long photoperiods have been found to cause increased vegetative growth of unchilled cranberry plants, but flower bud differentiation was reported as rare (Eaton and Ormrod, 1968).

The literature relating to the dormancy of woody plants has recently

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1 C.C. Doughty, personal communication, 1971.

2 The work published by Eady and Eaton (1969) forms a part of this thesis.

been reviewed (Perry, 1971). Other reviewers have discussed the subject of dormancy and vernalization of plants in detail (Hillman, 1969; Wareing, 1969; Lang, 1965; Vegis, 1964; Chouard, 1960; Samish, 1954; Doorenbos, 1953).

The morphogenetic effects of the gibberellins have been discussed by Brian (1959), who noted that "gibberellin will usually replace vernalization and allow development to proceed normally in plants kept in long day photoperiods." Gibberellic acid has been found to overcome the dormancy of buds in many tree species (Eagles and Wareing, 1963). Smith and Kefford (1964) have suggested that completion of chilling followed by long days stimulate the production of gibberellins which are involved in release from dormancy. "The possible role of native gibberellins in dormancy must be investigated by determining the gibberellin content of dormant and non-dormant tissue" (Wareing, 1961).

Gibberellic acid applied to V. macrocarpon during and after bloom caused a significant increase in fruit set but also interfered with terminal bud development (Devlin and Demoranville, 1967). However, the latter phenomenon was reported to have resulted in a marked reduction in flowering in the year after application (Mainland and Eck, 1968).

The importance of the winter dormant period to the overall growth and development of V. macrocarpon is generally recognized, but the actual effect of the dormant period has been little studied. The objectives of the present study are: 1) to investigate the effect of winter conditions on the growth and subsequent development of the terminal bud through the use of controlled environment facilities, 2) to investigate the possible role of gibberellin in the induction of flowering, 3) to correlate the above data with terminal bud development under field conditions, and 4) to provide

some basic information relating to the problem of frost injury.

In order to make maximum use of the available facilities, the present study was confined to one cultivar of V. macrocarpon. The use of additional cultivars would have resulted in a reduction of either treatments or replications. The cultivar McFarlin was chosen because it comprises 65 percent of the British Columbia plantings (Eaton, 1970) and therefore is both commercially important and readily available.

Because of varying usage, it is necessary to define some of the terms as used in this study. Dormancy is a "general term for all instances in which a tissue predisposed to elongate (or grow in some other manner) does not do so" (Romberger, 1963). Chilling requirement is used to refer to a need for the plant or organ to be exposed to a period of low temperature before the continuation of normal development following the onset of dormancy. There has been considerable variation in the use of the term vernalization. Many workers favour limiting the term to apply only to low temperature promotion of flowering (Salisbury, 1963); this is the usage adopted in the present study.

MATERIALS AND METHODS

Plant Material

Vaccinium macrocarpon Ait. cultivar McFarlin was used in all of the following experiments. The plants were obtained from a commercial planting[1] in Richmond, British Columbia. This planting was established in 1955 using cuttings of McFarlin plant material obtained from the Greyland area of Washington State, and has been maintained in commercial production since that time.

McFarlin cranberry vines were originally a selection from the wild made by Thomas H. McFarlin in 1874 at a natural bog near South Carver, Massachusetts. In 1885, Charles Dexter McFarlin, his brother, established the first commercial cranberry planting near Coos Bay, Oregon using mainly McFarlin vines (Chandler and Demoranville, 1958).

The commercial practice is to propagate cranberry vines by cuttings, which root easily; thus in the absence of detailed taxonomic or genetic studies, it is assumed that the present plant material is a reasonably direct descendent of the original selection, although some seedling mixtures are possible.

Anatomy of the Terminal Bud

One hundred sixty-two buds, collected between January and June 1969, and 53 buds collected between October 1969 and June 1970 were fixed, sectioned, stained and examined.

The first set of buds were fixed in a formalin, acetic acid, alcohol solution (FAA); those of the second set were freeze-dried. Dehydration and

1 Big Red Cranberry Co., Richmond, British Columbia.

paraffin infiltration were done according to Jensen (1962). The material was sectioned at a thickness of 10 microns. The staining procedure for all sections was that described by Sharman (1943), using tannic acid and iron alum with safranin and orange G.

After staining, the sections were examined and compared on the basis of gross anatomical differences.

Field Temperature

Temperatures in the field from which the plants were collected were recorded using a Short and Reed thermograph of the revolving-drum type. The instrument was placed in a Stevenson screen located directly on the bog surface; the sensor was thus located approximately at the level of the terminal buds.

Controlled Environment Studies

Three sets of experiments were done using a variety of controlled environment facilities. The details for the lighting systems of these facilities are described in Appendix 1.

1968-1969 Experiment

Budded uprights were collected on October 4, 1968. Each upright was trimmed 10 cm below the bud. The lower 4 cm of leaves were removed and the plants set in commercial peat moss. The collection was maintained in the greenhouse under a misting system until November 20, 1968 when the plants were transplanted to 15 cm pots, one plant per pot. These remained on a greenhouse bench until initiation of treatments on December 18, 1968. A selection of uniform plants were then assigned treatment numbers and

allocated random positions in the growth chambers.

Treatments were applied in two Sherer Model CEL-266-6 growth chambers using one-half lighting for a nine hour photoperiod. Selection of the chilling temperature was based on the observation of Chandler and Demoranville (1964) that V. macrocarpon responded to temperatures below 7°C. A limitation on the duration of the treatment temperature was imposed by the fact that the chambers were not designed for continuous low temperature operation. Treatment temperature was established as 20 hr at 5-6°C with a 4 hr midday period of 13°C to allow the machinery time to defrost. Treatment durations are listed in Table 1.

Following the treatment periods, the plants were transferred to another Sherer growth chamber with 15 hr of full light per day. The temperature was maintained at 19°C day and 13°C night. Frequent inspections were made to record the initiation of new shoot growth, following which shoot length was recorded. Numbers of flowers were also recorded.

This experiment was set up as a randomized complete block design with each of treatments 2-6 replicated three times in each chamber. The six untreated plants could not be included in this design as they had not been exposed to either of the chambers (blocks). When the data were analysed using the completely random design with six treatments and six replicates for a total of 36 plants, the untreated set could be included.

In all the experiments, Duncan's new multiple range test was used to compare mean values when the F value in the analysis of variance was significant.

1969 (Summer) Experiment

The plants used in these experiments were from the same collection as for the previous experiment. They had been maintained in the greenhouse at

Table 1.

Treatment Durations of Controlled Environment Experiments

Experiment	Treatment No.	Days Treated	Accumulated hr below 7° C
1968-1969	1 (control)	0	0
	2	33	650
	3	65	1300
	4	65	1700
	5	105	2100
	6	125	2500
1969 (Summer)[1]	1 (control)	0	0
	2	15	300
	3	33	650
	4	65	1300
	5	85	1700
	6	105	2100
	7	125	2500
1969-1970			
Regime 1	1 (control)	0	0
	2	25	175
	3	50	350
	4	75	525
	5	100	700
	6	125	875
	7	150	1050
Regime 2	1 (control)	0	0
	2	25	194
	3	50	387
	4	75	581
	5	100	775
	6	125	969
	7	150	1162
Regime 3	1 (control)	0	0
	2	25	262
	3	50	525
	4	75	787
	5	100	1050
	6	125	1312
Regime 4	1 (control)	0	0
	2	25	600
	3	50	1200
	4	75	1800
	5	100	2400
	6	125	3000
Regime 5	1 (control)	0	0
	2	25	0
	3	50	0
	4	75	0
	5	100	0
	6	125	0

1 Conditions and treatments were the same for the light and dark experiments.

temperatures above 18°C from November 20, 1968 until June 20, 1969 when they were placed into the treatment chambers. Immediately prior to being placed in the chambers, all lateral growth was removed from the plants. Treatment conditions were exactly the same as in the previous experiment except that one of the chambers was operated without lights, thus providing a dark treatment. Treatment durations are listed in Table 1.

The useful capacity of the treatment chambers was increased by transferring the plants to a set of photoperiod cabinets in the greenhouse following treatment. From 0830 hr to 1630 hr daily, the plants received natural daylight, the remainder of a 15 hr photoperiod being supplied by the photoperiod cabinets. Observations were recorded in the same manner as in the previous experiment.

The light chilled plants and the dark chilled plants were considered as two separate experiments. The completely randomized design with seven treatments and five replications was used with a total of 35 plants in each experiment.

1969-1970 Experiment

Budded uprights were collected on October 17, 1969. Plants were prepared as in 1968 and were set 30 per box in 30 cm x 20 cm x 7 cm cedar flats. These were allowed to root in the greenhouse until initiation of treatments on October 31, 1969.

Two growth chambers and two modified cold rooms were used to provide four different chilling regimes. A warm temperature regime was provided in the photoperiod cabinets. A nine hour photoperiod was used for all treatment regimes.

Temperature Regimes 1 and 2 were supplied in Percival Model PGC-78 growth chambers equipped with Partlow Model RC-15 thermal controllers.

Both regimes had maxima of 10°C and minima of 2°C. Regime 1 reached the minimum once in 24 hr and Regime 2 reached it three times (Fig. 1)[1].

Temperature Regimes 3 and 4 were applied in the two cold rooms. In each case, a corner area approximately 1.0 m x 1.6 m was partitioned from the rest of the room by a black plastic curtain suspended from the ceiling, thus enclosing the bench on which the plants were located. Temperatures for Regime 3 were 13°C day, 4.5°C night; for Regime 4, 3°C day and 0°C night (Fig. 1).

Regime 5 was applied in the photoperiod cabinets. The ambient temperature was maintained above 18°C for the duration of the experiment. Plants received natural daylight from 0830 hr to 1630 hr daily, with an additional hour of low intensity illumination given as one half hour before and after the natural daylight.

Following the treatments, the plants were transferred to a greenhouse bench. To ensure a minimum 15 hr daylength, supplemental lighting was provided from 0630 hr to 2130 hr.

Observations were recorded as for the 1968-1969 experiment. In addition, the mean shoot growth of the plants within each box was calculated and plotted against time. The data for the linear portion of the resulting growth curves were used to calculate the simple regression coefficient, b, from the formula $y = a + bx$, where y = shoot length and x = time (days) following termination of chilling treatments. These b values were then used in an analysis of variance to compare differences in growth rates.

Two boxes of plants were used for each regime-treatment combination. Twenty plants from each box were measured; thus a total of 1080 plants were involved in this experiment. With the omission of the 150 day treatments in Regimes 1 and 2 and the unchilled plants (which did not show a

1 Regime 2 was used to investigate the effect of a fluctuating minimum.

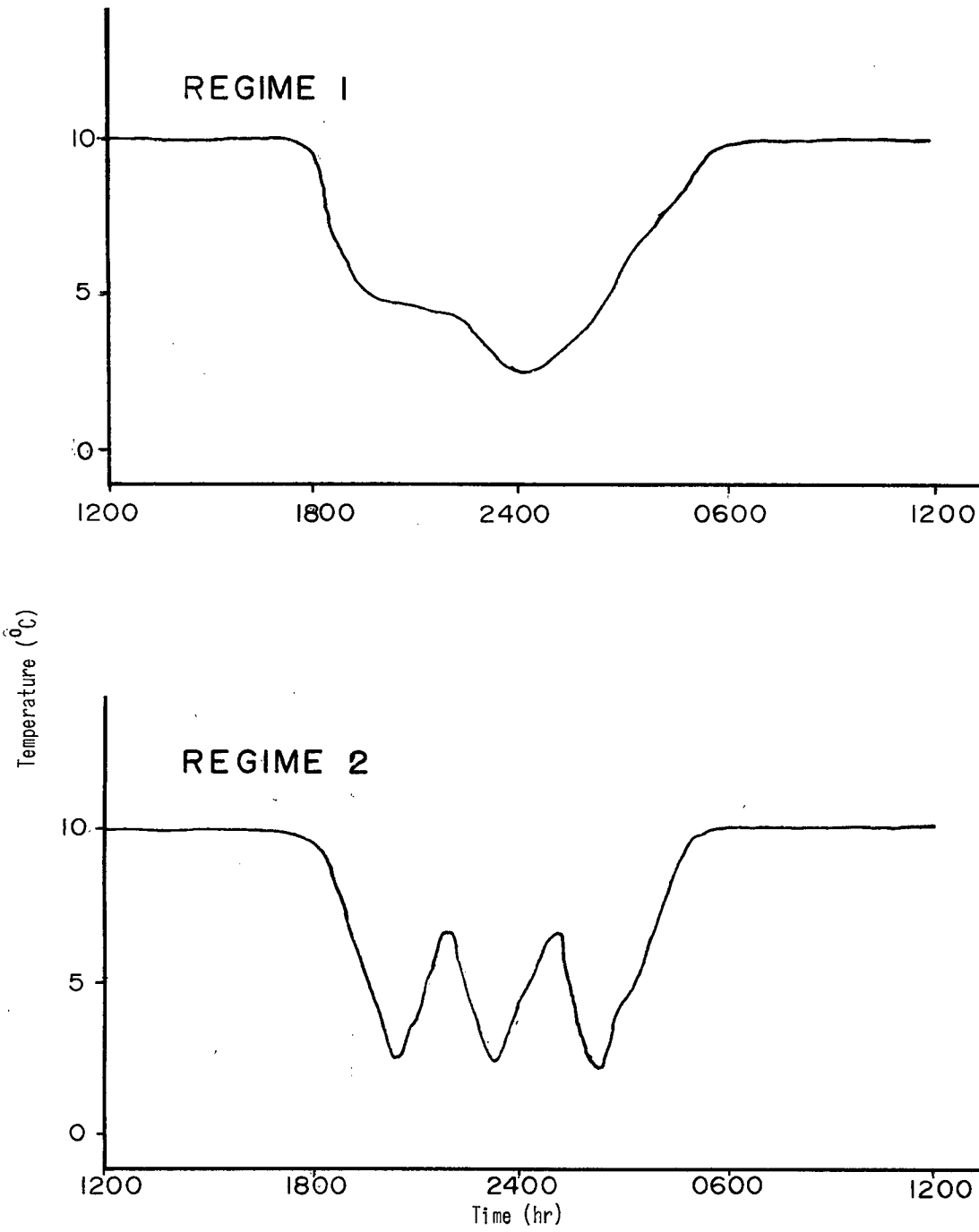
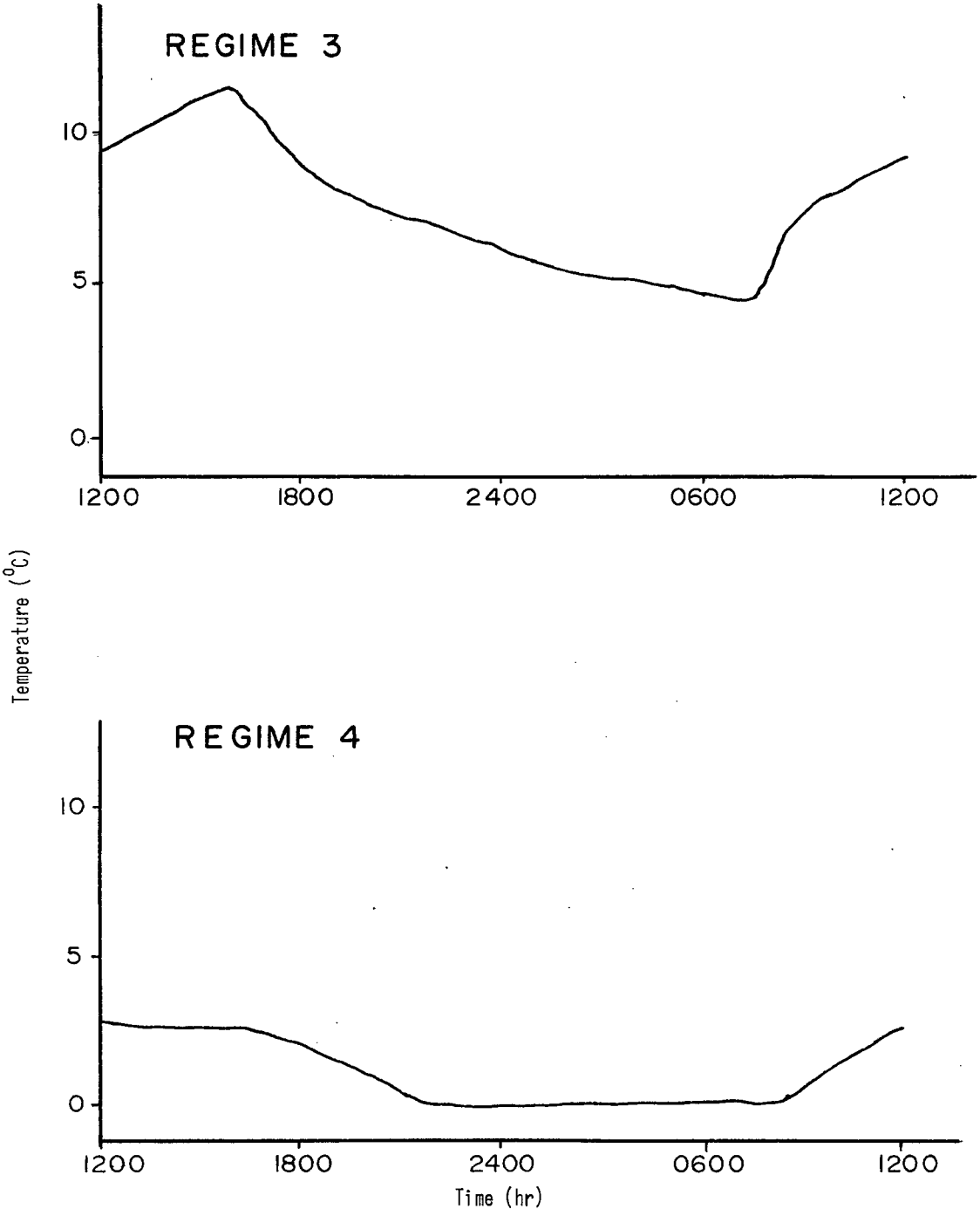


Fig. 1. Daily Temperature Curves for Chilling Regimes, 1969-1970 Controlled Environment Experiment.

Fig. 1. (Continued)



response), the data were analysed using a two-way analysis of variance. Because of the significant interaction between regime and treatment, a one-way analysis of variance within each treatment was used to compare the effect of the different regimes and vice versa to compare the effect of the different treatments.

Gibberellic Acid Experiment

Three sets of five plants, each collected October 4, 1968 and handled as previously described, were used for this experiment.

Treatments were initiated in the greenhouse on May 15, 1969 as follows:

Set 1	gibberellic acid aqueous spray 100 ppm
Set 2	" " " " 1000 ppm
Set 3	water control (0 gibberellic acid)

Daily application from May 15 to May 23, 1969 failed to have any visible effect on the plants. On June 8, 1969, a small incision was made 1 cm below the bud of each plant. One hundred microlitres of the appropriate treatment solution for the set was applied to each plant by means of repeated injections with a 10 microlitre syringe. The response of each plant was recorded.

Gibberellin-like Activity

Analysis of the quantity of compounds exhibiting gibberellin-like activity in V. macrocarpon was done on field-grown material for the 1969-1970 winter season.

Extraction, Purification and Separation of Gibberellin-like Compounds

Budded uprights were collected at various dates between October 1969 and June 1970. The material was collected directly onto dry ice and was stored at -20°C both before and after freeze-drying.

The quantity of terminal buds used for each extraction depended on the number present in the sample. Extraction of leaf tissue was made from 15-gram samples. A total of 11 bud samples and 12 leaf samples were done.

The extraction and purification of gibberellin-like compounds from the plant tissue is outlined in a flow diagram (Fig. 2). The first part is as adapted by Crozier et al. (1969) from Hayashi and Rappaport (1962).

All solvents with the exception of methanol were redistilled prior to use as a precaution against impurities which could cause gibberellin-like growth promoting activity (Hartley et al., 1969). Concentration to dryness was done in vacuo with a water bath temperature of 32°C.

The polyvinylpyrrolidone (PVP) column was adapted from that of Pharis[1]. A slurry of PVP was prepared in 0.1 M, pH 8.0 phosphate buffer, and a 19 mm glass column filled to a height of 20 cm. A layer of small glass beads was sprinkled on top of the PVP. The crude acidic fraction was taken up in phosphate buffer and transferred to the column. Three hundred ml of buffer were used to elute the column.

The charcoal-celite column[1] was prepared by mixing 10 g of Celite 545 (Fisher) with 5 g of Darco G-60 activated charcoal (Matheson). This was slurried with 80% acetone and placed in a 19 mm glass column. Glass beads were again used to receive the sample. The column was eluted with 250 ml of 80% acetone. To remove the water, it was necessary to add methanol when concentrating the sample.

Thin layer chromatography was done according to Reid et al. (1969), using Eastman-Kodak Silica-gel thin layer chromatography sheets. Aliquots of the acetone residue were taken up in methanol and streaked onto the sheets. The chromatogram was developed with ethyl acetate:chloroform:formic acid (50:50:1). The sheets were air-dried and cut into ten equal strips

1 R.P. Pharis, personal communication, 1970.

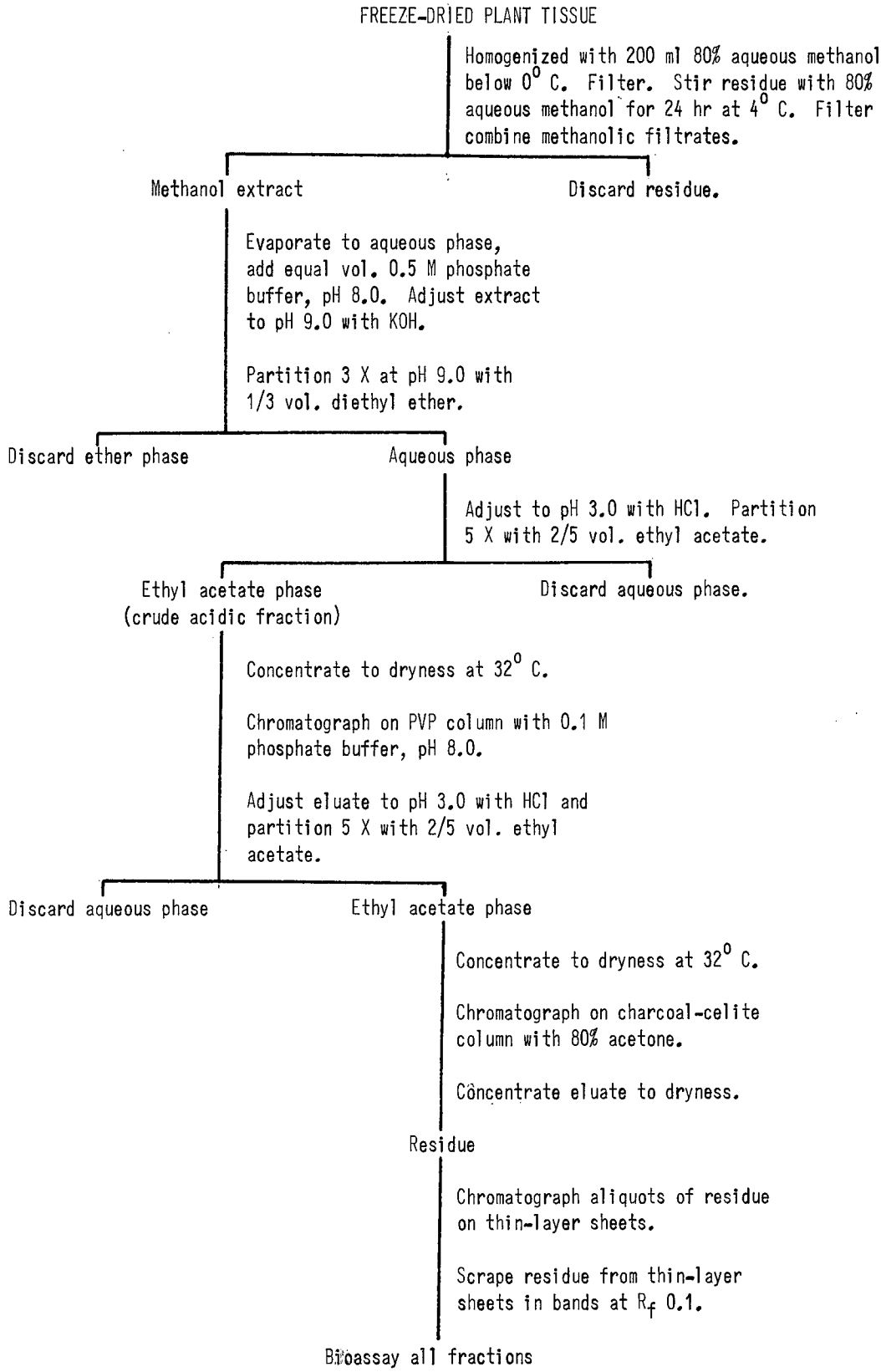


Fig. 2. Flow Diagram for Extraction, Purification and Separation of Gibberellin-like Compounds from Plant Tissue.

between the line of application and the solvent front; thus each strip represented $R_f = 0.1$. The silica-gel was scraped from the strips (including the starting line as a separate strip, $R_f = 0$) and used in the bioassay.

Bioassay

The lettuce hypocotyl bioassay (Frankland and Wareing, 1960) was used to estimate the amount of gibberellin-like activity in each fraction. Lettuce seeds cultivar Arctic were used. The silica-gel from the chromatograms was placed in 6.0 cm x 1.5 cm petri dishes lined with filter paper. These were moistened with 3.0 ml of a one-quarter strength nutrient solution (Machlis and Torrey, 1956). A series of gibberellic acid (GA_3) standards was made up using the same nutrient solution and were also added to petri dishes in 3.0 ml quantities.

The lettuce seeds were germinated in the dark for two days. Ten of these seedlings were added to each petri dish. Both the unknowns and the standards were kept under continuous light for three days, after which the hypocotyl lengths were measured.

Gibberellin Recovery

The recovery of known amounts of gibberellic acid (GA_3) was checked using the above methods. The rate of recovery using gibberellic acid alone or mixed in with plant material was 100%.

RESULTS

Anatomy of the Terminal Bud

Examination of the terminal bud sections for the two winter periods revealed that the flower buds had overwintered at different stages of development. The upper portion of Fig. 3 shows a longitudinal section of a flower bud collected January 28, 1969. The lower portion of the same figure shows a flower bud collected October 17, 1969. On October 17, 1969, the stamen and carpel primordia were more developed than those of the previous winter, even though the previous winter's flower buds were collected at a later time.

In each of the two winters for which terminal buds were examined, the flower buds appeared to be dormant until early March. Fig. 4 shows the stages of development observed for buds collected on March 9, May 4 and May 19, 1970. In comparing the March 9, 1970 section to that of October 17, 1969, it is readily seen that the ground meristem below the flower in Fig. 4A has more cell elongation. Cell elongation is most likely a phenomenon which marks the onset of flower development. Hence, it would appear that flower bud development begins in late February or early March.

By the end of April in each year, the flower buds had differentiated to about the same extent. The May 19, 1970 section shows the stage of development reached just prior to the elongation of the terminal buds in the field.

Field Temperature

Field temperature data were recorded from October 20, 1969 to May 4, 1970. Only the information relating to the plant material collected from the field is presented here. Accumulated hours below 7°C from October 20, 1969 (read from thermograph charts), along with the maximum and minimum

Fig. 3. Vaccinium macrocarpon flower bud (l.s.) to show winter dormant stages. A. Collected from field January 28, 1969; B. Collected October 17, 1969.

Abbreviations: c = carpel, s = stamen

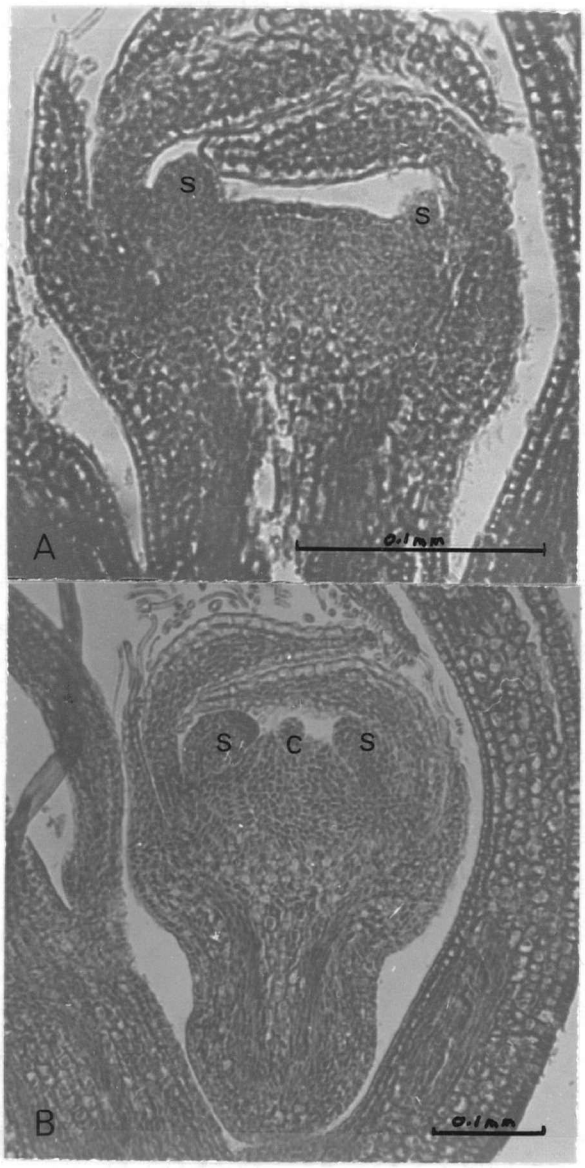
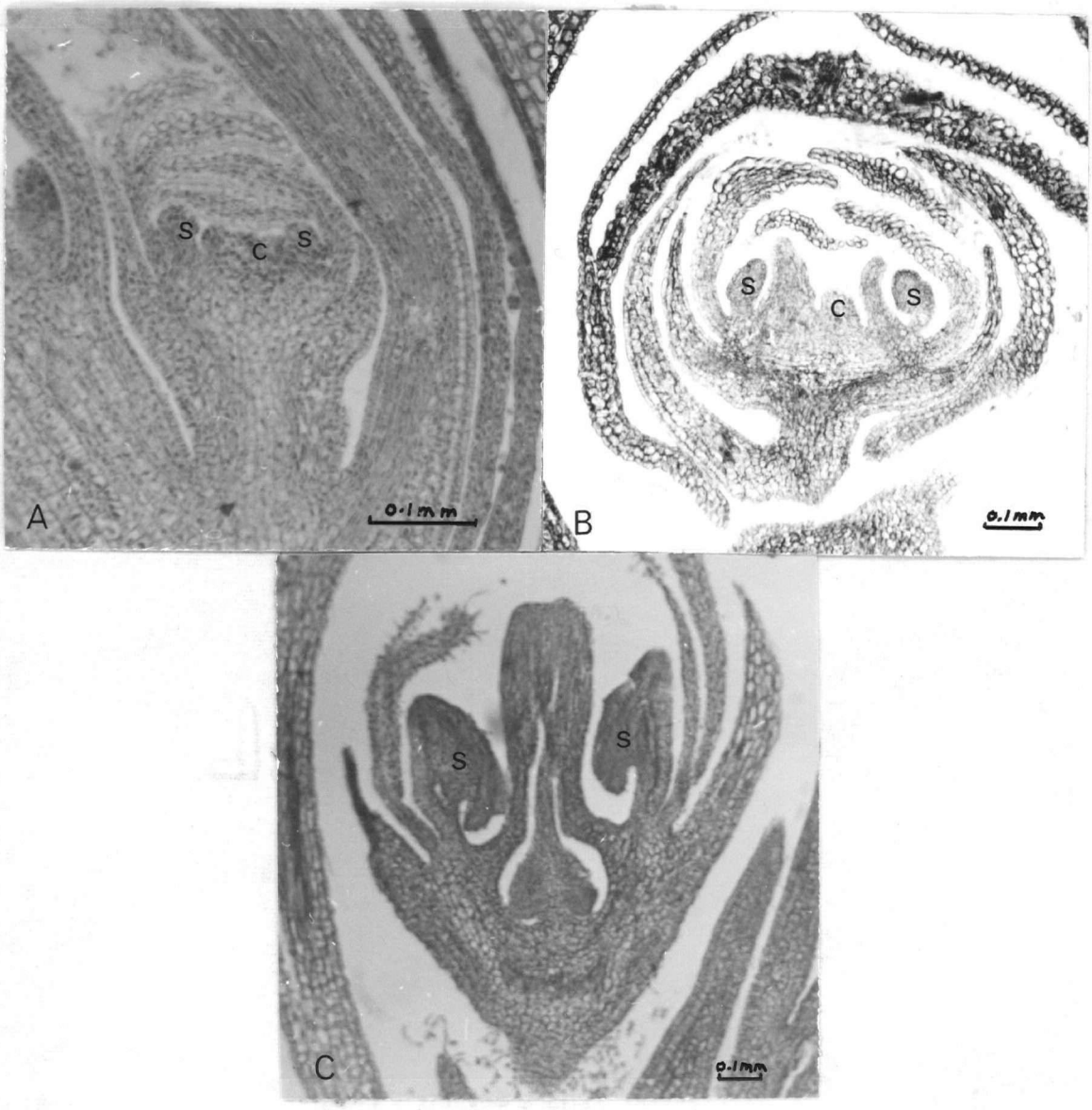

S
S
A
S
C
S
B

Fig. 4. Vaccinium macrocarpon flower bud (l.s.) to show stages of spring development. A. Collected from field March 9, 1970; B. Collected May 4, 1970; C. Collected May 19, 1970. Abbreviations: c = carpel, s = stamen


S
C
S
A
0·1mm
S
C
S
B
0.1mm
S
S
C
0·1mm

temperatures for the week preceding each plant collection date, are presented in Table 2. Records are missing from March 24 to 26, 1970 and were not available for the period immediately following May 4, 1970.

Controlled Environment Studies

1968-1969 Experiment

The portion of the data dealing with the mean number of days to bud break has been published (Eady and Eaton, 1969), along with some observations on the growth of the treated plants. The data from this experiment are summarized in Table 3.

No macroscopic evidence of terminal bud activity was observed in the unchilled plants. The mean number of days to bud break was decreased from 44 days after 650 hr chilling to 9 days after 2500 hr chilling (statistically significant, $P = 0.05$)[1]. Mean shoot length at the completion of the observation period (100 days after termination of chilling) and number of flowers per plant were not significantly different, with the exception of the unchilled treatment. The number of plants flowering under these conditions reached 100% after 1300 hr of chilling.

There was no significant difference in any of the plant responses between the two treatment chambers.

1969 (Summer) Experiment

Plant response to both the light and the dark chilling treatments was poor. Initiation of new shoot growth following treatments occurred only in a few instances (Table 4). Buds held for months without chilling had thus

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1 The 5 percent level of significance was used throughout this section. The term "significant" therefore indicates $P = 0.05$. The analysis of variance tables are presented in Appendix 2.

Table 2.

Field Temperatures[1] for 1969-1970

Plant Collection Date	Accumulated hr below 7° C (from Oct. 20/69)	Maximum Temp. (°C)	Minimum Temp. (°C)
Oct. 17/69	not available	-	-
Nov. 18/69	194	10.0	0.0
Dec. 18/69	680	9.0	0.0
Feb. 3/70	1564	8.5	-5.0
Feb. 23/70	1934	12.0	-5.0
Mar. 9/70	2177	12.0	-7.5
April 6/70	2438[2]	14.0	-3.5
April 27/70	2688	11.0	0.5
May 4/70	2777	18.0	1.0
May 19/70	not available	-	-
June 22/70	not available	-	-

1 Maximum and minimum temperatures are for a one week period preceding each plant collection date.

2 Records were not available for March 24, 25 and 26, 1970.

Table 3.

Mean Number of Days to Bud Break, Shoot Length, and Flowers per Plant in *V. macrocarpon*, 1968-1969 Chilling Treatments[1]

Chilling (hr)	Days[2] (mean no.)	Shoot Length (cm)	Flowers/Plant (mean no.)	Plants Flowering (%)	Plants Treated (no.)
0	no bud break	0	0	0	6
650	44 d	7.9	1.1	50	6
1300	27 c	8.4	1.7	100	6
1700	23 c	7.8	1.3	100	6
2100	14 b	9.1	1.3	100	6
2500	9 a	11.8	1.3	100	6

1 After Eady and Eaton (1969).

2 Means sharing the same letter did not differ significantly (P = 0.05).

apparently lost the ability to respond to the chilling treatments provided.

Table 4.

Number of Plants Initiating New Terminal Growth Following Completion of Chilling Treatments, 1969 (Summer) Experiment

Experiment	Chilling (hr)	No. of Plants Initiating New Growth (out of 5)
light	1700	1
"	2100	1
dark	650	2
"	1300	2
"	1700	1

No macroscopic evidence of flower bud development was found on any of the plants which had broken dormancy, their growth being entirely vegetative.

The light chilled plants retained their leaves both during and after chilling. Most of the dark chilled plants lost their leaves either during or shortly after the completion of the chilling treatments.

1969-1970 Experiment

In all of the chilling treatment regimes, there was an increase in the number of terminal buds breaking dormancy in response to longer chilling times (Table 5). The analysis of variance indicated a statistically significant interaction between the chilling regime and the length of time chilled. This indicates that the plants did not respond at the same rate for each regime. When the data were reanalyzed, the only significant difference between regimes occurred for the 75 day treatment, where Regimes 1

Table 5.

Average Percent Bud Break of V. macrocarpon, 1969-1970 Chilling Treatments[1]

Regime	Total Number of Days Chilled						Standard Error of the Mean
	25	50	75	100	125	150	
1	32.5 a	52.5 ab	77.5 bc	85.0 c	100 c	92.0 c	8.00
2	10.0 a	52.5 b	52.5 b	90.0 c	100 c	98.0 c	4.86
3	0 a	15.0 a	37.5 b	82.5 c	87.5 c	-	4.33
4	20.0 a	62.5 b	85.0 b	82.5 b	90.0 b	-	9.33
5	5.0	7.5	5.0	10.0	5.0	-	-

1 Means within rows sharing the same letter or letters did not differ significantly (P = 0.05). Data from Regime 5 were not included in the statistical analysis.

and 4 were significantly different from Regimes 2 and 3.

Some of the terminal buds in Regime 5 initiated new growth (Table 5). In all of these plants, the resulting growth was vegetative only and displayed the characteristics of runner growth rather than that of uprights. This would indicate that from 5-10% of the terminal buds were vegetative rather than mixed buds. The data for Regime 5 were not included in any of the statistical analyses.

The mean number of days from termination of the chilling treatment until the first 25 percent of the terminal buds had broken dormancy (all of the terminal buds where the percent bud break was less than 25) was plotted against length of time in the chilling treatments for each regime (Fig. 5). Again there was a statistically significant interaction. The mean values for each of the four regimes changed in their relative order depending on the length of chilling. The means that did not differ significantly after the same length of chilling are indicated in Fig. 5. A significant reduction in the number of days to bud break occurred with each increased chilling period for Regimes 1 and 2. In Regime 3, added chilling up to 100 days caused a significant decrease. In Regime 4, the treatment differences were not as pronounced; the 25 day treatment was significantly different from all the others, 50 days was not from 75, 75 days was not from 100, and 100 days was not from 125. In Regimes 1 and 2, a few plants initiated new terminal growth while still in the growth chambers.

Growth of the plants following removal from chilling was linear with time during the first phase (Fig. 6). The growth rates, which were calculated as the linear regression coefficient for this period, are listed in Table 6. There was not a significant interaction between regimes and chilling times, and the difference between regimes was not significant.

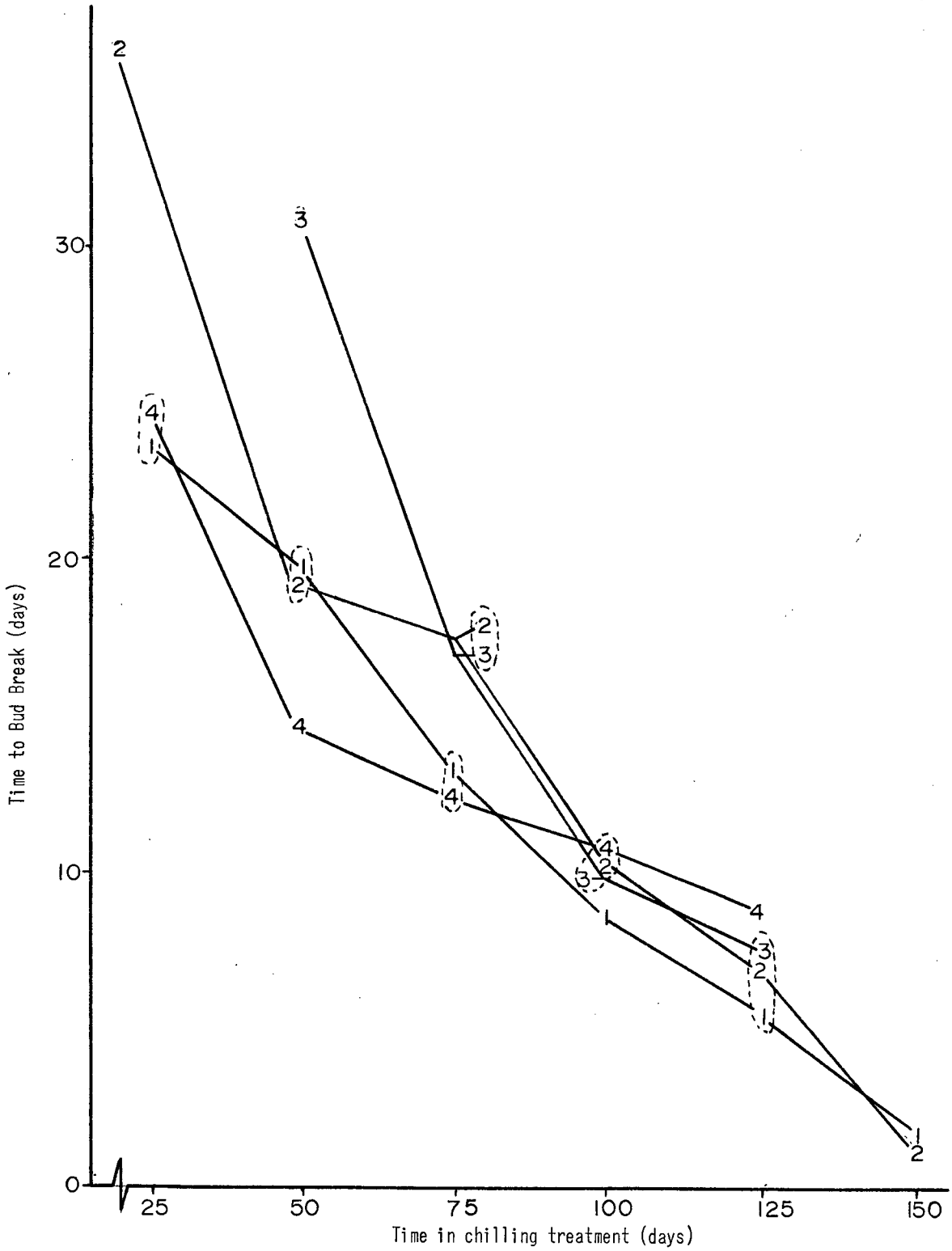

Fig. 5. Number of Days to Bud Break Following Termination of Chilling Treatments, Regimes 1-4, 1969-1970 Controlled Environment Experiment.
Means of symbols encircled by the same dotted line did not differ significantly ($P \doteq 0.05$).

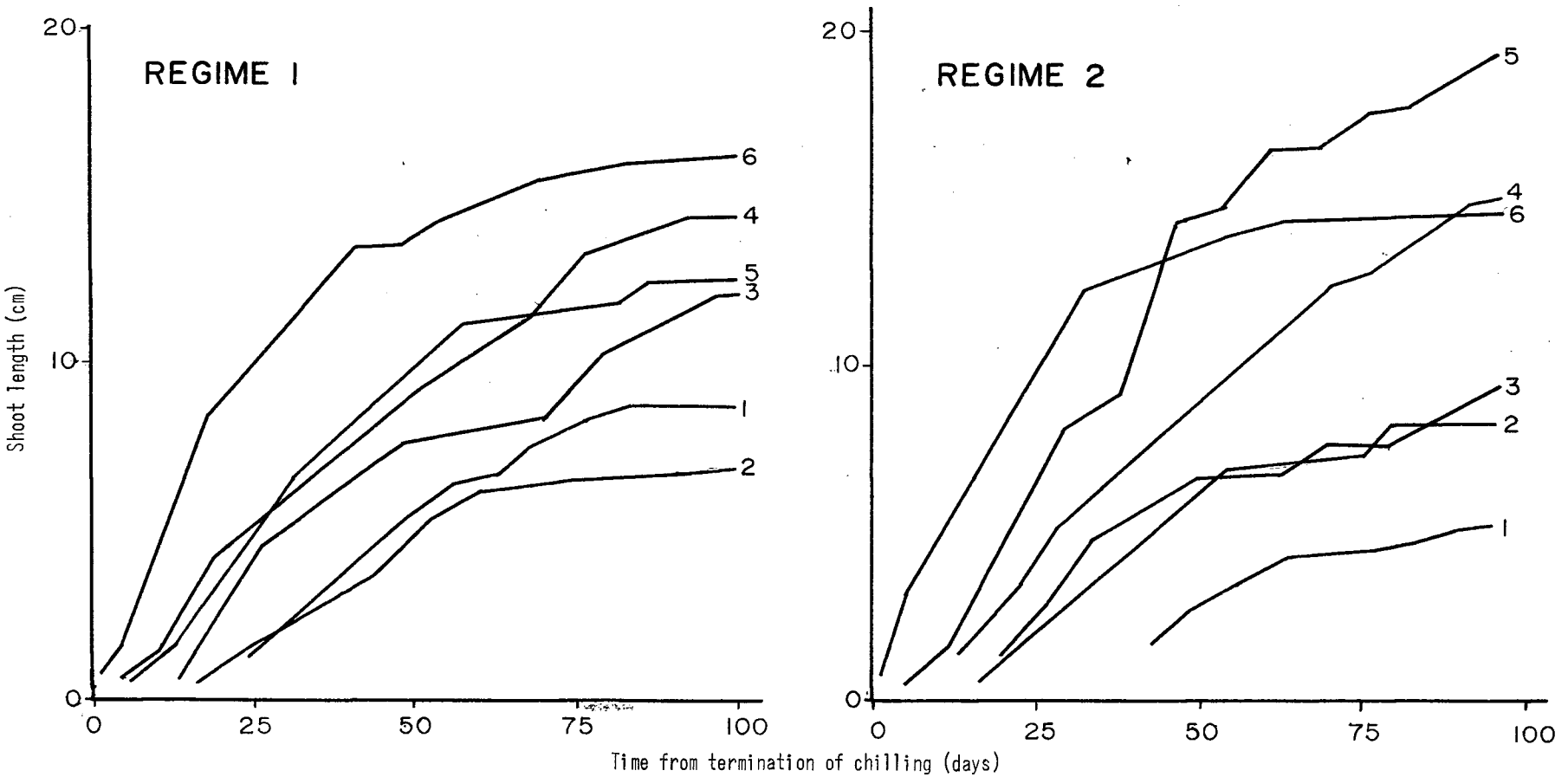

Fig. 6. Shoot Growth Following Termination of Chilling Treatments, 1969-1970 Controlled Environment Experiment.

Legend; 1=25 days chilling, 2 = 50 days, 3 = 75 days, 4 = 100 days, 5 = 125 days, 6 = 150 days.

Fig. 6. (Continued)

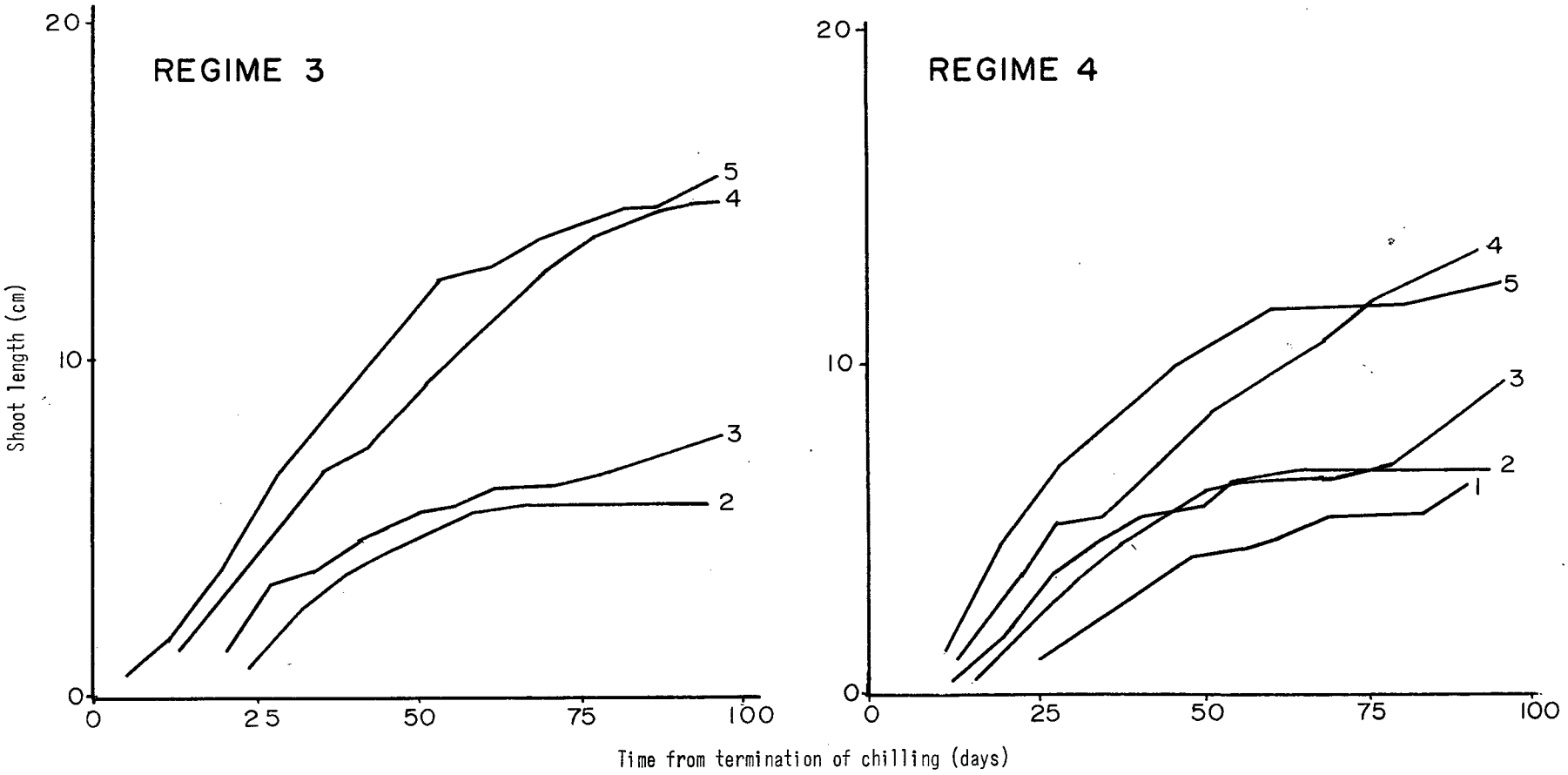

Table 6.

Growth Rates (cm/day) for the Linear Portion of the Growth Curve of *V. macrocarpon*, 1969-1970 Chilling Treatments[1]

Regime	Total Number of Days Chilled						Standard Error of the Mean
	25	50	75	100	125	150	
1	.151 a	.164 ab	.271 bc	.279 c	.241 abc	.407 d	.030
2	.113 a	.171 ab	.228 ab	.263 bc	.364 c	.344 c	.032
3	-	.241 a	.190 a	.249 a	.291 a	-	.056
4	.062 a	.180 b	.212 b	.214 b	.290 b	-	.031
Average	.082 a	.189 b	.225 bc	.251 cd	.296 d	-	.018

1 Means within rows sharing the same letter or letters did not differ significantly (P = 0.05).

There was a general increase in growth rate as the treatment time was increased. There was no bud break after only 25 days in Regime 3. The remaining treatment times in this regime were not significantly different from each other. In Regime 4, the only difference was that the 25 day treatment resulted in slower growth rates than any of the other treatments.

The mean length of the new shoot growth at the end of the experiment (100 days after removal from the chilling treatment) generally increased with longer chilling periods (Table 7). There was a significant interaction between regimes and the chilling times. The only significant difference in response between regimes for the same treatment times occurred for the 75 day treatment where the plants in Regime 1 had a mean length significantly greater than those of the other regimes.

Flowering did not occur after any of the 25 or 50 day treatment times and only occurred in the coldest regime (4) after 75 days (Table 8). The number of plants flowering was significantly increased with time chilled for Regimes 1, 2 and 3. The flowering response in Regime 4 was not increased significantly with the additional chilling.

Gibberellic Acid Experiment

There was no response to the aqueous foliar spray of gibberellic acid. When the same material was injected into incisions in the stems, the terminal buds of the gibberellic acid treated plants responded within 10 days. The terminal buds of the responding plants elongated and leaves developed, but there was no visible flower bud development.

Gibberellin-like Activity

The recovery of known amounts of gibberellic acid by the methods used

Table 7.

Mean Shoot Length (cm) of *V. macrocarpon* 100 Days after Removal from 1969-1970 Chilling Treatments[1]

Regime	Total Number of Days Chilled						Standard Error of the Mean
	25	50	75	100	125	150	
1	7.9 a	6.8 a	12.3 a	14.5 a	12.3 a	16.1 a	1.83
2	5.1 a	7.9 ab	9.3 b	15.0 c	19.2 d	15.5 c	0.83
3	-	5.7 a	7.6 a	14.9 b	15.2 b	-	1.42
4	3.1 a	6.7 ab	9.3 bc	13.2 c	12.2 c	-	1.44

1 Means within rows sharing the same letter or letters did not differ significantly (P = 0.05).

Table 8.

Mean Number of Flowers per 20 Plants, 1969-1970 Chilling Treatments[1]

Regime	Total Number of Days Chilled						Standard Error of the Mean
	25	50	75	100	125	150	
1	0	0	0	10.5 a	12.5 a	29.0 b	2.48
2	0	0	0	2.5 a	15.0 ab	39.0 b	6.07
3	0	0	0	6.0 a	14.0 b	-	1.00
4	0	0	4.0 a	2.0 a	14.0 a	-	6.07

1 Means within rows sharing the same letter or letters did not differ significantly (P = 0.05).

was 100 %. The minimum quantities of gibberellin-like activity detected with the lettuce hypocotyl bioassay were equivalent to 5×10^{-3} micrograms of gibberellic acid (GA_3) activity. The standard error of the estimate was approximately 2×10^{-2} micrograms of gibberellin-like activity.

In most of the samples, activity was established using 1/10th and 1/20th aliquots. The use of 1/4 aliquots gave unsatisfactory results probably because of the presence of inhibitors which were diluted out at the lower levels. The minimum detectable activity of any one sample was based on a 1/10th aliquot, and was directly related to the original dry weight of the sample. Sample dry weights and the calculated minimum detectable levels are presented in Appendix 3.

A considerable reduction in the dry weight of the sample after methanol extraction was obtained through the use of PVP and charcoal-celite chromatography columns (see Appendix 3).

Fig. 7 shows the gibberellin-like activity of extracts from both terminal buds and leaves. Where the activity was too low to be detected, the calculated value for the minimum detectable quantity was substituted to indicate that the true value was below that figure. The activities followed the same general pattern, with the notable exception of the March 9 and April 6 samples where a reduction in the leaf level was accompanied by an increase in the bud level. The reduction in the levels on February 23 coincided with a period of low night and fairly high day temperatures for the previous week, but there is no evidence for a direct causal relationship.

The gibberellin-like activity did not always occur at the same R_f values (thin-layer chromatography) for each sample. Much of the activity did occur between R_f 0.2 and 0.6, but activity in other fractions was not

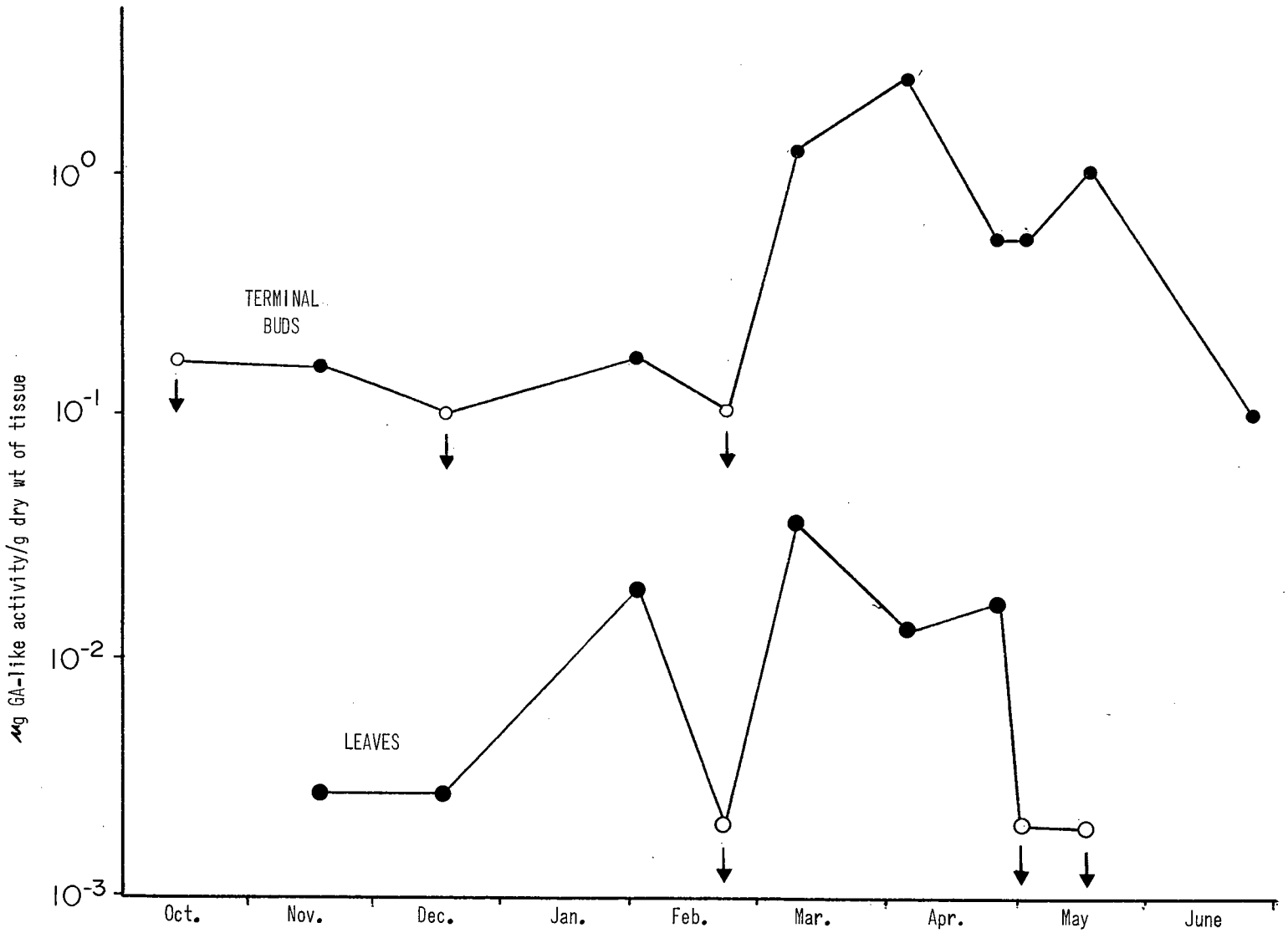

Fig. 7. Gibberellin-like Activity of Substances Extracted from Leaves and Buds of V. macrocarpon.

Legend: ● = observed data point, ○= activity not detected at the minimum level shown.

uncommon. Known samples of gibberellic acid (GA_3) showed activity between R_f 0.2 and 0.4 with the solvent system used. GA_7 showed activity between R_f 0.3 and 0.5.

Histograms of activity from the terminal bud and leaf extracts for March 9 and April 6 are shown in Fig. 8. The leaf sample for March 9 showed high activity at R_f 0.2 to 0.4, which decreased considerably by April 6 with a corresponding rise in the same region for the bud samples. It is possible that a transfer of these substances from the leaves to the terminal bud had taken place.

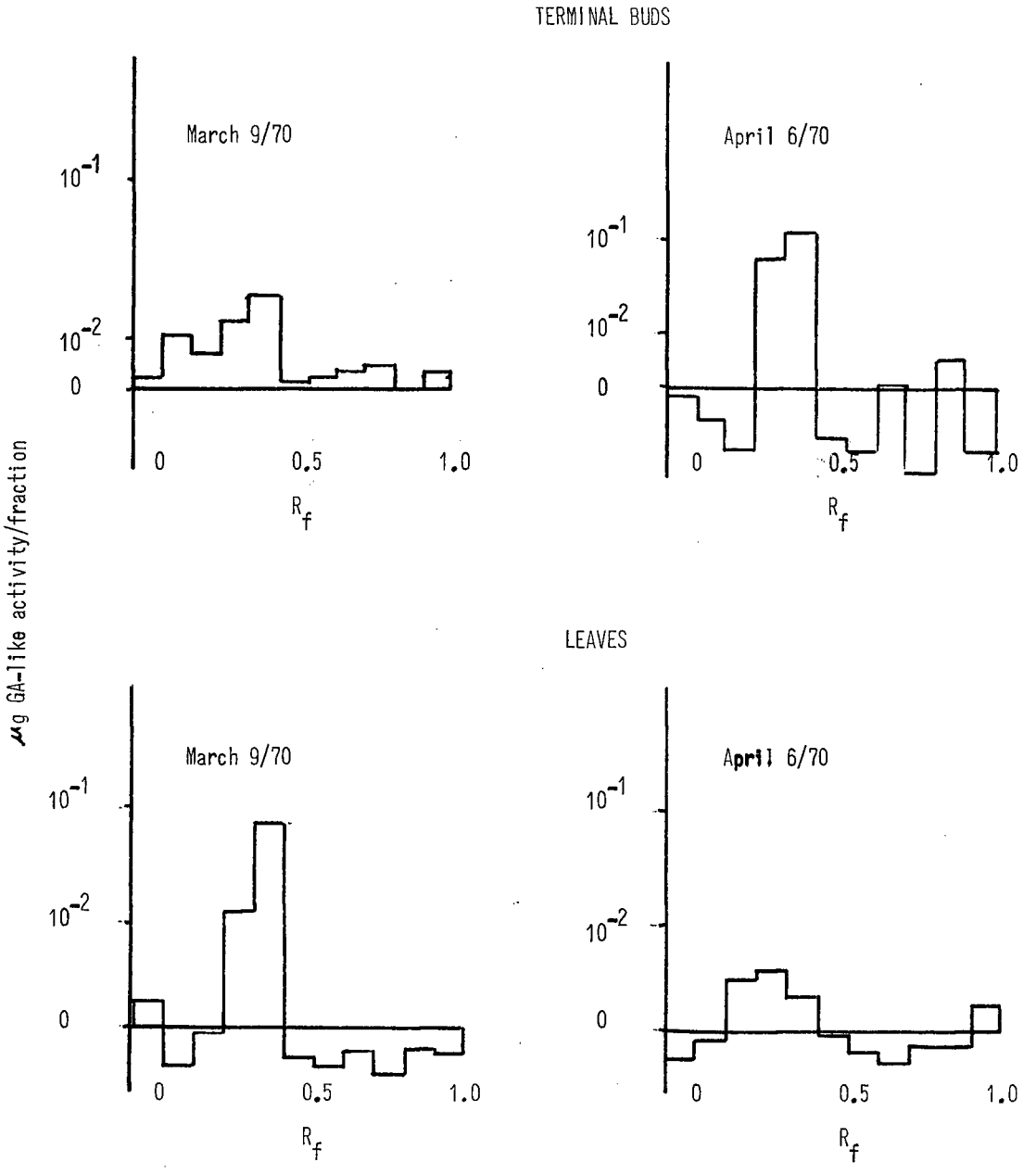

Fig. 8. Histograms of Gibberellin-like Activity of Substances Extracted from Leaves and Buds of V. macrocarpon. Samples illustrated were 1/10 aliquots, dry weights of tissue were as follows: Buds; March 9/70 = 0.3429 g, April 6/70 = 0.7051 g.
Leaves; March 9/70 = 15.00 g, April 6/70 = 15.00 g.

DISCUSSION

The post-dormant response of the terminal bud of *V. macrocarpon* to chilling temperatures during the dormant period is quite evident. The obligatory nature of the chilling requirement was demonstrated by the failure of the mixed terminal buds to break dormancy when maintained under non-chilling conditions during the winter period. The failure of the terminal bud to respond to varying photoperiods under non-chilling conditions was reported by Eaton and Ormrod (1968), who investigated the effect of photoperiod on the non-terminal vegetative growth.

Of the vegetative responses measured, the most evident effect of chilling was a decrease in the number of days to bud break. This response is the first visible result of the chilling treatment and is therefore probably the one which has the least number of intervening factors affecting it. The greatest responses were obtained in those chilling treatments which had a suitable balance between inductive chilling temperatures and a warm part of the cycle, which would allow a certain amount of activity to occur within the plant. This activity was evident in those plants which broke dormancy before completion of the 150 day chilling treatments. There is an apparent levelling off in the number of days to bud break with longer chilling times in Regime 4. One possible explanation is that in the absence of longer periods of higher temperatures, very little activity can occur during the chilling treatment period; therefore the time lag between termination of the chilling treatment and bud break is not reduced as rapidly as in the other regimes. The fact that some of the plants initiated new shoot growth before the termination of the 150 day chilling treatments in the 1969-1970 experiment is in itself evidence that some development took place under these particular chilling conditions. It is possible that as far as the

vegetative development of the plant is concerned, the chilling requirement is fulfilled quite early and that the additional response to further time in the chilling treatment is due to extra growth and development that took place during that time and not as a direct result of the additional chilling itself.

The rate of growth and the shoot length attained were less dependent on the chilling treatment received than was the reduction in the number of days to bud break. The more rapid rate of growth after some of the longer chilling periods is again more likely a result of the activity of the plants during warm periods in the chilling cycle rather than a direct effect of chilling itself. Both the rate of growth and shoot length attained would be extremely dependent on the post-dormant conditions, which were maintained reasonably uniform for these experiments.

The floral primordia responded somewhat differently to the chilling treatments than did the vegetative parts of the terminal bud. In all of the chilling treatments given in 1969-1970, the shorter treatment times resulted only in vegetative growth of the terminal bud. A longer period was necessary to induce further development of the floral primordia. This difference was not as evident in the 1968-1969 treatments. There are at least two possible reasons for this. First, there may have been an effect of spacing on the response of the plants and, secondly, the warm part of the chilling cycle was quite short in the 1968-1969 treatments, thus allowing for more actual hours of chilling in a day while still providing a period of reasonably high temperature.

It is possible that the fairly long exposure of the plants to higher temperatures during the chilling cycle in Regime 3 was resulting in a partial devernalization. Devernalization has been reported in other plant

species as a result of exposure to high temperatures immediately after vernalization. Salisbury (1963) noted that temperatures above 35°C are most effective for devernalization, but that in rye temperatures above 15°C begin to have a devernalizing effect. The exposure of the plants to 13°C during the light period could be a major factor in explaining the marginal response patterns for Regime 3.

Flowering occurred in V. macrocarpon following as few as 650 hr below 7°C during the 1968-1969 experiment. The longest actual exposure to temperatures below 7°C before induction of flower development occurred in Regime 4, where 1800 hr of exposure was required. The additional chilling requirement for flower formation suggests that the vegetative and floral portions of the terminal bud may not have closely integrated chilling requirements. There is also the possibility of a consecutive relationship whereby part or all of the vegetative requirement must be fulfilled before the floral portion of the bud becomes receptive to the chilling stimulus.

Examination of the development of terminal buds from the field revealed that a considerable amount of differentiation of the floral primordia occurred prior to bud break during periods when the temperatures in the field were quite low.

It has been suggested that vernalization takes place only in cells which are undergoing mitosis (Wellensiek, 1962, 1964). It is possible that the initial development of the flower bud primordia of V. macrocarpon must take place when the plant is still being exposed to chilling temperatures. If this is true, it would explain the lack of flowering in plants that were removed from the chilling treatments prematurely. The fact that a longer period of exposure to actual temperatures below 7°C was required for the plants chilled in Regime 4 to flower could be related to the retarding

effect of the overall temperatures of that regime. The maximum temperature of 7°C would not allow rapid development to take place within the plant.

For both vegetative and floral chilling requirements to be efficiently fulfilled, it would appear that not only is the amount of low temperature exposure of importance but also the fluctuating nature of the temperatures during the chilling period. In general, if the overall temperatures are too low, the chilling reauirement appears to become saturated, and additional chilling will not result in as great an increase in the response that would occur under warmer conditions. If overall temperatures are too high, even though a good portion of the daily cycle is low enough to satisfy chilling requirements from a thermal standpoint, there may be an effect of devernalization. A balance between exposure to low temperatures and a warm part of the cycle to allow some activity in the plant appears to be best suited to filling the dormancy requirement. Because of the varying effect of the chilling temperature cycles, it is not possible to say that either the vegetative or floral chilling requirement appears to be fully met by a certain time period under so many degrees C, unless the actual treatment conditions are specified. For this reason, it is extremely difficult to predict when these requirements would be fulfilled under field conditions.

That the floral and vegetative chilling requirements may be separate mechanisms in V. macrocarpon is supported by the results of the experiment in which unchilled plants were induced to initiate terminal growth in response to application of gibberellic acid. If the two chilling requirements were one and the same, it would be expected that flowering would also occur in response to the gibberellic acid. The fact that the induced growth was only vegetative also suggests that gibberellic acid is releasing a mechanism

that is separate from that involved in flower induction.

The role of an applied plant growth substance in breaking dormancy may or may not be related to the natural mechanism. There is a possibility that the action of applied gibberellic acid was a substitution effect. In order to determine the possible role of gibberellins in breaking dormancy, it was necessary to have some information on the amounts of endogenous gibberellin-like activity in V. macrocarpon for the dormant and immediate post-dormant period. There is a general increase in the levels of activity in both buds and leaves apparent in early March. The continued increase of this activity in the buds and the decrease in activity in the leaves demonstrated for the April 6, 1970 sample suggest a translocation of the gibberellin-like substances. Further support for this hypothesis is obtained by examining the histograms of activity in leaves and buds for the March 9, 1970 and April 6, 1970 sample dates. The increased activity of the buds on April 6 was in the same R_f as was the high activity for the leaves on March 9. Because this apparent translocation occurs after flower development has begun, it seems unlikely that gibberellins are involved in flower production following dormancy. This is further substantiated by the failure of unchilled V. macrocarpon plants to flower in response to gibberellic acid. Fontes et al. (1970) also have reported that gibberellins seem not to be involved in flower induction in broccoli. Halaban et al. (1965) concluded that the response of Ornithogalum apices to gibberellin is conditioned by the thermal regime. Jones and Stoddart (1970) concluded that the primary action of gibberellic acid in the shoot apex of red clover was not flower induction but the induction of synthesis of specific proteins.

The delay observed between application of gibberellic acid to dormant unchilled V. macrocarpon plants and the visible growth response could

occur if the immediate action of applied gibberellic acid was to induce protein synthesis in the shoot apex. It is difficult to predict how long a delay between the occurrence of increased amounts of endogenous gibberellin-like substances in the terminal bud and the initiation of visible activity would take, but some delay should be expected. Jones and Stoddart (1970) reported a delay of 4 days between application of gibberellic acid and protein synthesis.

The different stages of development reached by the flower bud primordia prior to the onset of dormancy in 1968 and 1969 suggest that their cessation of development is not under internal control, and that the initiation of dormancy in the terminal bud relies on the response of the vegetative portion to an external stimulus. Once this stimulus has caused the bud to enter the dormant state, the control of dormancy is internal until the chilling requirement has been satisfied.

Vegetative growth is initiated following shorter chilling periods than are required for the initiation of floral growth under artificial conditions. In the field, however, vegetative growth is not initiated until after the flower bud primordia have begun their development.

This flexibility in the order of vegetative and floral responses to chilling suggests that their respective primordia are under independent control mechanisms. The apparent translocation of gibberellin-like substances to the terminal bud prior to elongation further suggests that while the control of dormancy may remain within the bud itself, there is under natural conditions a reliance on the leaves to supply the necessary stimulus to initiate vegetative growth.

Vegis (1964) suggested that "for better understanding of the origin of the dormant condition in plant organs one should always take into

consideration that dormancy is the result of a highly useful adaptation to the environmental conditions which prevail where the species or variety originates." V. macrocarpon is indigenous to Eastern North America (Porsild, 1938); thus it is not surprising that the plant has a chilling requirement which ensures that dormancy is maintained throughout the winter. The effect of the chilling requirement on the range of this species has been twofold. The obvious effect is that chilling has been a factor in limiting the southern extent of the natural range of V. macrocarpon, which occurs occasionally as far south as Arkansas (Fernald, 1950). The chilling requirement may also play a role in limiting the northern range of the species.

Neither of these range limitations, as such, has been of concern in the cranberry production industry, and therefore they have not been extensively studied. The problem of southern range limitations due to lack of chilling has been studied in a number of other horticultural crops such as blueberries (Darrow, 1942), grapes (Magoon and Dix, 1943), and peaches (Weinberger, 1950a, 1950b). The general conclusion for all of these crops was that lack of chilling was the major factor in limiting the southern range for commercial fruit production. This is also true for V. macrocarpon.

The role of the chilling requirement as a factor in limiting the northern range of the species is somewhat more complex. In the more northern latitudes, the absolute minimum temperatures would most likely be responsible for the plants not surviving, but this may not be the case near the border of the northern limit for the species. Doughty[1] observed

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1 C.C. Doughty, personal communication, 1971

that after 557 hr below 4.5°C in the field, V. macrocarpon could withstand temperatures as low as -20.5°C before more than 25 percent of the buds were injured. This occurred on January 30, 1966 under Washington State conditions. By February 25, the temperature which would cause 25 percent injury was -12.5°C, and by March 17 was -4.0°C. The susceptible period to frost injury apparently occurs after the flower bud primordia have recommenced their development in the spring. This development could be induced by relatively warm day temperatures after the minimum chilling requirement has been met. It is quite possible that the northern range of the plant could be limited not by the absolute minimum temperatures encountered during the winter months but by the occurrence of low spring temperatures which would cause injury after early fulfilment of the chilling requirements. What may then happen is that the susceptibility of the plant to frost injury may increase at a faster rate than the rise in minimum temperatures for the region. Considerable damage to the buds could result if the susceptible temperature was higher than the regular minimum temperatures.

In British Columbia, development of the floral primordia is well under way in March and by the end of April considerable differentiation has taken place; thus the crop is quite susceptible to frost damage at a time of year when minimum temperatures sufficiently low to cause damage are not uncommon. The currently recommended practice in Canada is to irrigate until the danger of frost damage is past (Hall, 1969). Because of the complex nature of the factors involved, determination of the susceptibility of the crop to frost injury is difficult, and for this reason there are no real guidelines as to when frost protection should be initiated. This is of concern to the commercial grower in that the operation of the irrigation equipment while the plants are still hardy unnecessarily removes from his profits.

There are two possible approaches that might be used in decreasing the period of irrigation to correspond more closely to the actual minimum requirements consistent with sufficient crop protection. The first approach involves studying the response of the plant to the complexity of environmental factors and trying to predict the behaviour of terminal bud activity in the field. The second approach is to examine the plant itself for signs of activity and determine the minimum temperatures which will cause damage once this activity has been initiated.

More investigation is needed to define accurately the frost protection requirements for commercial cranberry bogs. It may be possible to devise a simple yet reliable method for measuring enlargement of the terminal buds accurately in the field. If bud enlargement could be shown to integrate both chilling and devernalization effects, it could be used in conjunction with environmental records to predict when frost protection becomes necessary.

SUMMARY

The purposes of this research were to obtain data on the effect of winter conditions on the subsequent growth and development of V. macrocarpon through the use of controlled environment facilities, to investigate the possible role of gibberellins in the induction of flowering, to correlate the data obtained with development of the terminal bud under field conditions, and to provide some basic information relating to the problem of frost injury. The main results are summarized below:

1. The overwintering stage of the floral primordia in the terminal bud of V. macrocarpon was more advanced in 1969-1970 than in 1968-1969.
2. Development of the floral primordia recommenced in late February or early March under British Columbia field conditions. These structures were well differentiated by the end of April in each of the two years studied.
3. Dormant mixed terminal buds that were not exposed to chilling temperatures did not initiate new growth even under long photoperiods.
4. The number of days to bud break following termination of the controlled environment chilling treatments was reduced as the length of the chilling treatments was increased. Rate of growth and final shoot length were also affected, but were less dependent on the length of chilling. The increased responses after longer chilling were possibly the result of other plant activities in addition to the fulfilment of the chilling requirement.
5. Longer chilling periods were required for the induction of floral development; shorter chilling periods resulted in vegetative growth only. It is suggested that the floral primordia may need to undergo a certain amount of development while cyclically exposed to relatively low temperatures.

6. It was not possible to define either vegetative or floral chilling requirements in the form of a certain number of hours below a certain given temperature regardless of other factors. There was an apparent action of the warmer part of the chilling regimes that caused an acceleration of the plant responses quite aside from the fulfilment of the chilling requirements.
7. Application of gibberellic acid to unchilled plants resulted only in vegetative growth of the treated plants.
8. The activity of endogenous gibberellin-like substances in both the leaves and buds of V. macrocarpon was found to have increased by March 9 of the sample year (1970). The continued increase of activity in the April 6 bud sample and the reduction of activity in the corresponding leaf sample suggested that a translocation of the gibberellin-like substances had occurred. The timing of this translocation was such that these substances are probably more important for vegetative than floral development.
9. The combination of results regarding the induction of vegetative and floral development in the terminal bud suggested that these two processes are under independent control. Shorter chilling periods resulted in only vegetative growth of the terminal bud, but under field conditions development of the floral primordia takes place prior to the initiation of vegetative growth. It is probable that once the vegetative chilling requirement has been satisfied, a stimulus from another plant organ, such as gibberellin-like compounds from the leaves, is needed to initiate elongation of the terminal bud.
10. Once the basic chilling requirement has been met, the floral primordia will, under warm daytime conditions, begin to develop. As they develop, they may become susceptible to frost injury at a rate faster than the

progressive rise of minimum temperatures. The resulting injury could be one of the prime factors in limiting the northern range of V. macrocarpon.

11. Further study of the morphological and physiological development of V. macrocarpon cultivars under field conditions is needed to develop adequate guidelines for commercial frost-protection programs.

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APPENDIX 1.

Light Sources of Controlled Environment Facilities

Facility	Number of Lamps[1]		Intensity (klux) at plant level
	Tungsten filament	Fluorescent	
Sherer Model CEL-266-6			
Full light	4	8	7.1
One-half light	4	4	3.6
Percival Model PGC-78	10	16	7.9
Cold Room	3	4	2.1
Photoperiod Cabinets	1	2	1.1

1 All lamps listed were 40 watt.

APPENDIX 2.

Analysis of Variance

1968-1969 Experiment

Days to Bud Break

Source	d.f.	SS	F
Chilling time	4	4381.53	119.6*
Chamber	1	0.30	0.03
Residual	24	228.87	
Total	29	4610.70	

Shoot Length

Source	d.f.	SS	F
Chilling time	4	63.139	2.23
Chamber	1	6.912	0.98
Residual	24	170.05	
Total	29	240.10	

Flowers per Plant

Source	d.f.	SS	F
Chilling time	4	3.199	0.26
Chamber	1	3.332	1.07
Residual	24	74.658	
Total	29	81.190	

1969-1970 Experiment

Percent Bud Break - Factorial

Source	d.f.	SS	F
Chilling time (CT)	4	31888.0	76.99*
Regime	3	3927.5	12.64*
Regime x CT	12	3104.7	2.50*
Residual	20	2071.0	
Total	39	40991.0	

Percent Bud Break - Regime 1

Source	d.f.	SS	F
Chilling time	5	6628.8	10.34*
Residual	6	769.5	
Total	11	7398.2	

Percent Bud Break - Regime 2

Source	d.f.	SS	F
Chilling time	5	12497.0	52.99*
Residual	6	283.0	
Total	11	12780.0	

* Statistically significant P = 0.05

Percent Bud Break - Regime 3

Source	d.f.	SS	F
Chilling time	4	12367.0	82.45*
Residual	5	187.5	
Total	9	12555.0	

Percent Bud Break - Regime 4

Source	d.f.	SS	F
Chilling time	4	6625.4	9.51*
Residual	5	871.0	
Total	9	7496.4	

Percent Bud Break - 25 Days Chilling

Source	d.f.	SS	F
Regime	3	1154.0	1.61
Residual	4	958.5	
Total	7	2112.5	

Percent Bud Break - 50 Days Chilling

Source	d.f.	SS	F
Regime	3	2634.4	4.46
Residual	4	787.5	
Total	7	3421.9	

Percent Bud Break - 75 Days Chilling

Source	d.f.	SS	F
Regime	3	2909.4	16.33*
Residual	4	237.5	
Total	7	3146.9	

Percent Bud Break - 100 Days Chilling

Source	d.f.	SS	F
Regime	3	75.0	4.00
Residual	4	25.0	
Total	7	100.0	

Percent Bud Break - 125 Days Chilling

Source	d.f.	SS	F
Regime	3	259.4	5.53
Residual	4	62.5	
Total	7	321.9	

Percent Bud Break - 150 Days Chilling

Source	d.f.	SS	F
Regime	1	36.0	1.80
Residual	2	40.0	
Total	3	76.0	

* Statistically significant P = 0.05

Days to Bud Break - Factorial

Source	d.f.	SS	F
Chilling time (CT)	4	456500.0	15798.5*
Regime	3	177590.0	8194.5*
Regime x CT	12	2239800.0	25838.7*
Residual	156	1126.9	
Total	175	2875000.0	

Days to Bud Break - Regime 1

Source	d.f.	SS	F
Chilling time	5	3109.1	99.6*
Residual	52	324.6	
Total	57	3433.7	

Days to Bud Break - Regime 2

Source	d.f.	SS	F
Chilling time	5	5082.3	311.7*
Residual	48	156.5	
Total	53	5238.8	

Days to Bud Break - Regime 3

Source	d.f.	SS	F
Chilling time	4	3514.6	82.1*
Residual	37	395.9	
Total	41	3910.5	

Days to Bud Break - Regime 4

Source	d.f.	SS	F
Chilling time	4	481.5	16.9*
Residual	40	283.7	
Total	44	765.2	

Days to Bud Break - 25 Days Chilling

Source	d.f.	SS	F
Regime	2	511.7	9.9*
Residual	14	359.8	
Total	16	871.5	

Days to Bud Break - 50 Days Chilling

Source	d.f.	SS	F
Regime	3	1018.5	19.9*
Residual	32	544.5	
Total	35	1563.0	

Statistically significant P = 0.05

Days to Bud Break - 75 Days Chilling

Source	d.f.	SS	F
Regime	3	193.5	40.38*
Residual	36	57.5	
Total	39	251.0	

Days to Bud Break - 100 Days Chilling

Source	d.f.	SS	F
Regime	3	22.1	3.02*
Residual	36	87.8	
Total	39	109.9	

Days to Bud Break - 125 Days Chilling

Source	d.f.	SS	F
Regime	3	45.7	7.09*
Residual	36	77.3	
Total	39	123.0	

Days to Bud Break - 150 Days Chilling

Source	d.f.	SS	F
Regime	1	1.8	1.23
Residual	18	26.4	
Total	19	28.2	

Growth Rates - Factorial

Source	d.f.	SS	F
Chilling time (CT)	4	0.21072	20.61*
Regime	3	0.010176	1.33
Regime x CT	12	0.04946	1.61
Residual	20	0.051109	
Total	39	0.32147	

Growth Rates - Regime 1

Source	d.f.	SS	F
Chilling time	5	0.08647	9.33*
Residual	6	0.01112	
Total	11	0.09759	

Growth Rates - Regime 2

Source	d.f.	SS	F
Chilling time	5	0.09505	9.12*
Residual	6	0.01251	
Total	11	0.10756	

* Statistically significant P = 0.05

Growth Rates - Regime 3

Source	d.f.	SS	F
Chilling time	4	0.10400	4.37
Residual	5	0.02975	
Total	9	0.13375	

Growth Rates - Regime 4

Source	d.f.	SS	F
Chilling time	4	0.05504	7.03*
Residual	5	0.00978	
Total	9	0.06482	

Mean Shoot Length - Factorial

Source	d.f.	SS	F
Chilling time (CT)	3	703.59	49.07*
Regime	4	50.98	4.74*
Regime x CT	12	110.92	2.58*
Residual	20	71.69	
Total	39	937.18	

Mean Shoot Length - Regime 1

Source	d.f.	SS	F
Chilling time	5	131.83	3.94
Residual	6	40.15	
Total	11	171.98	

Mean Shoot Length - Regime 2

Source	d.f.	SS	F
Chilling time	5	289.72	42.12*
Residual	6	8.25	
Total	11	297.97	

Mean Shoot Length - Regime 3

Source	d.f.	SS	F
Chilling time	4	332.08	20.65*
Residual	5	20.11	
Total	9	352.19	

Mean Shoot Length - Regime 4

Source	d.f.	SS	F
Chilling time	4	137.50	8.31*
Residual	5	20.68	
Total	9	158.19	

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* Statistically significant P = 0.05

Mean Shoot Length - 25 Days Chilling

Source	d.f.	SS	F
Regime	3	66.91	3.07
Residual	4	29.02	
Total	7	95.93	

Mean Shoot Length - 50 Days Chilling

Source	d.f.	SS	F
Regime	3	4.96	0.66
Residual	4	9.97	
Total	7	14.93	

Mean Shoot Length - 75 Days Chilling

Source	d.f.	SS	F
Regime	3	21.33	11.81*
Residual	4	2.41	
Total	7	23.74	

Mean Shoot Length - 100 Days Chilling

Source	d.f.	SS	F
Regime	3	3.75	0.39
Residual	4	12.95	
Total	7	16.70	

Mean Shoot Length - 125 Days Chilling

Source	d.f.	SS	F
Regime	3	64.94	4.99
Residual	4	17.34	
Total	7	82.28	

Mean Shoot Length - 150 Days Chilling

Source	d.f.	SS	F
Regime	1	0.35	0.04
Residual	2	17.50	
Total	3	17.85	

Mean Number of Flowers per 20 Plants - Regime 1

Source	d.f.	SS	F
Chilling time	2	412.33	16.72*
Residual	3	37.00	
Total	5	449.33	

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* Statistically significant P = 0.05

Mean Number of Flowers per 20 Plants - Regime 2

Source	d.f.	SS	F
Chilling time	2	1417.0	9.62
Residual	3	221.0	
Total	5	1638.0	

Mean Number of Flowers per 20 Plants - Regime 3

Source	d.f.	SS	F
Chilling time	1	64.00	32.00*
Residual	2	4.00	
Total	3	68.00	

Mean Number of Flowers per 20 Plants - Regime 4

Source	d.f.	SS	F
Chilling time	2	180.28	7.42
Residual	3	36.42	
Total	5	216.70	

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* Statistically significant P = 0.05

APPENDIX 3.

Dry Weights and Minimum Detectable Levels of Gibberellin-like Activity in Terminal Bud and Leaf Samples

Bud Samples

Sample Collection Date	Dry Weight (g) Initial	After MeOH Extraction	After PVP Column	After Charcoal -Celite column	Minimum Detectable Level of GA-like Activity (μg GA/g tissue)
Oct. 17/69	0.1873	0.1359	0.0177	0.0102	2.6×10^{-1}
Nov. 18/69	0.4990	0.0610	0.0273	0.0110	1.0×10^{-1}
Dec. 18/69	0.4860	0.0323	0.0110	0.0093	1.0×10^{-1}
Feb. 3/70	0.3864	0.1982	0.0220	0.0138	1.3×10^{-1}
Feb. 23/70	0.4930	0.0745	0.0073	0.0052	1.0×10^{-1}
Mar. 9/70	0.3429	0.0632	0.0117	0.0097	1.5×10^{-1}
Apr. 6/70	0.7051	0.1146	0.0072	0.0057	7.1×10^{-2}
Apr. 27/70	0.5335	0.1350	0.0224	0.0109	9.4×10^{-2}
May 4/70	0.5025	0.1216	0.0427	0.0163	9.9×10^{-2}
May 19/70	0.5460	0.1461	0.0244	0.0114	9.2×10^{-2}
June 22/70	1.6596	0.1656	0.0160	0.0010	3.0×10^{-2}

Leaf Samples

Sample Collection Date	Dry Weight (g) Initial	After MeOH Extraction	After PVP Column	After Charcoal -Celite column	Minimum detectable Level of GA-like activity (μg GA/g tissue)
Nov. 18/69	15.00	1.4705	0.1219	0.0504	3.3×10^{-3}
Dec. 18/69	15.00	1.3340	0.1190	0.0563	3.3×10^{-3}
Feb. 3/70	15.00	1.4647	0.0906	0.0520	3.3×10^{-3}
Feb. 23/70	15.00	1.2520	0.0794	0.0377	3.3×10^{-3}
Mar. 9/70	15.00	1.5090	0.0982	0.0641	3.3×10^{-3}
Apr. 6/70	15.00	1.6434	0.1216	0.0879	3.3×10^{-3}
Apr. 27/70	15.00	1.4729	0.1157	0.0866	3.3×10^{-3}
May 19/70	15.00	1.3771	0.0586	0.0443	3.3×10^{-3}