A COMPARISON OF THE PHYSIOLOGY AND MORPHOLOGY OF BLIND AND FLOWERING ROSE (ROSA HYBRIDA L.) SHOOTS

Dissertation for the Degree of Ph. D.

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ABSTRACT

A COMPARISON OF THE PHYSIOLOGY AND MORPHOLOGY

OF BLIND AND FLOWERING ROSE (ROSA HYBRIDA L.) SHOOTS

By

Terril Arnold Nell

Section I: High Intensity Lighting Effects on Blindness in Rosa hybrida L. cv. Tropicana

Flowers were harvested from 'Tropicana' plants during the fall and winter of 1975 and spring and summer of 1976. The plants were given 0, 5, 10, 20 days or supplemental high intensity lighting (Lucalox, 640 W/M^2, 12 hr daily) from lateral bud initiation to flowering (cut to cut). Following these lighting treatments, the plants were transferred to a Lab-Line growth chamber where the light intensity was maintained at 300 W/M^2. Plants grown in the growth chamber without supplemental lighting had the highest % of blind shoots regardless of time of year. Plants receiving 20 days of supplemental lighting had only 7% more blind shoots than plants grown with supplemental lighting from cut to cut. Maximum blindness occurred during the winter months regardless of lighting treatment. Blind shoot production decreased with increased supplemental lighting.

Section II: Prediction of Blind Shoots on Three Rosa hybrida L. Cultivars

Shoot length, bud diam and stem diam were evaluated as morphological indicators of blind shoot development of 'Tropicana', 'Forever Yours', and 'Cara Mia' roses. Shoot length proved to be an indicator of blind shoots as early as 10 days following lateral bud initiation on all cvs.

Bud diam and stem diam could be used after 16 days on 'Tropicana' and 'Forever Yours'. Stem diam failed to indicate blindness between 16 and 30 days on 'Cara Mia'. Bud diam was a reliable predictor on 'Cara Mia' 16 days after lateral bud initiation.

Section III: Floral Development and Blindness in Roses--An SEM Study

Fresh, unfixed rose meristems were viewed in the scanning electron microscope to determine morphological differences and organogenesis of flowering and blind shoots. Gluteraldehyde fixed, ethanol dehydrated and critically point dried tissue were severely desiccated with individual cells being concave. Fresh tissue was turgid for at least 10 min in the microscope.

Visible signs of initiation were evidenced by the presence of sepal primordia followed by differentiation of petals, anthers and stigma. No evidence of flower initiation was observed in the blind shoot.

Section IV: Histochemical Study of Blind and Flowering Rose Meristems

Activity of acid phosphatase, peroxidase, succinic dehydrogenase and the presence of starch and histones were determined in the blind and flowering rose shoots during the first 10 days after lateral bud initiation, at the time of floral initiation and following floral initiation. Histones were in both shoots during the first 10 days after lateral bud initiation. No increase was apparent in the blind apex. Enzyme activity was found to be present in both shoot types with all enzymes appearing to be evenly distributed throughout the apex. Insoluble polysaccharides were found in older cells of blind and flowering shoots. Starch was not observed in either type of shoot.

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Terril Arnold Nell

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TABLE OF CONTENTS

Page

SECTION III

FLORAL DEVELOPMENT AND BLINDNESS IN ROSES--
AN SEM STUDY

SECTION IV

HISTOCHEMICAL STUDY OF BLIND AND FLOWERING ROSE
MERISTEMS

APPENDICES

LIST OF TABLES

LIST OF FIGURES

Figure Page

SECTION III

SECTION IV

Guidance Committee:

The journal-article format was adopted for this dissertation in accordance with departmental and university requirements. Four sections were prepared and styled for publication in the Journal of the American Society for Horticultural Science.

LITERATURE REVIEW

The greenhouse rose, Rosa hybrida L., is the leading monetary cut flower crop in the United States (20) with wholesale value exceeding $68 million annually. The red rose is the most popular among consumers (19). Commercial production of cut roses incorporates flower quality as well as the total number of flowering shoots harvested. Grades and standards used to determine quality are based on stem length, bud size, and stage of flower development (1). Blind shoots are not marketable since they fail to produce a flower. Maximum economic profits are realized when cultural procedures result in the largest number of marketable flowers.

Blindness, under normal growing conditions, is highly dependent on environmental conditions. Several researchers (3, 4, 17) have shown that blindness increased during the low light levels of winter. Recently, Carpenter and Anderson (3) showed that high intensity supplemental lighting increased year-round rose production. They found that the absolute number of blind shoots increased with high intensity lighting, but the percentage declined.

Temperature (14) and carbon dioxide levels (10) also affect the number of blind and flowering shoots. Moe (14) found that 'Baccara' grown at 12°C had six times more blind shoots than plants grown at 18°C. Plants grown at 18°C for 21 days or longer were not affected by a transfer to 12°C while plants transferred prior to 16 days had a higher

percentage of blind shoots. Zieslin and Halevy (24) found that the average number of flowers per plant on 'Baccara' could be increased during each growing cycle from 2.7 flowers at 15°C to 3.7 flowers at 22°C.

Carbon dioxide levels can affect the percentage of flowering shoots. Hand and Cockshull (10) found that the addition of 1000 ppm carbon dioxide from December-April resulted in a 23 percent increase in marketable flowers. The increased flower production was due to a reduction in blind shoot formation and the stimulation of lateral shoot growth.

Failure of the greenhouse grower to maintain optimal environmental conditions results in an increase in the number of blind shoots, as well as reduction in flower quality. Blind shoots must be either pinched or completely removed from the plant at regular intervals (19). With rising labor costs, manual removal is expensive. Furthermore, the production costs and plant photosynthetic products required by blind shoots fail to result in monetary returns for the grower.

It is now possible to manipulate the greenhouse environment more effectively than ever before. There are greenhouse heating systems that provide uniform heat throughout the greenhouse and which minimize temperature differences during cycling periods. Greater efficiency of fan and pad cooling enable producers to control greenhouse temperatures during summer months (2). Also, high intensity lighting is available to supplement incident radiation during the reduced light levels of winter, especially in northern latitudes (3, 4). These environmental controls have increased rose production by providing near ideal environmental conditions year-round. Maximization of environmental factors does

not, however, result in 100 percent flowering shoots (14, 24). Some commercial rose cultivars still produce up to 50 percent blind shoots (12).

The blind shoots has been termed a physiological condition (11). Hubbel (11) found that the first differentiation in the rose apex occurred 8 days after lateral bud initiation. Lindstrom (13) using 'Better Times' attributed blindness to the abortion of the reproductive meristem beginning with necrosis of the sepals and terminating with abscission layer formation below the receptacle. When this occurred, death of the apex was evident 35 days after bud break.

Morphologically, the flowering shoot is different from the blind shoot (14, 23). Blind shoots are shorter when grown under the same conditions with exact differences dependent on environmental conditions and cultivar (14). Flowering shoots generally have 4 to 6 more leaves above the hook (14). Moe (14) has shown that the uppermost leaf on blind shoots is morphologically identical to the number five leaf above the hook on flowering shoots.

The probability of blindness is affected by position on the rose stem. Zieslin and Halevy (23) and Moe (14) have shown that the primary bud on 'Baccara' plants averaged 9 percent blindness (91 percent flowering) while positions 2, 3, and 4 from the apex were 34.5, 56.1 and 100 percent blind, respectively. Blind shoots originating at the primary position were longer and had more leaves than blind shoots produced at other positions.

Cultivar Response

Corbett (4) associated blind shoot production with the genetic composition of the rose cultivars. Hubbell (11) concluded that blindness

was a nutritional disorder and entirely physiological. As discussed in the previous section, environmental conditions play a major role in the determination of blindness in roses, indicating that blindness is not totally under genetic control. However, greenhouse rose cultivars vary in the production of flowering and blind shoots. Moe (14) observed that 'Baccara' and 'Super Star' produced fewer blind shoots than other cultivars as the temperature increased from 12°C to 21°C. 'Super Star' produced more blind shoots than 'Baccara' at each temperature. Another cultivar, 'Dr. A. J. Verhage' flowered at 12°C but did not flower at higher temperature. Carpenter and Anderson (3) found that 'Forever Yours' averaged 20-25 percent blind shoots during January, while 'Red American Beauty' produced 30-43 percent blind shoots and 'Electra' was intermediate in blind shoot production. 'Tropicana', an orange-red cultivar, is produced primarily in California and Hawaii, since it produces nearly 100 percent blind shoots during winter months in northern latitudes.

Histochemistry

Histochemical analysis has been used to study chemical changes associated with floral induction (7, 8, 18, 20). Procedures have been used to localize various metabolites and to quantify them within the shoot apex.

Changes in enzyme levels have been associated with cell division (7, 15, 16, 21) and differentiation of the meristem in *Chenopodium album* and *Allium cepa* (8, 9). Fosket and Miksche (7) found high acid phosphatase activity in the apical meristem of 5-day-old pine (*Pinus lambertiana*) seedlings. Initial activity was located at the extreme tip of the apex and the activity reached high levels in the peripheral zones after 8

days. Riding and Gifford (18) found high acid phosphatase throughout the apex of Pinus radiata prior to needle formation. Activity declined after needle formation. Wilson (22) has shown a positive relationship between acid phosphatase and development in cucurbit fruit. She suggested an association between acid phosphatase activity and differentation and a possible relationship between acid phosphatase and carbohydrate metabolism.

Peroxidase and succinic dehydrogenase have also been shown to change with apical differentiation. Riding and Gifford (18) found high succinic dehydrogenase levels throughout the meristem of Pinus radiata. Peroxidase activity was observed around the aleurone grains. Activity was localized in subsurface cells at the onset of needle formation. Goff (9) found high levels of peroxidase in cell walls, on the plasmalemma, in the Golgi apparatus cisternae and vesicles, in young and developing vacuoles, in the endoplasmic reticulum and on the ribosomes of developing onion (Allium cepa) root tips. He observed that even the most undifferentiated cells exhibited perioxidase activity.

Peroxidase activity precedes, and is a good indicator of, cell division. Van Fleet (21) found that in Zea mays peroxidase is present prior to cell division but then declines. Low levels were found in older tissue. He concluded that activation of peroxidase occurs in meristems and other centers of cell division. Poovaiah and Rasmussen (15, 16) have shown that increased levels of peroxidase, acid phosphatase and succinic dehydrogenase precede abscission layer formation in bean (Phaseolus vulgaris) which involves cell division.

Basic proteins (histones) and starch have been used as indicators of floral initiation. Gifford and Tepper (8) found that histone and

starch levels increased after photoperiodic induction in Chenopodium album. Starch increased in the apex after 2 inductive cycles while histone levels were highest after 4 cycles. The concentration of both substances declined after induction. Vegetative meristems contained a uniform concentration of histones prior to induction, while starch was most evident in the leaf primordia during this period. Emino (6) found high starch levels in both the leaf primordia and subapical regions of vegetative carnation apices. Starch was observed throughout the apex prior to and following initiation.

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SECTION I

HIGH INTENSITY LIGHTING EFFECTS ON BLINDNESS IN *ROSA HYBRIDA* L. CV. TROPICANA

HIGH INTENSITY LIGHTING EFFECTS ON BLINDNESS IN ROSA HYBRIDA L. CV. TROPICANA

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ABSTRACT. Flowers were harvested from 'Tropicana' plants during the fall and winter of 1975 and spring and summer of 1976. The plants were given 0, 5, 10, 20 days or supplemental high intensity lighting (Lucalox, 640 W/M^2, 12 hr daily) from lateral bud initiation to flowering (cut to cut). Following these lighting treatments, the plants were transferred to a Lab-Line growth chamber where the light intensity was maintained at 300 W/M^2. Plants grown in the growth chamber without supplemental lighting had the highest % of blind shoots regardless of time of year. Plants receiving 20 days of supplemental lighting had only 7% more blind shoots than plants grown with supplemental lighting from cut to cut. Maximum blindness occurred during the winter months regardless of lighting treatment. Blind shoot production decreased with increased supplemental lighting.

The use of high intensity discharge (HID) supplementary lighting has been shown to be beneficial for the production of geraniums (2, 3), bedding plants (3, 5), chrysanthemums (1, 3), and greenhouse roses (3, 4, 6, 7). The use of HID lighting on greenhouse roses from October to April resulted in higher yields, better quality flowers and a lower % of blind shoots (3, 4). Carpenter and Anderson (4) found that HID of 'Forever Yours', 'Red American Beauty', and 'Electra' with Lucalox lamps (6.2 W/ft^2) or a combination of Lucalox and multivapor lamps (6.0 W/ft^2) increased rose yields from 48 to 90%. The yield differences were dependent upon cv. and time of year. Natural low light conditions of winter produced higher % of blind shoots on all cv., with the unlighted plants having the most blindness. Moe (10) found that reducing natural light levels in Norway increased blind shoot production on 'Baccara'.

Additional research has shown that low temp (10, 12) and reduced CO_2 levels (8) increase blindness. 'Baccara' roses grown at 12°C had six times more blind shoots than those grown at 18°C (10). Those plants remaining at 18°C for 21 days or longer were not affected by a move to 12°C, while the plants transferred prior to 16 days at 18°C evidenced a high % blind shoots.

Continuous use of HID lighting reduced blindness in greenhouse roses (4). However, escalating energy costs have discouraged many northern growers from installing HID lighting fixtures. In the meantime, low light levels in late fall and winter continue to reduce crop yields and flower quality, increase blindness, and ultimately reduce grower profits. This study was conducted to evaluate the effect of various periods of HID lighting on blind shoot production of roses. 'Tropicana' was selected for the study, since commercially it is known to produce an unusually high % of blind shoots during periods of low light levels.

Materials and Methods

Cultural. Two-year-old 'Tropicana' plants were grown in 30 cm diam clay pots using a planting medium consisting of equal vol of soil, peat, and Turface. Plants were studied during the fall and winter of 1975 and spring and summer of 1976. Plants were irrigated as needed with a 200 ppm N solution using water soluble fertilizer (20-20-20) including micro-nutrients. Greenhouse day-night temp were maintained at $21^{\circ}\pm1^{\circ}$C and $19^{\circ}\pm1^{\circ}$C, respectively. All plants received continuous (24 hr daily) supplemental HID lighting (640 W/M^2) 4 weeks prior to the commencement of the treatments. All other procedures were performed using standard cultural practices (9, 11).

Experimental. The flowers were removed (bud initiation) from each plant at the commencement of the study. Plants were then given 0, 5, 10, 20 days or supplemental HID lighting (12 hr daily, 640 W/M^2, 2000 to 0800 hr) from lateral bud initiation to flowering (cut to cut). Following these lighting periods, the plants were transferred to a Lab-Line Controlled Environmental Room maintained at 300 W/M^2 with 70% of the input wattage from Cool-White fluorescent lamps and 30% from incandescent lamps. Those plants receiving HID lighting from cut to cut remained in the greenhouse during the entire growth cycle.

The weekly growth rate of blind and flowering shoots was measured beginning eight days after lateral bud initiation. Newly emerging buds were not measurable until this time. Shoot length was determined from the point of shoot origin to the tip of the shoot.

Results

HID lighting reduced blindness on 'Tropicana' rose in all seasons (Table 1). There appeared to be an inverse relationship between the length of the lighting period and the number of blind shoots.

Plants grown during the winter months had the highest % of blind shoots (Table 1). Values ranged from 94% on plants grown in the growth chamber with no HID lighting to 53% for those plants receiving HID lighting from cut to cut. The smallest % of blind shoots occurred during the summer months. In the summer HID lighting from cut to cut produced only 35% blind shoots--a 45% reduction over plants grown with no supplemental lighting.

Increased periods of HID lighting resulted in reduced blindness (Table 1). Those plants grown in a growth chamber with no supplemental lighting had the largest proportion of blind shoots regardless of

Table 1. The influence of various periods of high intensity lighting (640 W/M^2) on blindness in 'Tropicana' rose.

	Time of Year			
	Fall	Winter	Spring	Summer
	1975-76 Percentages of Blind Shoots			
No HID	77	94	80	80
5 Days	70	86	67	65
10 Days	64	73	61	56
20 Days	51	55	53	48
Continuous	50	53	40	35

season. The proportion of blind shoots on plants remaining in the greenhouse for 20 days before being transferred to the growth chamber was very similar to plants receiving supplemental lighting from cut to cut. Plants receiving 20 days of supplemental lighting averaged 7% more blind shoots than plants grown with Lucalox lighting during the entire growth cycle. Increases of 38, 26, and 19% were obtained when plants were grown with 0, 5, and 10 days of supplemental lighting, respectively.

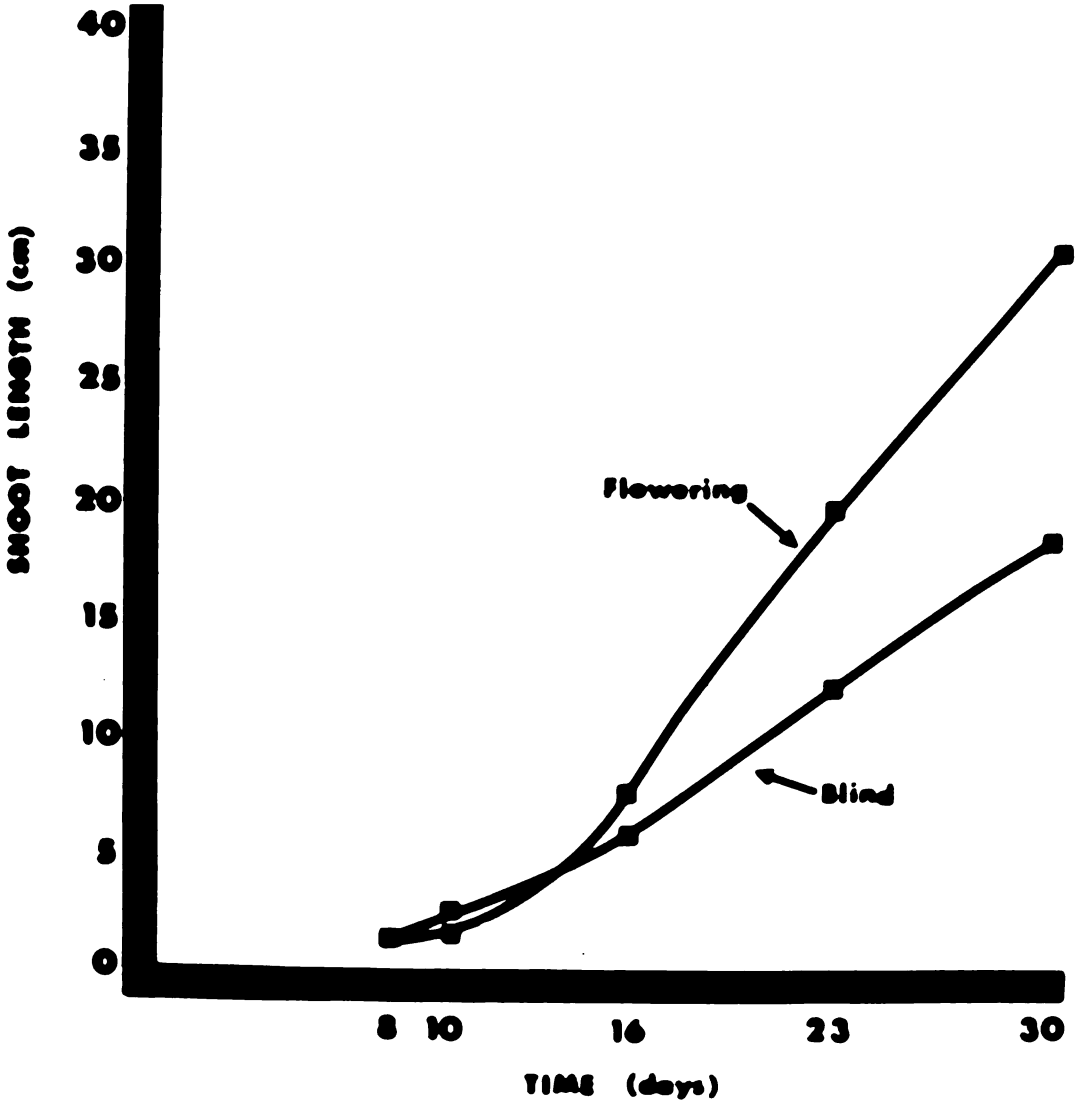
There were no differences in shoot growth of blind and flowering shoots prior to 16 days following flower removal (Fig. 1). Growth rate of blind shoots was considerably slower than the growth of flowering shoots after 16 days. The flowering shoots were nearly 50% longer than the blind shoots after 23 days.

Discussion

Use of continuous HID lighting daily on greenhouse roses from October to April has been recommended to improve flower quality and increase crop yields (3, 4). This study (Table 1) has shown that HID lighting for 20 days after flower removal will produce more flowering shoots than plants receiving fewer than 20 days of lighting. Blind shoot production in plants given 20 days HID during the fall and winter was similar to plants given continuous high intensity lighting from cut to cut.

The commercial rose industry has attempted to satisfy the demand for red roses, most popular among U. S. consumers. Commercial northern growers must select cvs. which not only have consumer appeal, but which also produce year-round. Unproductive cvs. are rarely grown regardless of consumer demand. Such is the case with 'Tropicana' rose. Northern production of 'Tropicana' is limited, due to the tendency for this cv.

Figure 1. Average shoot length of blind and flowering shoots on 'Tropicana' rose, 1975-1976.


SHOOT LENGTH (cm)
40
35
30
25
20
15
10
5
0
Flowering
Blind
8
10
16
23
30
TIME (days)

to produce a large proportion of blind shoots, especially during periods of low light intensity. Findings of this research, however, indicate that northern growers should be able to reduce blind shoot production on 'Tropicana' (and perhaps other cvs. as well) by using HID lighting. Once again it may be possible for northern growers to supply local and regional markets with an orange rose year-round.

Major emphasis in past research has been placed on the effects of light, temperature, and CO_2 on blindness in several rose cvs. (8, 10, 12). The results clearly indicate that unfavorable environmental factors promote blindness, which accounts for as much as 50% of all shoots on most commercial cvs. (9). Technology has enable researchers to control these three important variables, but even the most favorable environmental conditions have failed to produce 100% flowering shoots (4, 9, 10, 12). Maximization of flowering shoot production on commercial rose cvs. cannot be achieved until the morphological and chemical changes associated with the induction of blindness are identified with certainty and resolved.

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SECTION II

PREDICTION OF BLIND SHOOTS ON THREE *ROSA HYBRIDA* L. CULTIVARS

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ABSTRACT. Shoot length, bud diam and stem diam were evaluated as morphological indicators of blind shoot development of 'Tropicana', 'Forever Yours', and 'Cara Mia' roses. Shoot length proved to be an indicator of blind shoots as early as 10 days following lateral bud initiation on all cvs. Bud diam and stem diam could be used after 16 days on 'Tropicana' and 'Forever Yours'. Stem diam failed to indicate blindness between 16 and 30 days on 'Cara Mia'. Bud diam was a reliable indicator on 'Cara Mia' 16 days after lateral bud initiation.

The preponderance of plant-related research deals with long delayed post-treatment observations rather than short term physiological, morphological or chemical changes associated with a given plant response. To study the histochemical changes preceding a visual response such as blind shoot formation in greenhouse roses, a nondestructive technique must be developed to select blind shoots prior to the visible expression of the disorder.

Lindstrom (5) described blindness in roses as an abortion of a reproductive meristem, beginning with necrosis in the sepals and terminating with abscission layer formation below the receptacle. The final visible expression of blindness, a brown meristem, was generally not observable until the shoot was 30 to 40 days old. Blind shoots were generally shorter, had thinner stems and fewer leaflets than flowering shoots (6, 7).

Cultural requirements for the greenhouse rose, Rosa hybrida L., have been well established. Ideal environmental conditions for growth and flower production can be obtained by providing conditions of high light (1, 2), cool temp (6), and high CO_2 levels (3). Modern greenhouse environmental control systems enable greenhouse operators to control temp and CO_2 levels throughout the year. Light levels can be increased with high intensity discharge (HID) supplementary lighting, but the primary limiting growth factor in the northern United States is still the reduced light levels during the fall and winter seasons. Regardless of the cv., maintenance of the greenhouse environmental conditions does not produce 100% flowering shoots; blind or nonflowering shoots may account for as many as 50% of the total shoots produced by the plants (4).

This study utilizes morphological characteristics to predict blindness and evaluate prediction techniques on three commercial rose cvs.

Materials and Methods

Cultural. 'Tropicana', 'Forever Yours' and 'Cara Mia' plants were grown in equal vol. of soil, peat and Turface using standard cultural procedures (4, 8) including watering, fertilization, insect and disease control. Minimum day-night temp were maintained at $21^{o}\pm1^{o}C$ and $19^{o}\pm1^{o}C$ respectively.

'Tropicana' plants were studied during the fall and winter of 1975 and spring and summer of 1976. High intensity sodium vapor lighting (640 W/M^2) was used to supplement natural radiant energy on 'Tropicana' until the beginning of the study. 'Forever Yours' and 'Cara Mia' were studied during the summer of 1976.

Experimental. Flowers were removed (bud initiation) from 'Tropicana' plants. Some plants were moved to a growth chamber, while others were maintained in the greenhouse using supplemental sodium vapor lighting (640 W/M^2), 12 hr daily (2000-0800 hr). Light intensity in the growth chamber was 300 W/M^2 with 70% of the input W from Cool-White fluorescent lamps and 30% from incandescent lamps. All 'Forever Yours' and 'Cara Mia' plants remained in the greenhouse after the flowers were harvested.

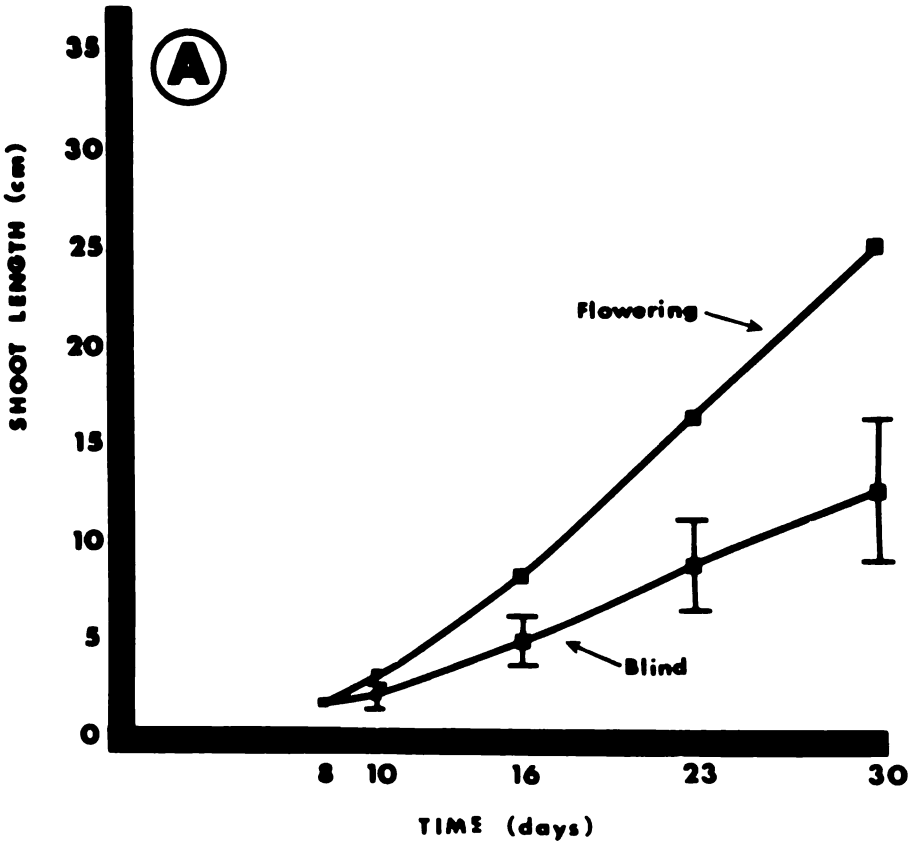
The morphological characteristics studied were shoot length, bud diam, and stem diam. The weekly growth rate of blind and flowering shoots was measured beginning eight days after lateral bud initiation. It was impossible to measure growth of the newly emerging shoots until eight days following lateral bud initiation, and bud diam and stem diam could only be ascertained 16 days after bud initiation. Shoot length was determined from the point of shoot origin to the tip of the shoot. The outside bud diam was measured at the widest point with a Helias caliper while the stem diam was recorded immediately distal to the first 3-leaflet leaf above the hook (7). Confidence limits were computed for the blind shoots at the 5% level (9).

Results

Morphological characteristics were reliable indicators of blind shoots as early as 10 days following lateral bud initiation in all three cvs. (Fig. 1, 2).

'Tropicana' rose was studied during all seasons with very few observable growth differences. Flowering shoots were 35% longer during the summer months than during the winter but bud and stem diam remained constant regardless of time of year. The increased flowering shoot length

Figure 1. Growth of blind and flowering 'Tropicana' rose shoots grown with and without supplemental high intensity lighting (Lucalox, 640 W/M^2, 12 hr daily) from bud initiation to flowering. Plants grown without high intensity lighting were grown in a Lab-Line growth chamber with the light intensity maintained at 300 W/M^2. A. 0 days high intensity lighting. B. Supplemental high intensity lighting from bud initiation to flowering.


A
SHOOT LENGTH (cm)
35
30
25
20
15
10
5
0
Flowering
Blind
8
10
16
23
30
TIME (days)

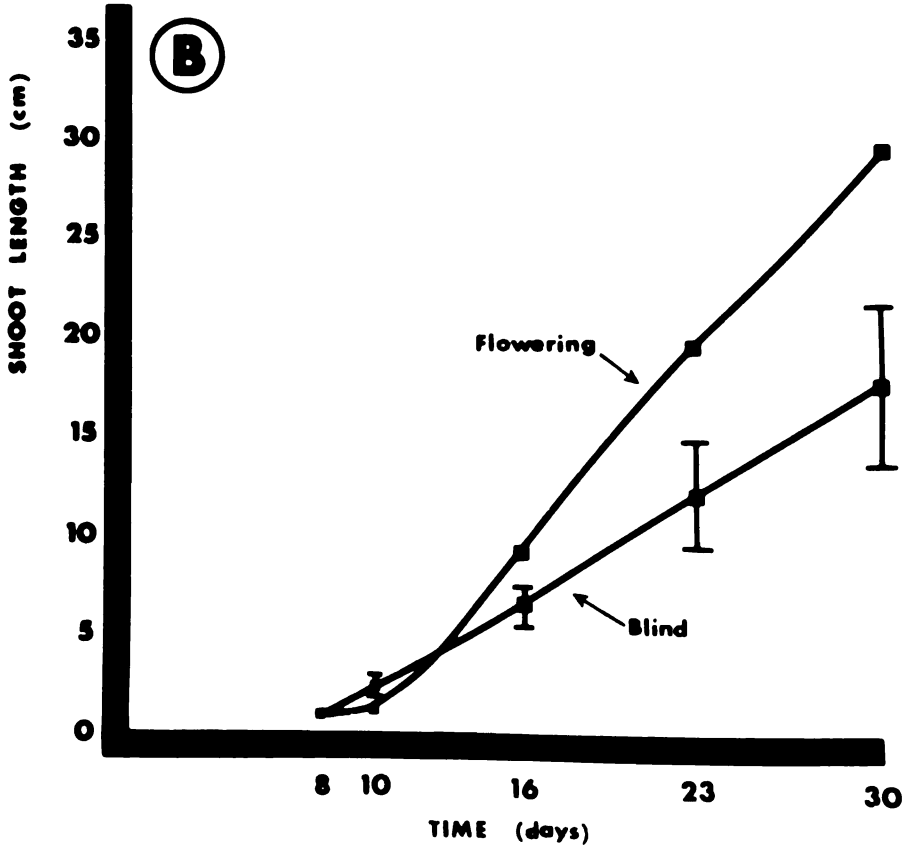
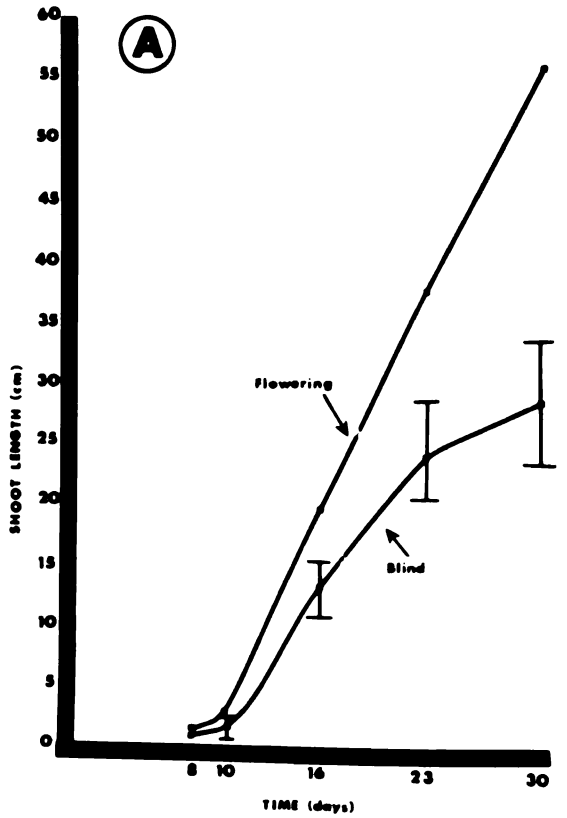

B
SHOOT LENGTH (cm)
35
30
25
20
15
10
5
0
Flowering
Blind
8
10
16
23
30
TIME (days)

Figure 2. Growth of blind and flowering 'Forever Yours' and 'Cara Mia' rose shoots, summer 1976. A. Shoot length, 'Forever Yours'. B. Shoot length, 'Cara Mia'.


A
SHOOT LENGTH (cm)
0
5
10
15
20
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35
40
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50
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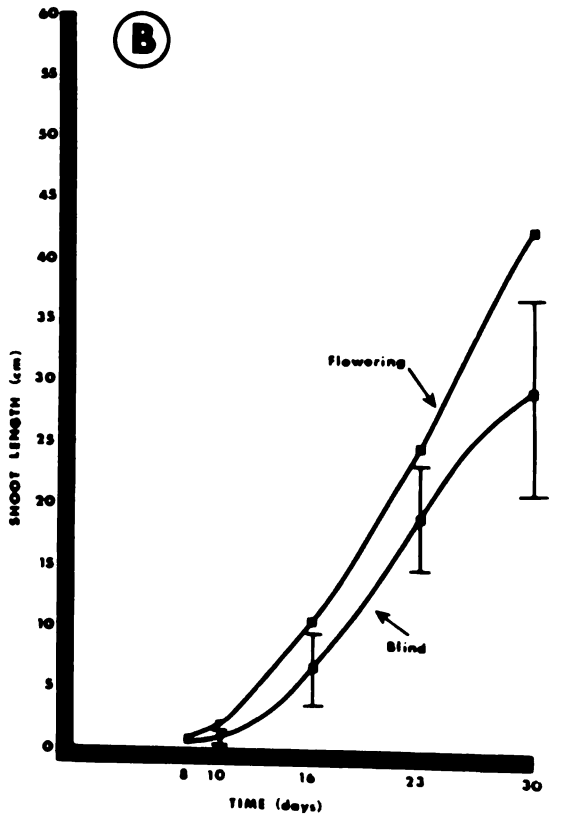
60
Flowering
Blind
8
10
16
23
30
TIME (days)


B
SHOOT LENGTH (cm)
0
5
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45
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Flowering
Blind
8
10
16
23
30
TIME (days)

observed during the later phases of shoot growth did not affect the early prediction of blind shoots. Data from plants grown during the winter are representative of other growth periods and will be the only 'Tropicana' data presented and discussed in this paper.

Both blind and flowering shoots on 'Tropicana' rose exhibit a typical sigmoid growth curve at maturity regardless of lighting treatment. The growth curves for 'Forever Yours' and 'Cara Mia' were similar.

There were no measurable differences in the growth of blind and flowering shoots during the first eight days, but the flowering shoots on 'Tropicana' and 'Forever Yours' were 66 and 65% longer after 10 days (Fig. 1, 2). Flowering shoots on 'Cara Mia' were 42% longer. 'Tropicana' flowering shoots averaged 2.5 cm after 10 days, while blind shoots were 1.7 cm. Increased flowering shoot length was maintained on all cvs. throughout the growth cycle, with flowering shoots measuring 50, 93, and 44% longer than blind shoots after 30 days on 'Tropicana', 'Forever Yours', and 'Cara Mia' respectively. Confidence intervals indicated that 95% of the shoots on 'Tropicana' between 4 and 6 cm long after 16 days would eventually become blind (Fig. 1). Flowering shoots averaged 8 cm after 16 days.

Bud diam and stem diam indicated blindness on 'Tropicana' and 'Forever Yours' between 16 and 30 days (Fig. 3, 4). Bud diam was found to be a good indicator on 'Cara Mia'. Stem diam on 'Cara Mia' did not indicate any difference in blind and flowering shoots between 16 and 30 days (Fig. 4). However, the original stem diam at the time of lateral bud initiation on blind shoots was smaller than flowering shoots on 'Forever Yours' and 'Cara Mia' (Table 1). Blind shoots on 'Cara Mia' averaged 4.6 mm while the mean for flowering shoots was 5.7 mm. A high

Figure 3. Bud and stem diam of 'Tropicana' rose shoots grown with and without supplemental high intensity lighting (Lucalox, 640 W/M^2, 12 hr daily) from bud initiation to flowering. Plants grown without high intensity lighting were grown in a Lab-Line growth chamber with the light intensity maintained at 300 W/M^2.
A. Bud diam, 0 days high intensity lighting.
B. Stem diam, 0 days high intensity lighting.
C. Bud diam, supplemental high intensity lighting from bud initiation to flowering. D. Stem diam, supplemental high intensity lighting from bud initiation to flowering.

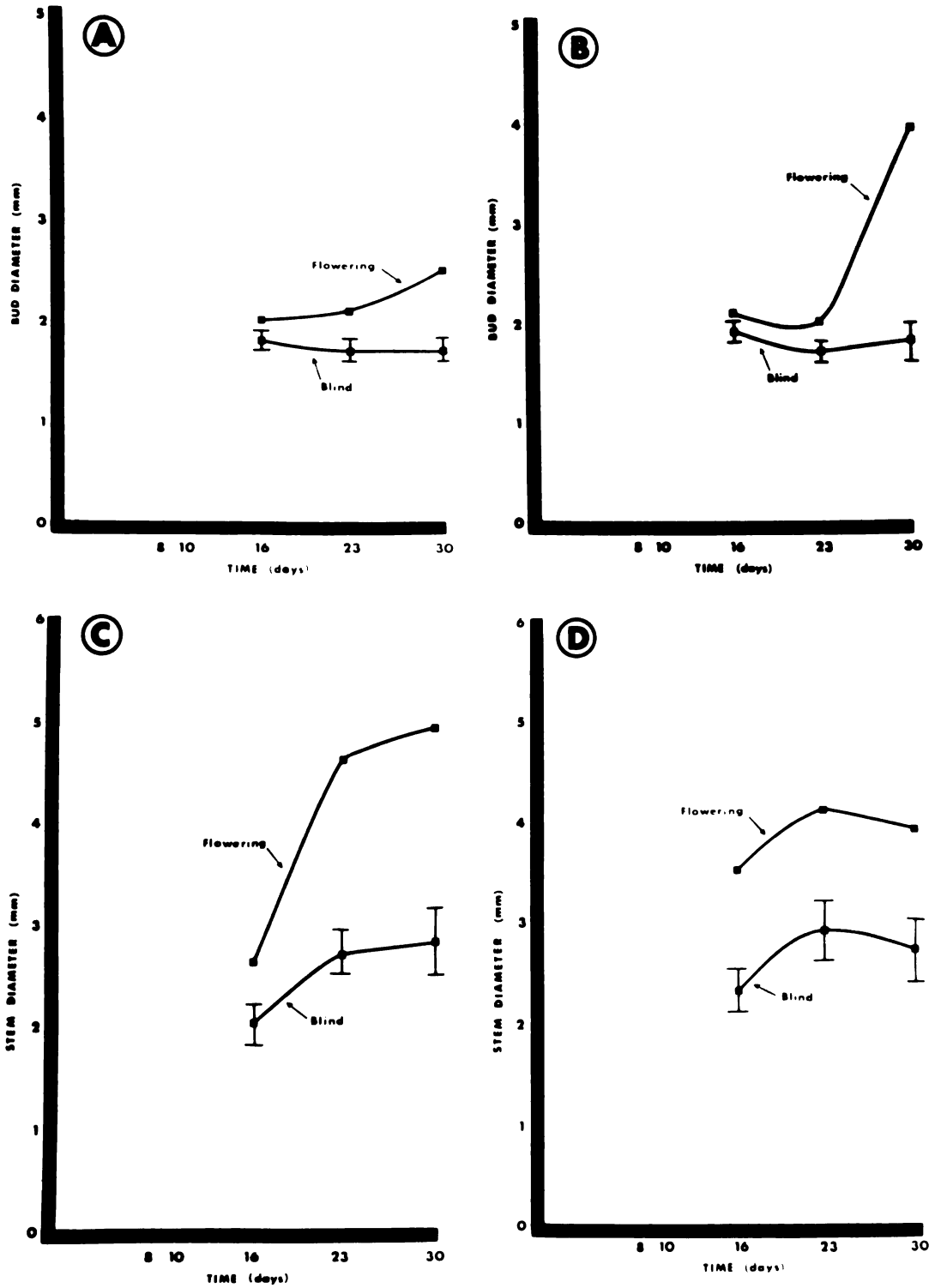
A
BUD DIAMETER (mm)
Flowering
Blind
8 10 16 23 30
TIME (days)
B
BUD DIAMETER (mm)
Flowering
Blind
8 10 16 23 30
TIME (days)
C
STEM DIAMETER (mm)
Flowering
Blind
8 10 16 23 30
TIME (days)
D
STEM DIAMETER (mm)
Flowering
Blind
8 10 16 23 30
TIME (days)

Figure 4. Bud and stem diam of 'Forever Yours' and 'Cara Mia' rose shoots, summer 1976. A. Bud diam 'Forever Yours'. B. Bud diam, 'Cara Mia'. C. Stem diam, 'Forever Yours'. D. Stem diam, 'Cara Mia'.

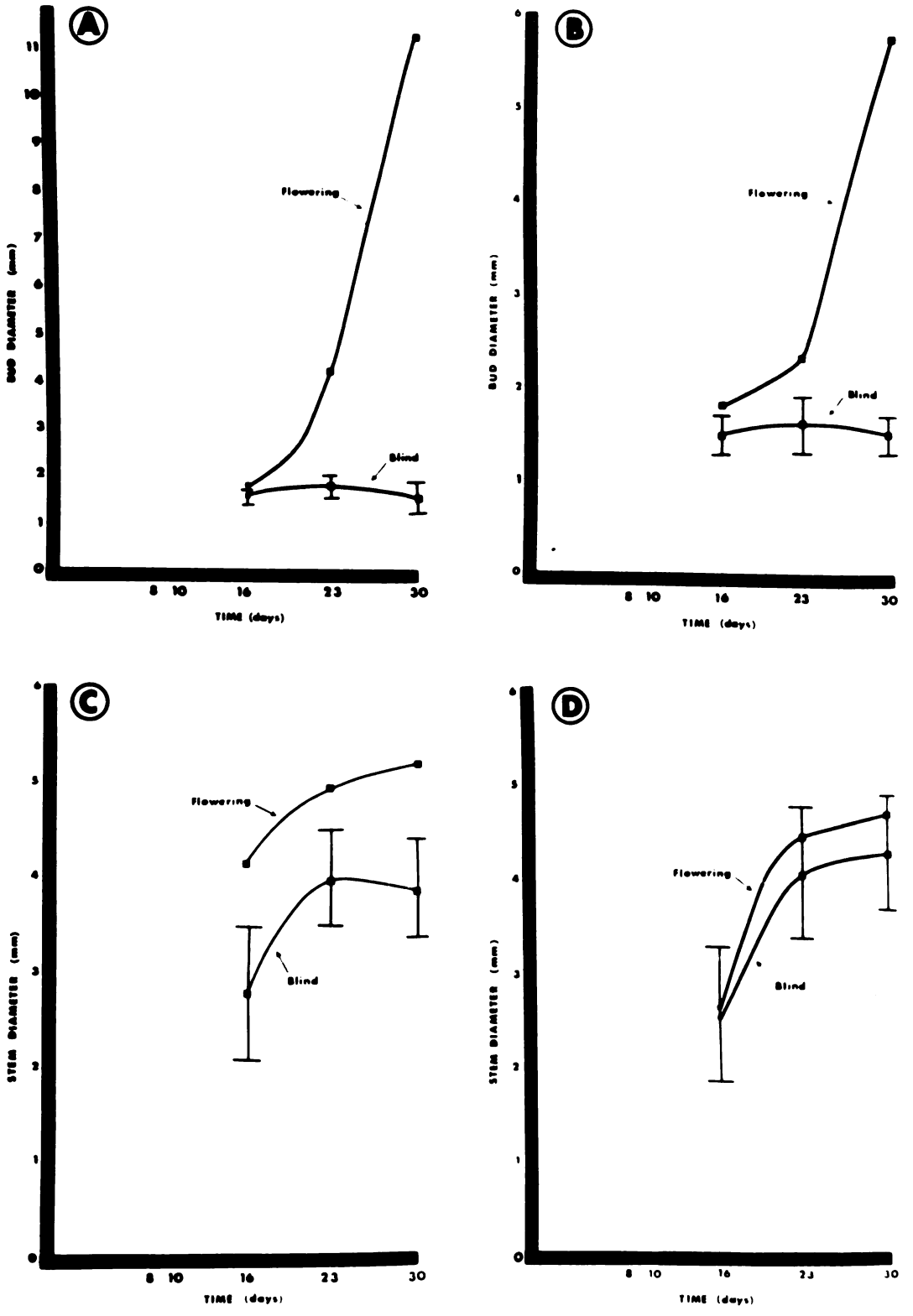
A
B
C
D
Flowering
Blind
BUD DIAMETER (mm)
STEM DIAMETER (mm)
TIME (days)
8
10
16
23
30

Table 1. The Effect of Stem Diam on Blind Shoot Production

Type of Shoot	Stem Diameter (mm)	
	'Forever Yours'	'Cara Mia'
Blind	4.5	4.6
Flowering	5.2[x]	5.7[x]

[x]F-test significant at 5% level.

correlation was found on both cvs. between the diam of the original stem and the new shoot diam after 23 days (Table 2).

Discussion

Quality of greenhouse rose flowers is generally determined by grades and standards which involve stem length, stem strength, flower size and stage of flower at harvest. The largest quantity of high quality flowers is generally produced during the summer months. During the winter months, stem diam is generally reduced due to the low light levels; consequently blind shoot production is increased. Early removal of blind shoots would reduce the total plant requirements for photosynthetic products and possibly result in thicker, stronger stems and larger flowers.

Previous work (7) has shown that 'Tropicana' rose plants grown with 20 days of HID lighting had 7% more blind shoots than plants grown with supplemental high intensity lighting from lateral bud initiation to flowering (7). Plants grown with 0, 5, and 10 days of HID lighting averaged 38, 26, 19% more blind shoots, respectively.

Moe (16) has shown that blindness is determined within the first 20 days after cut back on 'Baccara' rose. Shoot length, bud diam, and shoot diam indicate differences between blind and flowering shoots after 16 days and shoot length was a reliable indicator as early as 10 days following lateral bud initiation. These results suggest that blindness may be determined prior to 10 days.

The data indicate that shoots on 'Forever Yours' and 'Cara Mia' with small stem diam are predisposed to blindness. Research has clearly shown the dominant effects of environmental factors (1, 3, 4, 6) and

Table 2. Correlation of the Original Stem Diam with New Shoot Diam on Two Rose Cvs.

Cv.	N	R
'Forever Yours'	78	.85
'Cara Mia'	60	.89

position within the bench on the occurence of blindness (4). Although shoots may have a small stem diam, it does not necessarily indicate that the new shoot will become blind.

The prediction techniques discussed in this paper enable the prediction of blind shoots using morphological characteristics of shoot length, bud diam, and stem diam, 16 days following lateral bud initiation. The absolute values for each morphological characteristic will more than likely change with season and cv. These procedures could be applied during heavy cropping periods, allowing the commercial grower to remove certain shoot lengths 16 days after lateral bud initiation. Also, these prediction techniques will enable researchers to study chemical changes associated with the induction of blindness.

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SECTION III

FLORAL DEVELOPMENT AND BLINDNESS IN ROSES--

AN SEM STUDY

FLORAL DEVELOPMENT AND BLINDNESS IN ROSES--AN SEM STUDY

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ABSTRACT. Fresh, unfixed rose meristems were viewed in the scanning electron microscope to determine morphological differences and organogenesis of flowering and blind shoots. Gluteraldehyde fixed, ethanol dehydrated and critically point dried tissue were severely desiccated with individual cells being concave. Fresh tissue was turgid for at least 10 min in the microscope.

Visible signs of initiation were evidenced by the presence of sepal primordia followed by differentiation of petals, anthers and stigma. No evidence of flower initiation was observed in the blind shoot.

The greenhouse rose, Rosa hybrida L., is the leading monetary cut flower in the United States, with a wholesale value of over $68 million annually (14). Ideal environmental conditions for greenhouse roses include high light intensity (1, 2, 15), cool temp (7, 9, 15) and high CO_2 levels (6). The response of several cvs. to these environmental factors has been studied (1, 10), but there has been a limited amount of research relating morphological changes to these environmental manipulations (9).

Lindstrom (9) and Horridge and Cockshull (7) have studied the floral development of two greenhouse rose cvs. Lindstrom (9) has compared flowering and blind shoot development in 'Better Times', while Horridge and Cockshull (7) related apex organogenesis of 'Sonia' to shoot length and apical volume. In both studies, the light microscope was used to analyze floral development. Studies by De Hertogh et al. (3) and Emino and Rasmussen (5), related to apical development in Easter lily and

carnation, illustrated the value of the scanning electron microscope (SEM) in increasing depth of field, providing a means of viewing and photographing the entire apex and reducing sample preparation time.

In this paper, the morphological changes which occur in the blind and flowering rose apices during vegetative and reproductive organogenesis are presented.

Materials and Methods

Cultural. Two-year-old 'Tropicana' plants were grown in containers using standard greenhouse cultural practices (8, 13). Natural incident radiation was supplemented with high intensity Lucalox lighting (640 W/M^2) for 12 hr daily (2000-0800 hr). Minimum greenhouse night temp were $19^{o}\pm1^{o}C$.

Tissue Preparation. Rose meristems were prepared using two procedures. One group of excised meristems were placed in a 5% gluteraldehyde solution (buffered in a 1M phosphate buffer) for one hr. Leaf primordia were removed (under distilled water) to promote penetration of the fixative. The samples were post-fixed with 1% osmium tetroxide (OsO_4) followed by a 10-step graded ethanol series (10 to 100%), remaining in each solution for 15 min. Following three changes in 100% ethanol, samples were critical point dried (CPD Denton DCP-1) using clean, liquid CO_2.

The second technique utilized fresh unfixed rose meristems. Samples were collected, placed into distilled water and carried to the Michigan State University Electron Optics Lab (12) where leaf primordia were removed, under water, to prevent desiccation of the meristem.

When required, longitudinal sections were prepared with a single edged razor blade, then all meristems were mounted on aluminum SEM stubs

using Tube Coat (G. C. Electronics Co., Rockford, Ill.). CPD dried samples were sputter coated with 20-40 nm of gold; unfixed samples were placed directly into the sample chamber. Samples were viewed in the SEM (International Scientific Instrument Co. Super-Mini) using an accelerating voltage of 10 kv.

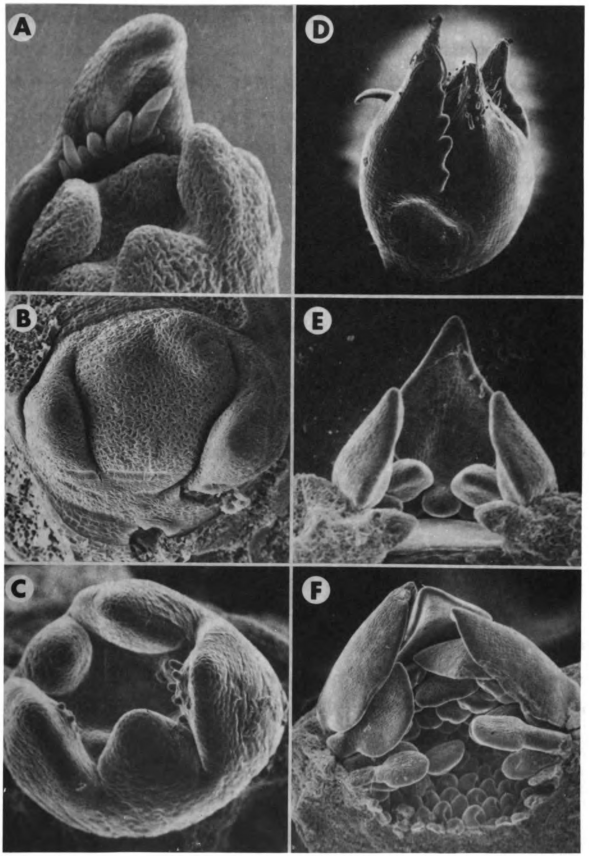
Results

The physical condition of the rose apex in the SEM was dependent upon the preparation technique. Samples fixed in gluteraldehyde, post-fixed in OsO_4, dehydrated and critical point dried were not satisfactory (Fig. 1A). All fixed samples were severely desiccated, and cell walls were concave. All other fixation and dehydration procedures were equally unsuccessful.

Fresh, unfixed samples (Fig. 1B-F) gave good detail and remained turgid for at least 10 min after being placed into the SEM. Fig. 1B illustrates a typical vegetative apex, while Fig. 1C-F illustrate stages in flower bud development.

The flat vegetative apex was radially symmetrical surrounded by young leaf primordia (Fig. 1B). Floral initiation was evidenced by the differentiation of sepal primordia in 8 cm long shoots (Fig. 1C) followed by differentiation of petals (Fig. 1E) and stamens and stigma (Fig. 1F). Petal primordia were first observed in 12 cm long shoots while stamens and stigma primordia were not present until the shoots were 22 and 28 cm. The early reproductive apex (Fig. 1C) was surrounded by a pentagonal whorl of sepal primordia (Fig. 1C) which elongated forming an enclosure over the hypanthium (Fig. 1D). Sepals were fused at the base with rounded points. The other floral parts, petals, stamens and stigma differentiated centripetally. Petals and whorls of stamen were found on the edge of the

Figure 1. Scanning electron micrographs of fixed and fresh intact apices of Rosa hybrida L. cv. Tropicana showing developmental changes between the vegetative and reproductive phase. A. Early reproductive meristem, gluteraldehyde fixation, 12 cm long. B. Vegetative meristem, fresh, 6 cm long. C. Early reproductive meristem, fresh, 10 cm long. D. Early reproductive meristem, fresh, 14 cm long, sepals not removed. E. Reproductive meristem, fresh, 3 layers of petals present, 18 cm long. F. Reproductive meristem, fresh, petals, anthers, stigma present, 28 cm long.


A
D
B
E
C
F

hypanthium and the stigma were borne in the center of the hypanthium developing toward the outside. The gynoecium was apocarpous with conduplicately folded styles. Light microscope observation showed that the stigma and style are not fused and originate separately from each ovary.

Blind shoots were selected at various morphological stages of development. Blind shoots were viewed 23 days after flower removal and were found to be vegetative (Fig. 2). Flowering shoots of the same age had several layers of petals differentiated and the bud was beginning to expand rapidly (Fig. 1E).

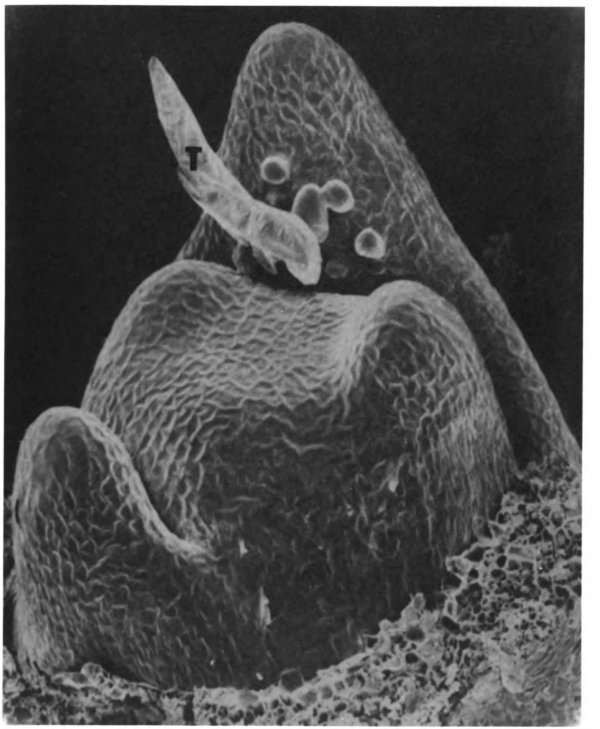
Discussion

The SEM has proven to be an excellent horticultural research tool. The sample preparation procedures generally vary with the type of plant tissue. Einert et al. (4) used freeze drying techniques while studies with carnations and chrysanthemums used fresh and chemically fixed tissue. The use of fixed tissue allows more latitude in the electron microscope, since fresh tissue must be viewed and photographed quickly. 'Tropicana' rose, however, gave unsatisfactory results with gluteraldehyde fixation.

Previous research described the blind shoot as an aborted reproductive meristem on 'Better Times' rose (9). Also, Horridge and Cockshull (7) found that events leading to the production of blind shoots on 'Baccara' occurred after flower initiation since vegetative shoots were never observed longer than 5 cm. In contrast, blind shoots on 'Tropicana' rose were found to be vegetative. These differences may simply be differences in cv. response to environmental conditions unfavorable for flower formation.

The actual mechanism controlling blindness is unknown. Several researchers have suggested that the occurrence of blind shoots is under

Figure 2. Scanning electron micrograph of the intact apex of a 23 day old blind shoot in Rosa hybrida L. cv. Tropicana. Vegetative meristem, 23 days old, 16 cm long.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hormonal control (10, 15, 16, 17, 18). Zieslin and Halevy (16, 17) and Moe (10) have studied this aspect of blind shoot production in 'Baccara' rose shoots 12-15 cm in length. However, work in our laboratory (11) suggests that blindness on 'Tropicana' rose can be predicted as early as 10 days after flower removal and when the newly emerging shoot is less than 2 cm long (11). Consequently, blindness in 'Tropicana' rose appears to be determined prior to the shoot being 2 cm long and approx one week before the first visible signs of floral initiation are evident.

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SECTION IV

HISTOCHEMICAL STUDY OF BLIND AND FLOWERING ROSE MERISTEMS

HISTOCHEMICAL STUDY OF BLIND AND FLOWERING ROSE MERISTEMS

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ABSTRACT. Activity of acid phosphatase, peroxidase, succinic dehydrogenase and the presence of starch and histones were determined in the blind and flowering rose shoots during the first 10 days after lateral bud initiation, at the time of floral initiation and following floral initiation. Histones were in both shoots during the first 10 days after lateral bud initiation then increased in the meristematic cells of the flowering shoot at initiation. No increase was apparent in the blind apex. Enzyme activity was found to be present in both shoot types with all enzymes appearing to be evenly distributed throughout the apex. Insoluble polysaccharides were found in older cells of blind and flowering shoots. Starch was not observed in either type of shoot.

Blindness in greenhouse roses is a major problem during winter months of low light in northern latitudes. As high as 50% of rose shoots may become blind. Moe (10) and Nell and Rasmussen (10) have shown that the blind shoot is shorter than the flowering shoot. Moe (10) studied the European cv. Baccara, while Nell and Rasmussen (11) compared 'Forever Yours', 'Cara Mia', and 'Tropicana'. Blind shoots had smaller stem diam and fewer leaves on these cvs. They found shoot length to be a reliable indicator of blindness as early as 10 days after lateral bud initiation on 'Tropicana', 'Forever Yours', and 'Cara Mia', suggesting that the chemical and hormonal changes causing blindness occur prior to 10 days.

Increased peroxidase and acid phosphatase activity have been shown to precede cell division and differentiation in the apices of several plants (12, 13, 16, 17). Fosket and Miksche (4) found high acid

phosphatase activity in shoot apical meristems of Pinus lambertiana prior to needle primordia initiation. High levels of peroxidase have been associated with differentiating cells in onion root tips (Allium cepa) (6, 16). Increased succinic dehydrogenase activity has been observed in areas of active cell division (13).

Gifford and Tepper (5) observed an increase in histone and starch levels after photoperiodic induction of Chenopodium album. Both substances were evenly distributed throughout the vegetative apex and they declined in conc. after initiation. Emino and Rasmussen (3) found increased starch levels in the carnation meristem, but histone levels did not change with the differentiating apex.

This study was conducted to investigate histochemical changes associated with blind shoot production in 'Tropicana' rose.

Materials and Methods

Cultural. Two-year-old 'Tropicana' plants (Rosa hybrida L.) were grown in 30 cm diam clay pots using a planting medium consisting of equal vol. of soil, peat, and Turface. Prior to starting each experiment, all flowers were removed and the plants were placed in a growth chamber (Lab-Line Controlled Environmental Rooms) or maintained in the greenhouse under high intensity supplemental lighting (640 W/M^2) for 12 hr daily (2000 to 0800 hr). Light intensity in the growth chamber was 300 W/M^2 with 70% of the input W from Cool-White fluorescent lamps and 30% from incandescent lamps. Min day-night temp were at $21^{o}\pm1^{o}C$ and $19^{o}\pm1^{o}C$, respectively.

Sampling. Meristems were collected daily, beginning 1 day after lateral bud initiation and continuing through 10 days. Blind and

flowering apices were also selected 16 and 23 days after lateral bud initiation to study the histochemical changes occurring prior to and following floral initiation. All samples were collected using the prediction techniques described by Nell and Rasmussen (11). Meristems used for peroxidase and acid phosphatase localization were plunged into liquid N_2 immediately following removal, while histone and starch samples were placed in Formalin-alcohol-acetic acid (FAA). Succinic dehydrogenase samples were placed into distilled water prior to examination.

Peroxidase. Longitudinal sections were cut at 25 µm on a cryostat (-20°C) and peroxidase localized using procedures of DeJong (2) and Poovaiah and Rasmussen (12). Equal parts of 1% H_2O_2 solution and 0.1M benzidene were placed on the microscope slide, the sections were incubated for 2 min and rinsed twice with distilled water. Heat treated samples (85°C for 15 min) and H_2O_2 deficient media were used as controls. Results were confirmed using the procedures described by Veech (16).

Acid Phosphatase. Acid phosphatase was localized with a post-incubation coupling procedure (8, 15). Longitudinal sections were cut at 25 µm on a cryostat (-20°C) and incubated for 30 min at 25°C in 25 mg of sodium 6-benzoyl-2-naphthyl phosphate and 2 g of NaCl dissolved in 80 ml of distilled water and 20 ml of 0.5M acetate buffer at pH 5.0. Samples were washed three times in cold water, placed in a freshly prepared solution of tetrozotized diorthoanisdine (1 mg/ml) in distilled water at 4°C, made alkaline with sodium bicarbonate, for 5 min. The tissue sections were washed in three changes of cold (4°C) 0.85% NaCl and preserved on the slide with glycine. Enzyme activity was evidenced by a red color. Control sections were treated with tetrozotized diorthoanisdine without substrate. Results were confirmed using the procedure described by Gomori (7, 8).

Dehydrogenase. Fresh, free hand sections were incubated in a mixture of 0.05M succinate, 0.1% of 2(p-iodophenyl-3-(p-nitro-phenol)-5-phenyl) tetrazolium chloride (Sigma Chemical Co., St. Louis) and 0.05M phosphate buffer at pH 7.3 (Defendi and Pearson, 8). Sections were incubated for 1 hr, washed in distilled water and enzyme localization immediately observed. Red coloration of the tissue was evidence of enzyme activity. Controls included heat treated tissue and tissue sections incubated without substrate.

Histones. Samples were dehydrated, paraffin embedded and cut at 10 µm on a rotary microtome. Staining procedures were those previously described by Alfert and Geschwind (1) and Jensen (8). Nucleic acids were removed by placing the samples in a boiling water bath consisting of 15% trichloroacetic acid for 30 min. Histones appeared green and localization was observed with a light microscope.

Starch. Sample preparation was the same as described for histones. Samples were stained using an IKI solution (8). Newly formed starch appears red using this procedure while older starch stains dark blue. Results were confirmed using the NaOH extraction of starch crystals (8) and Periodic Acid-Shiff's reaction for total insoluble polysaccharides (8).

Results

Histochemical analysis of 'Tropicana' rose revealed that both flowering and blind apices have high acid phosphatase, peroxidase and succinic dehydrogenase levels. The flowering meristem undergoes histone changes generally associated with conversion from a vegetative to a reproductive apex.

Acid phosphatase, peroxidase and succinic dehydrogenase were active in the blind and flowering shoots during the first 23 days after lateral

bud initiation. Enzyme localization was observed in the tunica and corpus with a decline in activity 80 mm below the apex.

Histones were concentrated in the nucleus of the tunica in blind and flowering vegetative meristems during the first 10 days after lateral bud initiation (Fig. 1A). Increased histone levels were observed in the tunica and corpus of the flowering meristem at floral initiation (Fig. 1B, 1C) but declined following initiation. Histone levels declined in the blind apex during the period of normal floral initiation and remained low after 23 days.

There was no evidence of starch in the meristem or adjacent stem tissue of blind or flowering shoots. Staining with KI solution failed to indicate the presence of starch crystals. However, insoluble polysaccharides were observed in the parenchyma tissue adjacent to the meristem. Extraction of the sections with 17.5% NaOH failed to remove the particles (Fig. 2).

Discussion

Low histone levels in the blind meristem and an accumulation of histones in the flowering apex prior to initiation confirm the earlier findings of Nell and Rasmussen (11) that the blind shoot in 'Tropicana' rose is a vegetative apex rather than an aborted reproductive meristem, as reported in previous research (9).

In earlier studies, we reported that blind shoots may accurately be predicted in three rose cvs. as early as 10 days following lateral bud initiation. The data in this study do not show enzymatic differences between blind and flowering shoots during this period. However, it may be possible that hormone differences or other enzymatic or chemical differences may occur in blind and flowering shoots.

Figure 1. Photomicrographs of the localization of histones in blind and flowering apices of 'Tropicana' rose (Rosa hybrida L.). A. A blind apex, 2 days after lateral bud initiation, showing presence of histones in the tunica layer. B. A flowering meristem, 14 days after lateral bud initiation, 6.5 cm long, showing presence of histones in tunica and corpus. Histones were localized above the black line, phase microscopy. C. Bright field microscopy of the meristem described in B. Histones were localized above the black line.

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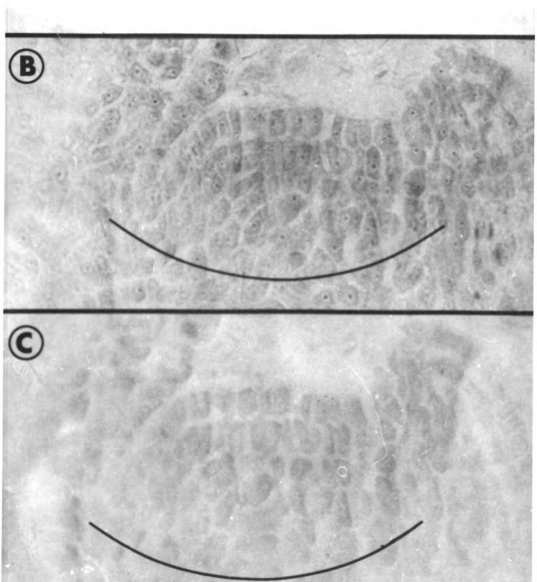
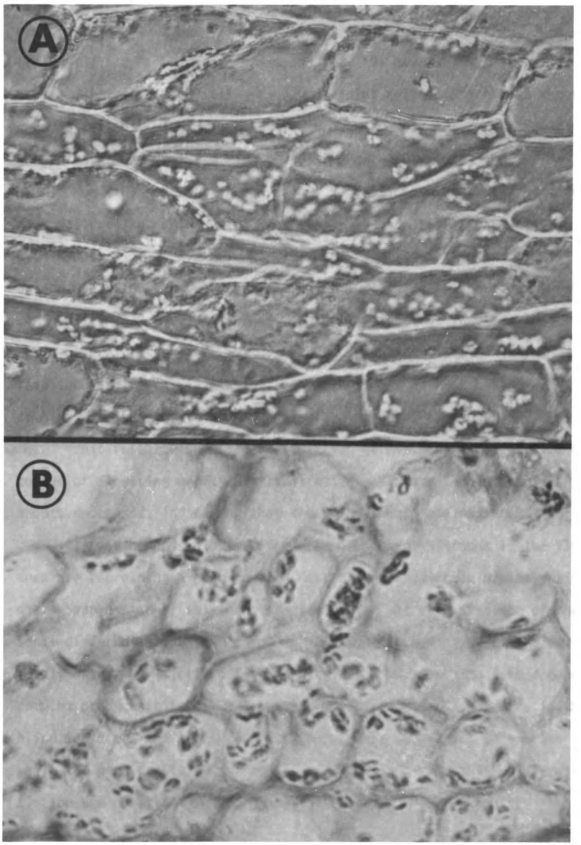


Figure 2. Photomicrographs of the histochemical localization of insoluble polysaccharides by IKI and Periodic Acid-Shiff's reaction in blind and flowering apices of 'Tropicana' rose (Rosa hybrida L.). A. A blind apex, 2 days after lateral bud initiation, showing insoluble polysaccharides in parenchyma cells. B. A flowering meristem, 2 days after lateral bud initiation, showing insoluble polysaccharides present after extraction with 17.5% NaOH.


A
B

The activity of acid phosphatase, peroxidase and succinic dehydrogenase in both types of shoot was not altogether unexpected. Van Fleet (16) and Poovaiah and Rasmussen (12, 13) have shown that these enzymes are associated with actively dividing cells. Blind rose shoots undergo cell division as part of the growth process, although flowering shoots have been shown to have a faster growth rate (11).

Moe (10) and Zieslin and Halevy (18, 19) have suggested hormones as a major factor determining blindness, based on research dealing with shoots 4 cm or longer. We (10) have reported that blindness is determined before the shoot reaches 2 cm in length, but have been unable to identify enzymatic differences in the two types of shoot at this age. Several researchers (1, 5) have shown an increase in histone levels during floral initiation, and have suggested that histones may combine with DNA molecules preventing synthesis of messenger RNA.

This research shows an accumulation of histones in the tunica and corpus of flowering meristem at initiation. Histone levels in blind shoots decreased, indicating that the blind shoot remains vegetative.

It may be possible that the accumulation of histones in the floral apex are repressing a gene complex controlling production of leaf primordia. Suppression of this gene complex may result in activation of the sepal differentiating gene complex. As each succeeding gene complex for petal, anther and pistil differentiation is activated, the former gene complex produces a factor which suppresses the latter. However, in the blind meristem, the leaf primordia gene complex continues to operate while the gene complexes for sepal, petal, anther and pistil differentiation are never activated.

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LITERATURE CITED

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APPENDICES

APPENDIX A1

Morphological Differences Between Blind and Flowering Shoots Fall and Winter 1975, Spring and Summer 1976

MORPHOLOGICAL DIFFERENCES BETWEEN BLIND AND FLOWERING SHOOTS FALL AND WINTER 1975, SPRING AND SUMMER 1976

An experiment was designed to study the gross morphological differences between blind and flowering rose shoots. Shoot length, bud diameter and stem diameter were measured on 'Tropicana' plants during the fall and winter of 1975, and spring and summer of 1976, while 'Forever Yours' and 'Cara Mia' were studied during the summer of 1976.

All plants were grown using the cultural practices described in the "Materials and Methods", Section II. Plants were grown in an all-glass greenhouse until the flowers were harvested. The plants were given 0, 5, 10, 20 days or supplemental high intensity lighting (Lucalox, 640 w/m^2, 12 hrs. daily) from lateral bud initiation to flowering. Following these lighting treatments, the plants were transferred to a Lab-Line growth chamber where the light intensity was maintained at $300w/m^2$. Measurements were begun 8 days after lateral bud initiation.

The following tables show the measurements for these morphological characteristics on 'Tropicana', 'Forever Yours', and 'Cara Mia' roses.

Influence of 0 Days of High Intensity Lighting[w] on Shoot Length, Bud Diameter and Stem Diameter of 'Tropicana' Rose.

	Number of Days After Lateral Bud Initiation																			
	8				10				16				23				30			
Confidence Interval[y]	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS
	Mean Shoot Length (cm)																			
Blind	1.5	1.0	1.0	1.0	2.2	1.7	1.6	1.3	6.5	4.8	3.6	2.6	12.0	8.5	14.9	9.4	13.3	12.0	24.3	15.0
Flowering	1.5	1.0	1.0	1.0	3.0	2.5	2.8	1.5	10.5	8.0	8.6	3.3	21.3	16.0	25.8	15.5	30.6	24.0	43.3	27.8
Confidence Interval ±	-	-	-	-	0.1	0.1	0.4	0.1	0.7	0.9	0.6	0.4	3.2	2.2	2.0	0.4	4.2	3.4	3.5	1.8
	Mean Bud Diameter (mm)[z]																			
Blind									1.4	1.8	1.6	1.6	1.5	1.7	1.6	1.5	1.6	1.7	1.6	1.5
Flowering									1.3	1.9	1.6	1.7	2.0	2.1	1.8	2.0	6.1	2.5	2.6	2.2
Confidence Interval ±									0.5	0.1	0.1	0.1	0.4	0.1	0.1	0.1	0.4	0.1	0.1	0.1
	Mean Stem Diameter (mm)																			
Blind										2.0	2.4	2.5		2.7	3.2	2.9		2.8	3.4	3.0
Flowering										2.6	2.8	3.0		4.6	3.8	3.5		4.9	3.8	4.5
Confidence Interval ±										0.2	0.2	0.1		0.2	0.2	0.2		0.3	0.2	0.2

[w]Plants were shifted to a Lab-Line growth chamber maintained at 300 w/m^2 following the supplemental high intensity lighting.

[x]Shoot Length determined from the point of shoot origin to the tip of the shoot. Bud diameter was measured at the widest point and stem diameter was recorded immediately distal to the first 3-leaflet leaf.

[y]Confidence intervals computed at .05 level of significance on blind shoots.

[z]Bud diameter and stem diameter were not measurable until 16 days after lateral bud initiation.

Influence of 5 Days of High Intensity Lighting[w] on Shoot Length, Bud Diameter and Stem Diameter of 'Tropicana' Rose.

	Number of Days After Lateral Bud Initiation																			
	8				10				16				23				30			
Confidence Interval[y]	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS
	Mean Shoot Length (cm)																			
Blind	1.5	1.0	1.0	1.0	1.9	1.8	1.1	1.5	5.0	5.1	2.7	3.5	13.6	9.0	10.5	8.4	20.0	11.4	18.0	12.8
Flowering	1.5	1.0	1.0	1.0	3.1	3.2	1.5	1.9	7.3	8.6	6.9	4.2	19.4	16.8	20.2	13.5	27.3	24.0	37.2	27.3
Confidence Interval ±	-	-	-	-	0.8	0.3	0.3	0.3	1.1	0.4	0.8	0.3	4.4	1.2	5.0	1.8	4.8	2.4	8.0	2.1
	Mean Bud Diameter (mm)[z]																			
Blind									1.4	1.8	1.5	1.4	1.5	1.7	1.7	1.5	1.6	1.8	1.6	1.5
Flowering									1.2	1.6	1.6	1.6	2.0	1.9	2.2	2.1	5.0	2.7	2.2	2.2
Confidence Interval ±									0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1
	Mean Stem Diameter (mm)																			
Blind										1.8	2.6	2.2		2.6	3.1	2.8		2.6	3.3	2.9
Flowering										2.7	3.1	2.9		3.3	5.1	3.6		3.5	5.5	3.8
Confidence Interval ±										0.2	0.3	0.2		0.1	0.9	0.2		0.1	0.5	0.2

[w]Plants were shifted to a Lab-Line growth chamber maintained at 300 w/m^2 following the supplemental high intensity lighting.

[x]Shoot Length determined from the point of shoot origin to the tip of the shoot. Bud diameter was measured at the widest point and stem diameter was recorded immediately distal to the first 3-leaflet leaf.

[y]Confidence intervals computed at .05 level of significance on blind shoots.

[z]Bud diameter and stem diameter were not measurable until 16 days after lateral bud initiation.

Influence of 10 Days of High Intensity Lighting[w] on Shoot Length, Bud Diameter and Stem Diameter of 'Tropicana' Rose.

	Number of Days After Lateral Bud Initiation																			
	8				10				16				23				30			
Confidence Interval[y]	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS
									Mean Shoot Length (cm)											
Blind	1.5	1.0	1.0	1.0	2.1	1.9	1.3	2.2	5.0	6.0	4.3	5.6	11.3	12.3	12.6	11.5	19.5	15.3	20.2	16.6
Flowering	1.5	1.0	1.0	1.0	2.8	1.7	3.5	3.5	9.9	7.2	8.9	10.3	23.4	15.2	28.8	19.5	30.6	21.2	32.0	32.0
Confidence Interval ±	-	-	-	-	0.3	0.4	3.2	0.4	1.8	1.2	3.2	1.0	4.3	2.0	4.4	1.3	5.8	1.2	6.2	1.8
									Mean Bud Diameter (mm)[z]											
Blind									1.4	1.8	1.4	1.5	1.5	1.8	1.5	1.5	1.6	1.7	1.5	1.6
Flowering									1.6	1.8	1.5	1.5	2.3	1.9	2.2	1.8	5.4	2.6	2.8	3.0
Confidence Interval ±									0.4	0.3	0.2	0.1	0.4	0.2	0.2	0.1	0.4	0.1	0.3	0.2
									Mean Stem Diameter (mm)											
Blind										1.9	2.5	2.3		2.9	2.9	2.7		3.0	3.1	2.7
Flowering										2.5	3.2	3.2		3.5	3.6	3.8		3.7	4.8	4.2
Confidence Interval ±										0.4	0.3	0.1		0.2	0.4	0.2		0.2	0.6	0.2

[w]Plants were shifted to a Lab-Line growth chamber maintained at 300 w/m^2 following the supplemental high intensity lighting.

[x]Shoot Length determined from the point of shoot origin to the tip of the shoot. Bud diameter was measured at the widest point and stem diameter was recorded immediately distal to the first 3-leaflet leaf.

[y]Confidence intervals computed at .05 level of significance on blind shoots.

[z]Bud diameter and stem diameter were not measurable until 16 days after lateral bud initiation.

Influence of 20 Days of High Intensity Lighting[w] on Shoot Length, Bud Diameter and Stem Diameter of 'Tropicana' Rose.

	Number of Days After Lateral Bud Initiation																			
	8				10				16				23				30			
Confidence Interval[y]	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS
	Mean Shoot Length (cm)																			
Blind	1.5	1.0	1.0	1.0	2.3	2.4	1.8	1.9	7.1	7.4	7.8	6.1	14.7	13.9	17.3	14.8	19.3	16.4	19.6	18.4
Flowering	1.5	1.0	1.0	1.0	3.2	2.0	1.5	2.5	10.5	7.1	14.5	10.8	27.8	18.4	30.9	19.2	39.0	26.4	36.0	24.2
Confidence Interval ±	-	-	-	-	0.3	0.7	0.5	0.3	2.4	1.5	2.1	1.0	5.4	2.1	3.2	2.4	6.9	3.9	5.3	2.6
	Mean Bud Diameter (mm)[z]																			
Blind									1.4	1.9	1.7	1.6	1.5	1.7	1.7	1.6	1.5	1.7	1.5	1.7
Flowering									1.6	2.0	1.6	1.7	1.7	1.7	2.3	1.9	3.3	2.3	2.8	2.2
Confidence Interval ±									0.4	0.2	0.1	0.1	0.1	0.1	0.3	0.1	0.2	0.2	0.2	0.2
	Mean Stem Diameter (mm)																			
Blind										2.1	2.7	2.3		2.8	3.0	2.7		3.1	3.4	2.8
Flowering										2.3	3.3	3.1		3.3	3.7	2.5		3.6	4.1	4.2
Confidence Interval ±										0.1	0.4	0.1		0.2	0.3	0.2		0.2	0.4	0.1

[w]Plants were shifted to a Lab-Line growth chamber maintained at 300 w/m^2 following the supplemental high intensity lighting.

[x]Shoot Length determined from the point of shoot origin to the tip of the shoot. Bud diameter was measured at the widest point and stem diameter was recorded immediately distal to the first 3-leaflet leaf.

[y]Confidence intervals computed at .05 level of significance on blind shoots.

[z]Bud diameter and stem diameter were not measurable until 16 days after lateral bud initiation.

Influence of Continuous High Intensity Lighting on Shoot Length, Bud Diameter and Stem Diameter of 'Tropicana' Rose.

Confidence Interval[y]	Number of Days After Lateral Bud Initiation																			
	8				10				16				23				30			
	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS	F	W	S	SS
	Mean Shoot Length (cm)																			
Blind	1.5	1.0	1.0	1.0	2.6	2.1	1.9	1.9	6.6	6.3	7.4	6.0	13.3	11.9	14.4	15.3	17.1	17.3	19.0	19.7
Flowering	1.5	1.0	1.0	1.0	2.3	1.5	1.4	2.6	8.6	7.1	12.8	9.5	20.4	19.8	17.3	21.5	26.4	28.0	30.0	37.0
Confidence Interval ±	-	-	-	-	0.4	0.3	0.4	0.4	1.5	1.0	1.3	0.9	4.4	2.7	2.6	1.5	6.2	4.0	4.3	2.3
	Mean Bud Diameter (mm)[z]																			
Blind									1.4	1.9	1.6	1.5	1.5	1.7	1.7	1.6	1.6	1.8	1.7	1.6
Flowering									1.6	2.0	1.5	1.6	2.0	2.0	2.1	2.3	4.3	3.9	3.5	3.5
Confidence Interval ±									0.6	0.1	0.1	0.2	0.4	0.1	0.2	0.2	0.3	0.2	0.2	0.3
	Mean Stem Diameter (mm)																			
Blind										2.3	2.4	2.5		2.9	2.4	2.8		2.7	3.3	2.9
Flowering										3.5	3.6	3.2		4.1	4.0	3.7		3.9	4.5	4.3
Confidence Interval ±										0.2	0.3	0.2		0.3	0.3	0.2		0.3	0.5	0.3

[w]Plants were shifted to a Lab-Line growth chamber maintained at 300 w/m^2 following the supplemental high intensity lighting.

[x]Shoot Length determined from the point of shoot origin to the tip of the shoot. Bud diameter was measured at the widest point and stem diameter was recorded immediately distal to the first 3-leaflet leaf.

[y]Confidence intervals computed at .05 level of significance on blind shoots.

[z]Bud diameter and stem diameter were not measurable until 16 days after lateral bud initiation.

Shoot Length, Bud Diameter and Stem Diameter Measurements on 'Forever Yours' and 'Cara Mia' Roses, Summer 1976.

	Number of Days After Lateral Bud Initiation									
	8		10		16		23		30	
Confidence Interval[y]	Forever Yours	Cara Mia	Forever Yours	Cara Mia	Forever Yours	Cara Mia	Forever Yours	Cara Mia	Forever Yours	Cara Mia
					Mean Shoot Length (cm)					
Blind	1.0	0.8	1.8	1.2	13.0	6.8	24.0	18.9	28.3	28.6
Flowering	1.1	0.9	2.9	1.7	19.2	7.7	37.5	24.1	54.8	41.2
Confidence Interval ±	-	-	0.4	0.4	2.0	3.0	4.1	4.3	4.7	7.8
					Mean Bud Diameter (mm)[z]					
Blind					1.6	1.5	1.8	1.6	1.5	1.5
Flowering					1.8	1.8	4.2	2.3	11.2	5.7
Confidence Interval ±					0.1	0.2	0.2	0.3	0.3	0.2
					Mean Stem Diameter (mm)					
Blind					2.7	2.5	3.9	4.0	3.8	4.2
Flowering					4.1	2.6	4.9	4.4	5.1	4.6
Confidence Interval ±					0.7	0.7	0.5	0.7	0.5	0.6

[x]Shoot Length determined from the point of shoot origin to the tip of the shoot. Bud diameter was measured at the widest point and stem diameter was recorded immediately distal to the first 3-leaflet leaf.

[y]Confidence intervals computed at .05 level of significance on blind shoots.

[z]Bud diameter and stem diameter were not measurable until 16 days after lateral bud initiation.

APPENDIX A2

Research and Commercial Implications of Blind Shoot Research

The greenhouse rose, Rosa hybrida L., is the leading monetary cut flower crop in the United States. Commercially, maximum economic profits are realized when cultural procedures result in the largest number of marketable flowers. However, even ideal environmental conditions fail to produce 100 percent flowering shoots. Blind (non-flowering) shoots comprise as many as 50 percent of all shoots. Maximization of flower shoot production on commercial rose cultivars will not be achieved until the morphological and chemical changes associated with the induction of blindness are identified with certainty and resolved.

This research has shown that blind shoots on 'Tropicana', 'Forever Yours', and 'Cara Mia' can be identified within the first 10 days following lateral bud initiation using morphological characteristics. A comparison of the organogenesis of the flowering shoot and development of the blind shoot on 'Tropicana' has shown that the blind shoot remains vegetative throughout the growth cycle and is not an aborted reproductive meristem, as previously reported. Histone level analysis in both types of shoot showed increased histone levels in flowering shoots and decreased levels in blind shoots at the time of floral initiation.

Future Research Implications: Previous research on 'Better Times' and 'Baccara' roses indicated that blind shoots are aborted reproductive meristems. This study produced conclusive evidence that blind shoots are vegetative. Future research studies should utilize the scanning electron microscope to determine whether blind shoots on other rose cultivars are vegetative or reproductive.

Enzyme analysis of blind and flowering rose shoots failed to show differences in either type of shoot at the tissue level. The transmission electron microscope should be used to further investigate

enzymatic differences in blind and flowering shoots at the cellular level. In addition, the transmission electron microscope could be used to study possible differences in organelle structure in both types of shoot.

Hormone changes have been presented as a possible explanation for blindness in other rose cultivars. Previous research has dealt with hormonal levels in rose shoots 12-15 cm. long. However, our results indicate that hormonal changes associated with blind shoot production occur within the first 10 days following lateral bud initiation or before the shoot is 2 cm. in length. Hormonal changes should focus on blind and flowering shoots during this stage of development.

Commercial Implications: Even under ideal environmental conditions, commercial rose cultivars produce as many as 50 percent blind shoots. Blind shoots must be either pinched or completely removed from the plant at regular intervals since they fail to produce a marketable rose flower. The prediction techniques described in this research could be used to allow early removal of blind shoots during heavy rose cropping periods. It should be possible to perfect a selection procedure permitting the removal of any shoot with a shoot length and stem diameter below a certain value.

Continuous use of high intensity lighting has been beneficial in reducing blind shoot production in commercial rose cultivars. However, high energy costs have discouraged many northern growers from installing high intensity lighting fixtures. This research has shown that 20 days of supplemental lighting (12 hours daily) produces only 7 percent more blind shoots than plants lighted during the entire growth cycle. It is now possible for commercial growers to minimize blindness by lighting

for only 20 days. Also, the use of supplemental lighting will enable growers to supply local and regional markets with cultivars generally produced only in high light areas.