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PROJECT NO. I-795-84

**Introduction of Plants from the Brodiaea
Complex as New Cut Flowers**

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B A R D

FINAL REPORT

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INTRODUCTION OF PLANTS FROM THE BRODIAEA COMPLEX AS NEW CUT FLOWERS

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C. ABSTRACT

About 160 accessions of plant material have been collected so far. They belong to 23 species of the 3 genera of the brodiaea complex: *Tritelia*, *Brodiaea* and *Dichelostemma*. The accessions have been grown and evaluated in Davis and Rehovot, and their main morphological and phenological properties determined. Twenty selected genotypes are now being propagated for testing under semi-commercial conditions.

A detailed developmental morphology study has been carried out with *Triteleia laxa* 'Queen Fabiola' (QF). The main axis of dormant corms harvested from the field in the summer is comprised of a leaf sheath, the two leaf primordia, and a vegetative meristem. Floral initiation, as indicated by formation of bracts on the flanks of the apical meristem, occurs after planting. The bracts enlarge and enclose the reproductive dome, which divides sequentially to form floral primordia of different ages. The daughter corm, which forms from the basal portion of the stem and whose meristem is derived from the primary axis, starts to enlarge early in the growing season. There is little competition for assimilates between the vegetative propagules (daughter corm and cormels) and the developing inflorescence until the start of fruit development. Seed dormancy of most species can be overcome by stratification of wet seeds at 6° for 6 weeks. Some species require longer treatment.

Flowering percentage, number of florets per scape and scape length were not affected by a 10-fold increase in corm size above the critical weight of 0.6g in *T. laxa* (QF). Diameter of the apical meristem in dormant corms increased up to the critical weight, but not above it. In contrast, the weight and the number of daughter corms and cormels were closely correlated with mother corm size. Similar behavior was observed with *T. bridgesii* and *T. ixioides* but not with *D. ida-maia*.

Flowering of *T. laxa* (QF) is only slightly affected by dry storage of corms, but great acceleration of flowering was obtained by vernalizing corms in wet soil at 7° for 8 to 12 weeks, with little effect on flower quality (stem length and number of florets). Flowering is also hastened by long photoperiods, but LD also decreased flower quality and flowering percentage. Scape length, which was not affected by photoperiod or mother corm size, was increased when plants were grown at low night temperatures.

Vernalization is the major factor advancing flowering of *T. bridgesii*, *T. lutea* and wild genotypes of *T. laxa*, *D. pulchellum*, *D. congestum* and *D. multiflorum*. These wild species responded also to dry cold storage of corms, but less than to wet cold storage.

In all these species flowering was also hastened by LD, but usually much less so than by cold storage.

Exposure of dormant corms of *T. laxa* (QF) to 20 ppm C_2H_4 for 7 days resulted in increased apical meristem size, early sprouting, early flowering, more flowers per inflorescence, and increases in fresh weight of daughter corms and cormels. The respiration rate of the C_2H_4 -treated corms increased to 4-5 times that of the controls during the 7-day treatment, declined markedly after termination of the C_2H_4 treatment, but remained higher than that of the controls. The C_2H_4 effects were associated with faster growth and greater final size of the apical meristem (determined by scanning electron microscopy). Leaves produced by C_2H_4 -treated corms were wider and longer than those of the controls. The consequent increase in photosynthetic capacity may be the cause of the increased fresh weight of the daughter corms and cormels.

Individual flowers of cut *Triteleia laxa* (QF) inflorescences senesced four days after opening. Best vase life was achieved by harvesting inflorescences 1-2 days before anthesis of the first flower and then holding in a vase solution containing 2% sucrose and 200 ppm 8-hydroxyquinoline citrate. Inflorescences treated in this way had a display life of 12 days. Decreasing the pH of the vase solution or pulsing inflorescences with 10% sucrose for 16 hr did not increase their longevity. *T. laxa* (QF) flowers pre-treated with 10% sucrose overnight could be stored for up to two weeks without significant reduction in vase life. Some wild genotypes of *T. laxa* had longer vase life than QF and stored for up to 3 months without loss of quality.

Unpollinated *laxa* flowers produced no measurable C_2H_4 during their entire life. The flowers are protandrous - the stigma was receptive only when the petals had started to senesce. Pollination induced C_2H_4 production, which peaked 9 hours after pollination. Treatment with C_2H_4 ($0.03 \mu l l^{-1}$) induced senescence of open flowers, completely inhibited opening of buds and petal growth, and promoted ovary growth. Silver thiosulfate (STS) had no effect on flowers kept in air, but counteracted the effects of applied C_2H_4 . The effect of C_2H_4 on ovary growth seems to be indirect, via promotion of petal senescence and mobilization of the metabolites of the petals to the ovary.

D. OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The following is a citation from the original research proposal:

"The objective of the proposed research is to introduce a range of cut flowers from

the native Californian flora to the floriculture industries of Israel and the USA. Although the brodiaea complex includes many native species and varieties of various colors and flower shapes, they are similar in their botanical characteristics and growth habit.

Instead of the single blue color and shape available now, we aim at developing a diverse group of varieties such as is available today for some other bulbous plants such as gladiolus, tulip, iris, freesia and lily. In each group of these plants, various species and interspecific hybrids are included.

The economic significance of the research may be considerable since the floriculture industry is constantly in search of new plants, which often obtain high prices because of their novelty. Although this proposal is primarily an applied project, it is hoped that it will also provide new basic information on the developmental and flowering physiology of geophytes. Most of the physiological work on bulbous plants has been done with Mediterranean and South-African plants. This project should provide information on the physiological and ecological responses of an important group of the Californian geophytes."

E. BODY OF REPORT

The nature of this research required constant cooperation of the investigators from both countries. All the sections of this research were carried out jointly by the two groups. This report is, therefore, written according to the various subject matters generally without distinguishing between the work done in the two countries.

1. Introduction

The genus *Brodiaea* was formerly called *Hookera* and included several subgenera. They are now generally separated into 3 genera: *Brodiaea*, *Dichelostemma* and *Triteleia*. Some botanists consider these plants to belong to the Liliaceae, others to the Alliaceae. But most authors now place them in the family Amaryllidaceae, tribe Allieae, subtitle Brodiaeinae (22, 23, 29). Detailed taxonomic, cytological, and morphological descriptions were presented in the introduction to our research proposal. The natural distribution of the plants of this complex is restricted to western North America from British Columbia to Baja California, from the Pacific Coast to the Sierra Nevada foothills. There are at least 40 species of the three genera, showing a wide variation in color, form, and probably physiological and ecological responses. Although the brodiaeas are closely related to

onion and other alliums, even being included in the Alliaceae by some taxonomists, they are free of the objectionable odor of the true alliums. The only member of the brodiaeas which is used as a commercial cut flower is a blue genotype of *Triteleia laxa*, named by the Dutch, 'Queen Fabiola' (QF).

The research plan as outlined in our proposal is comprised of two main lines: (a) collection of wild species and genotypes of apparent horticultural value and (b) study of the horticultural and physiological properties of the plants. Since the natural propagation rate of most of the native brodiaeas is quite slow, we decided to carry out most of the horticultural and physiological studies with *T. laxa* (QF), of which enough plant material could be obtained at the beginning of the research.

2. Survey and Collection of Plant Material

Since this is an introduction project of a native Californian plant, an important part of the project concentrated on collecting as many species and genotypes as possible. Most of the material was collected as seeds or corms of wild plants in their native habitat. Some plants were obtained from botanical gardens or amateur gardeners. The plant material collected was divided in most cases into two parts: one grown in raised beds with full light at the Department of Environmental Horticulture, UC Davis, and the other in a saran net house (15% shade) at the Department of Horticulture, HU Rehovot. In both locations the plants were protected from predatory soil rodents by dense net placed below the bed or by growing the plants in raised-isolated beds.

About 160 accessions have been collected so far; most of them are listed below. Species and genotypes of 3 genera are included: *Brodiaea*, *Triteleia* and *Dichelostemma*. In some cases the species could not be identified or could only be tentatively identified (Table 2.1)

3. Evaluation and Selection of Plant Material

Evaluation of the various accessions was carried out in Davis and Rehovot. Corms were graded according to size before planting, and detailed data was taken on various parameters of flower and corm production. Flowering date in Rehovot is a few weeks earlier than in Davis. It is mid-January to mid-April in Rehovot and late March to May in Davis for most genotypes.

Table 2.1. Plant material collected by July, 1988, presently in Davis or Rehovot.

Plant and Description	Source	Ac. #
<u><i>Brodiaea</i></u>		
<i>B. kinkiensis</i>	UCB Bot. Gard. (84)	52
<i>B. coronaria</i> (violet)	Dixon (85)	40
<i>B. coronaria</i>	UCB Bot. Gard. (84)	53
<i>B. coronaria</i>	Redding (87, 88)	107, 134
<i>B. jolonensis</i> (violet)	UCB Bot. Gard. (84)	63, 48
<i>B. elegans</i> (purple)	UCB Bot. Gard. (84)	54, 55
<i>B. elegans</i>	Red Bluff (86, 87, 88)	88, 125
<i>B. elegans</i> (selected genotypes)	Dusty Oaks (88)	128-133
<i>B. californica</i> (pink)	UCB Bot. Gard. (84)	57
<i>B. californica</i>	Tilden Park, Berkeley (84)	61
<i>B. californica</i>	Redding (86, 87)	91
<i>B. californica</i>	Sierra foothills (87, 88)	103
<i>B. californica</i> (selected genotypes, blue, pink, white)	Redding (87, 88)	105, 135-145
<i>B. filifolia</i>	Carlsbad, San Diego (85)	79
<i>B. minor</i>	Shasta Co. (85)	86
<i>B. volubilis</i>	Baccus, San Jose (87)	88
<i>B. terrestris</i>	Baccus, San Jose (87)	89
<u><i>Dichelostemma</i></u>		
<i>D. congestum</i> (purple)	Sierra foothills (83)	2, 5
<i>D. congestum</i> (purple)	Redding (85)	85
<i>D. congestum</i> (purple)	Red Bluff (83)	16
<i>D. congestum</i> (purple)	Red Bluff (84)	28
<i>D. congestum</i> (white)	Sierra foothills (84)	68, 31
<i>D. congestum</i> (dark lavender)	Cherokee Rd. (84)	66, 47, 77, 78
<i>D. pulchellum</i>	Table Mt., Oroville (83)	1
<i>D. pulchellum</i> (dark purple)	UCB Bot. Gard. (84)	49
<i>D. pulchellum</i>	Santa Cruz Island (86)	92
<i>D. multiflorum</i> (white)	Redding (84, 87)	69, 44, 118, 123
<i>D. multiflorum</i> (violet)	UCB Bot. Gard. (84)	50
<i>D. multiflorum</i>	Tilden Park, Berkeley (84)	62
<i>D. multiflorum</i> (long)	Red Bluff (87)	119
<i>D. multiflorum</i>	Sierra foothills (88)	126
<i>D. multiflorum</i> (white-pink)	Sierra foothills (88)	127

<i>D. multiflorum</i>	Redding (88)	131
<i>D. ida-maia</i> (red)	Redding (84)	46
<i>D. ida-maia</i>	Redding (85)	80, 73, 74
<i>D. ida-maia</i> (long)	Redding (87, 88)	109, 128
<i>D. ida-maia</i> × <i>D. multiflorum</i> (natural hybrid)	Redding (87)	108
<i>D. ida-maia</i> (very long)	Eureka (88)	153
 <u><i>Triteleia</i></u>		
<i>T. laxa</i> (Q. Fabiola)	Orig. - The Netherlands	18, 17
<i>T. laxa</i>	Rt. 128, Napa Co. (83)	59
<i>T. laxa</i> (blue)	Red Bluff (83)	33, 3, 14
<i>T. laxa</i> (blue, selected)	Sierra foothills (83)	11, 15
<i>T. laxa</i> (white)	Sierra foothills (83, 87)	7, 115
<i>T. laxa</i> (blue)	Sierra foothills (83)	8
<i>T. laxa</i> (white w/lt. purple)	Dixon (83)	19, 20
<i>T. laxa</i> (large w/dk. purple)	Dixon (83)	21, 23-27
<i>T. laxa</i> (blue)	Dixon (83)	70
<i>T. laxa</i> (white)	Dixon (83)	72, 71, 22
<i>T. laxa</i> (?) (dark blue)	Nicosia Dam, Marin Co. (84)	45, 64
<i>T. laxa</i> (white, do not brown)	Bear Valley (87)	122
<i>T. laxa</i> (tall)	Yosemite (87)	124
<i>T. colochortis</i>	Sierra foothills (83)	9
<i>T. ?</i> (yellow)	Sierra foothills (83)	13
<i>T. ?</i> (white)	Sierra foothills (83)	4, 6
<i>T. ?</i> (white)	Redding	32
<i>T. ?</i> (purple)	Sierra foothills (83)	10
<i>T. bridgesii</i> (selected blue)	Redding (84, 85, 87)	29, 83, 102, 104, 106
<i>T. bridgesii</i>	Sierra foothills (84, 87)	30, 110
<i>T. bridgesii</i> (pink)	Old Happy Valley (87)	117
<i>T. bridgesii</i> (lavender)	Eureka (88)	151, 152
<i>T. hyacinthina</i> (white)	Sierra foothills (84)	34, 38
<i>T. hyacinthina</i>	Shell Valley, Napa Co.	58
<i>T. hyacinthina</i>	Cherokee Rd. (84)	65, 43, 37
<i>T. hyacinthina</i> (selected)	Dixon (85)	39
<i>T. hyacinthina</i>	Sierra foothills (84)	67
<i>T. hyacinthina</i>	Redding (85, 86, 88)	75, 84, 90, 130
<i>T. hyacinthina</i> (long)	Old Happy Valley (87)	111, 112, 121
<i>T. hyacinthina</i> (pure white, long)	Redding (88)	150

<i>T. ixioides</i> (long)	Red Bluff (87)	116
<i>T. ixioides</i> × <i>T. laxa</i> (?)	Red Bluff (87)	112
<i>T. ixioides</i> (purplish white)	Red Bluff (87)	113
<i>T. ixioides</i> (white)	Red Bluff (87)	114
<i>T. ixioides</i> (yellow)	Hwy 49, Cool (84, 85, 87)	36, 100
<i>T. lutea</i> or <i>ixioides</i> ? (yellow)	Redding (84)	76, 42
<i>T. lutea</i>	Redding (85)	82
<i>T. peduncularis</i> (white)	UCB Bot. Gard. (84)	51
<i>T. peduncularis</i>	Shell Valley, Napa Co. (84, 85)	60
<i>T. peduncularis</i>	Lake Co. (85)	81
<i>T. lugens</i> (yellow)	Lake Berryessa (87)	101
<i>T. crocea</i> (?) (selections)	Redding (88)	146-148
<i>T. crocea</i>	Eureka (88)	154
<i>T. modesta</i>	Castle lake (88)	149

A comparison was done in Davis in the 1988 season between plants grown in full sun and those grown in 45% shade. No difference was found between plants grown under the two conditions of several accessions of *T. laxa*, *T. ixioides*, *T. bridgesii* and *B. californica*. In *T. hyacinthina* there was some reduction in percent flowering and number of florets per stem in shade as compared to full sun. Plants of *B. elegans* and *T. ida-maia* grew better in shade than in full sun. They were taller, with more florets per stem in shade.

Out of the vast plant material that was collected, 20 superior specimens have been selected for attempts to clone them for testing as potential cultivars. These genotypes excelled in their various properties as cut flowers: strong and long stems, large number of florets per inflorescence, bright and pure colors and attractive shapes. The selected plants belong to the following species: *T. laxa*, *T. bridgesii*, *T. hyacinthina*, *T. ixioides*, *B. elegans*, *D. congestum*, *D. multiflorum* and *D. ida-maia*. Flowers of some of the selected genotypes are shown in Plates 1 and 2.

Plates 1 and 2 - Some selected native genotypes of the brodiaea complex (photographs by Dr. R.A.H. Legro).

1. <i>Triteleia laxa</i>	6. <i>T. lugens</i>	9. <i>Dichelostemma multiflorum</i>
2. <i>T. laxa</i>	(bluish anthers)	10. <i>D. pulchelum</i>
3. <i>T. laxa</i> v. <i>alba</i>	7. <i>T. ixioides</i>	11. <i>D. ida-maia</i>
4. <i>T. bridgesii</i>	(white anthers)	12. <i>D. ida-maia</i> (left),
5. <i>T. lugens</i>	8. <i>T. hyancinthina</i>	<i>D. natural hybrid</i> (right)

4. Morphology and Developmental Morphology

All species of the brodiaea complex are summer-dormant geophytes having a fibrous-coated corm as their geophilic organ. The reproductive organ is an umbellate inflorescence borne on a slender scape. There are normally only 2-3 leaves, which commence growing after the rainfall and die soon after anthesis (23).

Since detailed morphological description of the plants and corms is not available in the literature, an artist was commissioned to draw some typical plants of several species. Plates 3 and 4 present descriptions of five different plants and Plates 5 and 6 present descriptions of the propagules of 11 species. It is clear that the number of propagules formed varied greatly among the various species and even genotypes. The cultivated genotype of *T. laxa* (QF) produces many cormels, while the wild plants normally produce very few cormels (Plate 4). In a few cases, such as in *B. californica*, droppers are formed on long, deep stolons in addition to the regular cormels (Plate 3).

A detailed study on the developmental morphology of *Triteleia laxa* (QF) is presented in the following section.

5. Developmental Morphology of *Triteleia laxa* (QF)

a. Growth and Development

In late Fall, previous season's corms of *Triteleia laxa* (average weight 1.32 grams) were planted outdoors at Davis. The corms were planted individually, 5 cm from the soil surface in slender PVC containers (8 cm diameter, 35 cm long) containing loamy sand. At 2 to 4-week intervals throughout the entire growth cycle, 3 replicate plants were harvested for analysis. Fresh and dry weight of each organ were recorded.

Plate 3 - Plant drawings of *B. californica* and *D. congestum*.

Plate 4 - Plant drawings of *D. ida-maia*, *T. hyacinthina* and *B. jolonensis*.

Plate 5 - Propagules of *B. californica*, *T. peduncularis*, *B. volubilis*, *B. elegans*, *T. laxa*, native genotypes -- bottom, left and center; *T. laxa* 'Queen Fabiola' -- right.

Plate 6 - Propagules of *D. multiflorum*, *D. congestum*, *B. terrestris*, *B. coronaria*, *T. bridgesii*, and *T. hyacinthina*.

Plate 3

Brodiaea californica (dropper)

Dichelostemma congestum

Plate 4

3.9 cm

Dichelostemma ida-maia

4.3 cm

Triteleia hyacinthina

1.8 cm

Brodiaea jolonensis

Plate 5

3 cm
Brodiaea californica

3.7 cm
Triteleia peduncularis

3.8 cm
Brodiaea volubilis

5.2 cm
Brodiaea elegans

2.2 cm
Triteleia laxa

2.8 cm
Triteleia laxa (Turner)

4.7 cm
Triteleia laxa
(Queen Fabiola)

Plate 6

3.4 cm

Dichelostemma multiflorum

3.5 cm

Dichelostemma congestum

2.4 cm

Brodiaea terrestris

2.8 cm

Brodiaea coronaria

2.4 cm

Tritoleia bridgesii

2.5 cm

Tritoleia hyacinthina

Corms lifted from the field in the summer remain dormant for a few months, then start to grow in early Fall, even if held dry at room temperature. Shoots of corms planted after dormancy is broken (visible shoot growth) emerged from the soil two weeks after planting. Each *Triteleia* shoot is comprised of two narrow leaves that continue to grow until the end of the growing cycle (Fig 5.1). The tips of the leaves started to senesce a few weeks prior to anthesis of the first flower in the inflorescence, but complete senescence did not occur until 4-5 weeks after anthesis. Storage reserves in the mother corm were continuously depleted throughout the growing cycle (Fig 5.2a). Depletion was most rapid between 4 and 9 weeks after planting, when shoots of the many axillary buds of each plant were growing rapidly, and the flowers and the daughter corm had initiated (Figs. 5.2a,b). Swelling just above the mother corm, indicating the future location of the replacement daughter corm, was visible 9 weeks after planting. Cormels formed at the base of each of the axillary shoots. At eighteen weeks after planting, the mother corm had lost 90% of its dry weight and had almost disintegrated.

Major dry weight accumulation in the inflorescence commenced when the scape started to elongate rapidly (week 21) and continued until anthesis (26 weeks after planting).

Figure 5.1.
Elongation of *Triteleia laxa* leaves.

Fig. 5.2a

Fig. 5.2b

Figure 5.2. Carbohydrate partitioning in various organs of *Triteleia laxa* during the growing period. (a) Dry weight changes and (b) dry weight to fresh weight ratio.

b. Microscopic Observation of Flower Bud Initiation and Development

The shoot axes were dissected and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated in ethanol series, critical point dried in CO₂ and examined and photographed using 1S1 DS130 SEM.

The apical meristem in dormant corms was vegetative, as seen in Figure 3a. The shoot axis, comprised of 2 leaf primordia and a vegetative meristem, surrounded by a leaf sheath (Fig. 5.3b), was embedded in the mother corm, approximately 5-10mm from the surface. Floral initiation, as indicated by initiation of bracts on the flanks of the apical meristem, occurred one month after planting. Concurrently, the apical meristem of the daughter corm originated near the apex of the mother meristem (Figs. 5.3d,e). By week 6 after planting, the 2 bracts enclosed the developing inflorescence (Fig.5.3e). An additional 3-4 bracts had been initiated before flower bud initiation. Three to 4 flower primordia were observed 12 weeks after planting; outer tepals were already evident in some (Fig. 5.3f). Three weeks later, the inner tepals had formed (Fig.5.3d), and the inflorescence was comprised of flowers at different developmental stages (Fig.5.3h). The first flower primordium continued to enlarge and differentiate floral organs while others were dividing and developing. The first flower in the inflorescence reached anthesis approximately 4 months after its initiation (Fig. 5.2a).

Figure 5.3. Scanning electron micrograph of *Triteleia laxa* apical meristem:

- (a) vegetative meristem
- (b) a shoot axis
- (c) formation of two bracts (B) and the daughter corm meristem (D)
- (d) the two bracts enclosing the whole inflorescence
- (e) relative position of the reproductive organ, daughter corm meristem, and the daughter corm (C)
- (f) initiation of the outer tepals (o) twelve weeks after planting
- (g) initiation of the inner tepals (i) fifteen weeks after planting
- (h) inflorescence with different stages of flower buds (F)

Fig. 5.3a

Fig. 5.3b

Fig. 5.3c

Fig. 5.3d

Fig. 5.3e

Fig. 5.3f

Fig. 5.3g

Fig. 5.3h

c. Formation and Enlargement of the Daughter Corm and Cormels

The daughter corm is formed by the enlargement of the basal portion of the stem. Unlike most other geophytes, in which the development of the daughter propagules occurs late in the growing season, the daughter corm of brodiaea begins to enlarge early in the growth cycle (Fig. 5.2a). Growth rate of the daughter corms is slow initially, but increases rapidly about 4 months after planting. They reached 80% of their final weight by anthesis, and continued growing until leaf senescence (Fig. 5.2a). The relatively constant dry weight to fresh weight ratio of the developing daughter corms (Fig. 5.2b) indicates a constant deposition of storage reserves into these propagules. These data suggest that, in contrast to other geophytes (8, 24), there is much less competition for assimilates in brodiaea between the daughter corm and cormels and the developing inflorescence. This hypothesis is consistent with the observation that removing the developing inflorescence at first appearance does not result in increased weight of the propagules as compared to removing it at anthesis.

When inflorescences remained on the plant throughout fruit development, the combined weight of the daughter corm and cormels was 20-30% less than those of plants from which the inflorescence was removed at anthesis. This suggests that competition for assimilates begins at the time of fruit development. When growing *T. laxa* for commercial corm production, growers could therefore harvest brodiaea cut flowers for marketing without reducing the corm yield.

6. Propagation

We have developed *in vitro* propagation methods for several of the selected genotypes. It is, however, clear now that various species have different requirements for micropropagation. We have not yet succeeded in propagating some of the promising selections. The main difficulties arose at the hardening stage and transfer from *in vitro* conditions. Most of the selected genotypes have a very low propagation index. For eventual commercial production of these selections, it will be necessary to improve the rate of vegetative propagation.

Members of the brodiaea complex set seed freely, but the seeds are often deeply dormant. Keeping seeds dry at 0° to 6° only slightly promoted germination as compared to uncontrolled room temperatures.

We were able to overcome dormancy of seeds from five species (*T. laxa*, *T. bridgesii*, *T. ixioides*, *D. congestum* and *T. ida-maia*) by stratification of seed kept moist at 5-7°. Stratification at 6° was more effective than at 0°. Stratification for 6 weeks was sufficient to induce full germination, in most species except *T. ida-maia*, which required 12 weeks of treatment for 50% germination. There was little or no difference between seeds germinated in light and in dark. Soaking seeds in GA₃ (100 ppm) for 2 days did not promote germination. Exposing seeds to ethylene (10 ppm for 1 or 7 days) had no effect on dry seeds, but slightly promoted germination of *T. ixioides* stratified for 2 weeks.

7. Hybridization

As far as we are aware, there has been no commercial breeding of brodiaeas. Each of the 3 genera of the complex has a different base chromosome number. In *Brodiaea*, $n=6$, whereas $n=7$ or 8 for *Triteleia*, and $n=9$ for *Dichelostemma*. Most species in each genus are diploid. In addition, many taxa in the 3 genera have one or more levels of autopolyploidy (for review see our proposal). Most species seem to be self-incompatible. We have found a few interspecific hybrids in nature in places where two species of the same genus grow in close proximity. These data indicate the potential for creating interspecific hybrids between horticulturally interesting genotypes of various species within the 3 genera. We are now in the initial stage of such a hybridization program.

During our studies with the effect of pollination on flowers (see below), we found that *Triteleia laxa* flowers are protandrous. Successful pollination is possible only when stigmas of flowers with senescing petals are pollinated.

8. Growth, Development and Flowering

a. Growth in the Wild and in Cultivation

Growing conditions may have a substantial effect on flower quality and production of the wild plants. The transfer of plants from the wild to cultivation, with a regular supply of water and nutrients, considerably improved the quality of the flowers. This is demonstrated by a comparison of quality parameters of *T. laxa* flowers from plants growing wild in Dixon, CA (Turner Field), to those from plants originally collected from the same field and grown for 2 seasons in the UC field at Davis, 30 km away, under the same climatic conditions (Table 8.1).

Table 8.1. A comparison of wild flowers of *T. laxa* and flowers produced from corms of the same source under cultivation.

	Scape length (cm)	Scape diameter (mm)	No. of florets per inflorescence	Vase life (days)
Wild	28.4	2.6	11.6	8.6
Cultivated	40.3	3.4	23.5	12.0

It should be noted that the values of the wild flowers are means of 40 of some of the best flowers in the field, while those of the cultivated flowers were means of all flowers.

b. Corm Size

It is generally accepted that in geophytes flower quality parameters (stem length, number of flowers per inflorescence, % flowering, etc.) are related to the size of the corm or bulb. Results with several members of the brodiaea complex showed that this is not always the case.

I. Experiments with *T. laxa* (QF)

Corms were graded by weight into 6 size classes, then planted individually in PVC containers containing loamy sand. They were grown outdoors in Davis, California. The inflorescences were harvested and weighed when the first floret reached anthesis. Propagule fresh weights and the number of cormels produced were determined when the leaves had senesced. There were 20 replications per treatment.

Apical meristems from dormant corms of different size were fixed and then examined and photographed by SEM. The diameter of the apical dome was measured at the point of insertion of the youngest leaf primordium. Although nearly all corms planted sprouted, below a corm weight of ca. 0.6g, the percentage of flowering fell rapidly. Only 50% of plants from corms weighing 0.25g flowered, and corms weighing 0.1g or less did not flower at all (Fig. 8.1b). The size of the apical dome in dormant corms increased rapidly as corm size increased from 0.1g to 0.6g; thereafter, it did not change over an 8-fold increase in mother corm size (Fig. 8.1a). Scape length was little affected by mother corm size (Fig. 8.2a), and the number of flowers per inflorescence was reduced significantly only in very small corms (0.6g or less) (Fig. 8.2a). As the mother corm size increased, the number and fresh weight of the daughter corms increased markedly (Fig. 8.2b). The combined weight of daughter corms and cormels was about 5 times that of the mother corm.

Figure 8.1. Effects of mother corm size on (a) apical dome size and (b) percentage of sprouting and flowering.

Figure 8.2. Effects of mother corm size on (a) number of flowers per inflorescence and scape length (b) combined fresh weight and number of daughter corm and cormels. Standard error bars smaller than the symbols are not shown

II. Experiments with wild plants

Corms of 3 species: *T. bridgesii*, *T. ixioides*, and *D. ida-maia*, were divided according to their weight into 5-6 groups and planted individually in PVC containers at the beginning of November, 1986. Plants were grown outdoors until early July, 1987, in Davis. There were 8 replications per treatment. Results are summarized in Table 8.2.

Table 8.2. The effect of corm size on flowering and corm production.

Species and corm size (g)	Flowering %	No. of stems/corm	Scape length (cm)	No. of florets/scape	Corm weight (g)	Total # corms/plant*
<i>T. bridgesii</i>						
A. 4.0	100	1.4	28.8	35.7	10.4	1.6
B. 2.5	100	1.3	34.4	28.4	8.9	1.3
C. 1.5	100	1	33.0	18.9	5.9	1
D. 0.8	100	1	30.0	11.8	3.3	1
E. 0.45	63	1	26.2	7.4	1.8	1
F. 0.2	50	1	18.8	6.5	1.2	1
<i>T. ixioides</i>						
A. 3.6	100	1.2	18.7	37.8	4.3	2.5
B. 2.1	100	1	16.2	21.3	1.2	1
C. 0.7	100	1	20.1	14.5	1.2	1
D. 0.3	100	1	18.1	15.1	1.1	1
E. 0.13	70	1	18.2	9.1	0.7	1
<i>D. ida-maia</i>						
A. 5.6	100	1.6	39.5	17.9	14.3	11.8
B. 3.1	100	1.1	49.6	10.9	10.0	2.4
C. 1.35	37	1.0	38.7	4.0	2.6	3.9
D. 0.4	0	-	-	-	1.6	3.3
E. 0.3	0	-	-	-	0.6	2.1
F. 0.1	0	-	-	-	0.4	1.0

*Total number of corms and cormels

T. bridgesii. Corms of 0.8g and up produced 100% flowering and 50% flowering was obtained even from corms of 0.2g. Scape length was not affected by 9-fold difference in corm size (size A to E), but 30 to 40% of corms larger than 2.5g produced 2 scapes per plant. The number of florets per scape and corm production were, however, directly related to corm size.

T. ixiooides. Seventy percent flowering was obtained, even from corms as small as 130mg, and all the corms of 0.3g and up flowered. Scape length was not related to corm size, but the number of florets per inflorescence and corm yield declined with the decrease in corm size.

D. ida-maia. The effect of corm size on flowering of this species was different from that of the other 2 species. Only one third of size C corms (1.3g) bloomed, and smaller corms produced vegetative plants. About one half of size A corms produced 2 scapes. As with the other species, the number of florets per inflorescence and corm yield were related to mother corm size.

In brodiaea, the daughter corm is formed by enlargement of the basal portion of the stem; cormels are formed from axillary buds on the mother corm. The number of cormels increased with increasing size of the mother corm (Fig. 8.2b, Table 8.2), presumably because larger mother corms had more axillary buds.

In most geophytes, flowering percentage and flower quality are correlated with the size of the mother propagule. In contrast, the percentage flowering and flower quality in several brodiaeas (except *T. ida-maia*), were independent of mother corm size for corms larger than a critical size. There is a disagreement as to whether it is the increase in storage carbohydrate or the enlargement of the apical meristem size with increased mother bulb size that determines the quality of the flowers. In iris and lily, bulb size is correlated with the apex diameter (3, 24); it has been suggested that there is a critical meristem size below which flower induction will not take place. According to this hypothesis, flower quality improves as the meristem increases in size because of the greater space available on the dome for the formation of floral primordia (24). In *T. laxa*, the apical meristem size in dormant corms increased rapidly as the mother corm size increased from 0.2g to 0.6g; thereafter, there was no significant increase over an 8-fold increase in corm size ranging from 0.6g to 5g (Fig. 8.1a). These changes were almost exactly mirrored in the changes in percentage flowering and flower quality with increasing corm size. These data strongly support the hypothesis that flowering and flower number

are determined by size of the apical dome.

c. Effect of Planting Depth

In late Fall, corms of *T. laxa* (QF) (average weight 1.1g) were planted individually in PVC containers containing loamy sand, on the surface, 5 cm, 10 cm, 15 cm, or 20 cm below the surface and grown outdoors in Davis. The percentage of flowering, number of flowers per inflorescence, and daughter corm and cormel fresh weight were collected. There were 15 replications per treatment.

Flowering percentage declined as the planting depth increased (Fig. 8.3a), but the number of flowers per inflorescence and the fresh weight of daughter corms and cormels were maximal in corms planted 10 cm below the soil surface (Fig. 8.3b).

(a)

(b)

Figure 8.3. Effects of planting depth on (a) percentage of flowering (b) number of flowers per inflorescence and combined fresh weight of daughter corm and cormels. Standard error bars smaller than the symbols are not shown.

d. Effect of Photoperiod and Cold Storage of Corms on Growth and Flowering

i. Experiments with *T. laxa* (QF)

a. *Photoperiod.* Corms of *T. laxa* (QF) were planted in pots on January 24, and held in a greenhouse (GH) with a minimum night temperature 15°. Treatments started February 9. Results of Table 8.3 show that LD enhanced flowering, but reduced the number of florets per scape.

Table 8.3. Effect of photoperiod, applied continuously from emergence, on flowering of *T. laxa* (QF).

Treatment	No. of days from planting to flowering	Scape length (cm)	No. of florets per scape
LD (24h), GH	86.7	30.0	9.0
SD (8h), GH	114.4	25.8	15.3
Natural day, GH	116.0	26.5	17.9
Natural day, Outdoor bed	127.0	26.0	23.8

The details of the effective photoperiod treatment (length of day, state of application of LD) were studied in a subsequent experiment (Table 8.4).

All the LD treatments promoted scape elongation, but only 24 h photoperiod considerable advance flowering (by 34d), with 18h day being less effective (21d). Night break of 3 or 6 h had little or no effect on flowering. The effect of LD is apparently on flower bud initiation, since lighting only until bud appearance was as effective as lighting continuously.

Table 8.4. Effect of various photoperiod treatments on flowering of *T. laxa* (QF). Corms were planted in pots on December 2 in a shade house and transferred to the greenhouse (15° min. temp.) on January 10. LD was applied as day extension following 8 h of natural light or as night break (NB) at midnight.

Treatment	Days from transfer to GH until blooming	Scape length (cm)	No. of florets per scape
Natural day	120	24.5	12.8
SD (8h) continuously	122	23.6	12.7
LD (24h) "	88	37.2	12.9
LD (18h) "	101	31.6	12.1
NB (6h) "	118	35.2	15.1
NB (3h) "	124	29.3	14.8
LD (18h) - bud appearance	97	29.5	13.4
LD (18h) - scape is 10 cm	94	30.2	13.6
LD (18h) - scape is 10-20 cm	92	32.3	14.9
Natural day - bud appearance then LD (18h)	116	34.5	17.4

b. Cold Storage of Corms. Low temperature had little or no effect on flowering of *T. laxa* (QF) plants, when applied to dry corms (data not shown). However, when corms were stored in moist soil at 0, 3, or 7° for 4, 8 or 12 weeks, considerable advancement of flowering was obtained. Cooling for 4 weeks had some promotive effect, but 8 and 12 weeks vernalization were highly effective. There was little or no difference between the 3 cold temperatures used. Table 8.5 presents results of 8 and 12 weeks at 7° as well as control (held dry at room temperature).

At the end of the 12 weeks vernalization period, flowers were already initiated within the corm. Vernalization advanced flowering by 2 months and had little effect on flower quality.

Table 8.5. Effect of vernalization of *T. laxa* (QF) corms (wet storage at 7°) on flowering. Following cold treatments, plants were grown in a greenhouse at minimum 13°.

Treatment	Flowering %	No. of days to flowering	No. florets per scape	Scape length (cm)
Control (Stored dry)	28	138	12.3	25.4
7° - 8 wks	88	101	10.3	26.1
7° - 12 wks	100	73	9.6	28.3

c. *Combined Effect of LD and Vernalization.* Corms (average weight 1.3g) were planted individually in PVC containers containing U.C. mix (sand, redwood bark, and peat; 1/1/1 (v/v/v)). The soil was watered and the plants were divided into two lots, one held at 20°C and the other at 5° for 5 or 12 weeks. Following the pretreatment, plants were placed in a greenhouse (24/18° day/night temp.) and given two photoperiod treatments; short-day (SD, 8 hr natural light), and long-day (LD, 8 hr natural light plus 16 hr of $5\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ incandescent light) throughout the growing period. Ten replications were used for each treatment.

Results of Table 8.6 show that both corm vernalization and LD accelerated flowering. It seems that each factor affected flowering independently and their combined effect was additive. LD reduced the number of florets per scape, especially when combined with long cold storage of corms (12w). In contrast to LD, cold treatment accelerated flowering with little or no effect on flower quality (number of florets per scape and scape length).

Table 8.6. Effect of cold treatment (5°C) and daylength on the flowering of *T. laxa* (QF). Data are means of 10 plants.

Treatment	Percentage of flowering	Flowering date	Number of days from end of treatment to anthesis	Number of florets per scape	Scape length (cm)	Scape diameter (cm)
No cold-SD	100	Apr. 31	153.4 ^{a*}	20.4 ^a	21.7 ^a	4.2 ^a
No cold-LD	80	Apr. 13	135.6 ^b	13.8 ^{bc}	31.0 ^b	3.8 ^a
5w cold-SD	100	May 7	126.5 ^c	17.4 ^{bc}	22.5 ^a	3.9 ^a
5w cold-LD	80	Apr. 12	101.6 ^d	11.5 ^{cd}	25.3 ^{ab}	3.2 ^b
12w cold-SD	100	May 10	80.3 ^e	21.6 ^a	26.9 ^{ab}	3.9 ^a
12w cold-LD	60	Apr. 24	73.2 ^e	6.7 ^d	23.9 ^{ab}	2.7 ^c

*Mean separation within columns by Duncan's multiple range test at the 5% level.

d. Effect of Night Temperature. Corms were planted and grown under 8 hr natural days in a 24°C greenhouse and moved to dark chambers at 5, 10, 21, or 25° for 16 hr each night throughout the growing period. Scape length of each inflorescence was measured at anthesis. There were 20 replications per treatment.

Low night temperatures promoted scape elongation. Scapes of plants grown at 5° and 10°C night temperature were more than twice as long as those of plants grown at higher night temperatures (Fig. 8.4).

This dramatic increase was not accompanied by any decrease in flower quality or increase in forcing time (data shown), suggesting that low night temperatures should be used in commercial production of brodiaea.

ii. Experiments with Several Wild Species

Experiments were conducted in Davis, with plants of *Triteleia bridgesii* (ac. 29), *T. laxa* (blue, ac. 14) and *T. lutea* (ac. 42), and in Rehovot, with 2 accessions of *T. laxa* (white), *T. laxa* (purple), *Dichelostemma pulchellum*, *D. multiflorum* and 2 accessions of *D. congestum*.

Figure 8.4. Effects of night temperature on scape length of *Triteleia laxa*.

a. Experiments in Davis. Corms were planted individually in PVC containers. The soil was watered, and the containers transferred to 5°C for 4 or 12 weeks. Following cold storage, plants were transferred to greenhouse with 18° night temperature and divided into 2 photoperiod treatments: short day (SD) of 8 h natural light and long day (LD) of 8 h natural light plus 16 h of 80-100 lux incandescent light, applied throughout the growing period. There were 10 replications per treatment. All corms were originally collected in the Redding-Red Bluff area.

T. bridgesii. (Table 8.7A). The average weight of the corms used was 0.75g. Cold treatment of corms was the major factor influencing flowering time. Cooling for 12 weeks reduced growing period until flowering to less than 50% of control plants, and even cooling for 4 weeks advanced flowering by 16 to 31 days. Scapes of cold treated plants were somewhat longer than controls. The number of florets per scape was also greater under LD conditions. Corm production was decreased in the cold treated plants grown under LD.

Photoperiod had a lesser effect on flowering than vernalization, and affected mainly the nonvernalized plants. Flowering was advanced 17 days by LD in control plants, but by only 2-7 days in cold-treated plants. LD promoted scape length, but had no consistent influence on the number of florets. Corm production was reduced under LD.

Table 8.7. The effect of corm vernalization (moist 5°) for 4 or 12 weeks and photoperiod during the entire period on flowering and corm production (Davis 1986/7).

Species and treatment		Flowering (%)	No. days to anthesis	No. florets per scape	Scape length (cm)	Corm wt. (g)
A. <i>T. bridgesii</i>						
No cold	- SD	60	108.2	6.2	19.1	2.8
	- LD	90	91.2	12.2	26.9	2.5
4 wks cold	- SD	100	77.0	9.1	22.4	2.6
	- LD	80	75.1	9.4	27.2	1.8
12 wks cold	- SD	70	47.5	9.4	25.2	2.6
	- LD	90	40.1	6.1	29.8	2.1
B. <i>T. laxa</i>						
No cold	- SD	100	101.9	17.6	27.5	4.0
	- LD	100	91.9	17.2	29.5	3.5
4 wks cold	- SD	80	75.3	15.6	32.6	4.3
	- LD	90	73.1	12.0	32.9	2.5
12 wks cold	- SD	80	44.4	15.2	34.5	2.8
	- LD	40	35.5	7.5	33.3	2.0
C. <i>T. lutea</i>						
No cold	- SD	100	98.0	24.3	20.4	2.2
	- LD	100	89.7	19.1	17.8	2.0
4 wks cold	- SD	100	74.2	20.1	23.9	2.2
	- LD	80	73.8	19.0	21.3	1.6
12 wks cold	- SD	80	41.9	23.1	26.3	1.9
	- LD	80	35.5	15.1	22.9	1.6

T. laxa. (Table 8.7B). The average weight of the corms used was 1.0g. Flower color - blue. Cold storage of corms advanced flowering considerably, by 18-26 days (4 weeks cold) to 56 days (12 weeks cold). LD affected mainly nonvernalized plants (20 days) and was less effective on cold treated plants (2-9 days). Vernalization promoted scape length, but reduced the number of florets per scape. LD reduced the number of floret in plants cooled for 12 weeks. Vernalization for 12 weeks reduced corm yield. LD reduced corm production in all temperature treatments.

T. lutea. (Table 8.7C). The average weight of the planted corms was 0.45g. As with *T. bridgesii* and *T. laxa*, vernalization was the major factor advancing flowering, and LD mainly promoted the flowering of non-vernalized plants. Scape length was somewhat promoted by cold treatments and reduced by LD. Number of florets and corm production were also reduced by LD.

b. Experiments in Rehovot. Corms of each accession were divided into 2 lots, one held under uncontrolled room temperature and the other stored dry for 10 weeks at 10°. They were then planted individually in pots (10 per treatment) and transferred (on October 1, 1986) to the various growing condition treatments.

- a. In the phytotron at 27/22° (day/night) temperature
- b. In the phytotron at 17/12° (day/night) temperature
- c. In a saran net house (15% shade) under ambient temperatures

In each temperature condition, plants were divided into 2 photoperiod treatments. In the net house: ND - the natural winter SD of 9-10.5 h, and LD-ND + incandescent light (100 lux) throughout the night. In the phytotron: SD - 8 h natural day, LD-SD + day extension to 16 h by incandescent lamps (150 lux).

The following accessions were included:

1. *T. laxa* - white with light purple, origin - Dixon (acc. 20).
2. *T. laxa* - dark purple, origin - Dixon (acc. 23).
3. *T. laxa* (?) - white, origin - Sierra foothills (acc. 6)
4. *Triteleia* sp. - white, origin - Sierra foothills (acc. 4).
5. *D. pulchellum* - white, origin - Table Mt. (acc. 1).
6. *D. congestum* - purple, origin - Red Bluff (acc. 16).
7. *D. congestum* - purple, origin - Sierra foothills (acc. 20).
8. *D. multiflorum* - purple, origin - Tilden park, Berkeley (acc. 62).

Table 8.8. Effect of 10° dry storage of corms for 10 weeks, photoperiod and growing conditions (27/22°, 17/12° - day/night temperatures and net house) on flowering of several brodiaea species (Rehovot 1986/87).

Treatment			<i>T. laxa</i>		<i>T. sp. White</i>		<i>D. congestum</i>		<i>D. pulchellum</i>		<i>D. multiflorum</i>	
Cold storage	Photo period	Temp. (°C)	Days to anthesis	Scape length (cm)	Days to anthesis	Scape length (cm)	Days to anthesis	Scape length (cm)	Days to anthesis	Scape length (cm)	Days to anthesis	Scape length (cm)
-	SD	17/12	130	52	92	38	116	35	91	45	138	41
-	LD	17/12	98	54	79	50	104	38	80	52	126	42
-	SD	27/22	109	36	84	31	101	34	88	22	112	41
-	LD	27/22	83	42	75	42	89	33	80	22	104	47
-	SD	net	154	49	148	53	143	46	86	41	141	51
-	LD	net	146	58	139	61	138	49	66	33	134	54
+	SD	17/12	88	36	73	49	89	36	68	32	120	42
+	LD	17/12	66	55	66	51	73	45	64	42	81	50
+	SD	27/22	70	51	83	38	51	38	48	27	88	43
+	LD	27/22	49	49	67	41	48	42	41	30	75	47
+	ND	net	86	52	81	46	84	39	58	33	132	49
+	LD	net	84	61	64	52	76	62	57	35	127	52

Table 8.8 summarizes the results. Only flowering date and length of scape were recorded in Rehovot. The data of the various accessions of one species were combined, since the response was similar. The basic response of the various species tested of both *Triteleia* and *Dichelostemma* was very similar, although the magnitude of the response varied. In all cases the major factor influencing flowering time was the cold treatment to corms. Dry storage of corms at 10° was effective in advancing flowering in all the species tested, although in most cases flowering was advanced less than by the storage of corms in moist conditions at 5° (Table 8.7). *D. multiflorum* and *D. pulchellum* were the least responsive to the cold treatment (7-39 and 11-39 days respectively), and *T. laxa* and *D. congestum* the most responsive (32-68 and 27-69 days respectively).

Flowering dates were advanced in all cases by long photoperiods, but the effect was less than that of the cold treatment (1 to 39 days). In most species and conditions, the promotion of flowering by LD was of less than 10 days.

Flowering was earlier in plants grown at higher temperatures (27/22°) than in those grown at low temperatures (17/12°), and generally latest under uncontrolled ambient temperatures (net house). However, growing temperatures affected flowering less than cold treatment to corms. Flowering was only 20 days earlier at 27/22° than at 17/12°.

Growing temperatures and cold treatment to corms had no consistent effect on scape length, but scape length was generally promoted a few cm by LD.

e. Effect of Ethylene Treatment to Corms on Growth and Flowering of *T. laxa* (QF)

Ethylene is a plant hormone having diverse effects on a wide range of plant tissues. Depending on the species, C_2H_4 may promote flower initiation, stimulate flower development, inhibit flower induction or cause abortion. Japanese bulb growers found that after burning iris fields in the autumn (to control leaf diseases), a greater proportion of the bulbs subsequently flowered and the crop flowered earlier (4). These useful responses were shown to be due to the smoke, and specifically to C_2H_4 in the smoke (15). Ethylene has been shown to affect both the sprouting date and the flowering of a range of geophytes (4, 7, 13, 15-17). However, the physiological responses of these organs to the C_2H_4 treatment and the mechanism by which C_2H_4 "breaks" the dormant state or affects flowering are still unexplored.

MATERIALS AND METHODS

1. Plant Materials

Triteleia laxa 'Queen Fabiola' corms, were grown outdoors in Davis, California, under natural conditions. Dormant corms (no visible shoots) with an average weight of 1.1g were used as experimental material.

2. Ethylene and Growth and Development

Corms were exposed to a flowing stream of air or of air containing C_2H_4 (20 ppm for 1 day, 20 ppm for 7 days or 40 ppm for 7 days) in the summer soon after lifting. Flow rates were sufficient to prevent significant accumulation of CO_2 . Individual corms were then planted 5 cm from the soil surface in PVC containers containing UC-mix (sand, redwood bark, and peat; 1/1/1 (v/v/v) and grown in a 18/10°C day/night temperature greenhouse under natural daylengths. The inflorescences were harvested when the first flowers reached anthesis. The fresh weight of the daughter corm and the cormels was measured when the leaves had senesced. There were 10 replications per treatment.

3. Determination of Respiration Rate

Individual corms were placed in 25ml vials ventilated (0.2 liter/h) with either air or 20 ppm C_2H_4 . One ml aliquots of the exit air stream were withdrawn and the CO_2 content of the air was determined using an ADC infra-red gas analyzer type 225-2B-SS.

4. Determination of Carbohydrate Mobilization

Ethylene-treated (20 ppm for 7 days) and control corms were planted in a 24/18°C greenhouse and grown under long photoperiod (8 hr natural light plus 16 hr of $5\mu\text{mol m}^{-2}\cdot\text{sec}^{-1}$ incandescent light). Plants were harvested at intervals and the dry weights of various organs and the carbohydrate content of the corm were determined. Each corm was divided into 2 sections, the inner ring (sampled by a 4mm cork borer) and the outer ring (the rest of the corm tissue). The tissues were freeze dried, and 100mg samples were ground to a fine powder with a mortar and pestle and extracted overnight in 5ml of 70% ethanol containing 2mM Ca-acetate. The samples were centrifuged, and the pellets were re-extracted three times. The supernatants were collected for determination of soluble sugars. The ethanol-insoluble residues were dried

in air for starch analysis.

The starch in the samples was solubilized by adding 1ml saturated $\text{Ca}(\text{OH})_2$ and autoclaving for 20 min. Samples were cooled to room temperature before adding 1.5ml Na-phosphate buffer (pH 4.6) and 5ml of a 10mg/ml solution of amyloglucosidase (Sigma Chemical Co., enzyme activity 12,000 unit/g protein; one unit will liberate 1.0mg of glucose from soluble starch in 3 min at pH 4.5 and 55°C). The samples were incubated in a 55° water bath with continuous shaking overnight, after which glucose released by hydrolysis was determined using the hexokinase method (1).

Total soluble sugar content was estimated by a modification of the anthrone method of Yemm and Willis (1954). The ethanol-extracted solutions were blow-dried and redissolved in deionized water (DI). Aliquots (20 μ l) of the dissolved sugars were layered on cold anthrone reagent, cooled for 5 min, heated in a boiling water bath for 10 min, and then cooled for an additional 5 min prior to determining absorbance at 600nm.

5. Determination of Apical Meristem Size

Samples of the shoot axis from corms of C_2H_4 -treated and control plants were collected at intervals after planting. They were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7) and dehydrated by passing through an ethanol series, critical point dried in CO_2 and examined and photographed using an ISI DS130 scanning electron microscope (SEM).

RESULTS

1. Effects of Ethylene on Growth and Flowering

Ethylene treatment reduced the number of days to sprouting (Table 1); one week exposure to C_2H_4 was more effective than one day. Leaves from the C_2H_4 -treated corms grew faster and were longer (Fig. 1), wider and had greater dry weight than the controls (Fig. 3b). Ethylene treatment reduced the number of days to anthesis of the first flower by 2 or 5 weeks for the 1 or 7 day treatments, respectively. Ethylene treatment for 7 days increased the number of flowers per inflorescence, but had no effect on scape length. This treatment also increased the combined fresh weight of the daughter corm and cormels (Table 1).

Table 1. Effect of ethylene on the sprouting, flowering and corm yield of *Triteleia laxa*.

Treatment	Number of days to sprouting	Number of days to flowering	Number of flowers per inflorescence	Scape length (cm)	Daughter corm F.W.* (g)
Control	104 ^a	274 ^a	15 ^a	40 ^a	7.4 ^{a**}
C ₂ H ₄ 20 ppm - 1 day	96 ^a	253 ^b	18 ^{ab}	44 ^a	8.1 ^a
C ₂ H ₄ 20 ppm - 7 days	78 ^b	231 ^c	27 ^b	43 ^a	9.9 ^a
C ₂ H ₄ 40 ppm - 7 days	81 ^b	228 ^c	26 ^b	45 ^a	9.2 ^a

* Combined fresh weight of the daughter corm and cormels.

** Means separated within columns by Duncan's multiple range test (P = 0.05)

Figure 1. Effects of ethylene (20 ppm, 7 days) on rate of elongation of *Triteleia laxa* 'Queen Fabiola' leaves. Standard error bars smaller than the symbols are not shown.

2. Effect of Ethylene on Respiration Rate

The respiration rate of corms treated with 20 ppm C_2H_4 increased rapidly (Fig. 2). Peak respiration rate of the C_2H_4 -treated corms was about 5-7 times that of the controls. Respiration fell markedly after the removal of C_2H_4 (Fig. 2), but was still 2-3 times higher than that of the controls 4 days later, and stayed slightly higher throughout the experimental period.

Figure 2. Effects of ethylene (20 ppm for 7 days) on respiration rate of *Triteleia laxa* 'Queen Fabiola' corms. Standard error base smaller than the symbols are not shown.

3. Effect of Ethylene on Carbohydrate Mobilization

The dry weight of the mother corms of C_2H_4 -treated plants decreased more rapidly than that of control corms (Fig. 3a). The dry weight of the leaves and the combined dry weights of the daughter corms and cormels in C_2H_4 -treated plants increased sooner than those of the controls and reached 160% and 130% that of the controls, respectively (Fig. 3b & 3c). Analysis of starch and total soluble sugars in the corms after planting did not reveal any differences between C_2H_4 -treated and control corms (data not shown).

Figure 3. Effects of ethylene treatment (20 ppm, 7 days) on growth (measured by dry weight) of *Triteleia laxa* 'Queen Fabiola': (a) mother corm, (b) leaves, and (c) combined weight of daughter corm and cormels (inset shows date of early

4. Effect of Ethylene on the Shoot Axis and the Apical Meristem

One week after planting, the main axis (comprised of a leaf sheath and 2 leaf primordia) of the C_2H_4 -treated corms was already longer (1.35 ± 0.03) and wider (1.17 ± 0.04) than that of the control (0.88 ± 0.08 vs. 0.82 ± 0.03 , respectively). A comparison of growth of the apical meristem of C_2H_4 -treated and control corms is shown in Fig. 4. The apical meristems of the C_2H_4 -treated corms were already larger than the controls after 1 week, and increased after planting at a rate of $22\mu\text{m}/\text{wk}$, reaching approximately $350\mu\text{m}$ in diameter before the change from vegetative to reproductive, 7-8 weeks after planting. In contrast, the apical meristems of the controls increased at a rate of $11\mu\text{m}/\text{wk}$ four weeks after planting and reached only $250\mu\text{m}$ before they changed from vegetative to the reproductive stage.

Figure 4. Effects of ethylene treatment (20 ppm, 7 days) on growth of the apical meristems. Standard error bars smaller than the symbols are not shown.

DISCUSSION

In the wild, mature plants of *Triteleia laxa* bloom once a year in late spring to early summer. Corms harvested after the leaves have senesced remain dormant until early- to mid-fall. Exposure of dormant corms to C_2H_4 caused early sprouting, early flowering, increased number of flowers per inflorescence, and increased combined fresh weight of daughter corm and cormels (Table 1). The optimum concentration and exposure time for flowering responses of geophytes to C_2H_4 varies between species (4, 13, 15, 16). In *T. laxa*, the response to C_2H_4 was maximal at concentrations above 20 ppm and exposure times longer than 1 day. In contrast, marked stimulation of flowering in small-sized (8.5-10.5 g) iris bulbs occurs after as little as 1 hr of exposure to 10 ppm C_2H_4 (17).

No explanation has yet been advanced for the striking effect of C_2H_4 on the flowering of geophytes. The pronounced effect of C_2H_4 on sprouting of potatoes is associated with increased respiration rate (26) and mobilization of carbohydrates (14). If the soluble carbohydrate content of dormant geophyte propagules increased following C_2H_4 treatment, this could be the cause of stimulated growth and flower induction. Induction of flowering by chemical or environmental factors has been suggested to be related to their influence on assimilate supply and distribution (27). Although the respiration of dormant *T. laxa* corms increased rapidly in response to C_2H_4 treatment (Fig. 2), no significant effect of C_2H_4 on their carbohydrate content could be measured. These data suggest that changes in carbohydrate mobilization are unlikely to be an initial mechanism for the effects of C_2H_4 on growth and flowering in these geophytes.

Examination of the effects of C_2H_4 on the morphology of the apical meristem of *T. laxa* corms suggests an alternative mechanism for the action of C_2H_4 in geophytes. The apical meristems of C_2H_4 -treated corms grew twice as fast as those in control corms (Fig. 4). The fact that the treated meristems were already considerably larger than the controls one week after the end of the C_2H_4 treatment suggests that meristem expansion was a very early event in treated corms. More detailed examination of the time course of the effects of C_2H_4 on meristem size might indicate whether meristem enlargement is the primary mechanism of C_2H_4 action. Electron micrographs of control and C_2H_4 -treated meristems immediately after treatment did not indicate any major change in cell size (data not shown), suggesting that the rapid enlargement of the apical meristem in C_2H_4 -treated corms may be the result of stimulated cell division.

We showed previously (Fig. 8.1) that the number of flowers per inflorescence in *T. laxa* is strongly related to the size of the apical meristem. The increased number of flowers per inflorescence in C_2H_4 -treated corms (Table 1), therefore, probably reflects the enhanced growth of their apical meristems (Fig. 4).

The stimulatory effect of C_2H_4 on the apical meristem of *T. laxa* was accompanied by early enlargement of the shoot axis, which resulted in longer (Fig. 1) and wider leaves. The increased photosynthetic capacity resulting from this increased leaf area probably explains the increased weight of the daughter corms and cormels produced by C_2H_4 -treated corms (Table 1).

9. Postharvest Physiology

Most of the studies on postharvest physiology of cut flowers were carried out with *T. laxa* (QF), since for these studies we required large quantities of uniform flowers. Comparative studies were also conducted with wild *T. laxa* and other species.

In postharvest studies, outdoor-grown flowers were used. Inflorescence maturity was defined as the number of open flowers in the inflorescence. Unless otherwise specified, inflorescences were harvested from the field when their first flower reached anthesis (Stage 1). The inflorescences were placed individually in sterile polyethylene vases containing ca. 80ml of vase solution. Their opening and vase life were evaluated in a controlled environment ($21^{\circ}C$, 12 h $15\mu mol \cdot m^{-2} \cdot s^{-1}$ light each day, 60% R.H.). The vase-life was considered terminated when the number of senescent flowers exceeded the number of open flowers.

a. General

A direct positive correlation was found between scape diameter and number of buds per scape and the longevity of the cut flower. This is demonstrated by results of Table 9.1, presenting only an example of two scape sizes. Scape diameter may thus serve as an indicator of the potential quality and longevity of the cut flowers.

In tests with *T. laxa* (QF) it was found that the vase life of inflorescences harvested at Stage 4 was 4-5 days less than that of those harvested at Stage 0 (Table 9.2). Flowers opened steadily at a rate of one flower per day during vase life of the inflorescence (Fig. 9.1), and the first flowers senesced on day 4. The life of the individual flowers (horizontal distance between opening and senescence curves in Fig. 9.1) was about 4

days throughout the vase life, and the life of the inflorescence (day when 50% of the open flowers were senescent) was 12 days.

Table 9.1. Relationship between scape diameter, number of florets per scape and vase life of the cut flowers (*T. laxa*).

Scape diameter (mm)	No. of florets per scape	Vase life (days)
2.9	16.8	10.9
1.9	5.8	6.3

Figure 9.1. Time course of the opening and senescing of *Triteleia laxa* flowers in an inflorescence.

Table 9.2. Effects of harvesting stage and sucrose concentration in the holding solutions on vase life of *Triteleia laxa* (QF). All vase solutions contained 200 ppm HQC.

Treatment	Vase life (days)	
	Stage 0	Stage 4
1% sucrose	11.5 ^a	6.0 ^{b*}
2% sucrose	11.5 ^a	5.8 ^b
4% sucrose	10.5 ^a	6.0 ^b
6% sucrose	11.0 ^a	6.8 ^b
8% sucrose	9.3 ^a	5.8 ^b

* Means separated within columns by Duncan's multiple range test ($P = 0.05$).

Rates of water uptake were monitored continuously using a computer connected to the digital output of an electronic balance (Fisher Scientific Model 8301A). A beaker containing water (and covered with a sheet of Parafilm perforated in the center) was placed on the balance. An inflorescence was lightly held in a bench clamp so that its base dipped into the water, but did not touch the beaker or the Parafilm. The decrease in weight of the beaker was taken as a measure of water uptake by the inflorescence. The contribution of different portions of the inflorescence to water uptake was determined by measuring water uptake after removal, first of the flower buds, then of the peduncles.

The typical steady rate of transpiration by inflorescences was 12ml/day. By sequentially removing organs from an inflorescence, we determined that 60% of the water was transpired by the flower buds (at a rate of 0.9g H₂O/g fresh wt. day⁻¹), 16% by the peduncles (0.5g H₂O/g fresh wt. day⁻¹), and 24% by the scape (0.4g H₂O/g fresh wt. day⁻¹).

The water loss of *T. laxa* flowers is only 1/5 that of rose flowers, since the inflorescence is leafless. It is therefore probable that water supply is seldom a problem. This may also explain the lack of response to biocides and pH (11, 12).

In tests with wild flowers of *T. laxa*, inflorescences had a longevity of about two weeks in tap water or deionized water. The oldest flowers on the periphery of the inflorescence senesced with the development of the ovaries. Evidently, these flowers were pollinated by bees before harvesting. Pollinated flowers became brown, but non-pollinated ones (those without developing ovaries) retained some of the blue color as they senesced. The problem becomes one of harvesting flowers before pollination occurs. This requires picking inflorescences without any mature flowers, when they are being grown in open field.

In harvesting from the field, stems are often pulled from the corm. The part below ground is white and terminates in a point at the contact with the corm. Tests showed that the white portion should be removed by cutting just before placing them in water. Cut stems kept 14 to 16 days; those uncut kept only seven days.

b. Cut-Flower Solution Components (Holding, Bud-Opening, Pulsing)

i. Biocides. Inflorescences were placed in opening solutions containing 2% sucrose and a biocide. The biocidal solutions tested were 200 ppm HQC (8-hydroxyquinoline citrate), 100 ppm Physan, 50 ppm AgNO_3 . In addition, the effect of 100 ppm Physan was tested for flowers whose base was first dipped for 10 sec in 1000 ppm AgNO_3 .

None of the different biocides tested had any significant effect on vase life. Phytotoxicity was observed, however, on scapes placed in solutions containing either Physan or AgNO_3 . Silver nitrate caused total blackening of the portion of the scape in the solution. The immersed portion of scapes held in Physan developed small lesions which rapidly spread so that the whole scape decayed. Scapes placed in solutions containing 200 ppm HQC showed no sign of phytotoxicity.

ii. Sucrose Concentration. Inflorescences were placed in solutions containing 200 ppm HQC and 0%, 2%, 4%, or 8% sucrose or were pulsed overnight with 10% sucrose and then placed in 200 ppm HQC solution without sucrose.

Fifty percent of the buds on the inflorescences (Stage 0-1) placed in water failed to open, even if they were pulsed for 16 hr with 10% sucrose (Table 9.3). The percentage of open flower buds and the vase life of inflorescences placed in solutions without sucrose were less than those placed in solutions containing sucrose. The addition of

sucrose to the vase solution greatly improved flower opening and, consequently, vase life. One percent sucrose was as effective as higher concentrations in improving the vase life of *T. laxa* (Table 9.2). Pulsing inflorescences with 10% sucrose slightly increased bud opening and vase life (Table 9.3).

Table 9.3. Effects of sucrose concentration on the vase life of *Triteleia laxa*. All opening solutions contained 200 ppm HQC.

Sucrose concentration	Vase life (days)	Percentage of flower buds opened
0%	5.1 ^{a*}	46.3 ^{b**}
2%	13.0 ^{b***}	86.0 ^a
4%	13.5 ^b	87.7 ^a
8%	12.5 ^b	88.1 ^a
10% pulse 16 hr then 0% sucrose	7.0 ^a	58.9 ^b

* Partially open flower buds were not considered as opening flowers when determining the vase life of the inflorescences.

** Including buds that were partly opened.

*** Means separated within columns by Duncan's multiple range test ($P = 0.05$).

iii. **pH.** Inflorescences were harvested at Stage 2 or 3 and placed in vase solutions containing 2% sucrose and 200 ppm HQC whose pH was adjusted to 3, 4, 5, 6, and 7 with KOH or HCl. There was no significant effect on vase life of changing the pH of the vase solution at the beginning of the experiment.

c. Storage

Inflorescences of *T. laxa* (QF) harvested at closed-bud stage were wrapped in perforated plastic bags. Some inflorescences were pre-treated overnight with 10% sucrose. They were then stored (dry) in the dark at 1°C for 2 or 4 weeks. After storage,

inflorescences were placed in solutions containing either 2% or 4% sucrose and 200 ppm HQC. Control flowers were placed in these same solutions immediately after harvest.

The vase life of inflorescences stored for two weeks and then placed in a 2% sucrose vase solution was the same as fresh flowers, but fewer flower buds opened (Table 9.4). Increasing the sucrose concentration in the vase solution to 4% or pre-treating the inflorescences with 10% sucrose before storage substantially improved bud opening of inflorescences stored for 2 weeks, but had no effect on inflorescences stored longer. Small flower buds (bud length of 0.8 cm or less) aborted after prolonged storage.

Table 9.4. Effects of dry storage and sucrose supplied after the cold storage on the vase life and opening of *Triteleia laxa* (QF). All opening solutions contained 200 ppm HQC. Pulsing treatments were done before storage.

Storage (weeks)	Opening solution (% sucrose)	Vase life (days)	Percentage flower buds opened
0	2%	13.0 ^{a*}	73.5 ^a
	4%	12.7 ^a	76.2 ^a
2	2%	12.8 ^a	48.0 ^b
	4%	12.8 ^a	63.3 ^a
	10% pulse - 2%	11.0 ^{ab}	68.0 ^a
4	2%	9.2 ^{bc}	31.5 ^c
	4%	8.8 ^{bc}	32.2 ^c
	10% pulse - 2%	8.3 ^c	38.4 ^{bc}

* Means separated within columns by Duncan's multiple range test (P = 0.05).

In another experiment, flowers harvested at various stages (1 to 5 open florets), were stored for 2 weeks and then opened in solution containing 2% sucrose. Results of Table 9.5 show that storing flowers with 4 open florets was better than storing bud cut flowers.

Table 9.5. Flower bud opening of *T. laxa* after 2 weeks storage at 3°C as related to stage of cut. Data were recorded 2 weeks from start of opening treatment.

No. of open florets when cut	Average scape diameter (mm)	Number of buds per scape	Total flowers opened per scape	Percent flower buds opened
1	3.6	27.8	16.5	59
4	3.7	32.0	23.2	73
5	3.4	32.3	23.3	74

In a further experiment, *T. laxa* (QF) flowers cut when about half the florets were open were stored for up to 3 months at 1°C. Storage for 1 week reduced longevity from 14 to 11 days, but longer storage of up to 8 weeks did not further reduce vase life. Flowers stored for 4 or more weeks became infected with botrytis.

In experiments conducted with wild *T. laxa* flowers, it was found that their postharvest life and storage ability often surpassed those of QF. In one experiment, flowers of *T. laxa* (Redding) cut tight (only one bud open) proved to have a remarkable storage life. It was possible to hold them at low temperature for up to 3 months and after opening, their quality and vase life was comparable to those of fresh-cut flowers.

d. Postharvest Behaviour of Other Species

T. bridgesii is an excellent cut flower species. Unlike *T. laxa*, the florets do not discolor when senescent but remain blue. Vase life was approximately 9 days, and all the florets in the scape opened in water when cut at Stage 1.

T. lutea. Flowers of this species (as well as the related sp. *T. ixiooides*) are sensitive to sucrose. Even 2% sucrose in the holding solution may cause stem collapse. The best sucrose concentration for holding solution was 0.5%. Vase life of untreated flowers was 10 days and that of flowers held in 0.5% sucrose, 13 days. Overnight pulsing with 10% sucrose solution caused collapse of 50% of the scapes.

e. Respiration Rate of *T. laxa* (QF) Flowers

Flower buds were harvested and placed in 25ml glass vials ventilated (2.4 l/hr) with CO₂-free air. The CO₂ content of the exit air stream was determined using an ADC infrared gas analyzer type 225-2B-SS.

The respiration rate of young *T. laxa* flower buds was very high and decreased rapidly until they reached anthesis. The respiration rate of open flower buds continued to decline, at a slower rate, reaching a steady rate three days after anthesis (Fig. 9.2).

Figure 9.2. Respiration rate of flowers of *Triteleia laxa* at different developmental stages.

f. The Role of Ethylene in Petal Senescence of *T. laxa* (QF)

Most geophyte flowers that have been tested are 'non-climacteric'; they produce very low levels of ethylene throughout their life and are mostly only slightly or not at all affected by exposure to C₂H₄ (6, 12, 25). Nothing is known on the response of brodiaeas to ethylene and the endogenous production of the gas by the flowers.

MATERIALS AND METHODS

Triteleia laxa (QF) flowers were grown outdoors in Davis. Unless otherwise stated, inflorescences were harvested when the first flower in the inflorescence opened. In experiments using single flowers, flower buds showing color were collected from intact plants and opened and held in the laboratory (21°C, 12 h $15\mu\text{mol m}^{-2}\text{s}^{-1}$ fluorescent light each day, 60% R.H.) in solutions containing 2% sucrose and 200mg l^{-1} 8-hydroxyquinoline citrate (HQC). Most experiments used 10 replications of single inflorescences or flowers.

Flower longevity was determined with flowers held in 2% sucrose and 200mg l^{-1} HQC under the conditions stated above.

Fresh samples of stigmas were collected at various stages from anthesis to senescence. They were fixed as described above and examined and photographed with a scanning electron microscope (SEM).

1. Effect of Ethylene, Aminooxyacetic Acid (AOA) and Silver Thiosulfate (STS)

Individual flowers with their bases in opening solution were exposed to a flowing stream of air or air containing various concentrations of C_2H_4 . Entire inflorescences or individual flowers were treated with STS by immersing the base of the stem or pedicel in STS solutions. Unless otherwise stated, 4mM STS was applied for 30 min. After treatment the bases were rinsed with water to remove the residual STS. In experiments examining the effects of inhibitors of C_2H_4 production on pollination responses, stigmas of individual flowers were pretreated with $2\mu\text{l}$ of either 1mM AOA or 2mM STS, 30 min. prior to pollination.

2. Determination of Ethylene Production

Ethylene production of individual flowers was measured by placing them in 35ml glass vials, sealed with serum caps, for 1 h. In experiments in which the time course of C_2H_4 production was measured, the vials were ventilated with C_2H_4 -free air every 1 or 2 hours. Three ml aliquots of the air in the vials were withdrawn and the C_2H_4 content of the air was determined using a gas chromatograph fitted with a photoionization detector (minimum detectable C_2H_4 1 nl l^{-1}).

RESULTS AND DISCUSSION

1. Effects of Exposure to Ethylene and Treatment with STS on the Longevity of the Flowers

The normal lives of inflorescences and of individual flowers were 9-10 days and 5-6 days, respectively (Table 1). No detectable C_2H_4 was produced from non-pollinated flowers during their entire life span. All open flowers in the inflorescences exposed to C_2H_4 ($10\mu l\ l^{-1}$) wilted within one day and C_2H_4 -treated flower buds failed to open. Pretreatment with STS ($0.5\mu mol\ stem^{-1}$) counteracted these C_2H_4 effects.

When inflorescences harvested with one open flower were exposed to C_2H_4 (1, 5, or $10\mu l\ l^{-1}$) for 2 days, more than 50% of the buds never opened, even if placed after treatment in a bud-opening solution containing 2% sucrose and $200g\ l^{-1}$ 8-HQC.

The sensitivity of the flowers to C_2H_4 was tested by placing individual open flowers and buds in containers ventilated with 0.03, 0.1, 0.2, 0.5, or $1\mu l\ l^{-1}$ C_2H_4 . All open flowers exposed to C_2H_4 concentrations higher than $0.1\mu l\ l^{-1}$ wilted within 10 hr (Fig. 1). Treatment with $0.03\mu l\ l^{-1}$ C_2H_4 reduced the vase life of the flowers to one-third that of the controls and prevented petal expansion, elongation, and opening of flower buds.

Table 1. Effect of ethylene ($1\mu l\ l^{-1}$) and silver thiosulfate on longevity of *T. laxa* (QF) flowers. Individual flowers, buds, or inflorescences were placed in 2% sucrose + HQC. Data are the means of 10 flowers.

Parameter	Pretreatment Treatment	Longevity (Days)			
		Water		STS	
		Air	C_2H_4	Air	C_2H_4
Flower longevity		5.6	0.4	5.8	3.0
% Bud elongation (2 days after treatment)		58.3	16.6	66.6	66.6
% Petal expansion (4 days after treatment)		170.00	20.00	-	-

Figure 1. Longevity of individual *Triteleia laxa* flowers exposed to different concentrations of ethylene continuously.

Figure 2. Effects of ethylene ($1 \mu\text{l l}^{-1}$) on ovary growth of flowers of *Triteleia laxa*: (a) 1 cm buds, (b) buds showing color, and (c) flowers at anthesis. Arrows indicate time of anthesis.

In their response to C_2H_4 , *T. laxa* (QF) flowers behave differently than most other flowers studied so far (6, 25). Although they do not produce measurable C_2H_4 during their entire life, both open flowers and flower buds are very sensitive to C_2H_4 . Continuous exposure to $0.03\mu l\ l^{-1}$ C_2H_4 or a 2-day treatment with $1\mu l\ l^{-1}$ irreversibly prevented bud opening and induced rapid senescence of open flowers. Ethylene concentrations higher than $0.03\mu l\ l^{-1}$ are common in the atmosphere of various locations where flowers are held (12, 25); for longest vase life under commercial conditions, brodiaea flowers should therefore be treated with STS.

2. Effect of Ethylene Treatment and Petal Removal on Ovary Growth

The ovaries of *T. laxa* flowers grew mainly after anthesis, when the petals had reached their final size and the flowers had opened (Figs. 2b & c). There was no ovary growth at all in young buds with growing petals (which reached anthesis on the last day of the experiment) (Fig. 2a), and flowers harvested at anthesis showed constant ovary growth (Fig. 2c). Treatment with C_2H_4 ($1\mu l\ l^{-1}$) induced rapid enlargement of ovaries of flowers from all stages (Fig. 2).

There have been many studies on the physiology of senescing carnation flowers and there has been some controversy as to the role of carbohydrate movement between the senescing petals and the developing ovaries (2, 18-21). Carbohydrates are obviously required for ovary development, and it has been suggested that the ovary of C_2H_4 -treated carnations grows at the expense of carbohydrate reserves in the wilting petals (19). However, removal of the petals had no effect on ovary growth; it was therefore concluded that the growth of the ovary was not dependent on reallocation of carbohydrate materials from the petals (18). In other experiments, application of ethrel accelerated petal senescence and enhanced ovary growth. Ovaries from flowers with petals removed weighed less than those from intact flowers, suggesting that metabolites move from the senescing petals to the developing ovary (2).

To examine the role of petals and carbohydrates in ovary growth, flowers detached at various stages of development (with or without petals) were placed in solutions containing 0% or 2% sucrose and $200mg\ l^{-1}$ HQC. Ovary growth was always promoted by sucrose. In flowers harvested as buds, petal removal enhanced ovary growth. In flowers harvested at anthesis, after petals were fully expanded, petal removal retarded ovary growth (Table 2).

Table 2. Effects of petal removal on growth of ovaries in detached individual flower buds harvested at various stages and placed either in opening solution containing 0% or 2% sucrose with HQC for 7 days. Data are the means of 10 flowers.

	Ovary fresh weight (mg)					
	1 cm buds		Buds showing color		Open flowers	
	Petals		Petals		Petals	
	+	-	+	-	+	-
Initial weight	9.6 ^a	9.6 ^a	14.2 ^a	14.2 ^a	16.4 ^a	16.4 ^a
Final weight (H ₂ O)	10.5 ^a	15.7 ^b	16.9 ^a	29.0 ^b	47.3 ^c	25.1 ^b
Final weight (2% sucrose)	16.8 ^b	22.3 ^c	31.2 ^b	40.1 ^c	60.8 ^d	50.7 ^c

Ethylene treatment of intact flowers harvested at anthesis almost doubled ovary growth (Table 3). Removal of the petals eliminated the C₂H₄ response. STS partially counteracted the promotive effect of exogenous C₂H₄ on ovary growth in intact flowers.

Table 3. The effect of petal removal, exposure to ethylene (1 μ l l⁻¹) and pretreatment with STS (4mM, 30 min) on ovary growth. Individual flowers harvested on opening day were placed in 2% sucrose + HQC for 4 days. Data are the means of 5 flowers.

Treatment		Fresh weight (mg)	
Ethylene	STS	With petals	Petals removed
-	-	36.8 ^b	24.2 ^a
+	-	67.8 ^d	27.6 ^a
-	+	28.3 ^a	25.5 ^a
+	+	47.6 ^c	24.2 ^a

The data obtained from *T. laxa* flowers indicate time-dependent changes in competition among the floral organs for carbohydrate. In young flowers, removal of the petals increased ovary growth, indicating that the growing petals were the strongest sink for carbohydrate from the stem (Table 2). These data are similar to those obtained from carnation flowers pre-treated with chemicals that delay flower senescence, such as STS (2, 9, 18). Addition of sucrose to the vase solution increased growth of both organs, which supports this sink-strength hypothesis. In contrast, the ovaries of flowers with fully-developed petals grew only slightly if the petals were removed, but increased by 3 to 4 fold when the petals were present, or when the stems were provided with sucrose. These data are consistent with those from carnation flowers whose senescence was enhanced by pre-treatment with ethrel (2). The changed response to petal removal as the flower ages indicates that the ovary in the aging flower becomes a very strong sink for carbohydrate from either the stem or the senescing petals. These results suggest that ovary growth is a function of the relative sink strengths of the ovaries and petals for carbohydrate from the stem and the vase solution.

3. Pollination

Field observations indicated rapid ovary growth following insect pollination. In initial experiments to test the effect of pollination on C_2H_4 production and flower life, *T. laxa* flowers were pollinated at anthesis. There was no resulting production of C_2H_4 or any acceleration of ovary growth, and it seemed possible that flowers were dichogamous. Pollination of flowers at different stages of development demonstrated that *T. laxa* flowers are protandrous. Pollination of flowers with senescing petals caused increased C_2H_4 production and ovary growth. Ethylene evolution started to increase 4 hr after pollination, a peak of $24 \text{ nl g}^{-1} \text{ h}^{-1}$ 5 hours later (Fig. 3). No measurable C_2H_4 was produced by non-pollinated flowers or pollinated flowers whose stigma was pretreated with 1mM AOA (aminooxyacetic acid). Application of 2mM STS to the stigma stimulated C_2H_4 evolution (data not shown). A similar response to pollination has been reported for other species (6, 10, 25, 28).

At anthesis, the papillae on top of the stigma were tightly packed together (Fig. 4a). By five to six days after anthesis, when petals had started to senesce and the stigma was receptive, the papillae had elongated and separated (Fig. 4b), and pollen grains lodged between the papillae (Fig. 4c). Twelve hours after pollen application, the pollen grains had already germinated (Fig. 4d).

Figure 3. Rate of ethylene after pollination of *Triteleia laxa* flowers.

Fig. 4a.

Fig. 4b.

Fig. 4c.

Fig. 4d.

Figure 4. Scanning electron micrograph of a *Triteleia laxa* (a) non-receptive stigma (at anthesis), (b) receptive stigma (petals started to senesce), (c) receptive stigma with pollen grains lodged between the stigmatic papillae, (d) stigmatic surface with pollen tubes fully grown 12 hr after pollination.

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F. DESCRIPTION OF COOPERATION

During the entire period of the research, there was a very close collaboration between the two laboratories at every stage. The responsibility was not divided between the two teams, and each phase of the research was carried out as a joint endeavor of the collaborating partners. Each of the accessions collected in California was divided into two parts, and one was shipped to Israel. Close contact was maintained between the two teams by frequent visits and constant exchange of information by mail, fax and computer contact.

G. EVALUATION OF THE RESEARCH ACHIEVEMENTS

Most of the objectives outlined in the original proposal (see Section D above) have been achieved. We were able to collect a total of over 160 interesting accessions of about 25 species (including some not yet fully identified) from the three genera in the brodiaea complex. The collection is divided into two populations being grown in Rehovot and in Davis. Basic information on growth flowering, and horticultural characteristics has been collected for each accession. From the accessions, 20 individual plants with particularly interesting characteristics are presently being propagated *in vitro* to provide clonal material for semi-commercial evaluation.

In our original objectives, we stated that "although this proposal is primarily an applied project, it is hoped that it will also provide new basic information on the developmental and flowering physiology of geophytes." This hope has indeed materialized. In many geophytes, flowering percentage and flower size are strictly a function of corm or bulb size. This relationship was found to hold in *D. ida-maia*. In three species of *Triteleia*, in contrast, flowering percentage and scape length were similar over a 10-fold range of corm size, so that, for example, in *T. ixioides*, corms weighing 0.3 and 3.6g flowered equally and the flowers had the same length of scape.

Geophytes are rarely sensitive to photoperiod. In bulbous crops, a low temperature exposure is used to break dormancy and manipulate flowering. In cormous crops, temperature manipulation is used to break dormancy, but, with the exception of crocus, not to manipulate flowering time. In studies with several accessions of six species of the brodiaea complex (*T. bridgesii*, *T. laxa*, *T. lutea*, *D. pulchellum*, *D. multiflorum*, and *D. congestum*), we found that flowering could be advanced both by low temperature storage of the corms, and by growing the plants in long photoperiods. The magnitude of the response varied between species and sometimes even accessions.

An exciting lead to an additional means for manipulating flowering is our finding that exposing dormant corms to ethylene accelerated flowering, improved flower quality (number of florets, scape length), and increased the propagation index in corms of *T. laxa*. This effect was first apparent in the enlargement of the apical meristem.

In the postharvest physiology studies, we found that *T. laxa* flowers behave differently than almost all other known flowers. Although they produce almost no ethylene during their entire life and their longevity is not extended by treatment with STS, they are very sensitive to external ethylene, and STS protected the flowers from ethylene damage. Ethylene production could be detected only following pollination. This is a very rare case in which ethylene production in flowers can be controlled and in which ethylene sensitivity is totally independent of ethylene synthesis.

In our original proposal we noted that "research projects of this kind are long-term projects which cannot be completed in the 3-year period normally allotted for BARD projects." Indeed, the project has not been completed, and it requires extension for several more years in order to enable us to develop our very promising brodiaea genotypes into new commercial floricultural crops ready to be introduced into cultivation.

H. LIST OF PUBLICATIONS

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