THE RELATIONSHIP BETWEEN FLOWER BUD INITIATION AND RESPIRATION IN ASIATIC HYBRID LILY BULBS

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Abstract

Asiatic hybrid lily, *Lilium elegans*, cultivars were used to investigate the relationship between the respiration pattern, changes in soluble carbohydrates, and flower bud initiation. The respiration pattern in non-vernalized and vernalized bulbs was investigated. Each cultivar had a characteristic respiratory pattern and produced an initial CO_2 peak followed by a decline (CO_2 dip) before increasing to a second peak. This respiration pattern was dependent on the bulb vernalization temperature. It was concluded that flower bud initiation is completed before the CO_2 dip in vernalized bulbs. Further, the level and pattern of respiration can be used to determine the degree of cold bulbs received before harvest. Soluble glucose and fructose increased only in the shoot apex. This may be useful in understanding the physiology of flower bud initiation.

1. Introduction

Asiatic hybrid lilies, *Lilium elegans* Thunb., have been forced for cut flower and pot plant production. Roh (1985) observed that flower bud initiation is completed before shoot emergence.

Yearly variation in shoot emergence and the requirement for bulb vernalization in several Asiatic hybrid lilies was observed (Roh, unpublished data). A yearly variation was also reported in the Easter Lily (Lin and Wilkins, 1975). Payne (1967) concluded that the respiratory pattern of bulbs during vernalization or during stem elongation could not account for the yearly variation.

In *Iris*, respiration reached a low, steady state at 25° or 30°C and then suddenly increased corresponding with the differentiation of flower buds (Halevy, 1963, Rodrigues Pereira, 1962). Transition from the vegetative to the reproductive stage involved an increase in soluble carbohydrates in the buds (Rodrigues Pereira, 1962).

Information on respiration, soluble carbohydrate content, and flower bud initiation in Asiatic hybrid lilies is not available at present. Therefore, research was initiated to investigate the respiration, flower bud initiation, and carbohydrate changes in scales and in shoots as influenced by bulb vernalization.

2. Materials and methods

Non-vernalized bulbs were received from the Oregon Bulb Farm, Sandy, Oregon, and packed in 60% moist peat moss before storage at various temperatures. Bulbs were dipped in Benomyl (1.5 g/1) solution for 30 minutes. Respiration was automatically monitored at 20°C in the dark starting after 4 hours as specified in each experiment (Watada amd Massie, 1981). A bulb was placed in a 2.5 1 jar filled with 50 g of coarse vermiculite which had been treated with Benomyl.

Evolution of CO_2 was recorded. Every seven days, 5 ml of distilled water were injected into the sample jar through an air inlet.

2.1. Expt. 1

'Sunray' bulbs (13 to 15 cm in circumference), which were harvested in July 1984 after flowering at Beltsville, received 0°, 2.5°, 7.5°, 10°, or 20°C for 56 days. Four bulbs per treatment were used to measure respiration rate.

2.2. Expt. 2

Non-vernalized 'Inferno', 'Red Carpet', and 'Sunray' bulbs (13 to 15 cm in circumference) were received on 12 October 1983 and given 9 weeks of 5°C. Three bulbs were used for the respiration study. Ten bulbs of each cultivar were forced in a 21°/16°C greenhouse.

2.3. Expt. 3

'Red Carpet' and 'Sunray' bulbs (13 to 15 cm in circumference) were stored for 52 days at 0°C after 6 weeks of 5°C. Twelve bulbs per cultivar were used for the respiration study.

2.4. Expt. 4

'Connecticut Lemonglow' bulbs, (15 to 18 cm in circumference) were stored for 170 days at 0°C in peat moss. After storage, the following percent by weight of outer scales were removed: 0% (whole bulb), 60%, or 85%. Outermost scales were collected and the base of each detached scale was slightly embedded into vermiculite. The experiment was replicated four times.

2.5. Expt. 5

The 'Connecticut Lemonglow' bulbs used in Expt. 4 were stored for 190 days at 0°C and respiration and alcohol soluble carbohydrates were measured. Three bulbs were used for respiration and carbohydrate measurement. Ethanol soluble fructose, glucose, and sucrose were analyzed by gas chromatography of trimethylsilyl derivates. Two grams of tissue were placed in 10 ml 80% ethanol, boiled for 10 minutes and homogenized for 1 minute with a Polytron homogenizer. Homogenates were centrifuged and the supernatants were filtered through a column containing 1 ml layers of Dowex 1-X8 and Dowex 50 ion exchange resins (Gross, 1983). After washing the column, extracts were made to 12 ml. A one-ml aliquot was taken to dryness at 40°C using a stream of N_2 and the sugars derived according to the method of Li and Schuhmann. One µl was injected into a gas chromatograph equipped with a 30 m SP 2330 capillary column (Supelco) and a flame ionization detector. The internal standard was β-phenyl-D-gulcoside. Five bulbs were sampled for anatomical observation of the growing point to check the developmental stages at the times indicated in Figure 5. Developmental stages (Cremer, e t a l, 1974) are: I - vegetative, II - differentiation at the circumference of the shoot apex, III - loss of the spherical shape of the dome, IV - bud differentiation at the circumference of the shoot apex, V - formation of two whorls of three perianthus, and VI - formation of stamen.

3. Results and discussion

3.1. Expt. 1

Respiration was lowest when bulbs received 20°C followed by 10° and 7.5°C (Figure 1). Bulbs that received 0° and 2.5°C showed a similar level and pattern of CO_2 production. Results from 'Inferno', 'Sunray', 'Red Carpet', and 'Connecticut Lemonglow' bulbs harvested in Oregon in 1984 were similar to those that received temperatures lower than 7.5°C (Data not presented). Iris bulbs stored at 25°C showed a low but steady state CO_2 production, whereas, bulbs stored at 13°C showed elevated CO_2 production (Rodrigues Pereira, 1962). From these results, the low temperature that bulbs received before or after harvest can be monitored by the level and pattern of respiration.

3.2. Expt. 2

The respiration rate of bulbs increased initially and reached the first peak within 20 hours in 'Inferno' and 'Sunray' and then decreased for 30 hours in 'Sunray' and 10 hours in 'Inferno', at which time the rate reached a minimum (CO_2 dip) (Figure 2.) After this decline, CO_2 production increased again reaching a second peak rate after 70 hours in 'Inferno' and 95 hours in 'Sunray'. In 'Red Carpet', CO_2 production constantly increased for 85 hours after showing a brief initial CO_2 peak and dip.

The respiration pattern of various Asiatic hybrid lily cultivars differ. In Easter lilies, the respiration pattern was similar in two cultivars (Payne, 1967). Fully vernalized 'Inferno' bulbs flowered after 126 days and 'Sunray' in 143 days. 'Inferno' and 'Sunray' 1.7 and 0.8 leaves, respectively, were unfolded per day (Table 1). The late and low CO_2 second peak in 'Sunray' may be related to late shoot emergence and flowering. Also, a low number of leaves must be developed between shoot emergence and flowering.

3.3. Expt. 3

After an initial respiratory peak, CO_2 production decreased continuously for 210 hours in 'Sunray', reaching a minimum after 275 hours (Figure 3). After this CO_2 dip respiration increased, reaching the second peak or plateau in 500 hours. In 'Red Carpet', a CO_2 dip occured in 35 hours. Two bulbs were disected for observing the presence of flower buds after 12 days. The size of 'Red Carpet' buds was 3 to 4 mm in length and the size of the 'Sunray' buds was 1 to 2 mm in length. When the experiment was started, flower buds had not been observed.

The results of Expts. 2 and 3, suggest: a) the initial CO_2 peak is related to the initiation of flower buds or the hydrolysis of starch in the scale, b) the CO_2 dip is related to the initiation of stem elongation, and c) the second peak or plateau is related to the development of flower buds and leaves. The timing of the CO_2 dip after the first CO_2 peak in 'Red Carpet' as compared to the pattern observed in 'Sunray' (Figures 2, 3) might be due to an increased rate of leaf unfolding (Table 1). Payne (1967) reported that respiratory activity was not a good indicator of the rate of stem elongation and flowering in Easter lilies.

3.4. Expt. 4

Since the observed respiration pattern from a whole bulb (Figures 1, 2, 3) may not provide information on the respiration pattern of the growing point, various percentages of scales were removed and CO_2 production measured from the bulbs or detached scales in 'Connecticut Lemonglow'. Initial CO_2 production was highest in bulbs with 85% of the scales removed, followed by 60% and detached scales (Figure 4). The respiration from a whole bulb was the lowest. Although respiration rate in the detached scales was low, the pattern was very similar to that of various scale removed bulbs.

Since bulbs with 85% of the scales removed showed the highest respiration, the shoot apex is apparently very actively respirating where the major morphological changes were occurring; a transition from a vegetative to a reproductive stage. The similar pattern observed in detached scales indicates that the initial CO_2 peak is not related to flower bud initiation, but is related rather to the hydrolysis of starch. Starch hydrolysis in *Iris* bulb scales was responsible for increased respiration rate in the scales (Halevy, 1963, Rodrigues Pereira, 1963).

The presence of scales and primodial leaves promotes flower bud initiation in *Iris* (Rodrigues Pereira, 1962), possibly due to the translocation of carbohydrates from scales to the shoot apex in tulips (Hobson and Davies, 1977). Therefore, the CO_2 production pattern that was observed in bulbs with 85% of the scales removed may not be precisely correlated with the time of flower bud initiation.

3.5. Expt. 5

The experiment was terminated when respiration reached the CO_2 dip in order to check the timing of 'Connecticut Lemonglow' flower bud initiation. The respiration pattern from the whole bulb was similar to that shown in Figure 4, however, the respiration rate was higher, producing about 240 mg CO_2/kg. h. during the initial peak that occurred after 36 hours. The second CO_2 dip occurred after 160 hours.

In scales, sucrose content increased to a maximum level of 22.6 mg/g. fw. after 72 hours and then decreased for 192 hours (Figure 5). Glucose and fructose were detected at less than 0.8 mg/g. fw. In the shoot apex, sucrose decreased linearly from 13.7 mg/g. fw. to 7.4 mg/g. fw. By comparison, glucose and fructose contents increased linearly to a level of 4.7 mg/g. fw., and 2.4 mg/g. fw., respectively. In tulips, only glucose accumulated in shoots and scales when shoots began to grow rapidly (Davis and Kempton, 1975). Rodrigues Pereira (1962) concluded that the transition from vegetative to reproductive growth involves an increase in sucrose and reducing sugars in the buds. However, in Asiatic hybrid lilies, sucrose content in the scales and shoot apex decreased continuously. Flower buds were observed after 216 hours (Figure 5). The increase in fructose and glucose in shoot apex may be an early sign of flower bud initiation.

4. Conclusion

The respiration pattern in Asiatic hybrid lilies may be a useful marker to understand the relationship between temperature effect, shoot emergence, and flowering. Respiration of non-vernalized bulbs was lower than that of vernalized bulbs. Flower bud initiation was completed between the initial CO_2 peak and the CO_2 dip. The rapid rise in CO_2 production after the dip may results from rapid stem elongation.

It is concluded that the CO_2 production pattern of Asiatic hybrid lilies can be used: a) to detect the length of cold exposure that bulbs received before harvest, b) to understand flower bud initiation before buds can be microscopically detected, and c) to detect the time of stem elongation that accompanies development of flower buds and the unfolding of initiated leaves. Further, the increase in soluble glucose and fructose in the shoot apex is an early physiological sign of flower bud initiation before flower primordia can be observed under the microscope.

References

Cremer, A. C., Beijer, J. J., and de Munk, W. J., 1974. Development stages of flower formation in tulips, narcisi, iris, hyacinths, and lilies meded. Landbouwhogeschool Wageningan, Nederland. 74-15; 16 p.

Davies, J. N., and Kempton, R. J., 1975. Carbohydrate changes in tulip bulbs during storage and forcing. Acta Hortic. 47:353-363.

Gross, K. C., 1983. Changes in free galactose, myo-inositol and other monosaccharides in normal and non-ripening mutant tomatoes. Phytochemistry 22(5):1137-1139.

Halevy, H., 1963. Metabolic changes in Wedgewood iris as influenced by storage temperature of bulbs. Proc. XVI Inter. Hort. Cong. 1962:220-228.

Hobson, G. E. and Davis, J. N., 1977. Mitochondrial activities and carbohydrate levels in tulip bulbs in relation to cold treatment. J. Expt. Bot. 28(104):559-568.

Kamerbeek, G. A., 1962. Respiration of the iris bulb in relation to the temperature and the growth of the primordia. Acta Botanica Neerlandica 11:331-410.

Li, B. W., and Schuhmann, P. J., 1980. Gas-liquid chromatographic analysis of sugars in ready to eat breakfast cereals. J. Food, Sci. 45(1):138-141.

Lin, W. C., and Wilkins, H. F., 1975. Influence of bulb harvest date and temperature on growth and flowering of L i l i u m L o n g i f l o r u m. J. Amer. Soc. 100(1):6-9.

Payne, R. N., 1967. The influence of 40°F and 70°F storage temperature on respiration and forcing bulbs of L i l i u m l o n g i f l o r u m Thunberg, cultivars 'Ace' and 'Georgia'. Ph.D. Thesis, Ohio State University, Columbus, OH, USA.

Rodrigues Pereira, A. S., 1962. Physiological experiments in connection with flower formation in Wedgewood Iris (Iris cv. "Wedgewood"). Acta Botanica Neerlandica 11:97-138.

Roh, M. S., 1985. Response of Mid-Century hybrid lilies to bulb vernalization and short photoperiod HortScience 20(4):710-713.

Watada, A. E., and Massie, D. R., 1981. A compact automatic system for measuring CO_2 and C_2H_4 evolution by harvested horticultural crops. HortScience 16(1):39-41.

Table 1 - Number of days to shoot emergence and flower and number of leaves of three Asiatic hybrid lilies. Number of leaves unfolded was calculated by dividing the number of leaves by the number of days from shoot emergence to flowering.

Cultivar	Weeks at 5°	Days to: Shoot emergence (A)	Days to: Flowering (B)	From A to B	No. of leaves	No. of leaves unfolded per day
Inferno	8	80	126	46	80	1.7
Red Carpet	8	82	138	56	86	1.5
Sunray	8	85	143	58	48	0.8

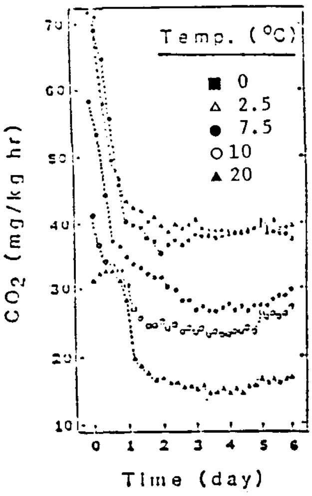


Figure - 1

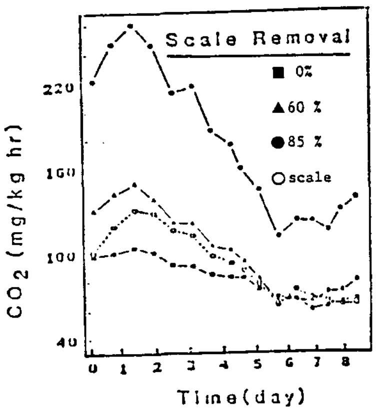
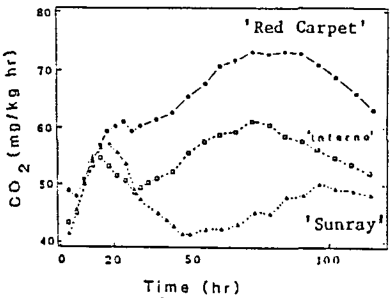


Figure - 2



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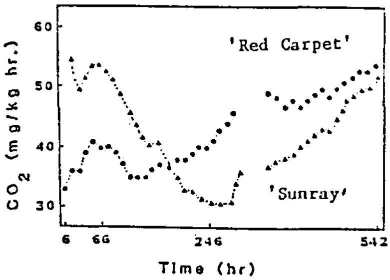

Figure - 4

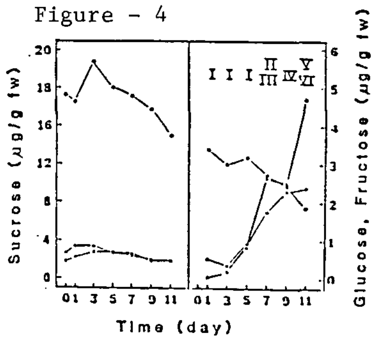

Figure - 5

Figures 1 to 5

1 - Respiration pattern of 'Sunray' bulbs that received various temperatures. Data was collected every 6 hours.
2 - Respiration pattern of three Asiatic hybrid lilies after 8 weeks of 5°C. Respiration was monitored at 3-hr intervals.
3 - Respiration pattern of 'Red Carpet' and 'Sunray' bulbs stored at 0°C for 52 days after 42 days of 5°C. Data were collected at 6.5-hr intervals.
4 - Respiration pattern of 'Connecticut Lemonglow' bulbs after removal of various amount of scales. Data were collected at 3.5-hr intervals.
5 - Changes in the developmental stages of the shoot apex and ethanol soluble carbohydrates in the scales or shoot apex of 'Connecticut Lemonglow' bulbs. Description of developmental stages explained in the text.