Morphological and Biochemical Effects of Ethylene on Tulips

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Summary. Tulip bulbs treated with ethylene at various concentrations, at different stages of development and during various lengths of time, exhibited various morphological abnormalities: open bud, stamen blasting, abnormal number of floral parts, increased number of second-year bulbs. These morphological changes were paralleled by rapid variations in the contents of proteins and RNAs, and a slow, yet noticeable, increase of the DNA content of flower buds.

Subsequent to the basic work on flower formation in tulips (*Tulipa gesneriana*, L.) by DIJK[2] in 1951 most research have centered around the influence of temperature on this process. However, recent investigations[3,4] have indicated that ethylene may be involved in these disturbances. In this study, the effect of ethylene on the morphology of tulips during the different stages of development was examined. In conjunction with this the levels of nucleic acids and proteins in the flower-buds were determined.

Materials and methods. Ethylene was generated by the action of sodium hydroxide on Ethrel (2-chloroethylphosphonic acid, Amchem Products, Inc.). The ethylene concentration was confirmed by gas chromatography[5].

Bulbs used in this study were cv. Darwin 33 and Paul Richter, purchased commercially, for preliminary experiments, and subsequently grown in a cold frome and selected on our laboratory. Bulbs at the different stages of growth were treated, in sealed containers, with ethylene concentrations ranging from 0 to 10 ppm for different periods. The morphological changes were noted, then the flower-buds were dissected out and analyzed for DNA, RNA and protein. The extraction procedure was essentially that of PILET and BRAUN[6]. DNA was determined by the diphenylamine method of BURTON[7], RNA by the orcinol method[8] and protein by the use of the phenol reagent[9].

Results. Effect of ethylene during the postharvest maturation period. a) Before G-stage. Gummosis occurred with Darwin 33 but not with Paul Richter even at ethylene concentrations up to 100 ppm. The gum blisters were localized on the external surface of the outermost scale, and did not penetrate to the subsequent scales. Morphologically, there was a random alteration in the number of tepals and stamens.

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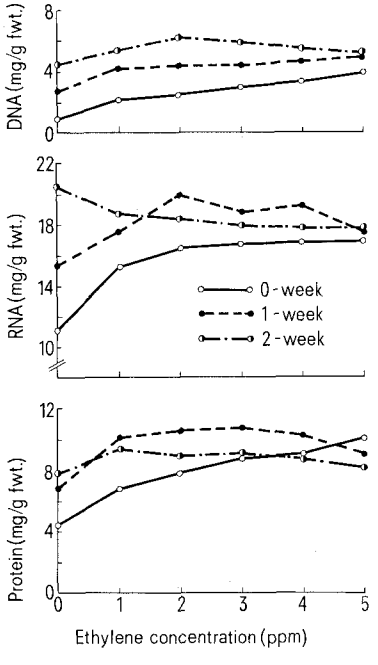

Fig. 1. DNA, RNA and protein levels (mg/g Fresh Wt.) at 0, 1 and 2 weeks after the removal of the 4-day ethylene atmosphere of 0, 1, 2, 3, 4 and 5 ppm.

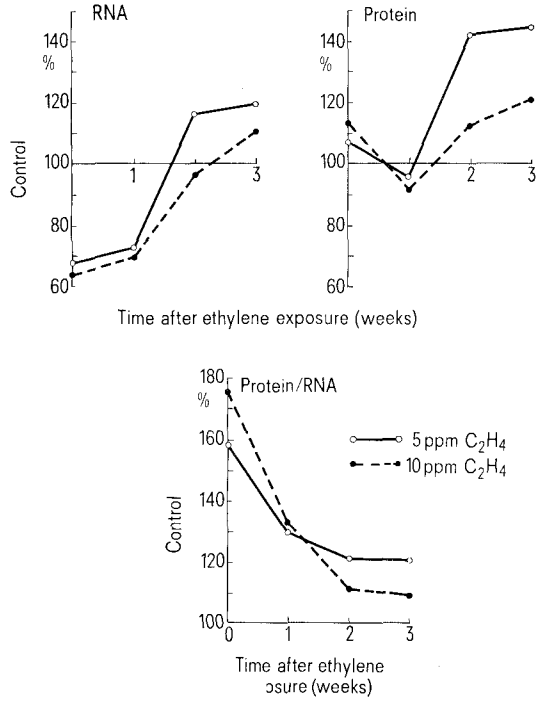

Fig. 2. RNA, protein and protein: RNA ratio (% of control) of buds at 0, 1, 2 and 3 weeks after the removal of the 4-week ethylene atmosphere of 5 and 10 ppm.

Table I. Effect of ethylene during the forcing period (day temp. 18 °C, night temp. 16 °C)

Ethylene concentration (ppm)	DNA		RNA		Protein	
	mg/g F. Wt.	Control[a] (%)	mg/g F. Wt.	Control[a] (%)	mg/g F. Wt.	Control[a] (%)
Day-2						
0.0	1.40		8.18		13.32	
0.1	1.82	130.00	15.18	185.57	14.41	108.18
0.3	2.03	145.00	15.67	191.56	16.89	126.80
0.5	2.34	167.14	16.13	197.19	17.78	133.48
Day-7						
0.0	4.47		12.11		12.89	
0.1	6.36	142.28	14.24	117.59	13.42	104.11
0.3	7.99	178.74	15.50	127.99	15.59	120.95
0.5	7.67	171.59	14.77	121.97	15.73	122.03
Day-10						
0.0	4.09		11.48		12.71	
0.1	6.88	168.22	13.17	114.72	13.19	103.78
0.3	7.28	177.51	14.67	127.79	14.97	117.78
0.5	5.98	146.21	11.89	103.57	14.60	114.87

Tulip plants (cv. Paul Richter) after the cooling period (12 weeks at 10 °C) were treated, in sealed plastic cabinets, during the forcing period with 0, 0.1, 0.3 and 0.5 ppm ethylene for 2, 7 and 10 days. The floral buds were analyzed immediately after the ethylene exposure for DNA, RNA and protein. The TCA-acetone method of extraction[6] was employed.
[a] Treated/control × 100.

b) After G-stage. 50 bulbs (cv. Paul Richter) were treated, in sealed jars with ethylene concentrations ranging from 0 to 5 ppm at 20 °C for 4 days. Half of these bulbs were then planted 'normally' to observe the morphological changes at blossom. From the other bulbs the flower buds were dissected out and analyzed for DNA, RNA and protein. The analyses were done immediately, then 1 and 2 weeks after the treatment (Figure 1).

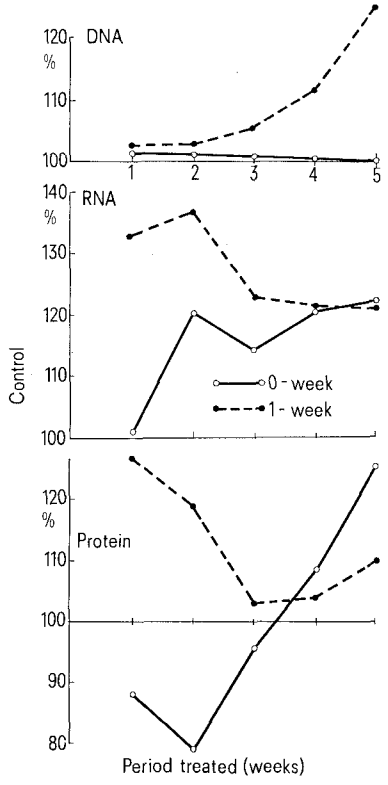

Fig. 3. DNA, RNA and protein levels (% of control) of buds at 0 and 1 week after the removal of the ethylene treatment (0.5 ppm for periods ranging from 1 to 5 weeks).

The main morphological change was the formation of 'open buds' (stamens longer than tepals and/or foliage leaves) was noted when the flowers blossomed.

2. *Effect of ethylene during the cooling period.* a) The effect of ethylene following fixed-length exposures. Bulbs (cv. Paul Richter, G-stage) were placed in cold storage (10 °C) and treated, in sealed jars, with 5 and 10 ppm ethylene for 4 weeks. In each sampling 0, 1, 2 and 3 weeks after the exposure period, the buds were dissected out and analyzed (Figure 2). The immediate effect was a lowering of the RNA level. Consequently the protein : RNA ratio showed a sharp increase. With time, the ratio of the treated samples decreased and came closer to, but nevertheless higher than, that of the control. No floral abnormalities were observed in these treatments.

b) The effect of varying the length of ethylene exposures. Bulbs (cv. Paul Richter, G-stage) were placed in cold storage (10 °C) and treated, in sealed jars, with 0.5 ppm ethylene for periods ranging from 1 to 5 weeks. In each instance, the buds were analyzed immediately and 1 week after the ethylene treatment (Figure 3). The most remarkable effects that the longer treatments gave higher increases in DNA concentrations. As in the previous experiment no floral abnormalities were observed.

3. *Effect of ethylene during the forcing period.* Ethylene concentrations used in this study were lower than 1 ppm because preliminary study showed that the plants were very sensitive to the gas during this period. Plants, in sealed cabinets, were exposed to 0.1, 0.3 and 0.5 ppm ethylene for 2, 7 and 10 days. Morphological changes in the flowers during the treatment included severe blasting of the flowers at the 0.3 and 0.5 ppm ethylene level in the 7- and 10-day treatments. However, once the flowers had blossomed, they were less susceptible to ethylene. Plants treated with ethylene during this period gave an increased number of daughter bulbs (Table II).

Discussion. Contrary to the prevailing opinion in existing literature[10] that the monocotyledonous plants are rather insensitive to ethylene, our results showed that

[10] S. P. Burg and E. A. Burg, in *Biochemistry and Physiology of Plant Growth Substances* (The Runge Press Ltd., Ottawa, Canada 1967).

Table II. Effect of ethylene during the forcing period (day temp. 18 °C, night temp. 16 °C)

Ethylene concentration (ppm)	No. of bulbs formed [a]
0	3
1	3
2	5
5	7
10	7

Tulip (cv. Paul Richter) plants treated during forcing after the flowers bloomed gave different number of daughter bulbs depending on the ethylene concentration. The plants were exposed to ethylene concentrations of 0, 1, 2, 5 and 10 ppm for a period of 1 week. The ethylene was then removed and the plants allowed to mature. After the foliage leaves had died down, the bulbs were lifted and the number of bulbs formed noted.

[a] Counting was done on a population of 5 bulbs for each concentration.

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tulips are very sensitive to the gas. The variety, growth phase and environmental conditions under which the bulbs are treated, however, determine to a large extent the concentration threshold for ethylene effectiveness. The bulbs appear to be most sensitive to the gas during periods of active morphogenesis – flower initiation and formation, and initial phase of the forcing period. In this study, we found most of the disturbances described by other authors[3,4]. Ethylene did not increase the 'femaleness' in tulips as it did in pineapples[11] and cucumber[12], instead it induced an abnormal elongation of the stamens resulting in the formation of 'open' buds.

The data obtained in the studies on the nucleic acids and protein are too scattered to lead to definite conclusions. However, three points may be stressed. Firstly, ethylene appears to act first on the somatic and phenotypical apparatus of the cell, and only subsequently on the DNA. Secondly, ethylene proved to be able to alter the nuclear genetic information. Thirdly, the altered genetic information appears to be irreversible. A study on the species of DNA and RNA of the treated plants would give a more definite answer to the question of the effect of ethylene on morphogenesis.

Growth and Development of the American Bollworm *Heliothis armigera* Hubn. under Laboratory Mass Rearing Conditions

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Summary. A kidney bean meal diet was the most satisfactory laboratory diet for the larvae of the American bollworm *Heliothis armigera* Hubn. Optimum rates of survival (63.5%) occurred and the larval growth was better than that of larvae reared on castor oil plant leaves. The pupal weight and the fecundity of the resulting adults were also much better than those on the control host plant.

Artificial diets have been devised for rearing a variety of insect species in the laboratory but many of these diets were developed with an eye to nutritional adequency and little consideration to cost. However, the present emphasis on methods of insect control which can supplement or replace control with insecticides has created a demand for large quantities of laboratory reared insects. The success of methods of control based on plant resistance pheromones and insect pathogens, and the eventual success of control by release of parasites or sterile male insects, depends on economical production of great numbers of the pest species. Development of rearing technique has been reported by authors[1–3] that would reduce the cost of rearing larvae of the tobacco budworm *Heliothis virescens* (F.) and the bollworm *H. zea* Boddie. Since these species consume large quantities of the diet, the cost of the medium is an important factor in production. Therefore manipulation of the ingredients in the diet was an obvious method of further reducing costs.

Table I. Constitution of an artificial diet for the American bollworm *Heliothis armigera* Hubn.

Ingredient	Quantities (g)	Total weight (%)
Dried kidney beans	236	26.4
Dried brewers yeast	37	4.1
Ascorbic acid	3	0.33
Methyl *p*-hydroxybenzoate	2	0.22
Agar	14	1.5
Water	600 ml	67.2

The purpose of this research was to produce a simple, highly reproducible artificial rearing medium for the American bollworm.

Material and methods. The soy bean protein diet developed by [4] and [5] as modified by the present author was selected for this study (Table I). The American bollworm larvae reared on this diet were compared with those reared on castor oil plant leaves. Adults were confined in one gallon wide-mouthed glass jars in the ratio of 3 males to 2 females. The moths were provided with 10% honey solution. Jars were covered with a black cloth on which the moths deposited their eggs. This method made egg collection easy as the cloth could be removed daily. Upon hatching of the eggs, the larvae were transferred to glass vials (7.5 × 10 cm) partially filled with the media, and were kept at 27 ± 1 °C. 1000 larvae were fed on an artificial diet and the same number were reared on castor oil plant leaves. 10 larvae were put in each glass vial from 1st to 3rd instar followed by a single larva per vial till pupation.

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