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Survey of wound-induced ethylene production by excised root segments

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Ethylene production was measured from excised 10-mm apical and subapical root segments from 50 cultivars in 19 species of 7 families. Monocotyledonous species tended to have much lower rates of ethylene production than dicotyledonous species. Ethylene production was generally higher in apical root segments than in subapical segments within 1 h of wounding. However, cultivars of *Cucumis melo*, *C. sativus*, *Helianthus annuus*, *Hibiscus esculentus*, and *Zea mays* had higher rates of ethylene production from subapical segments. In apical root segments, *Phaseolus aureus* cv. Berken had the highest ethylene production rate (76.7 $\eta l\, g^{-1}\, h^{-1}$), while *Zea mays* cv. Silver Queen had the lowest rate (0.6 $\eta l\, g^{-1}\, h^{-1}$). In subapical root segments, *Cucumis sativus* cv. Armenian had the highest rate (55.7 $\eta l\, g^{-1}\, h^{-1}$), while *Zea mays* cv. Silver Queen again had the lowest rate (0.6 $\eta l\, g^{-1}\, h^{-1}$). The many different responses in magnitude and kinetics of wound-induced ethylene production among the species, cultivars and tissues should provide interesting and useful systems with which to study wound responses and induced ethylene production.

Introduction

Ethylene is a plant growth regulator that affects essentially all phases of plant growth, development and senescence (Beyer 1981, Yang and Hoffman 1984, Yang 1985, Abeles et al. 1992). A variety of developmental and environmental stresses regulate C_2H_4 synthesis in vivo (Abeles et al. 1992, Yang 1985). For example, the rate of C_2H_4 synthesis increases during seed germination (Abeles et al. 1992), fruit ripening (Burg and Burg 1965, Lieberman 1975), and flower (Hanson and Kende 1976) and leaf senescence and abscission (Abeles et al. 1971, Kader 1985). Ethylene synthesis is also stimulated by environmental stresses such as wounding, chilling, waterlogging, drought, and toxic chemicals (Lieberman 1975, Saltveit and Dilley 1978, Yang 1985, Abeles et al. 1992).

The effects of wounding on the synthesis and evolution of C_2H_4 have been studied in a variety of plant tissues. These include cantaloupe (Hoffman and Yang 1981, McGlasson and Pratt 1964), banana (McGlasson 1969), morning glory flowers (Hanson and Kende 1976), etiolated pea stem segments (Saltveit and Dilley 1978), mung bean hypocotyls (Yu and Yang 1980), sweet potato storage roots (Saltveit and Locy 1982, Randle and Woodson 1986), and mesocarp tissues of winter squash fruit (Hyodo et al. 1983).

Wound-induced ethylene can either promote (Wang et al. 2002) suppress (Winz and Baldwin 2001), inhibit (Winz and Baldwin 2001), or have no effect (Geitmann et al. 1997) on other wound-induced responses. Wounding of *Nicotiana attenuata* produced a dramatic burst in ethylene and jasmonate, and treatment with methyl jasmonate induced nicotine biosynthesis. However, wound stimulated ethylene production, directly suppresses the biosynthesis of nicotine (Winz and Baldwin 2001). Inhibition of ethylene action in wounded sweet potato roots downregulated the ability of methyl jasmonate to enhance sporamin gene expression (Wang et al. 2002). Inhibition of wound-induced increases in ethylene synthesis in pea roots did not affect wound-induced microtubule re-orientation (Geitmann et al. 1997). In wounded and infected *Capsicum annuum* leaves, wound-induced ethylene may function as a strong signal elicitor in the activation of pathogenesis-related genes, eventually mediating the plant defense response (Kim and Hwang 2000).

Saltveit and Dilley (1978) studied C_2H_4 production from various tissues and reported that tissue excised from meristematic regions of stems or roots exhibited widely different rates of C_2H_4 production, but that apical tissue tended to produce more ethylene that subapical tissue. Burg (1973) hypothesized that higher C_2H_4

synthesis in apical tissues could be attributed to the high concentration of auxin that would stimulate C_2H_4 synthesis. Although the kinetics of C_2H_4 production is also variable among different tissues, the effect of wounding on C_2H_4 production was usually measurable within 30 min. For example, the rate of ethylene production from segments of tomato petiole increased after a delay of 20–30 min, and reached rates 10-fold higher than initial rates after 80 min (Jackson and Campbell 1976). They suggested that measurements made immediately after excision would better estimate production rates of intact tissue than those made later, but they do not provide rates from non-wounded tissue for comparison. The use of long collection periods employed in many studies prevented an analysis of the fine details of wound-induced ethylene production (e.g. the biphasic response characterized in pea by Saltveit and Dilley 1978). These rapid responses contrast with that reported for sweet potatoes (Saltveit and Locy 1982, Randle and Woodson 1986), and squash (Hyodo et al. 1983) where the maximum wound-induced C_2H_4 response occurred about 4 days after wounding.

Roots could serve as an effective system to study C_2H_4 production because they are quickly and easily grown, produce uniform tissue, and are metabolically active. However, little work has been reported on the physiology or kinetics of C_2H_4 production by root tissues (Abeles et al. 1992). A study of the dynamics of ethylene production from excised segments of *Zea mays* (cv. LG11) roots showed that subapical segments (10–20 mm) produced more wound-induced ethylene than did apical (0–10 mm) or other subapical (20–40, 40–60, and 60–80 mm) segments (Atwell et al. 1988). However, they collected ethylene for 60 min after excision of the segments so changes during that period were undetectable. A peak of wound-induced ethylene production was seen at 30 min for apical (0–2 cm) and subapical (2–4 cm) root segments of oilseed rape (*Brassica napus* L. cv. Primor) (Jackson et al. 1984). The excised apical segments produced about twice as much ethylene as did the subapical segments throughout the 50 min sampling period.

The literature on the kinetics of wound-induced ethylene production is sparse and limited to a few species. We felt that C_2H_4 production should be more extensively characterized from a uniform tissue (e.g. seedling roots) for a wide variety of plants in order to better understand the diversity of this wound response. This paper reports a survey of C_2H_4 production from apical and subapical segments of 50 cultivars of monocotyledonous and dicotyledonous plants from 19 species in 7 plant families. Results of this study will assist in the selection of tissue systems to study the effects of wounding on the induction and expression of ethylene biosynthesis.

Materials and methods

Plant material

Seeds were purchased from commercial seed companies. Seeds were imbibed in aerated deionized water for 10 h before being planted in moist vermiculite that had been soaked in deionized water for 8 h and drained for 2 h. Eighteen $\times$ 16 cm diameter plastic pots were filled with moist vermiculite to within 3 cm of the top. Imbibed seeds were scattered over the surface of the vermiculite and covered with 2 cm of moist vermiculite. Pots were kept at 20°C in a dark container continuously flushed with humidified, C_2H_4-free air. Root lengths of 40 seedlings of each cultivar were measured daily 3 days after planting in order to predict when to harvest seedlings with roots about 5 cm long for the wounding experiments. A new pot of seedlings was used each time. Seedlings usually took from 3 to 9 days to produce primary roots that were around 5 cm long. Seedlings were grown under identical conditions and roots were harvested at the same length to minimize effects of growing conditions and stage of development on the wound response. The apical segment would include tissue in the zone of cell division (root apical meristems) and zone of cell elongation, while the subapical segments could include tissue in the zone of cell elongation and in the zone of cell maturation, but not in the zone of cell division.

Ethylene measurements

Seedlings were gently removed from the vermiculite when their roots had reached 5 cm in length. Roots were washed with deionized water to remove any adhering vermiculite, and 20 straight roots, free of any visual defects were selected. Roots were laid on moist paper towelling and apical and subapical 10-mm segments were excised with a stainless steel razor blade and placed in folded 25 $\times$ 25 mm squares of aluminium foil. Each foil boat was immediately placed in a 16-mm diameter $\times$ 100-mm long glass culture tube (approximately 15.2 $\pm$ 0.1 ml) that contained a 5 $\times$ 10 mm piece of filter paper moistened with saturated KOH to absorb CO_2, and the tube was capped with a rubber serum stopper. Preliminary measurements with root segments from cultivars from a few species indicated that ethylene production was induced by excision of segments, and that a 3-h sampling period would include the initial burst of ethylene production (Saltveit and Dilley 1978). After 3 h, 1-ml gas samples were taken for C_2H_4 analysis with a Carle gas chromatograph equipped with an alumina column and a flame ionization detector (Abeles et al. 1992). Injection of 1 ml of a 1.1-$\mu l\,l^{-1}$ ethylene standard was used to calibrate the gas chromatograph (i.e. determine sensitivity and retention time). The gas chromatograph was able to consistently detect 0.01 $\mu l\,l^{-1}$ ethylene in a 1-ml air sample. All experiments contained at least two replicates and were repeated at least three times. Each experiment took place at least 1 week apart. Data presented are the means and standard deviations of all measurements (n = 6).

Kinetics of ethylene production

After an initial screen of the 50 species for rates of ethylene production from apical and subapical radicle segments, those species and cultivars that had widely

divergent rates and patterns of ethylene production were selected for a more detailed kinetic study.

The imbibed seeds were transferred to moist paper towelling sandwiched between two 15×30 cm Plexiglas plates that were held together with rubber bands. The seeds were orientated with the radicle down and the units were kept in a vertical position at 25°C in a humid, ethylene-free atmosphere until the radicles reached the appropriate length. The entire 5-cm long radicle was excised and about 10 radicles were gently rolled-up in moist tissue paper and transferred to a 7×1.5-cm diameter glass tube. Each end of the tube was capped with a rubber serum stopper and held vertically with the roots in their normal orientation. An 18-gauge $\times$ 7.5-cm long syringe needle that was attached by a 3-mm diameter plastic tube to a source of humidified, C_2H_4-free air was inserted through the top stopper. Another 18-gauge syringe needle was inserted through the bottom stopper sufficient to protrude 5 mm into the tube. The lure-lock end of the syringe needle was covered with a serum stopper and serves as a sampling port. The void volume of the tube was about 5 ml. A 3-ml plastic syringe was used to periodically remove 3-ml gas samples from the bottom sample port. In one series of experiments, sampling was done every 10 min for 180 min before and 230 min after wounding. In a later series of experiments with species of the Cucurbitaceae, samples were taken every 3 min for 21 min before wounding, and for 180 min after the wounding of whole 5-cm long excised radicles.

In most kinetic experiments, the 5-cm long radicles were sampled every 20 min for 40 min before and for 180 min after the segments were removed and quickly dissected into 10-mm apical and subapical segments. Dissection and redistribution of the segments took about 3 min. The aluminium foil boats was put into 50×16-mm diameter glass tubes that were capped at both ends with serum stoppers. After 10 min, a 3-ml gas sample was withdrawn and injected into the GC. A needle permanently inserted into the other end of the tube was connected through a thin flexible plastic hose to a reservoir of humidified, ethylene-free air that was drawn into the glass tube by the removal of the 3-ml gas sample.

The rate of passive ethylene diffusion from radicle segments was studied as follows. Sub-apical segments were held overnight until the wound response had dissipated and ethylene production stabilized. The segments were enclosed in a 5.0×1.6-cm diameter glass tube that was flushed with humidified air containing $10\,\mu l\,l^{-1}$ ethylene for 6 h. The segments were quickly transferred to the sampling apparatus and treated as previously described for the kinetic experiments, except that 3-ml samples were removed every 2 min for 90 min.

Results and discussion

Ethylene production from seedling before and after wounding

Diffusion of ethylene from radicle segments previously exposed to $10\,\mu l\,l^{-1}$ ethylene for 6 h was immediate and rapid (Fig. 1). It commenced as soon as measurements started, peaked in 6 min and exponentially declined to low, stable levels within 30 min, These data indicate that the ethylene evolved from excised segments was not the result of off-gassing of ethylene previously within the tissue when it was excised, but of ethylene synthesized in response to the wound of excision.

Evolution of ethylene from whole seedlings (data not shown) and from 5-cm long excised radicles remained fairly stable for at least 3 h (Fig. 2). Wounding the apical region of these whole excised radicles first produced a slight decline in ethylene production, which was then followed by a rapid rise that commenced around 20–40 min after wounding (Fig. 3). The slight decline and later transitory rise and fall in ethylene production shows that the response was not merely the result of enhanced diffusion of previously synthesized ethylene from the wounded tissue. Preparation of the excised 5-mm long radicle did not induce increases in ethylene synthesis within the 3 h duration of time used in these studies (Fig. 3). Limiting the collection period to 3 h insured that perturbations caused by preparation of the 5-cm long radicle did not influence the measurements. Ethylene collected during the 3 h sampling period therefore represented ethylene synthesized in response to excision of the 10-mm apical and subapical radicle segments.

Variability of ethylene production

Rates of ethylene production from both apical and subapical segments were consistent within each cultivar and segment type, but varied among cultivars. For example, in the Cucurbitaceae the coefficient of variability for measurements within each cultivar was around 10%, while measurements between cultivars in the Cucurbitaceae exceeded 65%. The uniformity of ethylene production

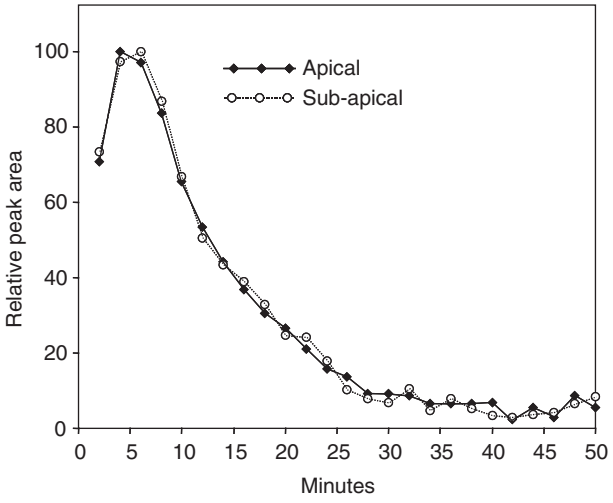

Fig. 1. Ethylene production from aged 10-mm apical and subapical radicles segments from *Cucurbita maxima* cv. Buttercup that were pre loaded with 10 $\mu l\ l^{-1}$ ethylene for 6 h. See Materials and methods for additional details.

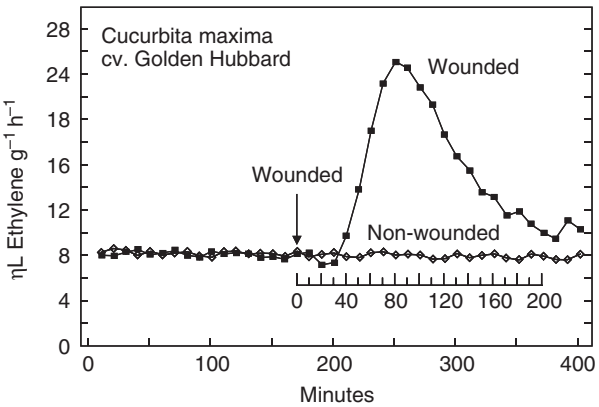

Fig. 2. Wound induced ethylene synthesis from excised 10-mm apical and subapical radicle segments of *Cucurbita maxima*. The entire 5-cm long seedling radicle was excised at time zero. Ethylene production was measured for 180 min before two cuts severed the 10-mm apical and subapical segments from the rest of the radicle. The arrow indicates the time of apical and subapical segment excision. See Materials and methods for additional details.

measurements within species was similar to that previously shown with excised stem segments from etiolated peas (Saltveit and Dilley 1978).

Apical and subapical patterns of ethylene production

The pattern of ethylene production between apical and subapical segments was consistent among cultivars within each species, but was variable among species. In general, the rate of ethylene production from 10-mm excised apical root segments was at least 50% higher than from subapical segments (Tables 1,2 and 3). This was true for 12 of the 19 species tested. In two species, the apical and subapical rates were about the same, while the subapical rate was higher in 5 species. Apical segments had higher ethylene production than subapical segments in most of the 6 monocotyledonous species (4 vs 1; with 1 being equal), and 13 dicotyledonous species (8 vs 4; with 1 being equal).

In the Cucurbitaceae, the apical ethylene production rate was higher than the subapical rate in cultivars of *Citrullus lanatus*, *Cucurbita maxima*, *C. moschata*, and *C. pepo*. In contrast, the apical ethylene production rate was lower than the subapical rate in species of *Cucumis melo* and *C. sativus* (Table 1). In the Gramineae, apical ethylene production was higher in *Triticolsecale* sp., roughly the same in *Triticum* sp., and generally lower than subapical in *Z. mays* (Table 2). All species and cultivars of the Leguminosae had higher apical than subapical ethylene production (Table 2), while most of the Malvaceae had higher subapical rates (Table 3). The remaining species had higher apical rates (Table 3).

In only a few species did ethylene production from some cultivars differ from the general pattern for that species. Ethylene production from *Cucurbita moschata* (Burpee's best), and *Z. mays* (Mandan Black and Silver Queen) did not follow the pattern shown by other cultivars of these species. It appears that the pattern between apical and subapical ethylene production is generally species specific, but that variability exists among species, genera and families.

Ethylene production by monocotyledonous plants

Segments of the 3 species of Gramineae, 2 species of Liliaceae and one species of Iridaceae had low rates of ethylene production compared to the dicotyledonous plants (2.4 vs 18.9 $\eta l\, g^{-1}\, h^{-1}$) (Tables 1,2 and 3). *Allium sativum* had the highest rates of ethylene production for both apical (6.48 $\eta l\, g^{-1}\, h^{-1}$), and subapical (3.99 $\eta l\, g^{-1}\, h^{-1}$) segments, while *Z. mays* cv. Silver Queen had the lowest ethylene production rates for both apical (0.6 $\eta l\, g^{-1}\, h^{-1}$) and subapical (0.6 $\eta l\, g^{-1}\, h^{-1}$) segments. In most of the monocotyledonous cultivars, the variability between apical and subapical ethylene production was less than that found in dicotyledonous cultivars.

Effect of growth habit

Seven species had cultivars that grew as bushes or as vines. The 13 cultivars that grew as vines produced more ethylene from both apical (32.2 ± 16.7 $\eta l\, g^{-1}\, h^{-1}$) and subapical ($19.3 \pm 15.0\, \eta l\, g^{-1}\, h^{-1}$) segments than did the 11 cultivars that grew as bushes (24.2 ± 15.0 $\eta l\, g^{-1}\, h^{-1}$ for apical and $10.9 \pm 5.7\, \eta l\, g^{-1}\, h^{-1}$ for subapical segments). Although these differences are large, the high degree of variability prevented the differences between ethylene production from cultivars with the bush or vine growth habit from being statistically significant. To reduce variability, cultivars were compared from the 3 species that had more than one cultivar with each growth habit. Again, there was no significant difference between ethylene production from bush or vine plants. There was also no significant difference between the ratio of apical to subapical ethylene production from the two growth habits. The average ratio between apical and subapical ethylene production for the 11 cultivars with a bush growth habit was $3.0 \pm 2.5\, \eta l\, g^{-1}\, h^{-1}$, while it was $2.6 \pm 2.4\, \eta l\, g^{-1}\, h^{-1}$ for the 13 cultivars with the vine habit.

Ethylene production by the Cucurbitaceae

Apical segments of *Cucurbita maxima* had the highest rates of ethylene production (49.8 $\eta l\, g^{-1}\, h^{-1}$), followed by *C. pepo* (31.1), *Cucumis sativus* (20.8), *Citrullus lanatus* (18.3), *Cucurbita moschata* (16.0) and *Cucumis melo* (13.9). The highest rate was observed from Green Hubbard Squash (56.1 $\eta l\, g^{-1}\, h^{-1}$), a cultivar of *Cucurbita maxima*. The lowest ethylene production rate was from Persian cantaloupe (6.7 $\eta l\, g^{-1}\, h^{-1}$), a cultivar of *Cucumis melo*.

Sub-apical segments of *Cucumis sativus* had the highest ethylene production rate of 35.5 $\eta l\, g^{-1}\, h^{-1}$, followed by *Cucurbita pepo* (24.4), *Cucumis melo* (22.3), *Cucurbita*

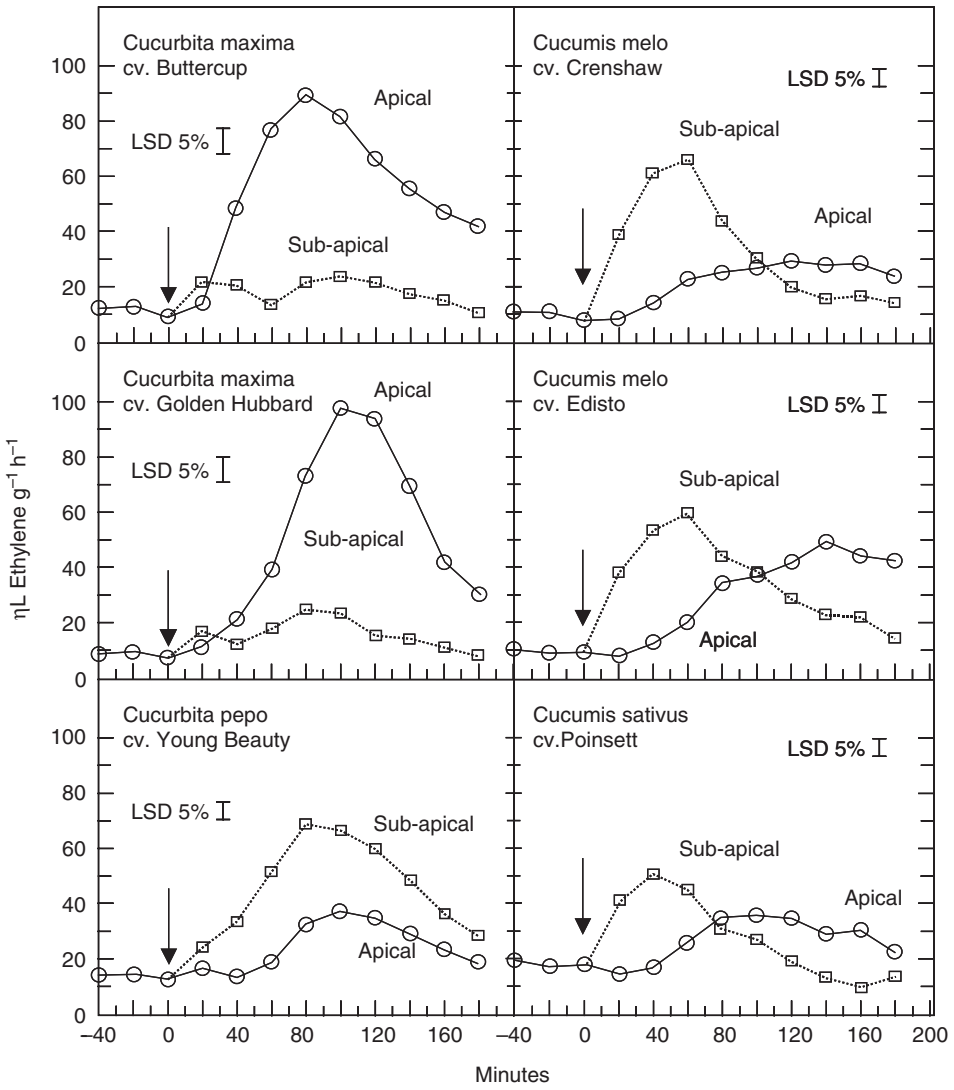

Fig. 3. Wound induced ethylene synthesis from excised 10-mm apical and subapical radicle segments of various species and cultivars of the Cucurbitaceae. The tissue was from *Cucurbita maxima* cv. Buttercup, Golden Hubbard, *Cucurbita pepo* cv. Young Beauty, *Cucumis melo* cv. Crenshaw, Edisto, and *Cucumis sativus* cv. Poinsett. The arrow indicates the time of apical and subapical segment excision. See Materials and methods for additional details.

moschata (11.7), *C. maxima* (11.0) and *Citrullus lanatus* (8.8). *Cucurbita maxima*, which had the highest rate of ethylene production in the apical segments, had the fifth highest rate in subapical segments. Armenian cucumber, a cultivar of *Cucumis sativus* had the highest ethylene production rate in the subapical segments (55.7 $\eta l\, g^{-1}\, h^{-1}$); close to the maximum ethylene production rate by apical segments. The lowest ethylene production rate was in Sweetheart watermelon (3.1 $\eta l\, g^{-1}\, h^{-1}$), a cultivar of *Citrullus lanatus*.

Ethylene production by the Leguminosae

Apical segments of *Phaseolus aureus* cv. Berken had the highest ethylene production rate of all cultivars (76.7 $\eta l\, g^{-1}\, h^{-1}$) (Table 2). *Phaseolus vulgaris* cultivars produced about 30% as much ethylene (25.7 $\eta l\, g^{-1}\, h^{-1}$) as *P. aureus*, while *Pisum sativum* cultivars produced only 12% as much ethylene (8.9 $\eta l\, g^{-1}\, h^{-1}$). The same relationship existed among Leguminosae cultivars for rates of subapical ethylene production; *Phaseolus aureus* cv. Berken was highest (32.4 $\eta l\, g^{-1}\, h^{-1}$), followed by *P. vulgaris* (16.8 $\eta l\, g^{-1}\, h^{-1}$), and *Pisum sativum* (1.7 $\eta l\, g^{-1}\, h^{-1}$). In all the cultivars of the 3 Leguminosae species, apical segments produced more ethylene production than did subapical root segments.

Ethylene production by the Malvaceae, Fagaceae, and Compositae

Excised subapical root segments of Malvaceae species produced about the same amount (*Gossypium barbadense*) or more ethylene production (*Hibiscus esculentus*) than

Table 1. Wound induced C_2H_4 synthesis ($\eta l\, g^{-1}\, h^{-1}$) from 10-mm long apical and sun-apical root segments excised from 5-cm long roots of six species of the Cucurbitaceae. Twenty excised segments were enclosed in 15.2 ± 0.1-ml glass tubes for 3 h before 1-ml samples were taken for analysis by gas chromatography. Data presented are the means and standard deviations of all measurements (n = 6).

Family/species	Cultivar	Habit	Apical 1 cm	Sub-apical 1 cm
CUCURBITACEAE			27.0	15.5
Cucurbita maxima	Blue Hubbard	bush	38.0 ± 3.8	13.1 ± 1.4
	Buttercup	bush	55.0 ± 9.0	6.2 ± 1.1
	Banana	vine	44.0 ± 1.9	17.4 ± 2.0
	Golden Hubbard	vine	55.9 ± 4.1	5.9 ± 0.3
	Green Hubbard	vine	56.1 ± 9.8	12.3 ± 0.7
			49.8	11.0
Cucurbita moschata	Burpee's best	bush	13.6 ± 0.3	15.7 ± 1.6
	Waltham	vine	20.5 ± 2.1	11.3 ± 1.3
	Butternut	bush	43.7 ± 3.4	13.4 ± 1.6
	Early Butternut	semibush	13.9 ± 1.8	8.1 ± 1.4
			16.0	11.7
Cucurbita pepo	Bush Acorn	bush	33.1 ± 3.2	11.4 ± 2.3
	Table King	bush	35.6 ± 3.7	16.0 ± 0.7
	Huicha	vine	30.2 ± 1.5	27.0 ± 0.6
	Lady Godiva	vine	38.9 ± 2.0	28.3 ± 3.6
	Small Sugar	vine	11.1 ± 1.4	9.4 ± 1.0
	Table Queen Acorn	vine	48.3 ± 3.8	36.4 ± 1.3
			31.1	24.4
Cucumis melo	Crenshaw	vine	8.3 ± 0.7	24.6 ± 1.4
	Edisto	vine	16.9 ± 3.4	24.3 ± 1.0
	Golden Beauty	vine	27.4 ± 1.1	39.6 ± 1.3
	Persian	vine	6.7 ± 0.8	8.1 ± 0.9
	Spear	vine	9.9 ± 0.6	15.0 ± 1.1
	Young Beauty	vine	20.2 ± 1.0	42.1 ± 4.5
			13.9	22.3
Cucumis sativus	Poinsett	bush	26.6 ± 1.8	40.3 ± 5.1
	Spacemaster	bush	10.4 ± 1.1	10.7 ± 0.1
	Armenian	vine	25.4 ± 2.1	55.7 ± 1.1
			20.8	35.5
Citrullus lanatus	Jubilee	bush	32.4 ± 2.8	18.9 ± 2.9
	Sweetheart	bush	10.7 ± 0.8	3.1 ± 0.7
	Crimson Sweet	vine	12.0 ± 1.2	4.6 ± 0.5
			18.3	8.8

Table 2. Wound induced C_2H_4 synthesis ($\eta l\, g^{-1}\, h^{-1}$) from 10-mm long apical and sun-apical root segments excised from 5-cm long roots of species of the Leguminosae, Malvaceae and Fagaceae. Twenty excised segments were enclosed in 15.2 ± 0.1-ml glass tubes for 3 h before 1-ml samples were taken for analysis by gas chromatography. Data presented are the means and standard deviations of all measurements (n = 6).

Family/species	Cultivar	Habit	Apical 1 cm	Sub-apical 1 cm
LEGUMINOSAE			27.9	21.4
Pisum sativum	Alaska	bush	8.3 ± 1.0	1.3 ± 0.6
	Maestro	vine	9.4 ± 0.7	2.1 ± 0.2
			8.9	1.7
Phaseolus aureus	Berken	vine	76.7 ± 7.7	32.4 ± 2.1
Phaseolus vulgaris	Romano	vine	23.7 ± 1.7	15.8 ± 1.9
	Scarlet Runner	vine	43.5 ± 1.3	25.0 ± 2.1
	Green Crop	bush	12.7 ± 0.9	8.2 ± 0.4
	Blue Lake	bush	21.2 ± 3.2	18.1 ± 1.3
	Contender	bush	27.4 ± 3.5	17.1 ± 4.4
			25.7	16.8
MALVACEAE				
Hibiscus esculentus	Clemson Spineless		5.4 ± 0.3	13.5 ± 1.5
Gossypium barbadense			13.8 ± 1.8	13.5 ± 1.4
FAGACEAE				
Quercus suberin			2.4 ± 0.1	1.5 ± 0.2
COMPOSITAE				
Helianthus annuus	Hybrid 897		4.6 ± 0.5	8.5 ± 0.2

Table 3. Wound induced C_2H_4 synthesis ($\eta l\, g^{-1}\, h^{-1}$) from 10-mm long apical and sun-apical root segments excised from 5-cm long roots of monocotyledonous species. Twenty excised segments were enclosed in 15.2 ± 0.1-ml glass tubes for 3 h before 1-ml samples were taken for analysis by gas chromatography. Data presented are the means and standard deviations of all measurements (n = 6).

Family/species	Cultivar	Apical 1 cm	Sub-apical 1 cm
GRAMINAE		2.2	2.3
Zea mays	Mandan Black	1.6 ± 0.4	1.5 ± 0.3
	Silver Queen	0.6 ± 0.1	0.6 ± 0.1
	Miracle Yellow	2.8 ± 0.01	3.6 ± 0.2
	Earliglow	1.8 ± 0.1	3.3 ± 0.1
		1.7	2.3
Triticum sp.	Anza	2.1 ± 0.2	2.2 ± 0.3
	Siskiyon	2.8 ± 0.2	2.4 ± 0.1
	Tadinia	3.8 ± 0.3	3.4 ± 0.6
		2.8	2.7
Triticolsecale	Beagulita	2.6 ± 0.3	2.1 ± 0.2
	Juanillo 168	2.0 ± 0.3	1.6 ± 0.2
		2.3	1.8
LILIACEAE			
Allium sativum.		6.5 ± 0.3	4.0 ± 0.3
Hyacinthus orientalis		1.8 ± 0.2	1.4 ± 0.2
IRIDACEAE			
Gladiolus	Sunbeam	1.3 ± 0.2	0.8 ± 0.2

the apical segments (Table 2). Ethylene production from subapical segments of the Compositae species *Helianthus annuus* was higher than from the apical segments. The rates of ethylene production from these species were generally low, though not as low as from the monocotyledonous species.

Apical segments of the only species of the one Fagaceae tested produced more ethylene than the subapical segment (2.40 vs $1.54\, \eta l\, g^{-1}\, h^{-1}$). The rate of ethylene production from both segments was similar to that produced by the monocotyledonous species.

Kinetics of ethylene production

The induction of ethylene production by apical segments of *Cucurbita maxima* cv. Buttercup and Golden Hubbard occurred within 40 min of excision, while that from subapical segments increased within 20 min, but was over 4-fold less than from the apical segments. A different pattern existed in the other 4 cultivars shown in Fig. 3. In these two species, ethylene production from the subapical segments was immediate and higher than from the apical segments that did not show an increase until later. The biphasic response characterized in pea (Saltveit and Dilley 1978) was only evident in segments from the two *Cucurbita* species (*C. maxima* cv. Buttercup, *C. maxima* cv. Golden Hubbard and *C. pepo* cv. Young Beauty). The timing of the two peaks in ethylene production was surprisingly consistent among the three cultivars (Fig. 4). When samples were collected every 3 min, the first peak occurred between 20 and 30 min, while the second peak occurred between 80 and 100 min. Induction of ethylene synthesis was rapid and consistent among the replicates within these experiments.

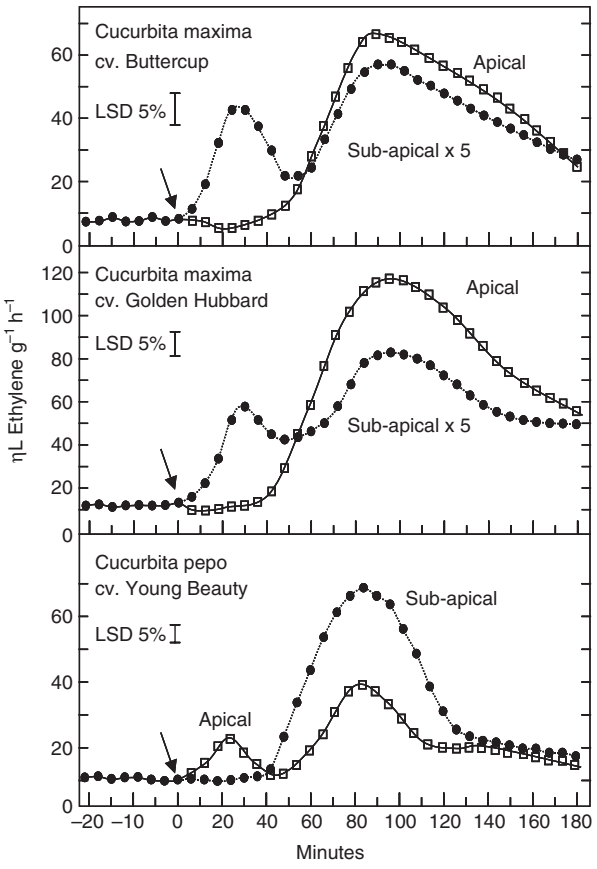

Fig. 4. Wound induced ethylene synthesis from excised 10-mm apical and subapical radicle segments of various species and cultivars of the Cucurbitaceae. The tissue was from *Cucurbita maxima* cv. Buttercup, Golden Hubbard, and *Cucurbita pepo* cv. Young Beauty. The arrow indicates the time of apical and subapical segment excision. See Materials and methods for additional details.

Conclusion

Rates of ethylene production were consistent within apical and subapical segments from each cultivar, but were variable between apical and subapical segments from the same cultivar, and among segments from cultivars within the same species, and within families. In contrast to a general pattern of higher rates of ethylene production from apical than subapical segments, ethylene production was higher from subapical than apical segments from all cultivars of *Cucumis melo* and *C. sativus*, two out of four cultivars of *Z. mays*, and one out of seven cultivars of *Cucurbita pepo* (i.e. cv. Young Beauty).

Wound-induced ethylene synthesis is thought to occur through the same biochemical pathway present in non-stressed tissues (Yang and Hoffman 1984, Kende 1993). The increased activity of ACC synthase and availability of ACC seems to be the key regulatory steps in the enhanced production of stress ethylene. Although ACC oxidase does not seem to be the rate limiting factor in ethylene biosynthesis, enhanced ACC oxidase gene expression (Ecker and Davis 1987) and activity (Hyodo et al. 1993, Dunlap and Robacker 1994) has been

reported to increase following wounding. The diversity in wound-induced ethylene responses between adjacent tissues, and among cultivars, species, and families makes excised root segments an attractive system with which to study the physiological cause of rapidly induced wound responses. Results of this study will assist in the selection of tissues with which we plan to examine the effects that wounding on the induction and expression of ethylene biosynthesis.

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