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ETHYLENE BIOSYNTHESIS OF CITRUS PEEL EXPLANTS AS INFLUENCED BY CYCLOHEXIMIDE[1]

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Abstract. **Using peel discs from fruit of 'Washington' navel (Citrus sinensis (L.) Osb.), it has been demonstrated that the flavedo tissue has apparent control over the ethylene production by albedo tissue. The nature of ethylene biosynthesis was examined by the use of cycloheximide (a protein inhibitor) on various types of explant systems. Radioactive cycloheximide applied to citrus peel remained primarily in the flavedo, where ethylene biosynthesis was inhibited, whereas it was stimulated in the albedo. Removal of the albedo from the flavedo also stimulated ethylene production by the albedo, demonstrating that the flavedo is in some way controlling ethylene biogenesis in the albedo. It is postulated that a proteinaceous factor or a product of protein synthesis is the transported control factor.**

Citrus is a non-climacteric fruit in that it does not synthesize large amounts of ethylene upon ripening (3, 19, 29). However, small amounts of ethylene are produced by citrus fruits at all stages of development (33). Citrus peel is capable of producing substantial amounts of ethylene as has been shown with whole fruits on trees sprayed with abscission chemicals (5, 18, 31). This is especially true of the albedo portion of citrus peel when separated from the flavedo (6, 22, 23, 26). The abscission zone and juice vesicles of citrus fruit, however, do not produce detectable levels of ethylene, whereas petioles produced 5 times more ethylene than leaf blades when treated with cycloheximide (11, 35).

Cycloheximide (CHI) is a well known inhibitor of protein synthesis (33). Citrus (23, 24), barley (15, 16), potato (26), and organs from other plant species exhibit a variety of responses to CHI, including fruit and leaf abscission of citrus (11). Cycloheximide inhibits protein synthesis at the translational level (8, 16, 18, 21), but may have other effects on plant tissues, particularly at high concentrations. Some argue that CHI does not act at the ribosomal level and that it can affect cellular metabolism other than by inhibition of protein synthesis (20, 33, 34).

Cycloheximide inhibited ethylene production in 'Valencia' orange peel discs when applied to the albedo side, indicating that enzyme synthesis was necssary for wound ethylene production in that tissue (23). Cycloheximide inhibited ethylene production in apple and pea stem tissues (1, 32), apples in cold storage (17), and carnation petals (37).

Cycloheximide has been found to have a dual action as an inhibitor and promoter of abscission of 'Valencia' oranges. When it is sprayed on the fruit it promotes abscission by causing an increase in ethylene content of the internal atmosphere of the fruit. However, the chemical inhibited abscission when injected near the abscission zone (14), or when uptake was through stems of citrus fruit explants (28). Cycloheximide inhibited abscission even in the presence of substantial quantities of ethylene (14). Application of CHI to separation zones of bean petiole explants also inhibited abscission (7), yet ethylene production was stimulated in citrus fruit explants dipped in CHI solutions. Acceleration of ethylene synthesis in this instance was only effective with mature fruits (28).

Cycloheximide droplets on citrus fruits cause injury of the rind (11, 12). Wounding of the peel is followed by a sudden increase in ethylene production and diffusion of the gas to the abscission zone where it initiates an abscission response (13). A tracer study using ^{14}C-labeled CHI showed that the distribution gradient of radioactivity from the peel surface to the juice indicated that neither CHI nor its degradation products were readily translocated into the endocarp (25).

Cycloheximide-induced injury of the flavedo, resulting from ethylene production, has been called a wound response (10). This interpretation is contradictory to the fact that CHI also inhibits ethylene production. Cycloheximide at 10 μM or higher, induced ethylene production and increased 1-amino-cyclopropane-1-carboxylic acid (ACC) levels in intact 'Washington' navel oranges. However, it inhibited excision-induced wound ethylene production, ACC accumulation, and ACC synthase activity when applied to flavedo discs (36). An alternative hypothesis would be that the flavedo exerts some control over the albedo, suppressing its potential ethylene producing capabilities and this control mechanism is blocked by CHI applications to the flavedo. This hypothesis is plausible if the control mechanism in the flavedo involves protein synthesis, as CHI is a protein inhibitor (8, 15, 21), and it would be the albedo that produces the major portion of the ethylene responsible for abscission of the fruit.

Materials and Methods

Peel discs were cut from the equator of 'Washington' navel oranges with a sharpened 15 mm cork borer. Some discs were kept whole while other discs were separated into albedo and flavedo sections with a razor blade. Discs were treated with 10 μl of 10, 20, or 30 ppm CHI applied to the flavedo side in the case of whole and flavedo discs, or the cut surface of albedo discs with an automatic micro-pipet. Controls were treated similarly with deionized water. Discs were then incubated at room temperature in loosely covered glass vials, 3 discs of uniform tissue-type per vial placed on moist filter paper.

Ethylene samples were taken from the vials at various times. One hr prior to each sampling the vials were flushed with air and sealed with silicon stoppers. At the end of the hr incubation period, 1 ml gas samples were taken by syringe and analyzed on a Hewlett Packard gas chromatograph, model 3710A, equipped with an activated alumina column at 90°C using a flame ionization detector.

Whole discs were treated with labeled ^{14}C-CHI in aqueous solution (obtained from Upjohn Co., Kalamazoo, Michigan, 1.15 mCi per mMol) 10, 20, and 30 ppm and 900, 942, and 986 dpm, respectively. Solutions were applied in a 10 μl drop placed within a ring of Dow Corning stopcock grease on the flavedo side to limit the area of application. Discs were then incubated for 72 hr at room temperature. Afterward, the flavedo was separated from the albedo with a razor blade and both sections were dried and combusted in a Packard B306 oxidizer to yield water and CO_2. The CO_2 was collected in a CO_2-absorber (Oxisorb) and then mixed with scintillation fluid (Oxiprep). Radioactivity was deter-

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Table 1. Ethylene production by whole (W), albedo (A), and flavedo (F) peel disc explants.

Treatment	nl C_2H_4/hr/3 • disc sample					
	24 hr			48 hr		
	W	A	F	W	A	F
H_2O	11.5 (± 0.3)[z]	26.1 (± 12.8)	10.2 (± 2.8)	22.6 (± 10.2)	32.8 (± 6.5)	4.4 (± 0.0)
10 ppm CHI[y]	4.4 (± 2.4)	1.3 (± 0.1)	2.3 (± 1.4)	27.8 (± 15.3)	1.2 (± 0.5)	4.7 (± 3.0)
20 ppm CHI	10.5 (± 3.7)	1.6 (± 0.4)	3.0 (± 2.4)	8.0 (± 3.1)	1.8 (± 0.6)	4.0 (± 2.6)
30 ppm CHI	27.9 (± 11.6)	0.4 (± 0.1)	7.6 (± 3.1)	35.2 (± 1.2)	0.2 (± 0.1)	4.0 (± 2.6)

[z]Values are means of 3 replications ± SE of the mean.
[y]CHI = cycloheximide.

mined on a Beckman liquid scintillation counter, model LS7500.

Statistical analysis of ethylene and ^{14}C labeling data consisted of analysis of variance and standard error of means.

Results and Discussion

Whole, albedo and flavedo peel discs, incubated for 48 hr with different concentrations of CHI and deionized water, showed differences in ethylene production across time periods (Table 1). The same data analyzed by treatment showed that for discs treated with deionized water there were no significant differences by tissue-type while for discs treated with CHI there were significant differences. Cycloheximide inhibited ethylene production in albedo and flavedo discs, especially at 20 and 30 ppm, and stimulated ethylene production in whole discs at 30 ppm.

Whole discs, which were treated with ^{14}C-CHI or deionized water (applied to the flavedo side) and incubated at room temperature for 72 hr, were separated into albedo and flavedo sections. These sections were then combusted separately to CO_2 and water. The CO_2 was trapped by a CO_2 absorber, mixed with scintillation fluid, and analyzed for radioactivity. Flavedo discs contained significantly more dpms than albedo discs (Table 2). Albedo discs contained 13% of the total dpm for the 10 ppm treatment, 11% for the 20 ppm treatment, and 14% for the 30 ppm treatment. For the 10 ppm treatment, 83% of the total radioactivity applied was recovered, 74% for the 20 ppm treatment, and 74% for the 30 ppm treatment. Overall, 77% of the applied radioactivity was recovered. There was significant treatment-tissue interaction.

Table 2. Radioactivity (dpm) recovered from whole peel disc explants separated into albedo and flavedo after 72 hr incubation with ^{14}C-cycloheximide.

Treatment	Type of explant	
	albedo	flavedo
H_2O	12 (± 1)[z,y]	12 (± 1)
10 ppm CHI[x]	97 (± 11)	624 (± 4)
20 ppm CHI	78 (± 6)	624 (± 10)
30 ppm CHI	104 (± 10)	647 (± 10)

[z]Ozidizer—95% recovery and less than 1% contamination.
[y]Values are means of 36 replications ± SE of the mean.
[x]CHI = cycloheximide.

Conclusion

Ethylene biosynthesis in citrus peel can take place in the flavedo and albedo (6, 23, 27). The effect of CHI on ethylene production of citrus peel is complicated by an interaction between the 2 tissue types. Cycloheximide, a well known inhibitor of protein synthesis (15, 16, 21), has been observed to inhibit ethylene production in many tissues including citrus peel (6, 23); yet, this same chemical has been shown to stimulate ethylene production when sprayed on citrus fruit in the field. This is thought to be a wound response. When CHI is applied to the flavedo side of whole citrus peel discs it stimulates ethylene production. Conversely, if the chemical is applied to the albedo side of the whole disc it inhibits ethylene production (23) as effectively as does aminovinylglycine (AVG) (6), an effective ethylene synthesis inhibitor (2, 30). When CHI is applied to separated albedo and flavedo discs it also inhibits ethylene production in both tissues (6).

Radioactive tracer analysis showed that 86% or more of the labeled CHI recovered from ^{14}C-CHI treated whole discs remained in the flavedo tissue after 72 hr (Table 2). In the field, fruit sprayed with CHI starts to loosen after 3 days (9, 28). An earlier study was done using whole fruits and labeled CHI with almost identical results but using a different analytical technique (25). Therefore, the effect of CHI is on the flavedo and most likely is not through a wound response but through inhibition of protein synthesis. The hypothesis is that CHI inhibits the synthesis of some protein or protein-dependent soluble factor in the flavedo that would normally be translocated to the albedo where it would inhibit albedo ethylene production. The albedo exhibits a high capacity for ethylene production when physically separated from the flavedo (22, 26). Carbon dioxide and ACC have been ruled out as correlative factors between the 2 tissue systems, and it apparently is not ethylene *per se* causing an auto-inhibitory response (4). Whether it is a protein or a product of catalytic protein action is not known at the present time. The strong action of CHI suggests it is one or the other.

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ABSCISSION AND ETHYLENE PRODUCTION IN MANGO (MANGIFERA INDICA L.) FRUIT CV. TOMMY ATKINS[1]

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Abstract. **Rates of fruit drop and remaining fruited panicles were monitored in the field for 96 days beginning on April 1, 1983. More than 90% of total fruit drop occurred within the first 4 wk, and final fruit set was 0.61%. Percentage of fruited panicles remained constant for the first 3 wk, decreasing sharply to 40% by the 6th week, and ending at 11.7% at harvest. Of the fruit which reached maturity, 42% were seedless. Samples of seeded and seedless fruit collected during the high drop period showed different abscission patterns in the laboratory. All seeded fruit separated within 72 hr after harvest, where as 22% of the seedless fruit were still attached 95 hr after harvest. Abscission of seeded fruit was accompanied by a rise in ethylene production, while parthenocarpic fruit abscised without an increase in the level of this hormone. In the field, the observation that seedless and seeded fruit are shed throughout the entire period of fruit development indicates that physiological factors occurring after pollination and/or ovule fertilization are involved in fruit abscission and consequently affect the final yield in mango.**

The mango is a tropical fruit crop which has a pronounced tendency to shed fruit at all stages of development until harvest (12). Monoembryonic types show this behavior more intensely than polyembryonic ones, a fact attributed to the development of nucellar embryos which apparently influence a greater retention of fruit (15).

The problem of low fruit set by mango trees has been well documented through the years. In Florida, the monoembryonic cultivar, 'Edward', had 3.8% fruit set in an 18-wk study of 160 panicles (15). The 'Haden' mango of Florida is well known for its high unfruitfulness and erratic bearing habits. Young (17) showed that only a small fraction of the perfect flowers normally set and mature fruit, which is attributed to a high percentage of fruitlets that drop early in the season due to abortion of ovules. In a study by Naik and Rao (8), mango panicles produced 2000 to 4000 flowers, with only 1 to 35% being hermaphroditic. Furthermore, 13 to 28% set fruit and only 0.1 to 0.25% reached maturity. Some cultivars have been noted to develop one fruit to maturity of 150 apparently fertilized flowers, particularly with a heavy fruit set (7).

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