UNIVERSITY OF FLORIDA

Cooperative Extension Service

Institute of Food and Agricultural Sciences

PACKINGHOUSE NEWSLETTER

Mark Ritenour
Indian River REC
2199 South Rock Road
Ft. Pierce, FL 34945-3138
Phone: (772) 468-3922
FAX: (772) 468-5668
Email: mritenour@.ifas.ufl.edu

Packinghouse Newsletter No. 196
September 30, 2002

Key Index Words: Degreening, ethylene measurement, *Diplodia* stem-end rot (*Diplodia natalensis*), Anthracnose (*Colletotrichum gloeosporioides*)

All previous and present Packinghouse Newsletters (PHNL) are available on the Internet at the University of Florida's postharvest web site (http://postharvest.ifas.ufl.edu) and can also be accessed through our citrus resources web site (http://flcitrus.ifas.ufl.edu).

Ethylene Measurement for Florida Citrus Degreening

William M. Miller
UF-IFAS, Citrus Research and Education Center, Lake Alfred

Degreening is commonly used for citrus grown in semi-tropical or tropical climates. Over 50% of fresh Florida citrus is degreened because fruit meet or exceed internal maturity standards but may remain green from chlorophyll in the peel. The driving factor in chlorophyll breakdown is ethylene (C_2H_4) which is a natural hormone produced by essentially all plants and their fruits (6). Ethylene is involved in numerous physiological responses including abscission, ripening, senescence and certain physiological disorders. Some properties of ethylene are presented in Table 1. It has been observed that late season oranges that have regreened don't degreen very successfully, interestingly chlorophyll B is predominant in these regreened fruit while chlorophyll A is more abundant at other times of the year.

Supplemental ethylene is supplied to control either ripening or color change or both for various produce items. In citrus, as well as banana and tomato, ethylene gas is introduced in a closed room or chamber. However, for banana and tomato, the rooms typically are much tighter (i.e., minimal air exchange) as required ethylene levels are higher (e.g. 100 to 150 ppm). Recommended gas concentrations are 5 ppm for Florida citrus degreening (5), but some packers have tried to lower levels to 2-3 ppm. Besides

temperature, humidity and fresh air exchange in the degreening rooms, accurate control and monitoring of ethylene in citrus degreening is also essential as levels greater than 10 ppm ethylene have been shown to enhance decay (1). With a recommended one air exchange per hour to reduce carbon dioxide levels, ethylene usage can be significant. Use of higher ethylene levels or more frequent air exchange rates increases ethylene consumption and operating costs without providing any benefit.

The types of sensors that may be used to measure ethylene include: chemical (gas chromatography), physio-chemical [metal oxide semiconductors (mos), conductive plastic, proton exchange membranes], spectrometric (infrared absorption, color change of absorption media) or spectrometric-chemical (chemical luminescence). Some of the techniques are rather elaborate and expensive and only suitable for laboratory use (e.g., gas chromatography) while others are easy and inexpensive (e.g., mos devices) and while suitable for use in detecting ethylene leaks, do not provide sufficient sensitivity for measurements in a degreening room. Infrared absorption and chemical luminescence units have been tried in the industrial workplace but haven't performed well in the dirty, high humidity environment of a citrus degreening room. Other sensor issues include price, reliability and needed sensitivity. For example, to achieve a +/- 5% accuracy at 10 ppm full scale, a required sensitivity of +/- 0.25 ppm is needed. Therefore, the industry practice continues to be the colormetric media gas sampling tube (5). These devices can be satisfactory but they do not provide any electronic output and are highly dependent upon the operator to achieve good readings. All readings should be taken when a degreening room is in steady state conditions which may require a 4 hr equilibration or more after initial start-up. The sampling tubes should be fresh because they tend to degrade much like photographic film and an expiration date typically is provided. A sampling location that has minimal disturbance from outside influences such as curtain openings, forklift traffic, etc. should be identified within each room.

If ethylene levels are found to be high or low, the ethylene flowrate requires appropriate adjustment. The fresh air exchange rate, recommended as one air exchange per hour (5), is the critical factor in determining the required ethylene flow. With 2 air exchanges per hour, the ethylene consumption is doubled. If a degreening room is operating at 5 ppm and the ethylene flowrate is known, an estimate of the fresh air exchange rate can be made readily. An example follows:

Room Size (RS) = 20 ft x 30 ft x 50 ft = 30,000 ft^3
Air Exchange Rate (AER) = 30,000 ft^3/hr
Known Concentration of Ethylene (KCE) = 5 ppm
Ethylene required (@ 1 air exchange/hr) = (AER) x (KCE)
Flowrate = 30,000 x $5x10^{-6}$ = 0.15 ft^3/hr (ethylene)

If the actual flowrate, typically measured with an in-line flowmeter, is higher, for instance 0.4 ft^3/hr, then the air exchange rate is actually 0.4/0.15 or approximately 2.7 air exchanges/hr. Units for most flowmeters are given as "standard cubic feet per hour" (scfh) which represents ft^3/hr at standard conditions.

Tighter sealing of the room will reduce operating costs for heating, humidity and ethylene. Frequency and duration of opening the curtain should be minimized also. The above calculation requires that the room is actually at 5 ppm ethylene and hence, a proper measurement of ethylene concentration is critical.

Precise ethylene measurements will become more important to Florida packers as ethylene needs are matched with specific varieties and temperature regimes in more controlled degreening processes. Degreening time and environmental condition may become part of the traceability log with respect to fruit quality. Advanced control packages will include temperature, humidity, ethylene and carbon dioxide sensing. More detailed information on automated systems can be found in additional references (2,3,4).

Table 1. Ethylene Physical Properties Data

Parameter	**Value**
Chemical Formula	C_2H_4 or $H_2C{:}CH_2$
Molecular Weight	28.054
Freezing Point	-169.2°C
Boiling Point	-103.7°C
Vapor Pressure @ 21.1°C	8,274 kPa (gauge) (1200 psi)
Relative Density, (Air = 1)	0.975 @ 1 ATM, 0°C
Solubility in Water @ 1 ATM, 0°C	0.226 cm^3/1 cm^3 water
Flammable Limits in Air	3.1-32.0% by volume (31,000-320,000 ppm)
Auto Ignition Temperature	490.0°C (914°F)

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Biology and Control of Diplodia Stem-End Rot and Anthracnose on Early Harvested Florida Citrus

Jiuxu (John) Zhang, Florida Department of Citrus, Lake Alfred
Mark A. Ritenour, UF-IFAS, Indian River REC, Ft. Pierce

Introduction

Postharvest diseases of citrus fruit can cause significant economic losses when environmental and fruit conditions are conducive to pathogen infections and disease development. *Diplodia* stem-end rot caused by *Diplodia natalensis* and anthracnose caused by *Colletotrichum gloeosporioides* are two major decays related to ethylene degreening on early harvested fruit. High temperatures and humidity during the summer months often result in early internal fruit maturation, but the coloration of fruit peel is not fully developed. Early harvested fruit are often subjected to ethylene degreening to improve fruit color and market value. This practice greatly increases the incidence and severity of *Diplodia* stem-end rot and anthracnose. Decay incidence and severity increase with the length of degreening and concentration of ethylene used. *Diplodia* stem-end rot occurs on all types of citrus fruit, but certain varieties are much more susceptible to anthracnose. The greatest severity of anthracnose is often observed on Robinson, Sunburst, and Fallglo tangerines, navel and Ambersweet oranges, and grapefruit from September to November.

Symptoms

Diplodia stem-end rot: *D. natalensis* usually infects fruit from the button at the stem-end of the fruit. It proceeds through the core more quickly than the rind, leading to a soft brown to black decay that appears at both ends of the fruit. The pathogen usually develops unevenly in the rind, forming finger-like projections of black to brown discolorations at the margin between the segments (Fig. 1). The fungus may also infect fruit through injuries to cause a soft brown to black lesion that enlarges very rapidly from the site of the injury.

Fig. 1. Diplodia stem-end rot on 'Valencia' orange.

Anthracnose: The lesions initially appear silvery gray and leathery (Fig. 2), and are similar in firmness and elevation to adjacent healthy rind. The infected rind becomes brown to grayish black and softens as the rot progresses. Lesion sizes and shapes are irregular. Pink spores may form on the lesion surface in humid environments. Lesions may be tear-drop in shape due to the distribution of spores (e.g. in water drops) at the time of infection. This decay may also develop at rind injuries on any type of fruit and produces firm, sunken, dry lesions.

Pathogen infection and disease cycle

Fig. 2. Anthracnose on Grapefruit.

Diplodia stem-end rot: *D. natalensis* grows and sporulates in deadwood of citrus trees. Water (e.g. from rain or irrigation) transmits fungal spores from deadwood to the surfaces of immature fruit. The fungus then colonizes dead tissue at the fruit button (calyx and disk). These fungal colonizations remain latent or quiescent, and do not cause any decay before harvest. The latent infections cause decay after harvest, especially under commercial degreening conditions.

Anthracnose: *C. gloeosporioides* also grows and sporulates in tree deadwood. Water transmits fungal spores from deadwood to the surfaces of immature fruit. The fungus proliferates and forms appressoria on the surface of immature fruit. These appressoria do not germinate but remain latent (quiescent) and do not cause decay before harvest. Appressoria germinate to form infection hyphae after harvest and especially under commercial degreening conditions.

Possible mechanisms for ethylene-degreening-related increase in *Diplodia* stem-end rot and anthracnose

D. natalensis and *C. gloeosporioides* are present on fruit before harvest as latent infections. The fungi remain dormant until changes in fruit physiology and/or environmental conditions trigger growth and development of infection resulting in decay. Ethylene has been shown to stimulate the growth of *Diplodia* stem-end rot and anthracnose during fruit degreening practices. Mechanisms of this stimulation are largely unknown. However, degreening-enhanced development of *Diplodia* stem-end rot may be due to:

1) ethylene stimulating the formation of an abscission zone between the fruit and button, which allows the pathogen to infect fruit tissue;
2) ethylene directly stimulating the growth of *D. natalensis* in combination with a degreening temperature of 85°F, which is optimum for fungal proliferation;
3) ethylene reducing natural fruit resistance to the fungus by triggering certain biochemical changes in the fruit rind; and/or
4) ethylene possibly stimulating the production of cell wall-degrading enzymes by the fungus.

Degreening enhanced anthracnose development may be due to:

1) ethylene stimulating renewed growth of infection structures called appressoria (which are the swollen tips of fungal hyphae or germ tubes that facilitate attachment and penetration of the host rind by the fungus);

2) ethylene reducing natural fruit resistance by changing chemical components of fruit rind; and
3) ethylene stimulating the production of cell wall-degrading enzymes by *C. gloeosporioides*.

Control of *Diplodia* stem-end rot and anthracnose

Control of *Diplodia* stem-end rot and anthracnose is most effective when several approaches are combined to reduce fungal inoculum, prevent infection, and eradicate existing infections during both preharvest and postharvest stages. To accomplish this, practice the following:

1) Perform good cultural practices to minimize the amounts of deadwood in citrus trees.
2) Delay harvest or spot pick for fruit with better natural color development.
3) Spray with benomyl preharvest (if available) and/or drench postharvest with thiabendazole (TBZ) before degreening. Heated Imazalil solution (100 –120 ^{o}F) might also be used for postharvest drenching before degreening, but TBZ is generally more effective against stem-end rot and anthracnose. The production of benomyl has been terminated by the producing manufacturer, and we are currently looking for alternative preharvest chemicals. Current results suggest that Topsin® may provide similar postharvest control when applied preharvest.
4) Minimize degreening time (< 36 hrs) and concentration of ethylene (no more than 5 ppm).
5) On the packingline, apply TBZ on fruit in aqueous (1,000 ppm) or in water wax (2,000 ppm) treatments. Imazalil might also be used in combination with TBZ to effectively control green and blue mold.
6) Precool or store fruit immediately after packing at 50 ^{o}F or below. The lowest "safe" temperature will depend on many preharvest (e.g. time of season) and postharvest (e.g. wax or fungicide) factors.

More information on these two diseases can be obtained from the following references

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