imium CPE of 8.0% (5), and vegetative chrysanthemums with 180 growing points/m² had a CPE of 6% (3).

Literature Cited


Ethylene-releasing Compounds and the Laboratory Modeling of Olive Fruit Abscission vs. Ethylene Release

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Abstract. A laboratory system was developed to study olive (Olea europaea L.) organ abscission (21). An improvement of the use of ethylene-releasing compounds in this system is described to provide a model for field abscission responses and characterization of ethylene release. Olive fruit began the separation process as early as 7 to 13 hr after treatment with CGA-15281 (CGA), but not until 19 to 25 hr after treatment with ethephon (ET). CGA is characterized by an immediate, substantial breakdown to ethylene, whereas ET reaches its maximum ethylene release at 12 to 18 hr after application. Ethylene release was much greater from CGA than from equimolar concentrations of ET throughout the abscission initiation period. The relation of ethylene release characteristics to control of olive fruit and leaf abscission is discussed, with the suggestion that fruit respond more rapidly to, and at shorter durations of applied ethylene than do leaves.

One of the most agriculturally important ethylene effects on plant tissue is the stimulation of flower, fruit, and leaf abscission. Indeed, after 30 years of field experiments for selection of abscission-inducing chemicals to aid in mechanization of olive fruit harvest, compounds which release ethylene have proven to be most promising. Even so, widespread use of ethylene-releasing compounds (ERC's) has not occurred because of the inconsistency of treatments in loosening fruit suitably without promoting excessive leaf loss (16, 26). This lack of treatment consistency is matched by a lack of understanding of ethylene promoting excessive leaf loss (16, 26). This lack of treatment consistency in loosening fruit suitably without promoting excessive leaf loss (16, 26) offers little chance of obtaining a definitive understanding of the physiology involved in the abscission process.

In order to study ERC-induced olive organ abscission under controlled conditions, others have proposed the use of olive fruit explants (8, 15). However, examination of representative data (15) indicates that nonsenescent integrity of the abscission zone (AZ) was not maintainable over the data collection period (3 to 5 days). Our use of this system found senescent changes in abscission within 36 hr after excision (19). Another system, developed by Lavee and Martin (21) used olive shoots placed in vials of water, with transpirational columns intact. Various ERC's were applied either as sprays or as solutions taken up from the vial through the xylem (22, 23, 24). The xylem-feeding technique suffers in usefulness due to its artificiality, and both techniques are nonspecific. In laboratory studies, treatment specificity is desirable to reduce possible physiological complications from the inadvertent treatment of tissues distant from the site of importance, i.e., the AZ.

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Therefore, in this work a modification of this system was sought which would allow specific treatment at the site of activity. The modified system was used for a descriptive, comparative study of ethylene release from ERC’s and a determination of the time of olive fruit abscission. Integration of these data with field trials reported in the literature is used to propose a possible regulatory role of ethylene in ERC-stimulated olive organ abscission.

Materials and Methods

Plant material. Fruiting shoots of ‘Manzanillo’ olive were cut in the field during periods of low transpirational demand and placed in water for transport to the laboratory. Fruit on the collected shoots were thinned to 1 per peduncle. Care was taken to select only those fruit with a perpendicular orientation of the pedicel attachment to the fruit in order to decrease variability in fruit removal force (FRF) measurements. Shoots with 4 to 6 fruit remaining were recut under water to about 25 to 40 cm and placed in 75 ml Kimax narrow mouth plain reagent bottles filled with 60 ml of deionized water. One to 4 shoots were placed in each bottle. These were placed on laboratory benches at about 25°C with a quantum flux density of about 5.5 μmol s⁻¹ m⁻². Treatments were applied in a completely randomized design, with individual shoots considered to be single replications. Field experiments were conducted on intact shoots with fruit selected as described above.

One experiment was conducted in a flowing air system. Excised shoots in Kimax bottles were placed in 9.5 liter jars which were connected to a 325 ml·min⁻¹ flow of humidified air. Treatment consisted of “bleeding” ethylene into the air flow to achieve a 5 μl·liter⁻¹ concentration. Quantum flux density in the system was 19.0 μmol s⁻¹ m⁻². The olive ‘Mission’, which is similar to ‘Manzanillo’ in size, initial FRF, and response to ethylene (unpublished data), was used.

Chemicals. Ethephon, 2-(chloroethyl)phosphonic acid, was donated by Union Carbide Agricultural Products, Inc., Ambler, PA 19002. CGA-15281, [2-(chloroethyl)-methylbis-(phenylmethoxy)-silane] was provided by Woolfolk Chemical Works, Inc., Fort Valley, GA 31030. Treatment solutions were made with deionized H₂O (pH ~ 4.5).

When ERC’s are applied as field sprays, “pooling” of the compound in the olive fruit/pedicel cavity can increase the effective dose delivered to the AZ, similar to the situation with apple fruit (14). Consequently, to treat the specific site of activity, yet prevent treatment of plant tissues proximal or distal to the fruit AZ, 2.5 μl ERC droplets were applied via micro-syringe to the fruit/pedicel cavity. This volume is just great enough to wet the fruit/pedicel surface exterior to the AZ, thereby giving a localized, quantitated application of chemical near the site of activity. Preliminary experiments with serial dilutions of ET and CGA identified suitable concentrations of each to evoke abscission activity.

Determination of FRF and ethylene evolution. In the discussion of ethylene-stimulated abscission, it is necessary to refer to (1) the chemical stimulation of the abscission process, and (2) the physical evidence that the separation process is occurring. We will refer to the former as “induction” of abscission, i.e., the reception of adequate stimulus to ensure the “triggering” of abscission. We will refer to the latter as “initiation” of abscission, i.e., the biochemical hydrolytic processes occurring in the separation layer as reflected by the decreased force required for separation (FRF). A Chatillon Model DPP-1KG push-pull force gauge equipped with a metal claw was used for measurement of FRF. Readings were taken immediately after the enclosure period for ethylene sampling.

Ethylene evolution was determined by enclosing detached fruit (with attached pedicels) from each treatment replication in 58 ml test tubes. These were stoppered with serum caps for 1 hr, after which the tube was briefly shaken. A 5 ml headspace sample volume was withdrawn into a 12 ml syringe while pressing the serum cap downward to alleviate negative pressure in the syringe. Samples were reduced to 4 ml for injection into a Carle Model 211 analytical gas chromatograph equipped with a 2.0 ml sampling loop, an alumina column at 80°C, and a single burner flame ionization detector.

Results and Discussion

Laboratory abscission system. In our testing of a laboratory system to quantify ethylene evolution and related abscission from ERC-treated olive fruit, we noted that ET droplet treatments to excised fruit (with attached pedicels) began to reduce FRF between 18 and 36 hr (19). We felt that if this rapid response could be related to field results, it would greatly decrease the time necessary to evaluate ERC treatments. Upon rejecting the explant system for reasons described previously, we applied 69 mm ET droplet treatments to fruit on excised olive shoots, since this system has been reported to maintain physiological integrity at the AZ for 5 to 7 days (21).

Fig. 1. Comparison of fruit removal force curve characteristics after treatment with 2.5 μl droplets of 69 mm ethephon to fruit on excised shoots in the laboratory or intact shoots in the field. Data presented are the means of 4 replications.
Comparing the field and laboratory FRF reductions induced by ET reveals parallel curves over different time bases. The difference in response times presumably can be accounted for by the constant light and temperature in the laboratory and a Q_{10} = 2 for abscission metabolism (2). Thus, as fall field temperatures reached those in the laboratory (about 25°C) only about one-third of the time each day, one would expect, as Fig. 1 implies, nearly 3 times the diurnal metabolic activity in the laboratory system when compared to the field. This difference in temperature also would be expected to favor accelerated decomposition of ET to ethylene in the laboratory, since ET is reported to have a Q_{10} of 4 to 6 (6, 27). The effect such differences in chemical decomposition would have on abscission physiology, however, remains to be elucidated.

Using excised shoots, the reduction in FRF of fruit treated with a 69 mM ET droplet was compared to that of fruit in which the whole shoot was sprayed to runoff with 13.8 mM ET, a concentration effective in field spray trials (26). Both showed a reduction in FRF by 31 hr (19). Other experiments, in which ET treatments were only applied to leaves, to the distal end of the fruit or to the peduncle/shoot juncture, showed no apparent abscission induction (19). This result substantiates earlier reports (15, 17) that ERC treatments must be delivered to tissue near the AZ for induction, since ET and CGA translocation is apparently minor (11, 14, 18). The similarity of the fruit response between the droplet and spray treatments, and of the lab and field trends (Fig. 1), demonstrates that the excised shoot/centered droplet technique provides a valid response model to ERC field spray-induced fruit abscission.

ERC ethylene release. Ethylene release and FRF were measured every 6 hr from both droplet and whole shoot spray ERC treatments in the excised shoot system. ET was applied with a 69 mM droplet or a 13.8 mM spray. CGA was applied with a 3.1 mM droplet or 1.6 mM spray. Ethylene production by untreated fruit was negligible compared to ethylene evolution from treated fruit. Ethylene release from ET reached a maximum at 12 and 18 hr for the droplet and spray, respectively, and both showed a delayed and reduced release peak (Fig. 2). The magnitude of ethylene release from the ET spray was much larger than for the droplet, which would be expected since the spray constitutes a substantially increased amount of chemical applied over a greater surface area for uptake and subsequent decomposition. The characterization of 2 peaks of ethylene release also has been made from field studies (3, 16, 17), with several investigators suggesting that the 2nd peak is due to autocatalytic ethylene production (4, 12). However, recent studies discount the possibility of significant ET-induced autocatalytic ethylene production in olive (8, 22, 24). It has been suggested that long term effects of ET, including the 2nd peak, are due to changes in the tissue buffering capacity and/or ET conjugation with mono- and di-saccharides (24).

Ethylene release from both CGA treatments reached a maximum quite rapidly (2 hr), followed by a precipitous decline for the 3.1 mM droplet treatment (Fig. 3). The 1.6 mM spray treatment maintained a high release rate for 12 hr before declining sharply. This may be due to a buffering of the solution taken up by the tissue (23); the greater surface area treated may allow more chemical to be buffered and consequently released over a prolonged period of time. Ethylene evolution from CGA not only peaked earlier than that from ET, but was much greater at the peak and generally thereafter, in spite of the substantially increased ET treatment concentrations used. There was no significant 2nd peak of ethylene release for the CGA treatments.
ERC field trials (4, 19, 26) and laboratory work (22, 23, 31) suggests that increasing initial ethylene release from ERC’s may reduce total leaf abscission while maintaining satisfactory fruit removal force. Data presented are the means of 3 replications.

Fig. 3. Effect of CGA-15281 application to fruit on excised shoots (3.1 mm droplet or 1.6 mm spray) on ethylene release and fruit removal force. Data presented are the means of 3 replications.

The ethylene release data presented here offer further support for the existence of olive fruit sensitivity thresholds to short durations of ethylene. The field observation that CGA induces less leaf drop than ET, yet promotes adequate fruit drop, seems to be a function of its rapid and short duration of ethylene release, not of overall ethylene quantities. CGA actually releases much more ethylene at lower molar concentrations than ET over the time frame reported here (Figs. 2, 3). It is recognized that mutually-exclusive ethylene-induced processes in the same plant can exhibit differential sensitivity to ethylene durations. For example, a short exposure to ethylene is all that is needed to induce flowering in pineapple (Ananas comosus Merr.), but a longer exposure is required to promote fruit ripening (25). Thus, it is reasonable to postulate that the abscission of 2 different organs on the same plant should exhibit different sensitivities to ethylene stimuli, the seasonal reproductive organ being more sensitive to a transient ethylene burst than the photosynthetic organ genetically programmed for a 3-year existence.

Consequently, as a final experiment, a flowing air system was modified to a 5 µ-liter-1 C2H4 flow, and excised shoots were placed in the system for 0, 12, or 18 hr to provide simultaneous leaf and fruit exposure to the same ethylene concentration. After the appropriate exposure period, flow was returned to pure air. After 18 hr, all shoots were moved to laboratory benches. Measurements of FRF and the percentage of initiated leaf abscission were made, the latter by removing leaves physically and dividing the number separating at the AZ by the total leaf number. The initiation of fruit abscission was evident at 9 hr, again much earlier than any previous reports (Table 1). The 18-hr exposure caused a slightly greater decline in FRF by 31 hr than the 12-hr exposure, though the values were within a SE of each other.

Initiation. Leaf abscission initiation was not apparent until 104 hr for either treatment. At this time, the 18-hr exposure had initiated more abscission than the 12-hr exposure, 31% vs. 13%. From the previous discussion of sensitivity to ethylene duration, as well as from work with other tissues (5), this would be expected. It is unfortunate that the experimental period was not extended beyond 104 hr to record possible long-term effects of the treatment on leaf abscission. Even so, it is remarkable that such a short period (12 to 18 hr) was required to induce abscission. This is only 25% to 36% of the required duration reported by Blumenfeld et al. (8) and Lavee and Martin (23) for olive leaf abscission, apparently due somewhat to the large dose applied and the optimal conditions in the laboratory. Yet, abscission initiation was not seen until about 3 days after the induction stimulus had been received. This time contrasts with the rapid initiation of fruit abscission during the 1st 9 hr of ethylene stimulus. Hence, these 2 abscission processes, which

Table 1. Fruit removal force (FRF) on excised olive shoots exposed to a 5 µ-liter-1 ethylene gas flow for two different durations. Data presented are the means of 4 replications.

<table>
<thead>
<tr>
<th>Duration of ethylene exposure (hr)</th>
<th>Time after ethylene exposure began (hr)</th>
<th>FRF as % of control (SE)</th>
<th>Initiated leaf abscission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>26 ± 19</td>
<td>27 ± 9</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>41 ± 13</td>
<td>15 ± 19</td>
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*Not determined.

The ethylene release data presented here offer further support for the existence of olive fruit sensitivity thresholds to short durations of ethylene. The field observation that CGA induces
are nearly identical anatomically (29), present an enigma in processing efficiency of the induction signal. Further investigation of the ethylene-induced initiation process, i.e., hydrolytic enzyme species and activities, is needed to account for the different abscission kinetics displayed by the 2 organs. The results from this study seem to confirm the idea that olive fruit abscission may be induced and leaf loss minimized by short-term exposure to large doses of ethylene. This response would tend to favor CGA over ET as a mechanical harvest treatment, for ethylene release from CGA was of a greater magnitude and displayed an earlier release peak than ET, as tested here. Quantitation of olive fruit and leaf ethylene thresholds during the harvest season, and the subsequent modification of either ERC formulation to release ethylene accordingly, ultimately may achieve a treatment consistent enough for field use.

**Literature Cited**