References and Notes

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Ethylene: A Factor in Defoliation Induced by Auxins

Abstract. *Aerial sprays of synthetic auxins defoliate many species of tropical trees. Treatment of* Euonymus japonica *leaves with the* n*-butyl ester of 2,4-dichlorophenoxyacetic acid causes premature senescence and leaf fall and stimulates ethylene production by the blade 5- to 25-fold. Exposure to ethylene alone similarly accelerates senescence and leaf fall. Evidence indicates that the defoliant action of auxin is mediated through the enhanced amounts of ethylene in the blade.*

The esters of synthetic auxins have been used as defoliants of tropical trees and shrubs for the past 20 years (*1*). Foliage sprayed with preparations of the *n*-butyl ester of 2,4,-dichlorophenoxyacetic acid (2,4-D) or of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) becomes pale green or yellow within a few days, and the leaves of susceptible species may be shed a week later.

Under natural conditions a leaf undergoes abscission only when the blade and petiole are yellowing or senescent, or when the leaf is subjected to environmental stresses which lead to changes in the synthetic activity of the blade, similar to those that occur during normal senescence. A young green leaf which is synthetically active does not abscind (*2*). Young leaves have a relatively high auxin content; during aging and senescence the amount of auxin decreases. If auxin is applied to a debladed petiole stump or to the distal end of an isolated abscission zone (explant), abscission is retarded. Auxin can therefore substitute for a young leaf blade in preventing abscission, and much evidence supports the view that as long as the auxin content of a leaf blade is high, yellowing and senescence of the blade and petiole do not occur and the leaf does not fall (*2, 3*). How then does the addition of auxin to a leaf blade lead to premature abscission?

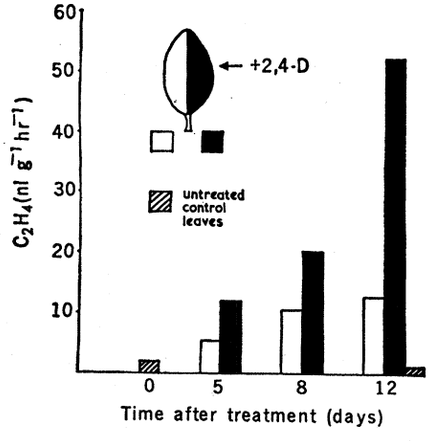

Fig. 1. Branches of *E. japonica* were maintained under greenhouse conditions. On day 0, the right half of the upper surfaces of about 600 2nd-year leaves were painted with the *n*-butyl ester of 2,4-D in methanol (6.67 mg/ml); control branches were untreated; 5, 8, and 12 days later leaves were removed and divided down the main vein. The half leaves were packed loosely in the dark glass jars and a current of moist ethylene-free air was passed through them for 18 to 24 hours at 26°C. The ethylene was collected in 0.25*M* mercuric perchlorate in 2*M* $HClO_4$ and was subsequently analyzed by gas chromatography. On day 0 and day 12 ethylene was also collected from control leaves from untreated branches. Values at day 0 represent ethylene production from 2nd-year (green) or 3rd-year (yellow) leaves. Values for control leaves on day 12 refer to 2nd-year leaves.

We have investigated this problem in the temperate evergreen shrub *Euonymus japonica.* Second-year leaves are induced to abscind within 10 to 14 days by application of droplets of the *n*-butyl esters of either 2,4-D or 2,4,5-T in methanol to the upper surface of the blade, and the pattern of events leading to leaf fall seems identical with that occurring in the tropical species examined earlier (*4*). The treated part remains green, but by day 4 after treatment, yellowing begins at the periphery of the treated area and subsequently extends outward. Abscission takes place when the petiole and major part of the blade are yellowing. After abscission, the treated area remains as an island of green tissue in the yellow blade (*5*). Experiments with 2,4-D labeled with ^{14}C have shown that the label (2,4-D as such) is restricted to the areas which remained green (*6*).

The areas of the leaf to which auxin is applied (and within which it remains) are characterized by an enhanced rate of metabolism. Protein and RNA synthesis and respiration are increased, and both carbon- and nitrogen-containing compounds move into the treated area (*4*). In contrast, in the untreated parts of the leaf, chlorophyll is lost progressively, total protein and RNA decrease, and the incorporation of precursors into both protein and RNA is reduced (*6*). Initially we suggested (*4*) that the enhanced metabolism in the parts of the blade treated with auxin leads to a withdrawal of metabolites from the untreated regions (depletion occurring first around the perimeter of the treated area), giving rise to premature yellowing and senescence. Although this situation must contribute to the premature senescence of the blade, we now propose that the senescence and subsequent abscission of leaves treated with 2,4-D is due to the increased synthesis of ethylene that occurs in the regions treated with auxin (*2, 6*).

Treatment with auxins promotes ethylene production in a range of plant tissues (*7*), and ethylene itself induces premature senescence of leaves and leaf fall in many species of plants (*8*). Painting half the surface of leaves of *E. japonica* with 2,4-D increases the production of ethylene by the treated halves nearly 25-fold in 12 days (Fig. 1). Yellowing of the leaf at the periphery of the treated half was visible by the 4th day, and leaves began to fall on the 11th day. Since there is no movement of auxin from the treated area, and since the amount of ethylene collected from naturally yellowing

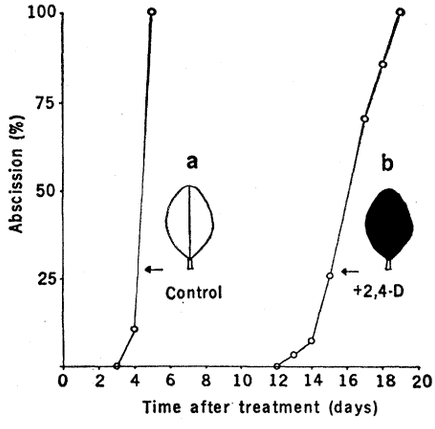

Fig. 2. Effect of ethylene (500 ppm) on abscission of 2nd-year leaves; (a) untreated leaves; (b) leaves treated with 2,4-D ester over entire upper surface. Branches were enclosed in glass containers that were maintained under greenhouse conditions; gas phase was renewed approximately every 3 days.

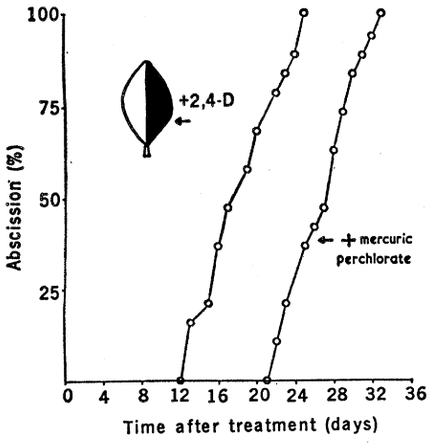

Fig. 3. Effect of removal of ethylene from ambient air on the abscission of 2nd-year leaves. The 2,4-D ester was painted on one half of the blades. Treated branches were maintained under greenhouse conditions either over water or over 0.25*M* mercuric perchlorate in 2*M* $HClO_4$; gas phase was renewed daily.

leaves of *E. japonica* is no greater than that from green leaves (Fig. 1), it is likely that much of the ethylene collected from the untreated halves had diffused in from the area treated with auxin. We suggest that it is this ethylene which induces premature aging in the untreated regions, leading to the subsequent abscission of the leaf. The following experimental evidence supports this proposition.

1) Two samples, A and B, of equal numbers of 2nd-year leaves were halved along the main vein, to give four lots of leaf halves designated A′ and A*, B′ and B*. The B′ halves were painted with 2,4-D ester in methanol (6.67 mg/ml) and the B* halves were painted with methanol. Both A′ and A* samples were left untreated. The A′ halves were enclosed with B′ halves and A* halves were enclosed with B* halves in glass containers of equal volume. In neither vessel were the A and B samples touching. The A′ halves became yellow and senescent earlier than the A* halves, an indication that some volatile product of the sample treated with auxin (B′) accelerated their senescence.

2) Exposing branches of 2nd-year leaves to ethylene (500 parts per million) causes complete defoliation within 5 days (Fig. 2a).

3) When branches of leaves treated with 2,4-D ester are enclosed above an ethylene absorbent (*9*) so that ethylene is removed from the ambient gas phase, defoliation occurs 9 days later than in branches enclosed in the absence of an absorbent (Fig. 3).

4) For many days after leaf fall, the treated areas remain green, and it seems probable that the high auxin content of the area protects it against the senescence-inducing effects of the ethylene produced. For example, branches bearing leaves treated with 2,4-D ester over the whole of the upper surface and then exposed to ethylene (500 ppm) are defoliated 9 days later than control branches exposed to ethylene but not receiving auxin (Fig. 2, a and b). By day 5 the abscinding control leaves show some loss of chlorophyll, but those treated with auxin retain their initial deep green color until a few days before they are shed.

5) Confirmation of the antagonism between ethylene and auxin in the separation process of abscission can be demonstrated in isolated abscission zones of other species. For example, in explants of the primary leaves of *Phaseolus vulgaris*, 2 μl of either water or aqueous solutions of the sodium salt of 2,4-D of increasing concentrations were applied to the distal end of freshly cut explants; all were then exposed for 24 hours to ethylene (500 ppm) and were finally transferred to air. At 47 hours nearly 60 percent of the explants treated with water had abscinded, but only 20 percent of those treated with 2,4-D (1 mg/liter); concentrations of 2,4-D of 10 mg/liter and higher completely inhibited abscission (*10*).

6) If the yellowing of the leaves treated with auxin is primarily dependent on the diffusion of ethylene from the edges of the treated region into the rest of the blade, then a measured volume of the ester applied as a single drop should be less effective in inducing abscission than the same volume applied in several smaller drops (since the sum of the perimeters for several small drops is greater than the perimeter for the single larger drop). As predicted, the abscission of leaves treated with six 1-μl drops occurs more rapidly than that of leaves treated with one 6-μl drop (Fig. 4); yellowing of the blade occurs sooner after the treatment with several drops, and as expected, is more uniform.

We conclude that in *Euonymus japonica,* and in the tropical species previously examined, defoliation after application of esters of synthetic auxins is due to an accelerated senescence induced by ethylene. Ethylene production is stimulated in those discrete regions of the blade in which the auxin is retained. In these areas the effects of ethylene are offset by the high levels of applied auxin, but in the surrounding tissue the ethylene diffusing from the treated area is antagonized only by the endogenous auxin. As a result of the diffusion of ethylene, premature senescence is induced in the untreated parts of the blade leading directly to the initiation of the processes of abscission.

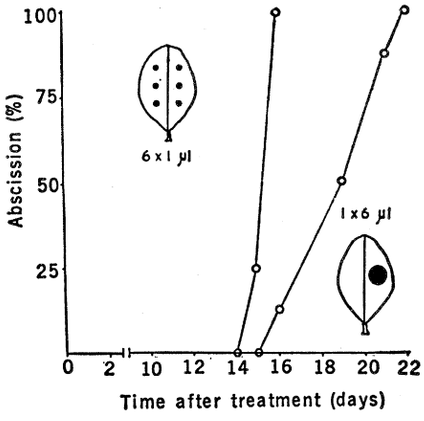

Fig. 4. Effect of distribution of 6 μl of 2,4-D ester (6.67 mg/ml in methanol) on abscission of 2nd-year leaves. Leaves received an application of either one 6-μl drop or six 1-μl drops to the upper surface of blade. The branches were maintained in greenhouse conditions.

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