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Mode of Leaf Shedding from Avocado Cuttings and the Effect of its Delay on Rooting

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Abstract. **Leaf shedding (LS) of 3 avocado *(Persea americana* Mill.) clones ('Northrop 28/5' 'Fuchs 20' and 'Nahlat 7') differing in rooting ability was studied. Two distinct processes, leaf blade senescence and formation of the abscission layer, were found to be associated with LS. The rate of these processes differs among the 3 clones, and their combination determines the speed of LS. Leaf senescence was simulated by measuring chlorophyll destruction over time in leaf discs incubated in the light. The speed of abscission layer formation was estimated by measuring the time required for petioles to shed from incubated internodes. The results obtained in these tests were in agreement with the behavior of LS from cuttings of the 3 clones under mist. Auxin and cytokinin alone, or in combination, as measured in the 2 test systems, affected these leaf blade senescence and abscission layer formations at different rates. Six cuttings of the clone 'Fuchs 20' were sprayed weekly with combinations of NAA and BA. The optimal combination (1.10^{-4} M NAA + 5.10^{-4}M BA) caused a significant delay in LS and improvement in rooting. Relatively simple tests of leaf senescence and petiole abscission are discussed as potential methods for predicting rooting ability of leafy cuttings.**

The number of leaves retained on avocado cuttings and their rooting ability have been reported to be correlated (3). The question arose whether by delaying leaf shedding (LS), rooting might be improved. It was noticed that in different clones, LS does not follow a uniform pattern. Leaves of some clones senesce and shed a short time after cuttings are harvested; in other clones, shedding occurs while leaf blades are still green. It was thought, therefore, that the speed of LS must relate to 2 distinct processes: abscission layer formation and blade senescence. The aims of the present study were to observe the roles of these 2 processes, separately and in combination, in controlling LS from avocado cuttings while under mist. Differences among avocado clones were studied with respect to 2 processes, as were the effects of auxin and cytokinins. Results obtained in the *in vivo*

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studies led to an experiment designed to extend leaf retention time and to study its influence on rooting of cuttings under mist.

Studies were carried out with 3 clones which vary in rooting ability: 'Northrop 28/5', easy to root with leaves retained under mist for a long time; 'Fuchs 20', difficult to root with leaves shedding under mist while still green; and 'Nahlat 7', which does not root at all and whose leaves senesce and shed after a short time under mist (3).

Chlorophyll degradation was determined using the procedure of Osborne and McCalla (2), with some modification. Discs 9 mm in diameter were cut from freshly harvested avocado leaves after washing and blotting dry. Discs taken from 10 individual leaves constituted a replication. Four replications per treatment were incubated on Whatman No. 3 filter paper in petri dishes, after adding 4 ml. of glass-distilled water or a test solution. Incubation was carried out in a growth chamber under a light intensity of 80 µmol s^{-1} m^{-2} for 3 days at 28°C.

Following incubation, the leaf discs in each petri dish were extracted for 24 hr in the dark at 4°C with 20 ml. acetone: methanol, 7:2 (v:v) saturated with $CaCO_3$. Readings were taken with a Bausch & Lomb spectrophotometer at 645 nm and 665 nm against the extraction solution as a blank. Chlorophyll degradation was computed as a percentage of the initial optical density at 665 nm of the relevant sample.

The petiole abscission technique of Halliday and Wangerman (1) was adopted with slight modifications. The same branches as those used for chlorophyll degradation studies were used in abscission tests. The branches were cut into nodal segments, containing 1 petiole and 1 axillary bud. The segments were placed in an aerated, humid chamber to prevent ethylene buildup. The chambers were maintained under the same light and temperature conditions as described above.

Growth substances were applied by paper discs (6 mm diameter, Whatman No. 3 filter paper) dipped in the test solutions and placed on the upper side of the petioles after a fresh cut was made. Each paper disc absorbed 0.015 ml. Daily observations were made, and results were calculated as the average number of days to abscission.

The rooting experiment was carried out as described previously (3). Growth substances were applied as a weekly spray in order to obtain a prolonged effect of these substances. Spraying solutions contained 0.05% Tween 20. The bases of the cuttings from a 9-year-old 'Fuchs 20' tree were dipped in 1% benomyl in talcum powder. Bottom heat was kept at 30°C ± 1°. Planting was on 11 June and the trial was terminated on 21 Sept. The rooting medium was peat moss : scoria (Volcanic ash), 3:1 (v:v) There were 10 cuttings per replication and 4 replications per treatment in randomized blocks. Rooting data were analyzed statistically using Duncan's multiple range test.

Chlorophyll retention was high in the clones 'Northrop 28/5' and 'Fuchs 20' and low in 'Nahlat 7' (Table 1).

NAA (10^{-8}M – 10^{-4} M) in the incubation solution of the leaf discs did not improve chlorophyll retention. The cytokinin BA delayed chlorophyll degradation especially in the low-chlorophyll-retaining clone 'Nahlat 7' (Table 2).

Petioles were retained for 24 days on the intenodes of 'Northrop 28/5' while they shed in 13 days from 'Fuchs 20' and 'Nahlat 7' (Table 1). A different rate of delay in petiole

abscission was achieved by treating the segments with NAA. The responsive clones were 'Nahlat 7' and 'Fuchs 20' (Table 3). Treating the petioles with BA also delayed abscission, but only in 'Nahlat 7' (Table 3).

Table 1. Initial optical density at 665 nm, chlorophyll retention after 3 days of incubation, average time for petiole abscission, rooting percentage and leaf retention on cuttings of 3 avocado clones.

Clone	Initial optical density at 665 nm ($\pm S_{\bar{x}}$)	Chlorophyll retention % $\pm S_{\bar{x}}$	Time to petiole abscission	Rooting[z] (%)	Leaf retention[z] (%)
Northrop 28/5	0.59 ± 0.010	100 ± 0.0	24 ± 2.4	89	69
Fuchs 20	0.60 ± 0.010	97 ± 1.5	13 ± 0.7	17	4
Nahlat 7	0.66 ± 0.022	61 ± 2.5	13 ± 1.0	0	0

[z]As found previously (3).

Table 2. Chlorophyll retention (%) in leaf discs of 2 avocado clones after 3 days of incubation as affected by various concentrations of BA.

BA concentration (M)	Clone		
	Northrop 28/5	Fuchs 20	Nahlat 7
0	82 ± 2.5	63 ± 4.3	72 ± 18.5
10^{-7}	92 ± 13.3	62 ± 5.1	82 ± 16.3
10^{-6}	92 ± 14.7	58 ± 3.6	85 ± 14.5
10^{-5}	85 ± 1.8	74 ± 5.7	100
10^{-4}	85 ± 5.8	76 ± 5.1	100
10^{-3}	85 ± 4.2	86 ± 4.0	100

The effect of NAA and BA together was tested with petioles of 'Nahlat 7'. A synergistic effect of BA and NAA was found in delaying petiole abscission (Fig. 1).

Repeated applications of NAA and BA improved rooting and leaf retention of 'Fuchs 20' cuttings. A delay in LS was achieved by high (5 x 10^{-4}M) BA and (10^{-4}M) NAA concentrations (Fig. 2). Only the combination of high concentrations of both BA and NAA delayed LS and increased rooting of cuttings (Table 4).

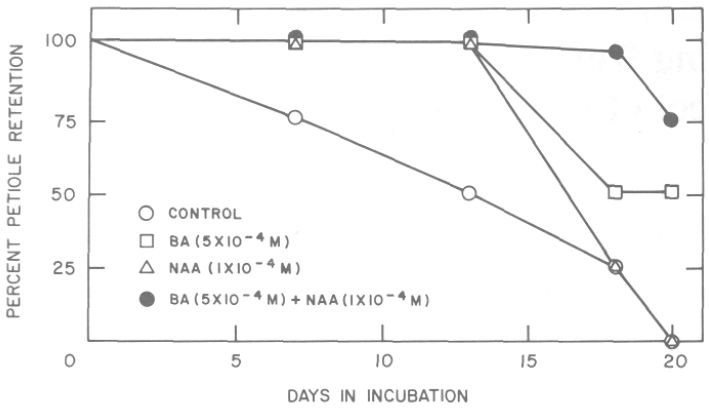

Fig. 1. Petiole retention on internodes ('Nahlat 7') as affected by BA and/or NAA application.

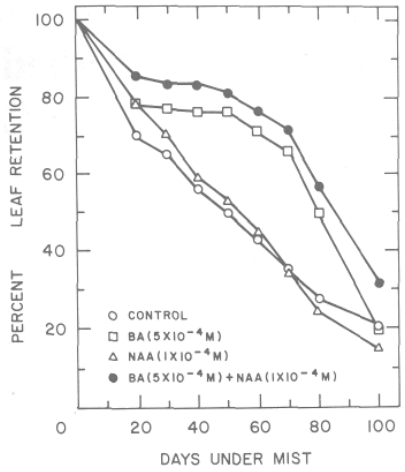

Fig. 2. Leaf retention of avocado cuttings ('Fuchs 20') under mist as affected by weekly sprays of BA and/or NAA.

Leaf senescence and petiole abscission of the 3 avocado clones measured *in vitro*

followed the same pattern as in cuttings of the same clones under mist (Table 1). In the easy to root clone 'Northrop 28/5', which retains the leaves under mist for a long time, chlorophyll degradation was slow (Table 1) and B A does not modify this behavior (Table 2). Petioles were retained for more than 40 days on segments of this clone and neither BA nor NAA prolonged this period (Table 3).

The difficult to root clone 'Fuchs 20' shed its leaves under mist while they were still green. Chlorophyll degradation was slow and petiole abscission fast (Table 1). Treating the petiole of this clone with NAA brought about a delay in abscission (Table 3).

Table 3. Petiole retention (days) on internodes of 3 avocado clones as affected by various concentrations of NAA and BA.

	Clone		
Concentration (M)	Northrop 28/5	Fuchs 20	Nahlat 7
0	43 ± 6.7	15 ± 1.6	14.5 ± 2.5
NAA 10^{-6}	45 ± 8.6	13 ± 1.9	21 ± 6.0
NAA 10^{-4}	46.5 ± 1.4	18 ± 1.1	21 ± 6.0
0	40 ± 4.1	15 ± 1.2	14 ± 1.5
BA 10^{-5}	35 ± 4.0	20 ± 4.6	17 ± 1.7
BA 10^{-4}	40 ± 2.0	18 ± 3.4	21 ± 4.2
BA 10^{-3}	47 ± 5.7	16 ± 1.5	28 ± 6.5

Table 4. Percentage of leaf retention and rooting 102 days after planting stem cuttings of avocado ('Fuchs 20'), as affected by weekly sprays of BA and/or NAA at different concentrations.

Treatment	Leaf retention (%)	Rooting (%)
Water (control)	18	15 b[z]
1×10^{-5}M NAA	13	5 b
1×10^{-4}M NAA	13	13 b
5×10^{-5}M BA	18	20 b
5×10^{-4}M BA	16	8 b
5×10^{-5}M BA + 1×10^{-5}M NAA	14	8 b
5×10^{-4}M BA + 1×10^{-4}M NAA	31	43 a

[z]Mean separation by Duncan's multiple range test at $P = 0.01$.

In the difficult to root clone, 'Nahlat 7', leaf senescence and shedding occurred after a short time under mist. Chlorophyll degradation and petiole abscission were rapid (Table 1). Chlorophyll degradation in this clone was inhibited by BA (Table 2) and petiole abscission was delayed by BA and NAA (Table 3).

The results suggest that in different clones different levels of plant growth regulators are needed in order to delay blade senescence or petiole abscission.

Based on the effects of BA on chlorophyll retention and of NAA on petiole abscission of 'Fuchs 20', an experiment was conducted to delay LS from cuttings and to test its effect on rooting. The results obtained (Table 4) support the assumption that it is possible to delay LS and that this delay improves rooting. The high rooting percentage achieved by using BA and NAA together might be explained by 1 to 2 factors: BA and NAA together have a synergistic effect on leaf retention in 'Fuchs 20' (Fig. 2), and/or NAA has a specific effect on rooting.

The results obtained in this study suggest that it is possible to predict the potential of leaf

retention by *in vitro* tests.

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