EFFECTS OF GROWTH REGULATORS ON HEALING AVOCADO PRUNING WOUNDS

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Additional index words, callus development, bud growth, *Persea Americana* L.

Abstract

Several growth regulators applied to terminal pruning wounds of small, container-grown avocado seedlings stimulated callus formation. The best treatments often caused almost complete coverage of the wound with callus before untreated controls had produced any. Some growth regulators inhibited bud growth near the wounds and some were phytotoxic at high concn. Gibberellic acid (GA), 2,2-4 dichlorophenoxyacetic acid (2,4-D) and benzyladenine (BA) at certain concn were the best treatments on the basis of callus formation, lack of phytotoxicity and bud growth near the wound area. Improvement of healing of large wounds on mature trees in the field was not attained.

Callus formation is the first step in healing. After a time a cambium or meristematic layer forms in the callus. From this time on progress in covering wounds extending into the woody cylinder is by formation of new phloem, xylem and cork along the sides (4). The importance of selecting wound dressings which encourage callus development has been recognized by a number of authors (10, 11, 13). Rapid formation of callus at the edges of the wound protects cambium cells from death due to desiccation and thereby prevents enlargement of the wound. Temp (1) and moisture (1, 2) influence callus formation.

Wound dressings have traditionally been applied to pruning cuts to prevent drying of tissue around the edges and surface of the wound, to sterilize the wound, to prevent the entrance of decay organisms, and to hasten wound healing. No commercial wound dressing accomplishes all of these objectives. Black asphalt wound dressings aid healing by preventing desiccation but dieback has occurred when used on wounds subjected to intense sunlight. White latex paint was superior to black asphalt compounds under such conditions (12). There are no reports of commercial wound dressings stimulating callus formation.

Haberlandt (7) postulated the production of a wound hormone in damaged tissue that aids in healing. Growth of new shoots in the vicinity of the wound is recognized as enhancing healing, possibly through growth regulators produced in the expanding leaves. Several growth regulators have increased callus in tissue cultures but attempts to increase callus and thereby aid healing of woody plant wounds have been largely unsuccessful. The work has, however, been sparse. The purpose of this work, therefore, was to evaluate a wider range of kinds and concentrations of growth

regulators than have been previously investigated for their influence on callus formation and wound healing.

Materials and Methods

Four related experiments were conducted to evaluate the influence of growth regulators on wound healing. Young, container-grown avocado seedlings in the greenhouse were used in Experiments I. III and IV. Seedlings of 'Waldin' were used in Expt I and 'Duke' were used in Expt III and IV. Large 'Gainesville' trees growing in the field were used in Experiment II.

A randomized block design was used throughout with single plant plots in greenhouse experiments and single branch plots in the field. There were 3 replications in Expt I, 5 in Expt III and IV and 10 in Expt II.

Treatments are listed in the tables of results for Expt I, III and IV. All growth regulators and combinations of growth regulators mixed with lanolin (LA) were first dissolved in minimum quantities of ethyl ether (EE). Then appropriate amounts of these solutions were mixed with a fixed amount of LA in beakers to obtain the desired concentrations on a weight basis. These mixtures were stirred daily for 3 days, kept under a fume hood for a week to dissipate the EE and stored under refrigeration until used. Wounds were made on the small seedlings by removing the tops with a slanting cut that started at an internode and extended 3 to 5 cm upward, terminating just above a node on the opposite side of the stem. Treatments containing lanolin were, applied to the wound surface with a spatula. Other treatments were applied with a small brush. Treatments in Expt II consisted of 500, 1000 and 2000 ppm IBA in LA, the same concn of the potassium salt of IBA (KIBA) in water solution, and a white latex paint. These were compared with untreated (UC) and LA controls. Wounds on the large trees were transverse cuts of limbs 2.5 to 9 cm in diameter.

Wound healing was determined by measuring the length and width of the wound area not covered with callus or wound tissue. The percentage of the wound area covered was calculated from these measurements. Bud growth in Expt I was rated from 1 to 5 in which 1 indicates buds dead in the vicinity of the wound, 2 buds fully dormant, 3 buds swelling, 4 slightly elongated shoots, 5 vigorous shoot growth. This rating system was changed for Experiments

III and IV with 1 indicating buds fully dormant, 2 buds swelling, 3 emergence of shoots, 4 slightly elongated shoots and 5 vigorous shoot growth. No measurements were made in Experiment II because no visual responses to treatments were obtained.

Results and Discussion

The effects of auxins were evaluated first (Table 1) because they seemed to hold the most potential for success. Auxins stimulate growth through cell division and cell elongation (8. 9). Attempts to use them to enhance wound healing of trees have been inconsistent (5, 6) but limited success has been reported. The results in Expt I (Table 1) show all of the auxins increased callus formation at non-phytotoxic concn. Part of this

response, however, was due to the LA carrier. Wounds of the untreated control produced no callus after 45 days. Wounds covered with LA had 13% of the wound callused within 7 days and 40% of the wound had healed over in 45 days. LA + EE gave similar results indicating the EE was not harmful. The effect of LA is assumed to be due to its prevention of desiccation. Moist sphagnum was not as effective as LA, even though it was superior to the UC. This is understandable because it was difficult to keep the sphagnum continuously moist. Statistical precision was poor because of the small number of plants. Even so, several treatments caused more callusing than the LA control and these mean differences were statistically significant. The best treatment was 1000 ppm IBA which resulted in 51% of the wound covered with callus in 17 days and 83% covered in 45 days.

The higher concentrations of all auxins caused death of tissue. No callus formed in such cases or it formed only in isolated spots of undamaged tissue. NAA was more toxic than IAA and IBA.

Bud growth was also inhibited by all auxins (Table 1). NAA was more inhibitory than IAA and IBA, which were similar in this respect. Inhibition of IAA and IBA at 1000 ppm was not severe.

An attempt was made in Experiment II to enhance callus formation of large tree wounds in the field with IBA, since results with 'Waldin' seedlings were successful. Concn higher than 1000 ppm were included on the assumption the material might not be as effective under field conditions as in the greenhouse. KIBA in a water solution was also used because LA melts at temp commonly occurring in the field. None of these treatments caused any response. The edges of all wounds died back slightly and no callus was evident. It is not known why the treatments failed but it is possibly because the weather was very hot and dry, the lanolin melted rapidly and it was impossible to keep the wound covered with it for long periods. The water solution of KIBA Dried within minutes after application and absorption by the tissues was probably poor. The failures of the field experiment lead to screening other growth regulators so that broader field experiments can be conducted in the future.

Table 1. Effect of growth regulators on callus formation and bud growth on terminal pruning wounds of 'Waldin' avocado seedlings.

Treatment	Wound surface callused[z]				Bud growth[z]		
	Mar 21	Apr 1 (%)	Apr 8	Apr 14	Mar 21	Apr 8 (rating)	Apr 14
Untreated	0f[z]	0g	0g	0e	4.0a	4.6a	4.6a
LA	13def	30de	32cde	40cd	4.0a	4.6a	4.6a
LA + EE	12ef	35cd	38bcd	41cd	3.6ab	4.0ab	4.0ab
Moist sphagnum	0f	20ef	26de	29d	3.6ab	4.0ab	4.0ab
IAA 1000 ppm	43ab	53bc	61a	64b	3.0bc	3.3bcd	4.0ab
IAA 2000 ppm	27cd	40bcd	43bc	50bc	2.6cd	3.3bcd	3. bc
IAA 4000 ppm	17cde	30de	32cde	40cd	1.6e	1.6fg	1.6ef
IAA 8000 ppm	2f	10fg	11fg	11e	1.3e	1.3g	1.3ef
IBA 1000 ppm	51a	66a	72a	83a	3.0bc	3.6bc	3.6bc
IBA 2000 ppm	30bc	44bc	47b	56b	2.6cd	3.0cde	3.0bcd
IBA 4000 ppm	9ef	11fg	22e	27d	1.6e	2.3ef	2.3cde
IBA 8000 ppm	0f	0g	0g	0e	1.6e	1.6fg	1.6ef
NAA 1000 ppm	8ef	12fg	22e	36cd	2.0de	2.6de	2.6cd
NAA 2000 ppm	0f	10fg	11fg	12e	2.0de	2.3ef	2.3cde
NAA 4000 ppm	0f	2g	2g	6e	2.0de	2.0f	2.0de
NAA 8000 ppm	0f	0g	0g	0e	2.0de	1.0g	1.0f

[z]See text for explanation of ratings.
[y]Means followed by the same letters are not significantly different at p=0.05 level by Duncan's New Multiple Range Test.

IBA at 1000 ppm continued to be very effective (Table 2), causing 85% of the wound surface of 'Duke' seedlings to be covered with callus in 53 clays while no callus was evident in the untreated control. Lower concn of IBA were much less effective. G A at 50, 100, and 200 ppm and 2,4-D at 10 ppm were equally as effective as 1000 ppm IBA. Moreover it not only was equal to IBA in callus formation but slightly stimulated bud growth, while 1000 ppm OBA inhibited it. There was a slight inhibition of bud growth by 10 ppm 2,4-D, another auxin, but it was superior to 1000 ppm IBA in this respect. Both asphalt paint (ASP) and white paint (WP) wound dressings were almost as effective as the LA control and superior to the UC which did not form callus during the expt.

Another experiment with 'Duke' seedlings was then conducted to evaluate higher concn of GA and 2,4-D, BA and combinations of these materials. BA, a cytokinin, is also known to cause cell division (3, 14) and it is usually not phytotoxic.

The UC produced appreciable callus within 35 clays, probably due to the warmer temp and more vigorous growth of the plants than in previous expts; however, it was still inferior to the LA control and all growth regulator treatments (Table 3).

GA continued to stimulate both callus formation and bud growth but GA at 800 ppm produced slightly less callus than lower concentrations. BA at 50 and 100 ppm caused slightly more callus growth than the best GA treatments but 200 ppm was not as good.

BA was also equal or slightly superior to GA in stimulation of new growth. Treatments with 2,4-D were about equal to GA and BA in stimulating callus formation but they continued to inhibit bud growth slightly.

None of the combinations of growth regulators were superior to the individual materials. GA added to 2,4-D did not overcome the tendency of the latter to inhibit bud growth.

Table 2. Effect of growth regulators on callus formation and bud growth on terminal pruning wounds of 'Duke' avocado seedlings.

Treatment	Wound surface callused[z]			Bud growth[z]		
	Apr 18	May 3 (%)	May 19	Apr 11	May 3 (rating)	May 19
Untreated	0c[y]	0d	0d	2.2bcd	3.8abc	3.8abc
LA	30b	53bc	60bc	2.0de	3.6cd	3.8abc
ASP	—	—	49c	3.0bc	4.2ab	4.2ab
WP	—	—	52c	3.2b	4.0ab	4.0ab
IBA 250 ppm	35ab	52bc	56c	2.0cd	3.2bcd	3.4bcd
IBA 500 ppm	37ab	61abc	67bc	1.0e	2.2d	2.8cd
IBA 750 ppm	30b	63abc	72bc	1.0e	2.2d	2.4d
IBA 1000 ppm	45a	72a	85a	1.0e	2.2d	2.2d
2,4-D 10 ppm	43ab	70a	87a	2.6bc	3.2bcd	3.2bcd
2,4-D 20 ppm	46a	62abc	79ab	2.4bc	2.8bcd	3.0bcd
2,4-D 40 ppm	35ab	51ab	76ab	1.4e	3.0bcd	3.0bcd
GA 50 ppm	35ab	55bc	81a	2.6bc	3.2bcd	4.2ab
GA 100 ppm	47a	63abc	84a	4.4a	4.6a	4.8a
GA 200 ppm	54a	69ab	85a	3.0bc	3.6abc	4.0ab

[z]See text for explanation of ratings.
[y]Means followed by the same letters are not significantly different at p=0.05 level by Duncan's New Multiple Range Test.

Table 3. Effect of growth regulators on callus formation and bud growth on terminal pruning wounds of 'Duke' avocado seedlings.

Treatment	Wound surface callused[z]			Bud growth[z]		
	Jun 16	Jun 24 (%)	Jul 4	Jun 16	Jun 24 (rating)	Jul 4
Untreated	0e[y]	0f	19d	3.5ab	4.0ab	4.3bc
LA	22bc	38bcd	43c	3.5ab	4.0ab	4.5ab
GA 200 ppm	18cd	43abcd	56ab	2.8bcd	3.0cd	4.3bc
GA 400 ppm	33a	49a	55ab	3.0abcd	3.5bcd	4.5ab
GA 800 ppm	14cd	32cde	47bc	3.3abc	3.5bcd	4.8ab
2,4-D 40 ppm	22bc	40abcde	50bc	2.5cd	3.3cd	3.5cd
2,4-D 80 ppm	29ab	45abc	50bc	2.5cd	2.8d	3.0e
2,4-D 100 ppm	31a	47ab	55ab	2.5cd	3.0cd	3.0e
BA 50 ppm	24abc	37bcde	63a	3.8a	4.3a	5.0a
BA 100 ppm	33a	45abc	61a	3.8a	4.0ab	4.8ab
BA 200 ppm	28ab	42abcde	47bc	3.5ab	3.8abc	4.5ab
GA 200 ppm + 2,4-D 20 ppm	23abc	33de	51bc	2.3d	3.0cd	3.3de
GA 200 ppm + 2,4-D 40 ppm	11d	32e	51bc	2.5cd	3.0cd	3.3cd
GA 200 ppm + BA 50 ppm	25ab	43abcd	57ab	3.3abc	3.8abc	4.3ab
GA 200 ppm + BA 100 ppm	25ab	35cde	50bc	3.5ab	4.0ab	4.5ab

[z]See text for explanation of ratings.
[y]Means followed by the same letters are not significantly different at p=0.05 level by Duncan's New Multiple Range Test.

The results show conclusively that auxins, gibberellins and. cytokinins ail stimulate callus formation and enhance wound healing if placed in contact with meristematic tissue. Auxins, however, consistently inhibited bud growth. This inhibition might be advantageous where both wound healing and suppression of sprouting is desired but in other cases it would be undesirable.

The only field test to date was a complete failure; however, the favorable responses obtained in greenhouse experiments with several growth regulators over a fairly broad range of concn without phytotoxicity suggests they can ultimately be adapted to field use.

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