VEGETATIVE PROPAGATION OF *ACACIA KOA* GRAY

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In 1973, the U. S. Forest Service began a program of tree improvement of koa (*Acacia koa*). We began by setting up a list of arbitrary criteria that we felt defined a superior tree. We then went out into the forest and selected trees that met these stringent criteria. The trees were selected primarily for straightness of stem, lack of low branches, and lack of fluting. Forty-six trees that met our criteria--38 on Hawai'i and 8 on Kaua'i--were selected for propagation.

Since 1973, we have been gathering seed from these 46 trees to use in the eventual establishment of progeny tests and seed orchards. We have also attempted many trials of vegetative propagation, seeking a means of establishing clonal progeny tests. We recognize such tests as being a much speedier technique than that of using open-pollinated half-sib seedlings.

These vegetative propagation trials have included grafting, air layering, rooting of cuttings under mist, aseptic grafting, of seedlings, propagation from root cuttings, and tissue culture.

MATERIALS AND METHODS

The experiments were conducted on O'ahu and Hawai'i. Much of the work done on O'ahu was aimed at developing techniques that could be used with the superior trees on the Big Island and was done with material from O'ahu trees.

Juvenility has repeatedly proven to be a critical factor in rootability of many woody plant cuttings (Kormanik & Brown 1974). Our early work showed this to be the case also for koa. Fortunately many koa trees produce root suckers. These suckers have the juvenility factor that is shown in the species by its true-leaf or bipinnately compound leaf form. Root suckers were our main source of propagative material, although we also used adventitious stem shoots bearing the true leaf form and tried, unsuccessfully, to use phyllode and mature (flowering) cuttings as well as swellings from the roots and stems I call sphaeroblasts.

Grafting experiments were made using largely conventional techniques applied by five different grafters, three of whom are considered experts at the art. The techniques consisted of bench grafting and budding using pot-grown seedlings as root stocks and field grafting to young forest-grown trees as stocks. Buds and cuttings were from various ontogenetic stages of growth.

Air layering experiments were tried on root suckers, adventitious shoots, and small branches by using polyethylene wrappers and sphagnum moss. Both juvenile and mature material were tested. Girdles were usually 2 cm long, and IBA in talc was used as the rooting substance.

Root cutting experiments consisted primarily of wounding and chemical treatment of *in situ* roots in attempts to cause them to produce root suckers. Root cuttings were also held in flats and under mist.

Experiments of rooting cuttings under mist were carried out in five different mist room facilities. Only one facility had light and temperature control and bottom heat. Mist was supplied intermittently at rates that would keep the leaves wet without dripping. For the most part, perlite was used as the rooting medium, and IBA in talc as the rooting substance. The cuttings were contained in styrofoam cups of rooting medium to reduce contamination.

We compared the rooting of various ontogenetic stages of cuttings, different mist cycles, different auxins, seasons of collection, and bottom heat or lack of it. Cuttings were from 3 to 20 cm tall and ranged from very succulent root suckers to hardened phyllodinous branch tips.

Aseptic organ culture was carried out in completely controlled conditions in a laboratory by using the basal nutrient medium of Murashige and Skoog (1962) and adding growth regulators. Surface-sterilized shoot tips of seedlings and root suckers were inoculated to test tube slants of agar media and held under continuous light at 25°C in attempts to induce rooting. Aseptic grafting of seedling shoot tips to aseptically-grown seedling stocks was also attempted.

Propagules obtained from the experiments were planted at two field locations--the Laupahoehoe Section of the Hilo Forest Reserve at 4800 feet elevation and within the 200-acre Kilauea-Keauhou koa regeneration area on Keauhou Ranch at 5000 feet. Both sites are fenced from cattle and the Laupahoehoe site is also pig-proof. Progeny tests at Laupahoehoe have been underway for 2 years and at Keauhou for about 7 months. Propagules have been observed at irregular intervals. We observed form, height, ontogenetic leaf stage, and susceptibility to the rust *Uromyces koae*.

RESULTS AND DISCUSSION

Many of the results of the propagation experiments were negative.

Grafting

All grafting experiments failed. These experiments included a total of 202 grafts (Table 1). Except for grafts 6, 8, 9, and 11 to 15, all grafts in the table were made by using polyethylene wrappings. The others were made by using raffia and grafting wax.

The basic reason for failure was that koa would not form callus at the grafts. Even when cuttings taken from a root stock were grafted back to the same plant, the grafts failed (Table 1, no. 10). The closest to a successful graft was achieved with an approach graft of a root sucker propagule to a seedling root stock (Table 1, no. 12). This graft held briefly but then broke apart a few days after it was cut free from the potted scion and unwrapped.

When the young potted seedlings used as root stocks were grown for 3 to 4 months in a very warm greenhouse at Waimanalo, they developed peculiarly shaped phyllodes over 30 cm long and only 1.5 cm wide. Trees of the same age grown at Makiki nursery in normal conditions did not begin phyllode formation (of normal shape) until they were 7 months old. When some of the potted trees were moved from the greenhouse back to Makiki, they reverted to the true-leaf habit and later developed normally shaped phyllodes. We found that this situation occurred repeatedly with koa of several provenances. High temperature and partial shading induce early phyllode formation in koa, and the phyllodes are highly elongated.

Young trees held under mist frequently developed roots on their lower stems up to a distance of 30 cm from the root crown, or soil line. This development suggests that some koa may have a latent adaptation to survive siltation resulting from flooding by producing new roots from the stem in new deposits of soil much as does redwood.

Aseptic organ culture

As in the grafting experiments, all aseptic organ culture tried also failed. The primary cause of failure was the difficulty of adequately surface sterilizing material collected in the field. Endogenous bacteria would usually erupt from tissue under culture after about 2 weeks.

Shoot tips were placed on media containing various levels of benzyladenine in attempts to make them elongate. They were also placed directly on media containing the rooting hormones indole-butyric acid and napthaleneacetic acid.

Aseptic grafting consisted of placing shoot tips of aseptically-grown seedlings and surface sterilized root sucker tips onto decapitated aseptically grown seedlings. All these grafts also failed, although in one instance two new shoots formed and grew from a decapitated seedling. This development suggests a possible method of propagating seedlings of the species.

Air layering

In the experimental work with air layering, which was initially all done on O'ahu, wrappings were applied to phyllodinous adventitious shoots, true-leaf shoots, and root suckers. A total of 39 trees and 24 root suckers were treated. On these 63 plants, 27 air layers were made on phyllodinous laterals, 26 on partly phyllodinous laterals, and 37 on true-leaf material.

The first air layers were made using a mixture of vermiculite and perlite. None of these was successful. All others were made by using sphagnum moss. As soon as sphagnum moss was used, successes began to occur, but only with true-leaf material. No air layers of phyllodinous material rooted. Among the 24 air layers of true-leaf root suckers, 5 rooted. This was considered sufficiently successful that we applied it immediately in the field on superior tree root suckers on the island of Hawai'i.

Among the 38 superior trees on Hawai'i, 16 have produced root suckers and 4 have produced adventitious stem sprouts. Some are prolific at producing root suckers; others produced only one in 5 years of observation. The formation of root suckers seems to be strongly influenced by rainfall patterns. They occur more frequently during wet seasons. They occur nearly as commonly under dense rain forest canopies as in open, logged-over stands.

A listing of all air layers made on superior tree root suckers (Table 2) illustrates the variation in root sucker production. Tree 15 produced so many root suckers that 57 of them were air-layered, while trees 1, 14, and 36 produced only one each. The 159 air layers made were applied to root suckers and stem sprouts of varying stages of stem hardness or age. Rooting achieved from certain of these indicated that the most success was achieved with material that had formed a round stem (initially stems of root suckers are square) which was "slightly woody" and just developing brown bark.

One reason for the low level success with rooting of air-layered root suckers is that feral pigs destroy them. Most root suckers are less than 30 cm tall. In this position near the ground, the air layers are destroyed by pigs seeking moisture in the wrapping. Another reason for the low level of rooting was that many of the air layers were applied to root suckers that were too succulent before this was determined to be an undesirable stage for rooting (Table 2).

Rooting of air layers was remarkably slow. Some took as long as 7 months to form roots. During this long interval, the layers were highly susceptible to pigs and to saturation of the medium by rainfall--another possible cause of low success in rooting.

The success in rooting of air layers was only 16%, but the success in aftercare of these rooted propagules was fairly good at 52%. The best technique of aftercare was found to be potting in a 50-50 mix of mica-peat and perlite and keeping the potted layer under mist, with the medium covered to prevent moisture entry, for a period of 3 weeks before placing on the bench.

The 13 surviving air layers were planted in a small clonal progeny test at Laupahoehoe. For the first year after planting, all performed poorly--the tallest reached a height of only 4 feet. During the second year, all produced phyllodes. Four of the 13 show plagiotropism, growing at an angle as though they were branches of a tree. Eight others are of normal growth habit except that three have been severely damaged by *Uromyces koae*. This rust is not particularly active on natural seedlings in the area. The largest air layer, which is now about 8 feet tall, has formed phyllodes at the terminal end of true leaves rather than in the normal fashion, which is in place of the petiole. The parent tree has normal phyllodes, but I have not observed phyllodes on any other of its root suckers to determine if this peculiar condition resulted in some way from the propagation technique or is common to other root suckers of the tree.

Attempts at propagation from roots

All experiments with root cuttings were unsuccessful. In all, 68 root cuttings were used. Eighteen of these had live root suckers growing from them, but did not re-root when kept in flats of rooting medium. Twenty-five were lengths of roots kept in nursery flats for more than 2 years. Forty-three others with and without suckers were kept under mist in perlite medium, but like the rest, did not root or produce shoots.

We tried to induce suckering of roots *in situ* by applying numerous treatments to the roots (Table 3). Most of these treatments, the knife wounding, the "chewing" with pliers, pounding, and exposure, were intended to simulate the actions of pigs and cattle. Nothing we did had any effect, except in one instance roots formed at the distal end of a girdled root.

The mechanism by which koa root suckers are induced remains a mystery. They have been observed to almost always form on roots that are at or above the soil line and from sphaeroblasts or swollen sections containing proliferations of bud tissue. They are much more common on roots in deep shade, or even hidden under grass than in roots exposed to direct sunlight. Most roots with suckers show no obvious scars of damage.

Rooting of cuttings under mist

Mist rooting of cuttings provided a higher percentage of rooting than did air layering, but the rooted cuttings had a much lower percentage of survival after transplanting than did the air layers. Therefore, it is difficult to determine which of these two successful methods of propagation is the better.

Most of the successes occurred when perlite was used as the rooting medium and 3000 mg/kg IBA in talc as the rooting substance (Table 4). Rooting was obtained only once on a phyllodinous cutting. One experiment (Nos. 7, 8, 9) indicated that rooting could be improved by supplying nutrients to the cuttings while they were under mist. In the potassium nitrate (KNO_3) solution portion of this experiment, one phyllodinous cutting in addition to half of the true-leaf cuttings rooted. Unfortunately, these results could not be duplicated when the experiment was repeated on two occasions. The difference may have been that in the first test the cuttings were placed under mist the same day that they were obtained. The other experiments were started with 1- to 2-day-old material.

Although an average of 20% of cuttings rooted when IBA and perlite were used, only 14 propagules survived transplanting. The low percentage resulted because koa cuttings were very slow to root and produced very few roots per cutting. Rooting usually took 2 months or longer. By the time the cuttings rooted, almost all the leaflets had dropped and when they were transplanted, the rest of the leaflets were usually lost.

The methods we found most successful were to collect root suckers that had reached the round rather than square stem stage. These were placed under mist the same day they were collected. We rubbed the freshly cut end in 3000 mg/kg IBA in talc and put the cuttings in individual styrofoam cups of perlite to reduce fungus contamination. Cuttings that rooted were transplanted as soon as discovered to black plastic bags containing a mixture of mica-peat and perlite. The bag was then closed and sealed around the stem to reduce moisture entry. The packaged transplant was then held under mist for about 3 weeks before transfer to a shade house.

Recently, we have obtained an excellent source of stool shoot and root sucker material from one superior tree that was mistakenly cut by loggers and using the methods described we have twice achieved the usual 20% rooting, and have increased the ultimate survival rate to 12% of the original cuttings started.

Seven rooted cuttings have been planted at Laupahoehoe and five at Keauhou. The seven at Laupahoehoe have grown in a very similar fashion to the air layers. One died a few months after planting. Three others have become infested with *Uromyces koae*, but are now growing in a normal upright fashion. Two others show plagiotropism. All have formed phyllodes, but all are so far much less vigorous than seedling trees growing in the same area.

Clonal variation

The percentage of rooting of cuttings and air layers indicates a possible pronounced clonal variation in rootability (Table 5). These data are only for rooting, not for ultimate survival of the propagules and are, of course, strongly weighted by the variation in numbers of cuttings worked. Only those trees that produced propagative material were listed. Six trees--numbers 1, 10, 18, 19, 20, and 22--provided a fair size sample of material which did not root. They can be compared with the quite good rooting performance of material from trees 2, 8, 9, 15, and 26. These results are only indicative of possible differences. They cannot be validly compared statistically because of the differences in conditions at each attempt in rooting.

Since these data were obtained, we have started more than 100 cuttings from tree number 2 of which 10 to 12 will survive. The key to success in all this propagation work is to use large amounts of material, and this has proved to be impossible with the superior trees up to now.

CONCLUSION

Acacia koa can be propagated by air layering or rooting of cuttings under mist. However, neither procedure has yet been developed sufficiently so that it can be considered a practical method of propagating forest-grown superior trees. So far only a few propagules of such trees have been produced, and many of them are showing slow growth, disease susceptibility, plagiotropism, or poor form.

The only practical way of propagating the species is from seed. This has several obvious disadvantages for tree improvement. Few seeds are produced by the tall, well-formed superior trees which have crowns above the general forest canopy where they are subjected to strong winds and, probably, low populations of insect pollinators. The seeds are open pollinated, so progeny expresses only a portion of the genotype.

Most of our tree improvement work with koa will be concentrated on seedlings. We are gradually building up a supply of sufficient superior tree seed to do a progeny test which will become a future seed orchard. We can also collect seedlings from beneath the trees to transplant to progeny tests.

We are continuing to explore ways of improving vegetative propagation of the species. The latest technique we are trying is to air-layer root suckers in the field and then bring them in as cuttings to root under mist once swelling has occurred above the girdle. This works well with southern hardwoods, but our trials of the method are not yet far enough along to report on.

LITERATURE CITED

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Table 1--Grafting trials of *Acacia koa*

Reference number	Grafts	Type of graft[1]	Scions[2]	Stocks and age	Remarks
1	36	Side veneer	Shoot tip (juv. & mature)	Seedling 4 mo.	18 (9 of each scion) kept under mist
2	30	Side veneer	Root sucker tip (juv.)	Seedling 5 mo.	One-half kept under mist
3	29	t-bud	Root sucker lateral (juv.)	Seedling 5 mo.	One-half kept under mist
4	12	Side veneer	Stem sprouts (juv.)	Seedling 6 mo.	Sprouts girdled, graft at swell above girdle
5	6	t-buds	Root sucker lateral (juv.)	Seedling 7 mo.	Raffia and grafting wax, expert grafter
6	6	Side veneer	Root sucker tip (juv.)	Seedling 7 mo.	Raffia and grafting wax, expert grafter
7	7	Top cleft	Root sucker tip (juv.)	Seedling 8 mo.	
8	15	Patch buds	Root sucker lateral (juv.)	Seedling 9 mo.	
9	15	Side veneer	Root sucker tip (juv.)	Seedling 9 mo.	
10	6	Side veneer	Seedling branch (10 mo. old)	Seedling 10 mo.	Scion and stock same plant
11	10	Top cleft	Seedling tip (juv.)	Forest trees to 1 yr.	Seedling tips to forest-grown tree stumps
12	4	Approach	Root sucker propagule	Seedling 5 mo.	Scions were potted cuttings and air layers
13	8	Side tongue	Seedling tip (juv.)	Seedling 7 mo.	
14	10	Whip	Seedling tip (mature)	Seedling 10 mo.	
15	8	Side veneer	Root sucker tip (juv.)	Seedling 10 mo.	

[1] Descriptive terms follow those by Hartmann and Kester (1975).

[2] Juv. indicates juvenile or true-leaf stage; mature indicates phyllode stage.

Table 2--Rooting and survival of air layered root suckers and stem sprouts of superior trees

Tree number	Air layers	Rooted	Surviving
1	1	0	0
3	13	2	1
5	18	3	2
8	32	6	4
9	6	2	1
10	2	0	0
12	7	1	0
14	1	0	0
15	57	9	5
19	6	0	0
20	2	0	0
22	3	1	0
23	5	0	0
26	2	1	0
35	3	0	0
36	1	0	0
Total	159	25	13

Table 3--Treatments applied to *Acacia koa* roots intended to induce suckering

Treatment[1/]	Trees	Treatments
Knife wounding (deep)	65	195
Knife wounding (shallow)	65	195
Expose root to sun	65	65
"Chew" with pliers	20	40
Pound with hammer	20	40
Heat with torch	20	40
Girdle root	32	32
Raise root on rock	9	18
Bury exposed root	11	11
Wound - kinetin (100 mg/ℓ)	31	48
Wound - NAA (500 mg/ℓ)	27	35
Wound - Ethrel (100 mg/ℓ)	15	17
Wound - B (500 mg/ℓ)	31	48
Wound - IAA (200 mg/ℓ)	15	17
Wound - GA (500 mg/ℓ)	31	48
Air layer (untreated)	6	6
Air layer B (500 mg/ℓ)	6	6

[1/] IAA = indoleacetic acid; NAA = napthaleneacetic acid; B = benzyladenine; GA = gibberellic acid.

TABLE 4. Mist rooting experiments conducted on *Acacia koa* cuttings.

Ref. No.	Number Cut-tings	Ontogenetic Stage	Source	Auxin[1]	Auxin Strength	Applica-tion Method	Rooting Medium	Number	Remarks
1	50	Succulent phyllode	Large trees	IBA	3000 mg/kg	Talc	1:1 vermiculite-perlite	0	One-half with bottom heat
2	50	Succulent phyllode	Large trees	IBA	100 mg/l	1 hr soak	1:1 vermiculite-perlite	0	One-half with bottom heat
3	50	Succulent phyllode	Large trees	None		- -	1:1 vermiculite-perlite	0	One-half with bottom heat
4	25	Phyllode	Stem sprouts	IBA	3000 mg/kg	Talc	Perlite	0	-
5	25	True-leaf	Stem sprouts	IBA	3000 mg/kg	Talc	Perlite	9	35% rooted
6	54	True-leaf & phyllode	Root suckers	IBA	3000 mg/kg	Talc	Perlite	8	15% of true-leaf rooted
7	20	True-leaf & phyllode	Root suckers	IBA	10 mg/l	In medium	0.002 M KNO_3 and perlite	6	50% true-leaf, 1% phyllode rooted
8	20	True-leaf & phyllode	Root suckers	IBA	10 mg/l	In medium	0.002 M KH_2PO_4 and perlite	0	-
9	10	True-leaf & phyllode	Root suckers	IBA	10 mg/l	In medium	Hoagland's solution and perlite	3	30% of true-leaf rooted
10	10	True-leaf & phyllode	Root suckers	None	-	-	Water and perlite	0	-
11	30	True-leaf	Root suckers	IBA	3000 mg/kg	Talc	Perlite	10	30% rooted
12	10	True-leaf	Root suckers	NAA	2000 mg/kg	Talc	Perlite	0	-

TABLE 4—Continued.

Ref. No.	Number Cut- tings	Ontogenetic Stage	Source	Auxin[1]	Auxin Strength	Applica- tion Method	Rooting Medium	Number	Remarks
13	62	True-leaf	Root suckers	IBA	3000 mg/kg	Talc	Perlite	7	11% rooted
14	37	True-leaf	Root suckers	IBA	3000 mg/kg	Talc	Perlite	10	27% rooted
15	9	True-leaf	Stem sprouts	IBA	1000 mg/kg	Talc	Running water	0	-
16	9	True-leaf	Stem sprouts	IBA	3000 mg/kg	Talc	Running water	0	-
17	9	True-leaf	Stem sprouts	IBA	8000 mg/kg	Talc	Running water	0	-
18	108	True-leaf	Root sucker	IBA	3000 ml/kg	Talc	Perlite	19	18% rooted
19	10	True-leaf	Root sucker	IBA	3000 mg/kg	Talc	Perlite after 10 min NaOH	3	-
20	10	True-leaf	Root sucker	IBA	3000 mg/kg	Talc	Perlite after 10 sec H_2SO_4	1	-
21	10	True-leaf	Root sucker	IBA	3000 mg/kg	Talc	Perlite	1	-
22	10	True-leaf	Stem sprouts	None	-	-	Perlite after 10 min NaOH	0	-
23	10	True-leaf	Stem sprouts	None	-	-	Perlite after 10 sec H_2SO_4	0	-
24	10	True-leaf	Stem sprouts	None	-	-	Perlite	0	-

[1] IBA = Indolebutyric acid; NAA = Napthaleneacetic acid.

Table 5--Clonal variation in rooting of root suckers and stem sprouts of superior trees

Tree number	Air layers	Rooted	Cuttings	Rooted	Percent both rooting methods
1	1	0	9	0	0
2	0	0	25	5	20
3	13	2	21	1	9
5	18	3	33	4	14
8	32	6	58	11	19
9	6	2	14	4	30
10	2	0	7	0	0
12	7	1	4	0	9
14	1	0	0	0	0
15	57	9	74	17	20
16	0	0	4	0	0
18	0	0	8	0	0
19	6	0	10	0	0
20	2	0	6	0	0
22	3	1	9	0	8
26	2	1	11	3	31
35	3	0	0	0	0
36	1	0	0	0	0