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THE PROPAGATION OF CASUARINA SPECIES FROM ROOTED STEM CUTTINGS

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Juvenile, mature softwood, and mature hardwood stem cuttings from 1- to 6-yr-old trees of the Casuarinaceae were tested for their capacity to form roots. Trials included mist-bench with bottom heat, water-culture jars, and, for large numbers, an aerated hydroponic system. *Casuarina equisetifolia, C. cunninghamiana, C. montana, C. suberosa,* and *Gymnostoma papuanum* were all successfully rooted. Greatest success (60%–100%) was achieved with mature softwood stem cuttings using auxin treatment (50 mg/liter indole-3-butyric acid for a 3-h soak) followed by water culture in $^1/_4$-strength Hoagland's solution minus nitrogen. Inoculating 4–6-wk-old rooted cuttings with nodule suspensions or pure cultures of *Frankia* HFPCcI3 gave 80%–89% nodule formation at 3 mo.

Introduction

Species of the genus *Casuarina* have been widely disseminated by man and now occur throughout the equatorial and subequatorial regions of the world, serving for fuel wood, land reclamation, dune stabilization, and shelter belts (NATIONAL ACADEMY OF SCIENCES 1980, 1984; MIDGLEY et al. 1983).

The plants are wind pollinated; they flower and fruit throughout their lives and have been propagated largely from seed. The roots of *Casuarina* are readily infected by the soil bacterium *Frankia* (Actinomycetales) and produce nodules that fix atmospheric nitrogen (TORREY 1982). When seeds are planted in new sites, *Frankia* must be introduced in soil samples taken from existing nodulated trees or from litter containing old roots or root nodules. Propagation by seed and nodulation by soil or leafy litter are proved methods for establishing large plantations of various members of the Casuarinaceae (MIDGLEY et al. 1983).

For selection and genetic improvement of species of Casuarinaceae, it is preferable to propagate selected trees vegetatively from superior specimens and to achieve nodulation with strains of *Frankia* known to be highly infective and to produce nodules with high rates of N_2 fixation, thereby maximizing the benefits of the symbiotic association.

DIEM and DOMMERGUES (1983), DIEM et al. (1983*a*, 1983*b*), and ZHANG et al. (1984) reported that pure cultures of *Frankia* isolated from *Casuarina* root nodules are available to use as inoculum. On the other hand, published accounts of vegetative propagation are sparse (cf. TORREY 1983), and the older methods reported, including air layering and rooting of cuttings, are relatively crude and unpredictable, with low yield. SOMASUNDARAM and JAGADEES (1977) and HUSAIN and PONNUSWAMY (1980) reported success in rooting softwood cuttings of *Casuarina equisetifolia* and *C. junghuhniana* using plant growth substance treatment. With a view to producing clonal material for experimental work, we undertook a study of the conditions favoring rapid propagation of young *Casuarina* plants from stem cuttings and their nodulation.

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Material and methods

SOURCE OF PLANTS

Three genera within the family Casuarinaceae were grown from seed. Plants were established in greenhouses at the Harvard Forest, Petersham, Massachusetts, from various seed collections (table 1). Trees up to 6 yr old were grown successfully in large containers in potting soil but required severe pruning at regular intervals to keep the plants within the greenhouse roof. Species of *Casuarina* were effectively nodulated in the greenhouse with nodule suspensions from field-collected nodules of *C. equisetifolia* collected in Florida. Plants were watered regularly with tap water and at intervals with $^1/_4$-strength Hoagland's solution (HOAGLAND and ARNON 1950) minus nitrogen. Species of *Allocasuarina* and *Gymnostoma* usually failed to nodulate in our greenhouses and were sustained by regular fertilization with $^1/_4$-strength complete Hoagland's solution.

TYPES OF STEM CUTTINGS

Three types of cuttings were tested for rooting capacity. "Mature cuttings" taken from trees between 1 and 6 yr old were either "softwood" (green stem) or "hardwood" (brown stem and woody) cuttings. In some species, 3–4-yr-old trees were cut off at the main trunk ca. 10–20 cm above the soil level. These trunks regenerated many new shoots from the stump. Cuttings taken from these regrown shoots were termed "juvenile cuttings." Experiments on rooting cuttings were performed throughout all seasons over a period of several years, using ambient greenhouse conditions.

TABLE 1

SCIENTIFIC NAMES AND SOURCES OF SEEDS USED FOR GREENHOUSE PLANTINGS

Plant species	Source	Donor
Casuarina cunninghamiana	Division of Forest Research, CSIRO, Canberra, Australia	D. BOLAND
C. equisetifolia	Clear Lake, Florida; Hilo, Hawaii	W. SILVER R. SKOLMEN
C. montana (= *C. cunninghamiana*)	Maui, Hawaii	P. WOOMER
C. suberosa (= *Allocasuarina littoralis*)	Lyon Arboretum, Honolulu, Hawaii	R. BAKER
Allocasuarina lehmanniana	King's Park Botanical Garden, Perth, W. Australia	DIRECTOR OF THE PARK
Gymnostoma papuanum	Waiakea Arboretum, Hilo, Hawaii	R. SKOLMEN

NOTE.—Nomenclature follows JOHNSON (1982).

METHODS OF PLANT GROWTH SUBSTANCE TREATMENT

In early experiments, cuttings ca. 7 cm long were excised from shoot tips and dipped into commercial rooting compounds including Rootone or Hormodin, which contain different levels of indole-3-butyric acid (IBA). These cuttings were then placed in sand in flats, in a rooting bed, or in water culture. In later more extensive trials, several plant growth substances were tested at a range of concentrations. Synthetic substances tested included: indole-3-acetic acid (IAA), IBA, and α-naphthaleneacetic acid (NAA). Most of these trials involved a 3-h dip in auxin solution; in others, shoots were placed in nutrient solution with added auxin for long-term treatment. In both cases rooting was performed in $^1/_4$-strength Hoagland's solution minus nitrogen at pH 7.

HANDLING THE TREATED CUTTINGS

In the early experiments, auxin-treated stem cuttings were planted in sand beds in the greenhouse and were provided bottom heat and intermittent mist.

A second procedure involved rooting cuttings in water-culture jars: 8-oz soft glass jars wrapped in foil were capped with lids covered with perforated opaque plastic film. The basal halves of 7–8-cm-long cuttings were treated with auxin solution and then transferred to jars containing $^1/_4$-strength Hoagland's solution without nitrogen at pH 7 (fig. 1). The basal ends were examined at intervals and responses recorded. Rooted cuttings were later transferred to pots (fig. 2).

A third technique involved trough culture. With a view to handling larger numbers of cuttings with single treatments, we experimented with a simple, aerated, polyethylene-covered plastic trough in which several hundred cuttings could be rooted simultaneously (figs. 3, 4). After auxin treatment and a tap water rinse, cuttings were transferred singly to the holes in the perforated plastic, and their bases were immersed in a common solution of $^1/_4$-strength Hoagland's solution minus nitrogen at pH 7.

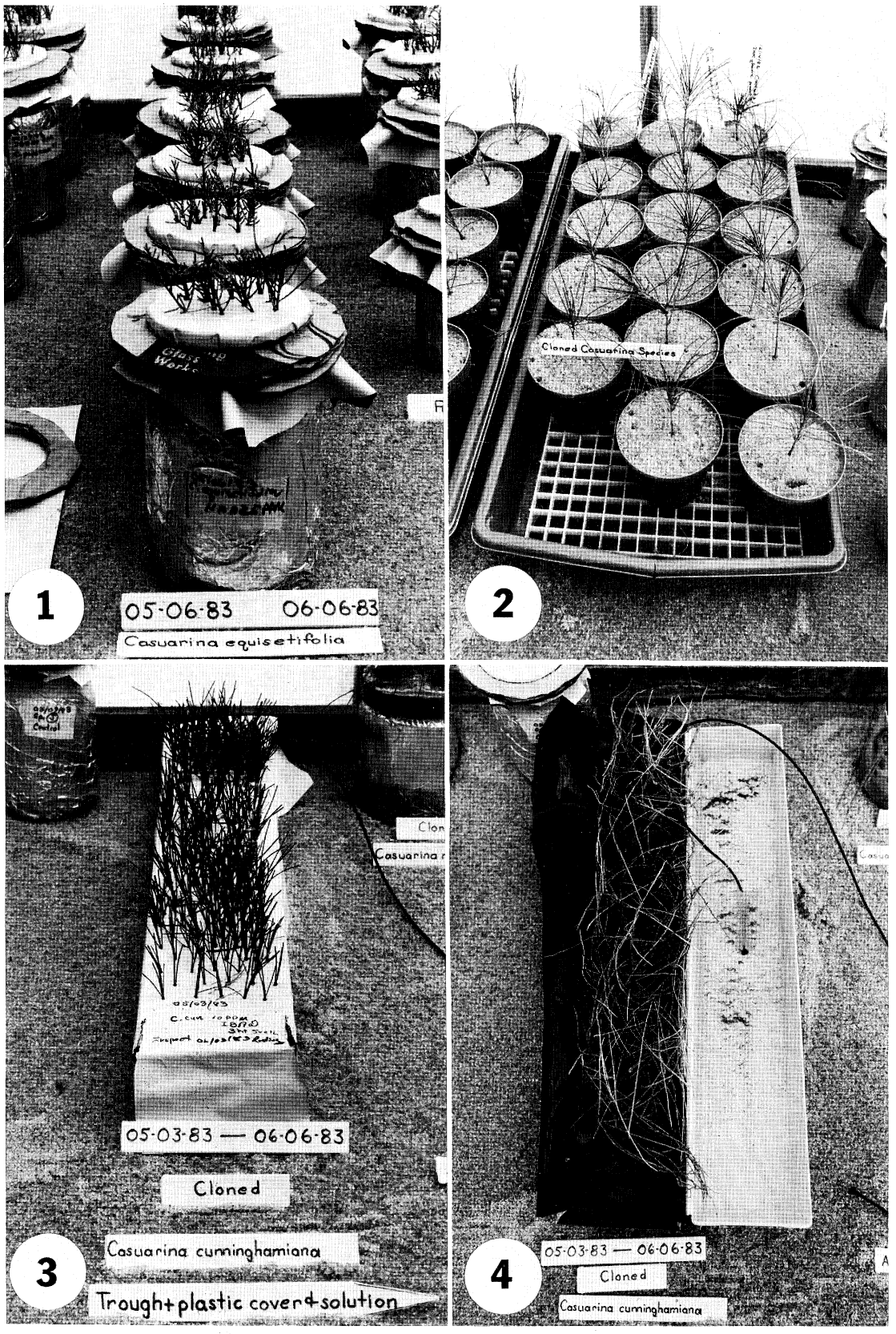
INOCULATION OF ROOTED CUTTINGS

Once successfully rooted, cuttings were transferred to pots or other containers for establishment. Upon transfer, the rooted cuttings were inoculated with microbial preparations of the actinomycete *Frankia,* which induces N_2-fixing nodules on roots by root hair infection (CALLAHAM et al. 1979). Suspensions were prepared by grinding in distilled water with a mortar and pestle fresh whole nodules from greenhouse-grown plants. Roots were immersed in the suspension before transfer of the cuttings to sand in pots. Later, when pure cultures of *Frankia* isolates were available (ZHANG et al. 1984), bacterial cultures were centrifuged, washed in inorganic solution to remove any combined nitrogen, homogenized, and then applied directly to the roots or to the stem base of established cuttings in sand in pots. Successful nodulation following inoculation was determined by direct examination for root nodules on the root systems after several weeks or by observing the successful establishment and growth of the inoculated cuttings when watered with nutrient solution lacking combined nitrogen.

Results

EXPERIMENTS WITH MATURE HARDWOOD CUTTINGS

Since we did not have access to fully mature trees growing in the field, experiments on the rooting of stem cuttings from fully grown trees were limited. In general, our experience was that older stem cuttings were more difficult and took longer to root than juvenile material but that both mature softwood and hardwood cuttings could be rooted with appropriate auxin treatment. Response to auxin treatment was slower in mature cuttings, and the species influenced the response markedly.



FIGS. 1–4.—Fig. 1, Mature softwood stem cuttings of *Casuarina equisetifolia* placed in water culture for rooting experiments on the effects of auxin treatment. Containers were 8-oz soft glass jars covered with foil. Lids were perforated, opaque plastic sheets held in place with cardboard rings. Fig. 2, Rooted cuttings of *Casuarina* species potted after inoculation and grown in sand-vermiculite in 3-inch pots. Fig. 3, Stem cuttings of *C. cunninghamiana* rooted in shallow trough with aeration of nutrient solution. Fig. 4, Inverted top of trough in fig. 3 with rooted cuttings in place, showing extensive rooting achieved in the aerated solution. Small black tube is the aeration line.

TABLE 2

PRELIMINARY EXPERIMENTS ON ROOTING AND CALLUS FORMATION OF MATURE HARDWOOD STEM CUTTINGS IN A SAND BED WITH PERIODIC MISTING IN THE GREENHOUSE

Species, no. of cuttings, and duration of experiment	Treatment[a]	Callused (%)	Rooted (%)
Casuarina equisetifolia:			
20, 5 wk	Rootone-F	60	0
20, 5 wk	Untreated	20	0
20, 8 wk	Rootone-F	10	90
15, 6 wk	Untreated	40	27
15, 6 wk	50 mg/liter NAA	20	47
15, 6 wk	50 mg/liter IBA	60	20
15, 6 wk	50 mg/liter IAA + 50 mg/liter IBA	0	0
C. montana:			
25, 4 wk	50 mg/liter IBA	4	0
25, 4 wk	50 mg/liter NAA	0	0
25, 4 wk	50 mg/liter IBA + 50 mg/liter NAA	0	0

[a] Rootone-F was used as a talc dip; auxins were given as a 3-h soak before placing cuttings in sand bed.

Results of some preliminary trials with mature hardwood stem cuttings, some treated with commercial root compounds in talc as a dip and others with pure plant growth substance treatments using a 3-h soak, were variable (table 2). *Casuarina equisetifolia* appeared to be most responsive to treatment. Callus formation usually accompanied any successful rooting response. Because of early success in rooting juvenile and mature softwood stem cuttings, work on older hardwood stem cuttings was discontinued.

JUVENILE AND MATURE SOFTWOOD MATERIAL

Among the various species tested under a wide range of conditions and treatments, softwood stem cuttings were most easily rooted and established as new plants. Our trials to determine the most effective root-inducing auxin, its concentration, and method of application were most extensive on juvenile and mature softwood material.

Softwood cuttings show two types of regenerative response: callus formation and/or root initiation. In many treatments, only callus was formed; in others, callus formation was followed by root initiation, usually at other than callus sites. In other treatments, roots formed without callusing.

ROOTING IN SAND ON MIST-BENCH

The juvenile cuttings from *C. montana* (possibly *C. cunninghamiana* or with close affinity to it) were easily rooted and showed little benefit from auxin treatment (table 3). Callusing was infrequent. Mature softwood cuttings of *C. equisetifolia* responded well to appropriate concentrations of auxin with optimum results with IBA or NAA at 50 mg/liter (table 3). Callusing was abundant even without auxin treatment and tended to be repressed by concentrations favorable to root initiation. Under mist-bench conditions, the plants were more susceptible to fungal attack, and misting was regarded as both unnecessary and unfavorable and was discontinued.

ROOTING IN WATER CULTURE

Among the *Casuarina* species tested in water culture, the mature softwood cuttings failed to root without auxin treatment, although some cuttings formed callus (table 4). IBA seemed to be consistently better than NAA; IAA was not tested in these trials as it was consistently less effective (table 3).

TABLE 3

ROOTING AND CALLUS FORMATION ON STEM CUTTINGS OF CASUARINA SPECIES PROPAGATED IN SAND BED WITH PERIODIC MISTING IN THE GREENHOUSE

	C. EQUISETIFOLIA (mature softwood)[a]		C. MONTANA (juvenile)[a]	
TREATMENT (mg/liter)	Callused (%)	Rooted (%)	Callused (%)	Rooted (%)
Water control	93	7	7	93
IAA:				
25	40	0	0	100
50	73	7	0	100
100	40	20	7	73
IBA:				
25	53	20	7	93
50	40	47	0	93
100	53	27	7	40
NAA:				
25	33	20	0	100
50	27	60	0	100
100	13	0	20	100

[a] Mature softwood or juvenile stem cuttings ca. 7 cm long were placed with basal 2 cm in treatment solutions for 3 h before planting in the sand bed, 15 cuttings/treatment. Data collected after 35 days.

TABLE 4

ROOTING AND CALLUS FORMATION ON STEM CUTTINGS OF SPECIES OF CASUARINACEAE PROPAGATED IN WATER CULTURE JARS IN THE GREENHOUSE

Treatment (mg/liter)	*Casuarina equisetifolia*[a]		*C. cunninghamiana*[a]		*Gymnostoma papuanum*[a]			*C. suberosa* (putative)[a]		
	Callused (%)	Rooted (%)	Callused (%)	Rooted (%)	Callused (%)	Rooted (%)		Callused (%)	Rooted (%)	
	(25 days)		(42 days)		(18 days)	(18 days)	(30 days)	(14 days)	(14 days)	(30 days)
Water control	93	0	0	0	73	0	47	0	0	0
IBA:										
25	20	60	7	3	7	67	80	0	30	60
50	20	60	0	27	0	73	80	10	40	80
100	7	20	40	13	13	73	80	0	40	90
NAA:										
25	7	27	0	27	7	53	80	9	36	55
50	33	33	13	0	13	60	60	10	60	70
100	7	27	20	7	33	47	67	0	30	50

[a] Mature softwood stem cuttings ca. 7 cm long were given 3-h soak in auxin solution, transferred to 1/4-strength Hoagland's solution minus nitrogen at pH 7; 15 cuttings/treatment. Measurements recorded after the number of days shown for each species.

Casuarina suberosa was induced to root at a range of concentrations of both IBA and NAA with maximum effectiveness at 50 mg/liter. *Gymnostoma papuanum* cuttings rooted without treatment, but the response could be increased and accelerated with plant growth substance treatment (figs. 5, 6). This species formed both callus and roots (figs. 7, 8) without auxin treatment.

ROOTING OF CUTTINGS IN TROUGHS

Large numbers of cuttings, as would be needed for clonal propagation of a specimen tree, could be rooted in hydroponic troughs (figs. 3, 4). As in earlier experiments with *C. equisetifolia,* basal callusing occurred spontaneously without auxin treatment (table 5). Rooting responses were best at low auxin concentration. Callus formation tended to be suppressed by auxin treatment in this species (see also tables 3, 4).

TABLE 5

ROOTING AND CALLUS FORMATION ON STEM CUTTINGS OF *CASUARINA EQUISETIFOLIA* PROPAGATED IN TROUGH CULTURE IN THE GREENHOUSE

Treatment (mg/liter)	Callused (%)[a]	Rooted (%)
Water control	80	7
IBA:		
25	7	80
50	13	60
100	0	40
NAA:		
25	0	53
50	0	53
100	7	40

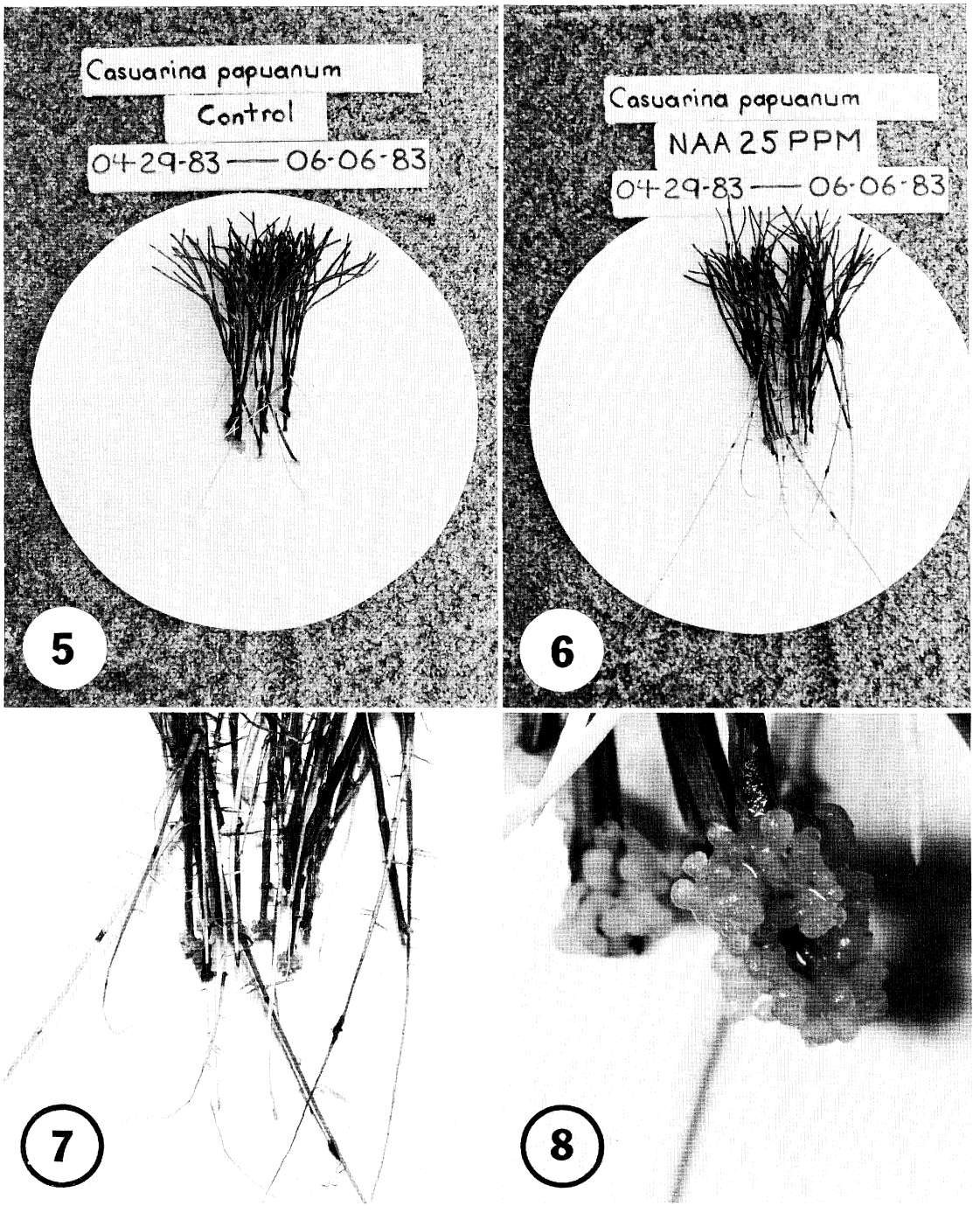
[a] Mature softwood stem cuttings ca. 7 cm long were given a 3-h soak in auxin solution, rinsed, and placed in trough with 1/4-strength Hoagland's solution minus nitrogen at pH 7 with aeration; 15 cuttings/treatment. Measured after 30 days.

INOCULATION TRIALS WITH ROOTED CUTTINGS

Rooted cuttings of *Casuarina* usually formed one or two major vigorous adventitious roots, which in turn formed numerous short lateral roots. When placed in pots, the main roots were bent around to fit the pot (fig. 2). Successful inoculation of the roots could be achieved by dipping the roots into a nodule suspension or a suspension of pure cultured *Frankia*. Using two separate series of rooted cuttings from two different plants of *C. equisetifolia,* inoculation with nodule suspensions and with Zhang's isolate HFPCcI3 resulted in nodulation rates of 83% and 89%, with a mean number of nodules of 4.1 in the first series and 10.7 in the second. Numbers of nodules per plant at 3 mo after inoculation varied from one per plant, usually quite large, to 37 nodules per plant (each quite small). Acetylene reduction trials indicated these nodules were effective in fixing dinitrogen, and the survival and growth of the nodulated cuttings confirmed this result. Rooted and nodulated cuttings could be established readily and in good numbers by these methods.

Discussion

These experiments lead to the recommendation of a simple, inexpensive, and effective method for the vegetative propagation of softwood stem cuttings of species of the Casuarinaceae. Young softwood cuttings taken from the growing parts of mature trees of *Casuarina* spp. respond to auxin treatment with callus and/or root initiation. IBA, the common component of commercial rooting powders, was the most effective of the plant growth substances tested. The concentration and the duration of treatment for optimum response should be determined first in small lots of cuttings, prefera-



FIGS. 5–8.—Fig. 5, Rooted stem cuttings of *Gymnostoma papuanum* after nearly 6 wk in nutrient solution in water-culture jars. Seedlings callused and rooted without auxin treatment. Fig. 6, Rooted stem cuttings as in fig. 5 but with a 3-h soak in 25 mg/liter NAA before transfer to water-culture jars. Fig. 7, Closer view of callus and roots of cuttings of *G. papuanum* in fig. 6. Fig. 8, Details of callus formation on stem cuttings of *G. papuanum* nearly 6 wk after treatment. Note that callus formation is distinct from root formation.

bly in water-culture jars; then large samples can be handled efficiently and effectively. No special arrangements for bottom heating or intermittent misting are required.

Rooted cuttings after 4–6 wk typically bear two to three robust elongating roots with many laterals. These roots can be inoculated effectively with *Frankia,* using suspensions either from pure cultures or from ground nodule preparations by dipping. The cuttings can be transplanted to pots or,

in large numbers, to conical tubes or Rootrainers where the nodulated seedlings develop rapidly.

Earlier studies reviewed by TORREY (1983) were rather pessimistic about methods for propagating *Casuarina*. Air layering, whether of large or small branches, was slow and inefficient, producing low yields (SYED 1957; CHAUDHARY and RAM 1961). Using long "tender" shoots, SOMASUNDARAM and JAGADEES (1977) found plant growth substance treatment essential for successful rooting of stem cuttings, and HUSAIN and PONNUSWAMY (1980) used shoot and root suckers for propagation of male plants of *C. junghuhniana,* for which seeds were unavailable.

KONDAS (1983) described methods of vegetative propagation of *C. junghuhniana* for mass plantings in India, using 10-cm-long stem pieces in red loam under mist tents. In 45 days rooting success following treatment with rooting substances varied from 40% to 90%, depending on the time of year. CHITTACHUMNONK (1983) reported experiments in Thailand on the effectiveness of IBA treatment in rooting stem cuttings of *C. junghuhniana*. Cuttings were given 24-h auxin treatment, planted in polyethylene bags containing topsoil, and maintained at high humidity for 30 days before hardening and planting.

DIEM et al. (1983*a*) reported procedures for inoculating seeds of *C. junghuhniana* applied in nursery plantations in Senegal. Their isolate of *Frankia* strain ORS 1106 was found to nodulate effectively seedlings of *C. cunninghamiana, C. equisetifolia, C. glauca,* and *C. junghuhniana*. The same methods could be applied to rooted cuttings.

Thus, there are now available suitable methods for propagation from stem cuttings of a number of *Casuarina* spp., using plant growth substance treatment and inoculating rooted cuttings with selected pure cultures of the effective actinomycete *Frankia*. From our studies (ZHANG et al. 1984) and from those of DIEM et al. (1983*a*), other genera of the Casuarinaceae, i.e., *Allocasuarina* and *Gymnostoma* (cf. JOHNSON 1982), may require still different strains of *Frankia* than have proved useful with *Casuarina*. Until these are isolated, nodule suspensions from the appropriate host plant should prove effective in inducing root nodulation.

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