suggest that ABA treatments may be useful for in vitro preconditioning of Stage III plantlets prior to ex vitro acclimatization. More importantly, this procedure may prove useful for ex vitro establishment of problematic species. Further studies need to address the after-effects of exogenous ABA on growth and development of in vitro produced plantlets under ex vitro conditions.

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EVALUATION OF THE CULTUSAK™ IN VITRO PLANT CULTURE SYSTEM

M. E. KANE, N. L. PHILMAN AND T. M. LEE
University of Florida, IFAS
Department of Environmental Horticulture
Gainesville, FL 32611

Additional index words. aquatic plants, culture vessel, micropropagation, woody plants.

Abstract. The Falcon CultuSAK™ (CK) is a commercially available heat sealable in vitro culture system consisting of an autoclavable 1.25 ml thick gas permeable (non-porous) polyethylene sheet partitioned into five cells each having the approximate volume of a standard 150 x 25 mm glass culture tube. Comparisons were made of the in vitro growth of the aquatic-wetland plants: Cryptocoryne becketii Thwaites ex Trimen, C. lucens de Witt, Myriophyllum heterophyllum Michx., Pontederia cordata L., Orontium aquaticum L.; the woody plants: Rosa 'Red Minimo', R. 'Royal Ruby', R. 'Red Summit', Aronia arbutifolia (L.) Pers., Photinia X 'Fraseri' Dress; and strawberry Fragaria X ananassa Duchesne cultured in CKs and standard 150 x 25 mm glass culture tubes. In 73% of the species tested, no significant differences in shoot regeneration rate were observed between cultures maintained in the CKs or culture tubes. Shoot regeneration of Myriophyllum was significantly greater in culture tubes. Both Orontium and Aronia exhibited significantly greater shoot multiplication rates in the CK system. No differences in either dry weight accumulation or rooting were observed in the eleven test species. The potential application of a totally sealed system for in vitro production and marketing of aquarium plants is discussed.

In vitro plant propagation systems require that culture vessels be used which are partially sealed to maintain sterility. Complete culture vessel closure either by tight capping or the use of sealing films can reduce gas exchange and alter water availability which can adversely affect multipli-

cation rate and induce undesirable morphogenic changes (3). *In vitro* physiological disorders such as vitrification and shoot tip necrosis have been, in part, attributed to restricted aeration (2,8,12). Hence, most cultures are grown commercially in vessels with loose-fitting closures so that adequate gas exchange occurs. However, these "open" culture systems are vulnerable to bacterial and fungal contamination when mites and thrips enter the culture vessels (1). Use of totally sealed but gas-permeable *in vitro* culture systems which prevent entrance of mites and thrips but allow sufficient gas exchange could be useful to Florida's tissue culture industry.

Recently, the Falcon CultuSAK™, a totally sealed but gas-permeable *in vitro* propagation system, has been made commercially available, but little information is available on the *in vitro* growth performance of plants cultured in this system. The objective of this study was to compare the growth of 11 plant species grown *in vitro* in the CultuSAK™ system and standard glass culture tubes.

Materials and Methods

Eleven species, representing a composite of herbaceous aquatic-wetland, fruit and woody plants were used (Table 1). All species had been previously established *in vitro* as actively growing stock cultures using the specific medium for each species as described in Table 1. Stock cultures were maintained on 12 ml medium contained in 150 x 25 mm glass culture tubes covered with clear polypropylene Margenta 2-Way Caps (Margenta Corp., Chicago, IL). Falcon CultureSAK™ (CK) containers were obtained from Agristar, Inc., Conroe, TX. The CK consists of a heat sea-lable and autoclavable 1.25 ml thick semi-permeable (non-porous) polyethylene sheet (24.4 x 5.3 cm) partitioned into five cells each having the approximate volume of a standard 150 x 25 mm glass culture tube (Figure 1). The medium for each species was adjusted to its respective pH (Table 1) with 0.1 N KOH before autoclaving at 1.2 kg·cm^{-2} for 20 min at 121 C. Explants (see Table 1) of each species were obtained from established stock cultures and transferred into individual CK cells and culture tubes containing 10 ml sterile medium. After inoculation, each CK sheet was sealed 2.0 cm below the top edge with an impulse heat sealer. There were 15 replicate cultures of each plant species per vessel type. All cultures were maintained in a Percival Model I-35LL Biological Incubator (Percival Manufacturing Co., Boone, IA) at 25 ± °C under a 16-hour photoperiod per day provided by cool-white fluorescent tubes (Philips F20T12/CW) at 45 μmol·s^{-1}·m^{-2} as measured at culture level. Cultures were scored for shoot growth, rooting and dry weight accumulation after 28 days except *M. heterophyllum* cultures which were similarly scored after 21 days. Data were statistically analyzed using the General Linear Model (GLM) procedure (11).

Results and Discussion

In 73% of the species tested, no differences in shoot multiplication rate were observed between cultures propagated in the CK system and culture tubes (Table 2). Similarly, 55% of the species screened exhibited no differences in shoot length between cultures maintained in the CKs and culture tubes (Table 2). A typical growth response for each culture vessel type is exemplified by *Cryptocoryne lucens*

Table 1. Plant species, explant type and media used to compare *in vitro* growth in culture tubes and CultuSAK™ cells.

Species	Explant	Basal Medium	Growth Regulators[u]	Gelling Agent	pH
Aquatic-Wetland Species					
Cryptocoryne becketii	basal bud	LS3[z]	4.4 μM BA 5.7 μM IAA	8 g/l agar[t]	5.7
Cryptocoryne lucens	basal bud	LS3	4.4 μM BA 5.7 μM IAA	8 g/l agar	5.7
Myriophyllum hetrophyllum	1 cm shoot tip	LS3	98.4 μM 2iP	liquid	5.7
Orontium aquaticum	basal bud	LS3	8.8 μM BA 0.54 μM NAA	liquid	5.7
Pontederia cordata	basal bud	LS3[y]	8.8 μM BA 5.7 μM IAA	8 g/l agar	5.7
Woody Species					
Aronia arbutifolia	3-node stem section	WPM3[x]	2.2 μM BA 0.57 μM IAA	8 g/l agar	5.7
Photinia X 'Fraseri'	3-node stem section	LS3	8.8 μM BA 0.54 μM NAA	8 g/l agar	5.7
Rosa 'Red Minimo'	3-node stem section	MS3[w] WPM vitamins[v,y]	2.2 μM BA 0.57 μM IAA	1.5 g/l Gelrite 4 g/l agar	5.5
R. 'Royal Ruby'	3-node stem section	MS3 WPM vitamins	2.2 μM BA 0.57 μM IAA	1.5 g/l Gelrite 4 g/l agar	5.5
R. 'Red Summit'	3-node stem section	MS3 WPM vitamins	2.2 μM BA 0.57 μM IAA	1.5 g/l Gelrite 4 g/l agar	5.5
Herbaceous Fruit Species					
Fragaria X *ananassa*	basal bud	LS3	2.2 μM BA	8 g/l agar	5.7

[z]Linsmaier and Skoog (6) medium with 30 g/l sucrose.
[y]Supplemented with 237.9 μM citric acid and 283.9 μM ascorbic acid.
[x]WPM3: Woody Plant Medium with 30 g/l sucrose.
[w]MS3: Murashige and Skoog (10) salts, 30 g/l sucrose and Woody Plant Medium vitamins (7).
[v]WPM organics: Woody Plant Medium vitamins.
[u]BA: N⁶-benzyladenine; 2iP: 2-isopentenyladenine; IAA: indole-3-acetic acid; NAA: ∝ naphthaleneacetic acid.
[t]Sigma AGAR Type A.

Fig. 1. Falcon CultuSAK™ (CK) culture system consisting of five cells each having the volume of a standard 150 x 25 mm glass culture tube. Scale bar = 1.0 cm.

(Table 2). Basal bud explants rapidly developed into clusters of basal shoots (Figure 2A and 2B). We have reported similar shoot development and multiplication rates for *C. lucens* using other culture vessels (5). Shoot multiplication in the aquatic plant *Myriophyllum heterophyllum* was greater in culture tubes (Table 2); but shoot production was ex-

tremely rapid in both culture vessel types (Figure 2C and 2D). The capacity for rapid shoot proliferation in *M. heterophyllum* has been attributed to its high capacity for both rapid axillary branching and adventitious shoot formation (4). The wetland plant *Orontium aquaticum* and the woody shrub *Aronia arbutifolia* (Figure 2F) exhibited significantly greater shoot multiplication rates in the CK system (Table 2). Of the species tested, only shoot length was greater in wetland species *O. aquaticum* and *Pontederia cordata* (Figure 2E) cultured in the CK system. Culture vessel type did not significantly affect total dry weight accumulation (Table 2) or rooting response (Table 3).

The CK system may be ideally suited for germplasm storage and maintaining the sterility of mother block cultures. Each cell of the five chambered CK has approximately the same volume as that of a 150 x 25 mm culture tube. Our results suggest that the sealed CK system can effectively be used as a replacement for culture tubes. However, the culture tube is not the standard culture vessel used for Stage II multiplication in the tissue culture industry. While culture vessel volume has a significant influence on shoot multiplication rate *in vitro* (9), additional studies are needed to compare shoot multiplication in the CK system with typically larger vessels used in the tissue culture industry for Stage II multiplication. Similarly, the

Table 2. Comparative *in vitro* growth of eleven species cultured in 150 x 25 mm culture tubes and CultuSAK™ cells for 28 days.[z]

Species	Shoot #[y]		Shoot Length (mm)[x]		Dry Weight (mg)	
	Tube	CultuSAK	Tube	CultuSAK	Tube	CultuSAK
C. becketii	4.9 a[w]	5.2 a	32.4 a	31.6 a	31.8 a	29.1 a
C. lucens	9.5 a	8.9 a	27.7 a	27.9 a	46.5 a	42.9 a
M. heterophyllum	161.5 a	127.7 b	57.7 a	57.5 a	158.6 a	154.5 a
O. aquaticum	2.8 a	9.5 b	41.2 a	56.2 b	52.7 a	50.8 a
P. cordata	20.7 a	19.1 a	80.9 a	100.9 b	148.1 a	141.8 a
A. arbutifolia	5.5 a	7.3 b	16.5 a	16.9 a	9.2 a	10.8 a
P. X 'Fraseri'	4.4 a	4.3 a	15.3 a	15.9 a	21.7 a	22.0 a
R. 'Red Minimo'	2.9 a	2.7 a	16.7 a	18.2 a	21.0 a	19.5 a
R. 'Royal Ruby'	3.3 a	3.4 a	15.4 a	12.9 b	22.9 a	22.7 a
R. 'Red Summit'	2.7 a	2.7 a	18.5 a	16.1 a	26.1 a	21.5 a
F. X *ananassa*	10.3 a	10.5 a	16.3 a	16.1 a	26.3 a	28.5 a

[z]Growth responses for *Myriophyllum heterophyllum* were recorded after 21 days.
[y]Mean response of 15 replicate cultures per vessel type.
[x]Mean length of the longest shoot per culture vessel.
[w]Values followed by the same letter for each species and culture method in each row are not significantly different; 5% level.

Table 3. Comparative *in vitro* rooting of species cultured in 150 x 25 mm culture tubes and CultuSAK™ cells for 28 days.[z]

Species	% Rooted		Root #[y]		Root length(mm)[x]	
	Tube	CultuSAK	Tube	CultuSAK	Tube	CultuSAK
C. becketii	100	100	5.5 a[w]	5.6 a	19.2 a	20.1 a
C. lucens	100	100	11.1 a	9.8 a	17.7 a	17.9 a
M. heterophyllum	40	60	2.1 a	3.4 a	1.1 a	3.1 a
O. aquaticum	73	73	1.3 a	1.0 a	2.8 a	3.6 a
P. cordata	87	60	8.0 a	4.8 a	5.7 a	3.1 a
A. arbutifolia	0	0	0.0	0.0	0.0	0.0
P. X 'Fraseri'	0	0	0.0	0.0	0.0	0.0
R. 'Red Minimo	0	0	0.0	0.0	0.0	0.0
R. 'Royal Ruby'	0	0	0.0	0.0	0.0	0.0
R. 'Red Summit'	0	0	0.0	0.0	0.0	0.0
F. X *Ananassa*	60	67	1.3 a	1.0 a	4.7 a	6.3 a

[z]Growth responses for *Myriophyllum heterophyllum* were recorded after 21 days culture.
[y]Mean response of 15 cultures per vessel type.
[x]Mean length of the two longest roots per culture.
[w]Values followed by the same letter for each species and culture method in each row are not significantly different; 5% level.
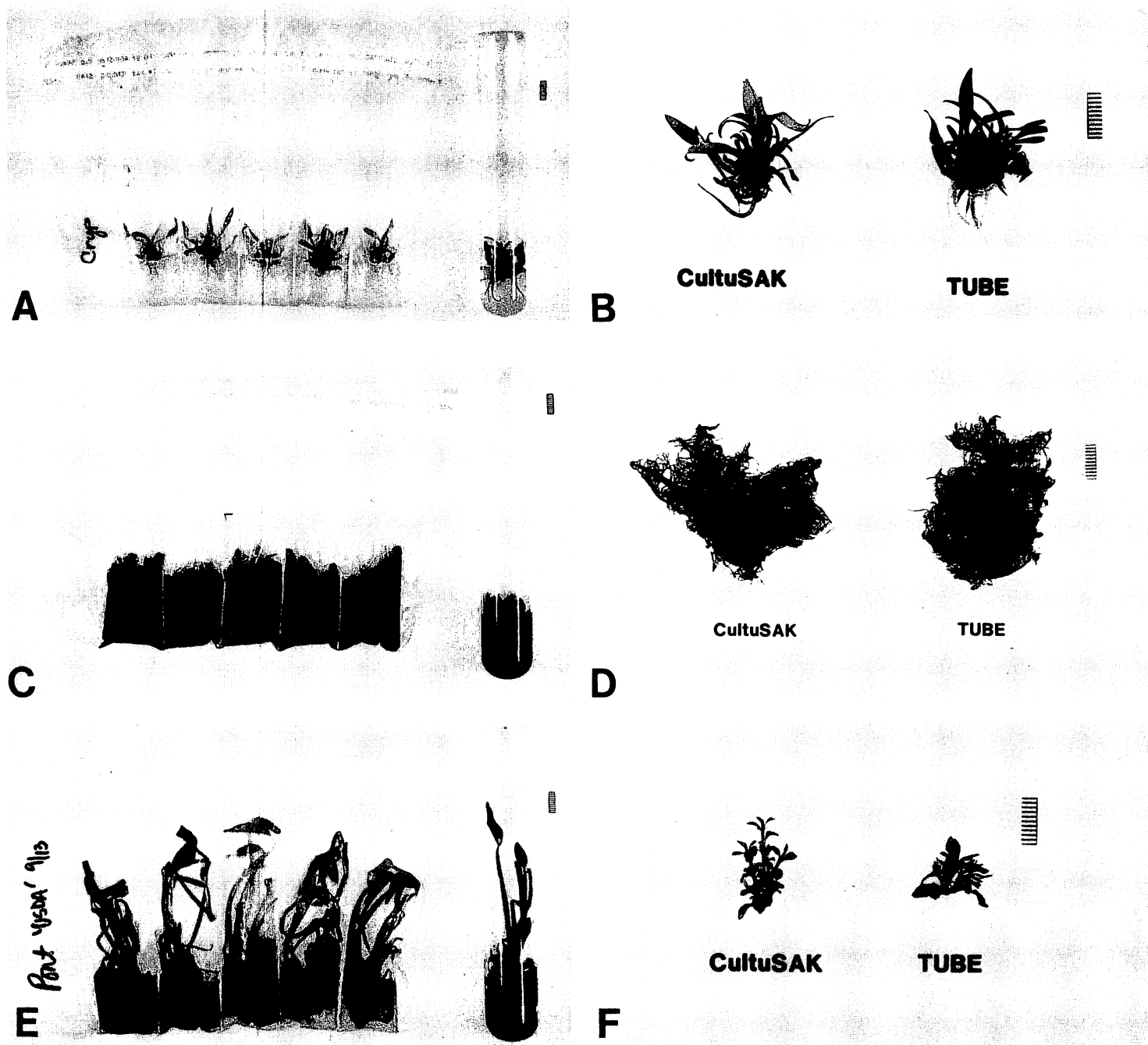
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Fig. 2. Comparative *in vitro* growth in the Falcon CultuSAK™ (CK) culture system and culture tubes. **A.** Growth of the aquarium plant *Cryptocoryne lucens* in culture vessels after 28 days. **B.** Comparative basal branching and morphology of *C. lucens* after 28 days. **C.** Growth of the aquatic plant *Myriophyllum heterophyllum* in culture vessels after 21 days. **D.** Comparative growth and morphology of *M. heterophyllum* depicting rapid axillary branching and adventitious shoot formation after 21 days. **E.** Growth of the wetland plant *Pontederia cordata* after 28 days culture. **F.** Axillary branching of the woody plant *Aronia arbutifolia* after 28 days. Scale bars = 1.0 cm.

consistency of shoot multiplication rate through repeated subculture using the CKs must be ascertained.

One limitation of the CK is that the cells are not reusable and cost $0.09 to $0.13 per cell depending on vendor contract price and quantity ordered (Fisher Scientific, Orlando, FL). We also had difficulty dispensing media into each CK cell with an automatic medium dispenser. The flexible walls of each unfilled cell tended to stick together. Following autoclaving, the CK cell walls tended to again stick together making it difficult to inoculate each CK cell with tissue. The multi-step procedure for surface steriliz-

ing and cutting the CK film prior to subculture of established cultures consumed more time than that for other culture vessels. However, we observed no culture contamination in the CK system.

Our results indicate that aquatic plants grow well in the CultuSAK™ system. One potential application for this system could be for the *in vitro* propagation, packaging and direct marketing of quality aquarium plants. Conceivably, each cell could be inoculated with a single sterile microcutting which would then develop into a larger rooted plant given the appropriate medium and culture time. Once the

plants had reached a salable size, they could be shipped *in vitro* direct to the consumer. We have observed that direct transfer of *in vitro* generated aquatic plants into the aquarium environment is possible (Kane, unpublished).

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AZADIRACHTIN FROM NEEM TREE (*AZADIRACHTA INDICA* A. JUSS.) SEEDS FOR MANAGEMENT OF SWEETPOTATO WHITEFLY [*BEMISIA TABACI* (GENNADIUS)] ON ORNAMENTALS

J. F. Price, D. J. Schuster and P. M. McClain
University of Florida, IFAS
Gulf Coast Research and Education Center
5007 60th Street East
Bradenton, FL 34203

Additional index words: natural insecticide, poinsettia, flowers, bedding plants, phytotoxicity.

Abstract. Egg, second to early third stage nymph, late third to early fourth stage nymph, late fourth stage nymph ("pupa") and adult sweetpotato whiteflies (*Bemisia tabaci* (Gennadius)) developing on poinsettia (*Euphorbia pulcherrima* Wild.) leaves were treated with Margosan-O® preparations of azadirachtin extracted from neem (*Azadirachta indica* A. Juss.) tree seeds. Single foliar spray applications of 20 ppm azadirachtin to these life stages resulted in 4.0%, 96.0%, 74.0%, 40.7% and 8.0% mortality respectively. Nymphs hatching from eggs treated with the spray were not killed. In another experiment, 20 or 40 ppm preparations were applied to second and third stage nymphs 1 to 3 times at 3-day intervals. Mortality of immature sweetpotato whiteflies was higher at 40 ppm than at 20 ppm. At 20 ppm, mortality was increased by a second application but mortality was not increased by a second application at 40 ppm. Azadirachtin (237 ml of a 20 ppm preparation) applied as a soil drench to 15 cm diameter pots of poinsettias infested with second stage nymphs did not increase mortality significantly among the insects. Four weekly applications of 28 ppm azadirachtin did not damage gerbera daisy (*Gerbera jamesonii* H. Bolus ex Hook.f.), Persian violet (*Exacum affine* Balfour), gloxinia (*Sinningia speciosa* Lodd. Hiern.) or African violet (*Saintpaulia ionantha* Wendl.). Four weekly applications of 38 ppm azadirachtin did not damage any of 5 poinsettia cultivars. Commercial azadirachtin can be a useful tool to manage sweetpotato whitefly on ornamental crops.

The azadirachtin extracted from seeds of the neem tree (*Azadirachta indica* A. Juss.) has been available for development as an insecticide for several years and properties of neem seed extracts to affect the behavior and development of insects recently have been summarized by Schmutterer (5). Effects of these extracts upon arthropods injurious to ornamentals have been reported by Knodel et al. (3), Larew et al. (4) Webb et al. (6), and others. Coudriet et al. (1) found that applications of 2% aqueous solutions of neem seed extract to sweetpotato whitefly (*Bemisia tabaci* (Gennadius)) resulted in reduced egg viability and oviposition, prolonged larval periods and larval mortality. They believed that the extracts acted as an antiecdysteroid or may have affected the neuroendocrine control of ecdysteroids. Flint and Parks (2) found that 160 ppm azadirachtin applied in aqueous sprays to sweetpotato whitefly on cotton resulted in 60% reductions in numbers of immatures, but at 20 ppm sprays were ineffective.

A commercial preparation of azadirachtin, Margosan-O (Grace-Sierra, Fogelsville, PA.), is registered for use on ornamental crops. This paper reports research conducted in 1988 and 1989 to determine the usefulness of the commercial preparation of azadirachtin for management of sweetpotato whitefly on poinsettia (*Euphorbia pulcherrima* Wild.) and other ornamental crops.

Materials and Methods

General. Insects used in these experiments were sweetpotato whiteflies from a laboratory colony held for ca. 2 yr