

The Use of an Agar Substitute in the Initial Growth of Boston Ferns *in Vitro*¹

Ron C. Cooke

Bailey's Nursery, Inc., Lodi, CA 95240

Additional index words. *Nephrolepis exaltata*, tissue culture

Abstract. There was no significant difference in growth (fresh weight) of runner tips of *Nephrolepis exaltata* (L.) Schott var. *bostoniensis* Davenport cultured on a modified Murashige and Skoog medium solidified by either agar or a new starch co-polymer agar substitute. The agar substitute requires no boiling to dissolve and appears to promote more shoot initiation than agar solidified medium.

In the large scale propagation of ferns and other plants by tissue culture procedures the solidification of medium by agar can become costly and time consuming due to the necessity of dissolving the agar by heating. It has been noted that agar may have toxic effects in the culture of certain plants (2). The use of liquid culture or support means in liquid culture is an option but may not lead to success. The search for an agar substitute is therefore a viable alternative. This study describes the preliminary work using powdered agar substitute that dissolves and gels without heating.

Runner tips (25 mm) were removed from Boston ferns and surface sterilized for 15 min in a 10% aqueous dilution of liquid Clorox (5.25% sodium hypo-

chlorite) plus 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate). Individual tips were cut to 5 mm length and rinsed with sterile tap water and placed flat on solidified medium in 25 × 150 mm test tubes. The medium contained the inorganic salts of Murashige and Skoog (1) plus the following (per liter): NaH₂PO₄·H₂O, 170 mg; sucrose, (cane) 30 g; myo-inositol 100 mg; thiamine HCl, 0.4 mg. The pH of the medium was adjusted to 5.7 ± 0.1 and solidified by a) agar, 9 g/liter of bacteriological grade dissolved by boiling or b) agar substitute, 12 g/liter of the powder suspended by stirring in room temp liquid medium. The agar substitute² is an insoluble graft co-polymer of a starch base material with no heavy metals except in trace quantities, as noted by conventional analytical procedures. Medium solidified by both means was dispensed to test tubes and sterilized at 1 kg/cm² (15 psi) for 15 min. The semi-solid medium containing agar substitute was somewhat difficult to dispense.

The tubes containing runner tips were put in a controlled environment of 27 ± 2°C and gro-lux lights (ca. 1 klx) for 16 hr per day. After 60 days the fern growth was removed individually from the tubes; any original runner tip was removed and discarded and the fern rinsed in distilled water, blotted dry and fresh weight recorded. The roots and aerial parts from each treatment were separated and pooled and fresh wt determined, then air dried and dry wt determined.

Thirty tubes of each treatment group were examined. On macroscopic observation it appeared that the "agar" group had more extensive root systems. The "agar substitute" group had smaller fronds, but more shoots. There was no significant difference between the fresh wt of the 2 groups ("agar" = 0.167 ± 0.023 g/tube; "agar substitute" = 0.137 ± 0.019 g/tube). The amount of root based on a fresh wt basis was "agar" = 27%, "agar substitute" = 19% with comparable results for dry wt.

The use of this agar substitute for initiation of Boston ferns from runner tips has been successful. This product may be conducive to shoot formation *in vitro*. The further use of this product for rapid shoot multiplication, rooting, growth, and final hardening *in vitro* needs to be evaluated.

Literature Cited

1. Murashige T and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15:473-479.
2. Romberger, J.A. and C. A. Tabor. 1971. The *Picea abies* shoot apical meristem in culture. I. Agar and autoclaving effects. *Amer. J. Bot.* 58:131.

¹Received for publication February 28, 1977.

²Available from Flow Laboratories, 1710 Chapman Ave., Rockville, MD 20852.

Tumid Spider Mite Control on Parlor Palm Grown in Containers¹

James A. Reinert and P. L. Neel²

University of Florida, IFAS, Agricultural Research Center, Fort Lauderdale, FL 33314

Additional index words. *Chamaedorea elegans*, *Tetranychus tumidus*, phytotoxicity, miticide

Abstract. The tumid spider mite (*Tetranychus tumidus* banks) is the most common and destructive spider mite species on palms in nursery and landscape plantings. Treatments with two applications of aldicarb, dienochlor, Tricyclohexyltin hydroxide (Dowco-213), propargite, oxythioquinox, and hexakis (beta, beta-dimethylphenethyl)-distannoxane, (SD-14114), left less than 1 mite per 3 leaflet sample on container-grown parlor palms (*Chamaedorea elegans* Mart.). This degree of control was also obtained but with phytotoxicity by chlormimeform, 0-[5-chloro-1-(methylethyl)-1H-1,2,4-triazol-3-yl] 0,0-diethyl phosphorothioate (CGA-12223), oxamyl, and S-tricyclohexyltin 0, 0-diisopropyl phosphorodithioate (R-28627). Other miticides which caused significant population reduction and no phytotoxicity were acephate and tetradifon. Treatments of chlorobenzilate, dicofol, and formetanate reduced populations significantly but resulted in plant damage.

It feeds on 70 host plants belonging to 4 plant families. This mite was originally described from Florida (3) and has been reported from Georgia (5), Tennessee (9), Louisiana (10), and westward from Texas (6), and Arizona to California (4). It has also been reported from Bermuda, Guam, Mexico, Puerto Rico, and Trinidad (8), as well as Hawaii (7).

Coconut palm (*Cocos nucifera* L.), Madagascar palm (*Chrysalidocarpus lutescens* Wendl.), Manila palm (*Veitchia merrillii* (Becc.) Moore), and parlor palm appear to be among the preferred hosts of this mite. It causes severe damage and can be a limiting factor in the quality production of *C. elegans* (Fig. 1) when this palm is grown under partial shade conditions in nurseries. This palm is widely grown in the nursery trade, and it was estimated that 84 million seedlings were produced in 1975 (12). Most of these are utilized as relatively inexpensive seedlings in dish gardens when but a few months old; however, plants only 1 m tall in pots may retail at \$40-\$50. Because it is a relatively slow-growing foliage plant,

The tumid spider mite is a serious pest of cotton (*Gossypium hirsutum* L.) throughout the south, but in Florida it is primarily a pest of palms and other ornamental plants in nurseries and landscape plantings. Saba (11) reported that

¹Florida Agricultural Experiment Station, Journal Series No. 437. Received for publication March 21, 1977.

²Associate Professor of Entomology and Assistant Professor of Ornamental Horticulture, respectively.



Fig. 1. Two-year-old, 80 cm tall, *Chamaedorea elegans*.

adequate protection of the foliage must be provided against its major pest, the tumid spider mite, throughout the production period of from 4 months to 4 years. Because most pesticide evaluations to find controls for this pest have been done on cotton (1, 2, 6) and because many of these pesticides are not presently labeled for use in the nursery industry, the following research was conducted to evaluate 15 miticides for possible use on *C. elegans* to control this mite.

Parlor palms heavily infested with tumid spider mites were obtained from a local nurseryman. Eighty 15 cm diam plastic pots containing 3 plants each 30–45 cm tall and infested with large mite populations were selected for efficacy testing. An additional 48 pots of plants each with several young developing leaves and with very few or no mites were also obtained to eval-

uate possible phytotoxicity caused by the treatments. This was necessary because mite damage was so severe on heavily infested plants that sometimes subtle phytotoxicity symptoms were masked. Three severely infested leaflets were clipped into Petri dishes from each of the 80 test pots and taken into the laboratory for counting. Mites and eggs were brushed from leaves with a leaf brushing machine (Manufactured by J. G. H. Edwards, RR-I, Okanagan Falls, B. C., Canada) and collected on greased glass discs and counted under 10 \times magnification of a dissecting microscope. Plants were then divided into 5 blocks according to sample populations of mites, and treatments were randomly assigned to plants within each block. Three of the lightly infested plants were randomly chosen to also be treated with each chemical. Test plants were sprayed to the point of runoff; twice with the appropriate miticide, once on Jan 8 and again on Jan. 22, 1976, using a 7.6 liter compressed air sprayer, except those treated with aldicarb, which was scattered on the soil surface and watered in on the same dates. Miticides and the rates tested are given in Table 1. Chemical definitions of the proprietary compounds used in the test which do not have approved common names (13) are: CGA-12223, Dowco-213,³ R-28627, and SD-14114.³

Phytotoxicity evaluations were made 3 days after each application and 21 days after the second treatment. The 3 additional low-mite infested plants as

³Dowco-213 = Plictran, SG-14114 = Vendex. The use of a trademark name does not constitute a guarantee or warranty of the product by the Univ. of Florida and does not imply its approval to the exclusion of other products that may also be suitable.

well as the 5 replicate plants for each chemical treatment were evaluated. Plant damage due to chemical injury was rated on a 0-5 scale, with 0 representing no apparent damage and 5 very severe damage. Notes on the type of damage observed were also made. Types of foliar injury symptoms included leaf burn (B), chlorosis (C), and distortion (D). A combined phytotoxicity rating for the 3 evaluations reflecting the types of damage observed during and after the treatments is reported.

All miticide treatments significantly (1% level) reduced mite populations compared to the untreated controls after 2 applications (Table 1). At 4 days after the initial application, aldicarb, CGA-12223, chlordimeform, Dowco-213, oxamyl, and R-28627 had produced significant control of mite populations. By 14 days oxythioquinox, SD-14114, and tetradifon also provided significant control but only Dowco-213, oxamyl, oxythioquinox, R-28627, and SD-14114 had reduced the egg level below 1 egg/sampled leaflet.

Populations were completely eliminated after the second application with Dowco-213, R-28627, and SD-14114, but severe plant damage resulted from the R-28627 treatments. Treatments of aldicarb, dienechlor, propargite and oxythioquinox reduced populations to less than 1 mite and 6 eggs per 3 leaflet sample with no phytotoxicity; CGA-12223, chlordimeform, and oxamyl produced equal control but caused phytotoxicity to the treated plants. Significant control was also obtained with acephate, tetradifon, chlorobenzilate, dicofol and formetanate, but the latter 3 also resulted in damage to the treated plants. An excessive number of apparently viable eggs which could result in a new population was also left

Table 1. Average number of tumid spider mites (eggs)/3-leaflet sample/plant of *C. elegans* treated with miticides on Jan. 8 and 22, 1976 (5 replicates).

Treatment	Active ingredients/ liter (g)	Mites ² (eggs) alive at days post initial treatment				Phyto- toxicity ³
		0	4	14	28	
Dowco-213 50WP	0.3	26.8(43.8)	6.4(13.2)a	0.8(2.8)a	0(0)a	0
R-28627 2EC	0.6	27.2(37.4)	6.8(13.0)a	3.8(2.0)ab	0(0)a	4B,C
SD-14114 50WP	0.3	36.0(69.6)	9.0(15.4)ab	4.2(2.6)ab	0(0)a	0
Oxamyl 2EC	0.6	32.2(71.0)	3.8(13.8)a	1.2(2.4)a	0.2(0)a	3C,D
Dienochlor 50WP	0.6	27.2(43.4)	13.4(20.8)abc	8.2(10.8)abc	0.2(0)a	0
Oxythioquinox 25WP	0.3	28.4(40.8)	10.2(26.4)ab	3.0(1.4)ab	0.4(0)a	0
CGA 12223 2EC	1.2	27.2(26.8)	6.0(13.0)a	5.6(5.4)ab	0.6(0)a	4B,D
Propargite 30WP	1.2	34.2(59.4)	24.8(33.0)c	28.6(17.2)d	0.4(0.4)a	0
Aldicarb 10G	9.0 ^x	26.6(61.0)	4.0(9.2)a	3.6(8.0)ab	0.8(1.4)a	0
Chlordimeform 4EC	0.6	27.8(61.8)	6.6(15.6)a	8.4(17.6)abc	0.8(5.4)a	3B,C
Chlorobenzilate 4EC	0.6	29.0(24.6)	9.8(14.0)ab	9.0(25.2)abc	1.8(4.8)a	3B,C
Tetradifon 50WP	0.3	28.8(35.2)	19.6(18.6)bc	7.2(45.4)ab	3.0(22.0)a	0
Dicofol 18.5%EC	0.6	26.2(31.4)	18.8(23.6)bc	20.0(47.0)bcd	6.2(11.0)ab	2B,C
Acephate 75WP	1.2	25.8(60.8)	14.4(15.2)abc	21.8(29.2)bcd	8.0(10.6)ab	0
Formetanate 95WP	0.6	26.8(31.6)	11.0(18.8)ab	8.6(16.0)abc	14.8(20.0)b	1C
Untreated Check	0	26.8(32.2)	17.4(15.2)bc	24.6(34.0)cd	33.4(32.4)c	0

²Mite mean separation in columns by Duncan's multiple range test, 1% level.

³0 = no observed phytotoxicity; 1 = very slight; 2 = slight; 3 = moderate; 4 = severe; and 5 = very severe damage. B = leaf burn; C = chlorosis; D = distortion.

^xAldicarb applied at rate of 3 kg a.i./ha.

by these 4 treatments. Egg numbers (Table 1) were not statistically compared but were included because of their potential to produce new mite infestations. Some acaricides are effective on eggs (ovicides), thus it was not known how many eggs were viable, and therefore remaining living mites were considered to be the most valid index of miticidal activity.

C. elegans appears to be very sensitive to many miticides and care should be taken to use an effective but non-phytotoxic miticide when treating this palm for tumid spider mites. *C. erumpens* H. E. Moore and *C. seifrizii* Burrett are being grown in increasingly large numbers by many Florida foliage nurseries because of their more rapid rate of growth as well as for their adaptability to interior environments. Although this work was done with but a single species, the other *Chamaedorea* species might be expected to react similarly to these

pesticides, considering their similar cultural requirements to *C. elegans*.

Literature Cited

1. Abid, M. K., and R. L. Ridgeway. 1969. Mortality, longevity, and fecundity of spider mites on cotton treated with systematic acaricides. *J. Econ. Entomol.* 62: 13-16.
2. Abo-El-Ghar, M. R., and H. B. Boudreaux. 1958. Comparative response of five species of spider mites to four acaricides. *J. Econ. Entomol.* 51:518-522.
3. Banks, N. 1900. The spider mites of the United States (*Tetranychus* and *Stigmaeus*). *USDA Div. Entomol.; Tech. Ser. Bul.* 8. p. 65-77.
4. Bibby, F. F., and D. M. Tuttle. 1959. Notes on phytophagous and predatory mites of Arizona. *J. Econ. Entomol.* 52:186-190.
5. Flechtmann, C. H., and P. E. Hunter. 1971. The spider mites (Prostigmata, Tetranychidae) of Georgia. *J. Ga. Entomol. Soc.* 6:16-30.
6. Ivy, E. E., and A. L. Scales. 1954. Are cotton insects becoming resistant to insecticides? *J. Econ. Entomol.* 47:981-984.
7. Prasad, V. 1967. Biology of the predatory mite *Phytoseiulus macropilis* in Hawaii (Acarina:Phytoseiidae). *Ann. Entomol. Soc. Amer.* 60:905-908.
8. Pritchard, E. A., and E. W. Baker. 1955. A revision of the spider mite family Tetranychidae. *Mem. Ser. 2, Pac. Coast Ent. Soc.*
9. Rodriguez, J. G., H. H. Chen, and W. T. Smith. 1957. Effects of soil insecticides on beans, soybeans, cotton, and resulting effect on mite nutrition. *J. Econ. Entomol.* 50:587-593.
10. Roussel, J. S., J. C. Weber, L. D. Newsom, and C. E. Smith. 1951. The effect of infestation by the spider mite *Septanychus tumidus* on growth and yield of cotton. *J. Econ. Entomol.* 44:523-527.
11. Saba, F. 1974. Life history and population dynamics of *Tetranychus tumidus* in Florida (Acarina:Tetranychidae). *Fla. Entomol.* 57:47-63.
12. Voster, J. 1975. Palm briefs - commercial use of *Chamaedorea elegans*. *Principes* 19:149-150.
13. Wiswesser, W. J., (ed.) 1976. Pesticide index 5th ed. Entomol. Soc. Amer. College Park, Md.

HortScience. 12(4):341-342. 1977.

Conifer Tree Seedling Response to Nursery Soil Amended with Composted Sewage Sludge¹

Francis R. Gouin²

Department of Horticulture, University of Maryland, College Park, MD 20742

Additional index words. *Picea abies*, *Pinus strobus*

Abstract. Seedlings of Norway spruce (*Picea abies* (L.) Karst) produced longer stems when grown in seedbeds amended with 224 dry MT/ha screened composted sewage sludge or top-dressed with 1 MT/ha of Osmocote 18-6-12 or 112 dry MT/ha of screened composted sewage sludge applied at the end of the first growing season. More seedlings of white pine (*Pinus strobus* L.) were produced in soils amended with 112 dry MT/ha of unscreened composted sewage sludge than in soils amended with 448 dry MT/ha of screened composted sewage sludge or in the 0 treatment levels. Soils amended with composted sewage sludge had higher pH, Mg, and P level than soils top-dressed with Osmocote 18-6-12 and mulched with pine sawdust.

Woody plants generally grow best when planted in fertile, well-drained soils. Because large quantities of soil are often removed when harvesting nursery plants and little crop residue is returned, the productivity of nursery soils decreases rapidly with each crop. To

maintain their soils productive, nurserymen rely on fertilizers, green-manure crops, peat moss and other readily available sources of organic matter.

Compost made from sewage sludge and wood chips may soon become an alternate source of organic matter available to nurserymen. With increased national emphasis on cleaning up the environment, composting appears to be an efficient and safe method for recycling sewage sludge (3, 6). A previous study has shown that quality dogwood and tulip trees can be grown from seeds in soils amended with screened composted sludge without any additional fertilizer (4).

The purpose of this study was to measure the germination and growth of Norway spruce and white pine, sown in beds treated with 3 levels of screened and unscreened compost made from wood-chips and dewatered digested sewage sludge.

The study was conducted on Evesboro Loamy Sand at the Buckingham State Forest Nursery near Glen Burnie, Md. In Nov., 1973, soil samples were collected to a depth of 13 cm and screened and unscreened compost was applied at 0, 2.5, 5 and 10 cm thick (equivalent to 0, 112, 224, and 448 dry MT/ha) and rototilled to a depth of 20 cm. The compost, supplied by Maryland Environmental Services, Annapolis, was made according to procedures outlined by Willson and Walker (6). At time of application the compost had a pH of 6.9, and contained (ppm): N (9,000); P (7,100); K (1,700); S (4,000); Ca (2,600); Mg (3,000); B (27); Zn (1,000); Cd (250); Pb (320). The screened compost contained wood-chips 3 cm or less in length while the unscreened compost contained chips up to 15 cm long.

The treatments were arranged in a randomized block design consisting of 4 replicates in one continuous bed. Each treatment plot was 3 m long and 1.2 m wide. An untreated buffer zone 0.3 m long the width of each bed was allowed between treatments. After leveling the seed beds, 2 blocks were sown with 35 g of Norway spruce seed per treatment and 2 blocks with 84 g of white pine seed per treatment. The seeds were broadcasted by hand and firmed into the soil with a tractor mounted floating roller and covered with 1 cm of a mixture of 1 coarse sand:1 pine sawdust (by vol). All treatments were covered with 5 cm of loose wheat straw held in place with chicken wire. In April, 1974, the wire and straw were removed.

During the 1974 growing season, sprouting tomato seeds created a weed problem in all compost amended plots. These and other weeds were hand pulled and the plots were irrigated when necessary.

¹Received for publication March 24, 1977. Maryland Agricultural Experiment Station, scientific article No. A2294, Contribution No. 5293.

²The author gratefully acknowledges the assistance of Dr. E. Epstein and technicians of the Biological Waste Management and Soil Nitrogen Laboratory, Beltsville, Md. and Dr. J. M. Walker, formerly of that laboratory, who advised and supplied the compost; the staff at the Buckingham State Forest Nursery in growing and harvesting the seedlings and the Soil Testing Laboratory, Dept. of Agronomy, Univ. of Maryland, College Park, for soil testing analyses; and to Dr. J. Kundt, Dept. of Horticulture, Univ. of Maryland, College Park, for harvesting and grading seedlings.