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Bermudagrass Germplasm Adaptation to Natural Pest Infestation and Suboptimal Nitrogen Fertilization

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Abstract. **Bermudagrass (*Cynodon* spp.) turf in subtropical Florida normally requires higher levels of N than other grasses and frequently requires pesticide applications. Three sequential 2-year cycles of clonal selection were performed in replicated field plots to recognize bermudagrass germplasm adapted to suboptimal fertilization and natural pest infestation. Low fertility, 19 to 25 g $N \cdot m^{-2}$, was applied yearly, including the establishment phase. No nematicides, fungicides, or insecticides were applied. Severely damaging mole cricket (*Scapteriscus* sp.) populations were left uncontrolled. Among 95 clones, 4 experimentals (FB-109, PI-291586, T-72-54, and FL-2400) survived repeated cycles with relatively high turfgrass coverage and quality. Among cultivars, only 'Tifgreen-II' and 'Ormond' performed well. African introductions and artificially-induced mutants of hybrid cultivars were the best sources of adapted germplasm. Although the mechanism of this adaptation is unknown, field tests were an effective prescreening method for clonal selection.**

Bermudagrass was introduced to the United States from Africa by 1751 (9). Its value as a turfgrass was recognized in the United States by 1917 (19), and it was planted on golf courses (4) and lawns (6) in Florida at least by the 1920s. Subsequently, bermudagrass cultivars, including the vegetatively propagated hybrids 'Tifway' and 'Tifgreen', were intentionally developed through breeding (2). Considerable germplasm was evaluated in the southern United States from 1955 through 1962 (10).

Bermudagrass turf requires high maintenance, particularly in subtropical Florida, where severe pest populations frequently require control. Pest problems encountered are mole crickets (15), tropical sod webworm (12), bermudagrass stunt mite (14), nematodes (11), and weeds. Nitrogen rates that provide maximum quality of hybrid bermudagrass in warm humid climates range from 116 to 180 g $N \cdot m^{-2} \cdot year^{-1}$ (1, 16). Bermudagrass turf grown on sandy soil requires additional N to compensate for losses due to leaching (17). In a one-growing season study in north Florida (5), the highest fertilization rate used (45 g $N \cdot m^{-2} \cdot year^{-1}$) provided the highest turf quality on 'Ormond' bermudagrass, and 25 g $N \cdot m^{-2} \cdot year^{-1}$ provided the lowest turf quality.

Because of its fine-leaved texture, high density, and traffic tolerance, bermudagrass is appropriate for sports turfs and lawns. With the limited use of bermudagrass in Florida home lawns and other low-maintenance areas (8), a search among bermudagrass introductions was undertaken to discover germplasm that would establish and persist as a turf under natural pest infestation and suboptimal N fertilization.

Materials and Methods

Cycle 1, 1976–1979. Stolons of 69 introduced clones of bermudagrass were obtained from W.R. Langford (ARS/USDA, Reg. Plant Intro. Sta., Experiment, GA 30212). Most had been collected by W.W. Huffine in Zimbabwe and the Republic of South Africa. During initial expansion, 26 clones were discarded due to duplications of vegetative traits, caterpillar damage, and ungainly stature, leaving 43 clones in Cycle 1. Other clones selected for comparison included 5 (FB-prefixes) that had persisted for several years without fertilization in plots at Fort Lauderdale, and cultivars 'Tifgreen' and 'Tifway'. Sprigs of these 7 clones were obtained directly from field plots (and not prepropagated), which may have affected their early performance.

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Table 1. Field performance characteristics of bermudagrass clones in Cycle 2, Fort Lauderdale Research and Education Center, 1980–82. Each value is the mean of 4 replications.

Clone	Source group	Origin	Unmowed height (cm)	Color[y] rating	Coverage[z] (%) 2 mo	Coverage[z] (%) 11 mo	Damage by mole crickets (%)	Quality[x] (12–24 mo)
PI-291584	Cycle 1	Zimbabwe	11.0	7.1	31	48	44	7.0
FB-109	Cycle 1	Tifton 6-31	8.5	7.3	65	29	44	6.9
PI-291586	Cycle 1	Zimbabwe	8.3	7.1	70	49	47	6.9
FL-2400	Misc.	'Uganda' contam.	5.8	7.8	56	39	38	6.5
PI-290659	Cycle 1	Rep. S. Africa	11.5	6.3	48	41	53	6.1
FB-119	Cycle 1	Rep. S. Africa	7.5	6.5	69	35	53	5.7
PI-290868	Cycle 1	Rep. S. Africa	6.5	6.5	50	5	53	5.6
PI-290899	Cycle 1	Rep. S. Africa	11.8	6.1	32	35	53	5.6
PI-291964	Cycle 1	Kenya	5.8	6.6	53	13	50	4.9
Tifgreen-II	Mutant (T-72-59)	Tifgreen	5.3	7.3	70	49	53	4.8
PI-224694	Cycle 1	Africa	5.3	6.9	43	38	53	4.8
T-72-54	Mutant	Tifgreen	6.0	6.1	59	36	72	4.6
Tifway-II	Mutant (T-72-114)	Tifway	7.0	7.5	49	29	44	4.6
CPI-60751	Misc.	Rep. S. Africa	9.0	6.6	38	9	56	4.3
PI-289912	Cycle 1	Rep. S. Africa	5.8	6.5	50	13	63	4.2
PI-226056	Cycle 1	Rep. S. Africa	10.3	6.9	20	11	59	4.2
PI-290885	Cycle 1	Rep. S. Africa	3.6	8.3	35	9	53	4.2
T-72-126	Mutant	Tifway	5.8	7.5	40	23	47	4.2
FL-1964	Golf course	Florida	9.3	7.8	61	51	33	4.1
T-56	Misc.	Tifton, Ga.	13.8	6.0	18	24	59	4.1
PI-290887	Cycle 1	Rep. S. Africa	3.5	6.8	35	26	66	3.7
Ormond	Cultivar	Florida	4.5	8.3	69	23	56	3.6
Tifgreen	Cultivar	Tifton, Ga.	2.9	6.9	18	11	66	3.6
T-72-16	Mutant	Tifgreen	5.3	6.8	39	18	66	3.4
Tufcote	Cultivar	Rep. S. Africa	8.0	6.5	41	14	66	3.0
FL-1877	Naturalized	Mississippi	4.5	8.3	56	15	63	2.8
FL-2016	Naturalized	Georgia	5.0	7.5	46	18	69	2.6
T-57	Misc.	Tifton, Ga.	10.6	6.3	24	8	66	2.5
PI-290898	Cycle 1	Rep. S. Africa	9.3	2.8	21	18	84	2.4
T221X21-17	Hybrid	Tifton, Ga.	9.8	5.3	27	5	75	2.1
FL-1879	Naturalized	Mississippi	4.0	6.8	56	1	69	2.1
T221X13-7	Hybrid	Tifton, Ga.	8.3	5.8	49	6	69	2.1
Tifway	Cultivar	Tifton, Ga.	6.0	5.0	46	23	78	2.0
T-53	Misc.	Georgia	7.8	5.9	44	1	63	2.0
FL-1837	Golf course	Florida	4.9	7.3	55	10	53	2.0
T221X16-13	Hybrid	Tifton, Ga.	14.0	5.4	10	8	69	2.0
FB-57	Cycle 1	Florida	6.0	4.9	43	8	56	1.9
FL-2063	Naturalized	Georgia	4.5	8.1	37	1	69	1.9
T221X18-17	Hybrid	Tifton, Ga.	8.8	6.5	31	19	62	1.9
Everglades	Cultivar	Florida	8.0	4.5	34	9	63	1.8
FL-1896	Naturalized	Louisiana	7.0	7.1	29	3	66	1.8
PI-291585	Cycle 1	Zimbabwe	6.8	6.3	50	11	56	1.8
FL-1902	Naturalized	Louisiana	3.4	6.4	47	11	72	1.7
FL-1892	Naturalized	Louisiana	7.5	6.9	31	4	69	1.6
FL-1915	Naturalized	Texas	3.6	6.1	39	8	63	1.6
T221X12-17	Hybrid	Tifton, Ga.	8.0	6.6	31	11	72	1.6
FL-1836	Golf course	Florida	4.3	6.4	61	11	75	1.5
FL-2075	Misc.	Guatemala	3.9	6.5	52	10	81	1.5
FL-1974	Golf course	Florida	6.3	5.3	66	1	88	1.5
FL-1973	Golf course	Florida	8.3	6.5	29	4	75	1.5
Nomow	Cultivar	Alabama	2.8	8.1	39	5	63	1.4
T221X12-16	Hybrid	Tifton, Ga.	7.0	7.0	37	8	69	1.4
FL-1842	Golf course	Florida	3.5	5.8	59	21	75	1.3
FL-1916	Naturalized	Texas	6.8	6.9	28	1	72	1.3
FL-1927	Naturalized	Texas	7.5	7.5	21	4	66	1.3
FL-2418	Misc.	Puerto Rico	2.6	5.5	43	3	84	1.3
T-10	Exotic	China	4.9	7.9	47	4	69	1.3
FL-1833	Naturalized	Georgia	6.8	5.5	30	4	75	1.2
PI-291981	Cycle 1	Kenya	7.3	4.3	21	3	81	1.2
FL-1976	Naturalized	Florida	4.8	5.9	36	1	66	1.1
FL-1899	Naturalized	Louisiana	3.4	6.1	18	16	94	1.1
T-32	Golf course	Georgia	1.6	6.9	33	9	56	1.1
FL-2194	Golf course	Florida	1.4	6.1	18	1	97	1.0
Tifdwarf	Cultivar	Tifgreen	1.4	6.3	23	3	94	1.0
MSD[w]			2.1	1.0	15	26	38	1.5

[z]Estimated visually. Between 2 and 11 months after planting, severe, untreated mole cricket infestation damaged the plots.

[y]10 = most blue-green; 1 = most yellow-green.

[x]Visual appraisal of overall turf quality (i.e., coverage, deepness of color, and uniformity of habit); 1 = plot dead; 7 = acceptable; and 10 = ideal. Mean of 5 dates of evaluation.

[w]Minimum significant difference by the Waller–Duncan k ratio t test; $k = 100$, $P \approx 0.05$.

Table 2. Summary of bermudagrass sources in Cycle 2, Fort Lauderdale Research and Education Center 1980–82, and performance means for traits that showed significant source variation.

Source group	No. of clones	Unmowed height (cm)	Coverage rating (%)			Mole cricket damage (%)	Quality rating[z]	
			Preplant[y]	11 mo	16–24 mo[x]		9 mo	12–24 mo[w]
Selections, Cycle 1	17	7.6 ab[v]	74 a	23 ab	33 a	57 ab	2.8 b	4.5 a
Artificial mutants[u]	5	5.9 bc	78 a	31 a	30 a	56 b	4.1 a	4.3 a
Named cultivars	7	4.8 c	71 ab	12 bc	11 b	70 a	1.8 c	2.3 b
Hybrids[u]	6	9.3 a	56 b	9 c	6 b	69 ab	1.7 c	1.8 b
Golf course off-types	8	4.9 c	71 ab	14 bc	7 b	69 ab	1.6 c	1.7 b
Naturalized collections[t]	13	5.3 bc	54 b	7 c	5 b	70 ab	1.4 c	1.7 b
Unclassified	8	7.6	72	18	27	65	2.7	3.9
Total/mean	64	6.5	67	15	17		2.2	2.9

[z] Quality rated visually, with 1 = dead; 7 = acceptable cover, deepness of color, and uniformity of habit; 10 = ideal.
[y]Rated visually in flats prior to field planting.
[x]Mean of 4 dates of evaluation.
[w]Mean of 5 dates of evaluation.
[v]Means separated by the Waller–Duncan Bayesian k ratio t test ($k = 100$, $P \approx 0.05$). Harmonic mean for number of clones per group was 7.8. Group means based on fewer clones should be treated with caution.
[u]Obtained from G.W. Burton. Hybrids were *C. transvaalensis* T-221 X Berlin progeny.
[t]Mostly *C. dactylon* from along roadsides.

Table 3. Performance ratings of bermudagrasses in Cycle 3, Fort Lauderdale Research and Education Center, 1982–84. Each value is the mean of 4 replications except as noted.

Clone	Soil coverage[z] (%)	Color rating[y]	Quality rating[x]	
			First year	2nd year
Medium to tall selections				
PI-291586	92 a[w]	7.4 a	6.5 a	6.3 a
FB-109	70 b	6.0 ab	6.5 a	6.1 ab
Ormond	32 cd	7.6 a	6.5 a	6.0 ab
FL-2400	52 bc	4.0 b	6.0 a	6.3 a
FB-119	73 ab	6.3 a	5.4 a	6.1 a
Tifway-II[u]	25	3.8	3.8	2.9
Tifway	28 d	4.0 b	2.8 b	4.1 b
Short selections grown separately				
T-72-54	58 a			
Tifgreen-II	43 a			
Tifgreen	12 b			

[z]Mean of 2 visual estimates.
[y]Rated visually 4 weeks after application of urea. 45N–0P–0K: 10 = deepest, bluest color, uniform; 1 = most yellow-green (chlorotic), mottled.
[x]10 = maximum possible cover, deepness of color, uniformity, density, and freedom from weeds; 1 = plot dead. Means of 2 and 4 dates of evaluation for first and 2nd years, respectively.
[w]Values within clone group separated by the Waller–Duncan k ratio t test, $k = 100$, $P \approx 0.05$.
[u]Only 2 replications, planted at the same time as other cultivars, but plant material received late and increased and preconditioned in trays for a shorter time than other cultivars.

The 50 clonal bermudagrasses were sorted into coarse- (30 clones) and fine- (20 clones) textured groups. Each group was planted in late Fall 1976 in 2 completely randomized blocks with 2 × 5 m plots. Plots were maintained until Jan. 1979. Stolons were planted in shallow slits in Hallandale fine sand—a siliceous, hyperthermic, Typic Psammaquent—of high (7.2 to 7.6) pH. Plots were clipped every 7 to 10 days with a reel mower set at heights varying from 25 to 32 mm, were irrigated nightly with 6 mm, and were fertilized 7 times per year with 19N–1P–5K ($g \cdot m^{-2} \cdot year^{-1}$) plus micronutrients. The N was 100% water-soluble. The only pesticides applied were *N*-(phosphonomethyl) glycine (glyphosate) to control bermudagrass encroachment between plots and (2,4-dichlorophenoxy) acetic acid (2,4-D) to control matchweed (*Phyla nodiflora* L.). A dense infestation of bullgrass (*Paspalum setaceum* Michx.) and purple nutsedge (*Cyperus rotundus* L.) crowded out most bermudagrasses.

Cycle 2, 1980–1982. Seventeen clones were selected from Cycle 1 based on their superior turfgrass coverage. All but FB-109 and FB-57 were from Africa. Three clones (PI-289912, PI-290868, and PI-291964) had been accessioned as 'Cape Royal'. Cycle 1 selections were supplemented with new collections and cultivars (Tables 1 and 2) to total 64 clones. Mutants induced by gamma rays and hybrids were obtained from G.W. Burton (ARS/USDA, Coastal Plain Sta., Tifton, GA, 31793). Clones (except for 'Tifgreen') were propagated and grown for 6 months prior to field planting in 260 × 520 × 60-mm-deep plastic trays, with 4 completely randomized blocks. Environmental variance in planting stock thereby was controlled. Plots 1.8 × 1.8 m were each planted in May 1980 from a single tray cut into eight 120 × 120-mm plugs, and were maintained until May 1982. A rotary mower was used at 35 mm, irrigation was applied every other night at 6 mm, and fertilizer was applied in 5 increments per year, with 25N–3P–10K ($g \cdot m^{-2} \cdot year^{-1}$). Two applications of monosodium methanearsonate (MSMA) and 4 of 2,4-D were made during this cycle.

Cycle 3, 1982–1984. From Cycle 2, 4 medium- to tall-growing bermudagrass clones were selected, based on superior coverage and quality ratings, and were reevaluated in 4 completely randomized blocks, along with 3 cultivars (Table 3). Plant materials were pre-propagated in trays, as in Cycle 2, and 2 trays were planted in Oct. 1982 in each 2.4 × 7.6-m plot. There were forty-eight 70 × 70-mm plugs per plot. Plots were maintained until Oct. 1984. A rotary mower was used an average of every 11 days at heights varying from 35 to 51 mm. Plots were irrigated 6 mm every other night and were fertilized 5 times per year with 24N–3P–10K ($g \cdot m^{-2} \cdot year^{-1}$). Three combined applications of 2,4-D and MSMA were made.

In addition, 2 low-growing bermudagrass clones (Table 3) were selected based on superior coverage and quality ratings

and were planted along with 'Tifgreen' in a separate experiment. There were 4 completely randomized blocks. Plots were 3.7 × 7.6 m, each planted with 2 trays, and 25 × 25 mm plugs were planted in Dec. 1982. Plots were fertilized with 20N–4P–8K ($g \cdot m^{-2} \cdot year^{-1}$).

Evaluation and analysis. Except for measurement of unmowed plant height, all observations were based on visual estimates. Soil coverage was estimated on a whole-plot basis. Turfgrass quality was rated on a 1–10 scale, with 1 = plot entirely dead; 7 = acceptable coverage, deepness of color, and uniformity of growth habit; and 10 = ideal. Analysis of variance (ANOVA) was performed on each variable individually and clones' means were separated by the Waller–Duncan Bayesian k ratio t test ($k = 100$, $P \approx 0.05$). Multiple dates of evaluation of the same trait (e.g., turfgrass quality) were combined as split plots in time. Main (clones) and split plot (dates) treatments were considered as fixed effects. Broad-sense heritability (on a clones' means basis) was estimated from the ANOVA as the ratio of genotypic (clones) variance to total phenotypic variance. Because this method may overestimate heritability due to general environmental effects, Falconer (7) prefers to call this ''clonal repeatability''.

Results and Discussion

Bermudagrass clones differed significantly across all variables in Cycle 1 (data not shown) and Cycle 2 (Table 1). Turfgrass quality ratings were chosen as the most appropriate measure of overall adaptive performance. Although there was clone × date interaction ($P < 0.01$) in the 12- to 24-month quality ratings, and the linear component for this interaction was also highly significant, the estimated variance component for the latter effect was small (0.33) compared to the overall clones effect (2.72). Thus, the 12- to 24-month quality ratings were fairly stable, and only the overall mean (average of 5 dates of evaluation) was reported (Tables 1 and 2).

Most African introductions performed significantly better than 'Tifway' and 'Tifgreen', although only PI-291584 had a quality of 7.0 or higher (''acceptable''). The vast majority of other clones were far from acceptable (Table 1). Cycle 1 selections had the highest turfgrass quality among source groups evaluated in Cycle 2 (Table 2), which indicated considerable progress from selection. The broad-sense heritability (a determinant of future progress from clonal selection) for coverage was 86%, 87%, and 94% in Cycles 1, 2, and 3, respectively, and for quality was 89% and 82% in Cycles 2 and 3.

Almost one-third of the clones had moderate coverage (30% or better) by 2 months but less than 10% coverage from 16 to 24 months. This reduction was due largely to mole crickets, which were active during the interim period (Table 1). Late coverage and quality ratings were negatively correlated with mole cricket damage rating ($r = -0.73$ and -0.74, respectively), but only weakly correlated with nonadapative features, e.g., color ($r = 0.30$). Although tall bermudagrasses may have had a selective advantage in competition with weeds under conditions of the relatively high (35-mm) cutting height, the correlation of height and turfgrass quality was weak ($r = 0.37$) and some medium- to low-growing plants (FL-2400 and 'Tifgreen-II') were relatively highly rated.

In Cycle 3, bermudagrass selections were again superior to certain cultivar standards (Table 3). Severe mole cricket damage that occurred was left uncontrolled. Nearly complete soil coverage was obtained within 5 months for PI-291586, which also had high turfgrass quality. Quality ratings were below acceptable overall under the N levels used. Accession FL-2400 was desirable in view of its relatively short stature, and 'Ormond' had the highest color ratings, as it did in Cycle 2 (Table 1).

Pest-resistance studies independently supported the present field observations. PI-290659, FL-2400, and PI-291586, which performed well in field plots, also had the highest resistance scores in a controlled mole cricket inoculation (13). Nematode susceptibility was documented in 'Tifgreen' and 'Tifdwarf' (18), cultivars that did very poorly in the present field tests, while several other strains showed no effect from nematode inoculation.

Fertilization rates of 19 to 25 g $N \cdot m^{-2} \cdot year^{-1}$ were applied in these experiments, including the establishment phase, during which relatively high nutrient requirements exist. These rates were comparable to those used on established bermudagrass fairways in Florida (8) and were low in comparison with optimal rates determined experimentally (1, 5, 16). Bermudagrass adaptative differences may be due partly to the low N fertilization, exacerbated by the severe weed competition observed.

The superior persistence of bermudagrass turfs, grown under suboptimal N fertility and in the absence of insecticides and nematicides, is associated with inherent differences in the susceptibility of bermudagrasses to insects and nematodes. Whether this association is due to specific resistance traits, or to the generalized adaptive traits (e.g., deep root systems) demanded in a harsh environment, is of academic interest only for the purpose of clonal selection. Repeated cycles of vegetative selection in a pest-infested environment showed repeatability and adequate stability in the 2nd year of evaluation and were an excellent method for intensive prescreening of turfgrass germplasm.

A superior selected source of germplasm—e.g., hybrids or artificial mutants of the better selections—might improve the proportion of outstanding turf types. At the same time, an understanding of the adaptive relationships of low-maintenance cultivars (e.g., stem and root morphology and pest resistance) would help to refine recurrent selection approaches to such a goal. Were improvement efforts to involve hydridization and selection among recombinants, and not just clonal selection, the inheritance of and possibly the mechanism for adaptation would be essential information.

This study resulted in the recognition (3) of the outstanding coverage and relatively high overall quality (Table 1) of T-72-59, thereafter released by the USDA as 'Tifgreen-II'; but, the tall growth habit of this cultivar suggested that it would be more appropriate for use on golf course fairways rather than on greens. Outstanding selections FL-2400 and PI-291586 would have potential for golf course fairways and roughs, respectively, and the latter is rapid-spreading and might even compete well as a lawngrass.

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Carbon Dioxide Enrichment for Stimulation of Growth of in Vitro-propagated Grapevines After Transfer from Culture

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Abstract. **The hypothesis was tested that the growth of in vitro-propagated, nonrooted grape shoots (*Vitis* L. hybrid) directly after transfer from culture (ex vitro) is limited by photosynthetic C supply and that growth would be stimulated by CO_2 enrichment (CDE). Plantlets were grown for 30 days in air with 350 or 1200 ppm (v/v) CO_2 in humidified, flow-through chambers at 26°C. Destructive growth analyses were made at 0, 10, 20, and 30 days after transfer from culture to soil. CDE had no significant effect on total plant dry weight increase in the first 10 days. By 20 and 30 days, CDE-treated plants were 2 and 4 times greater in dry weight, respectively, than controls. Root growth was most improved by CDE, being almost 6 times greater than controls by 30 days. Leaf area per plant and root : shoot ratio were both doubled by CDE at 20 and 30 days. Since these results were under nonstress conditions, the use of CDE for growth stimulation needs to be evaluated under stress-hardening regimes.**

The survival and growth of in vitro-propagated plantlets after removal from culture has long been a problem for some crops. Transfer from the protected environment of sterile medium, with sugars and a saturated atmosphere, to a nonsterile medium, with no supplied C and reduced humidity has been reported to lead to plant loss (2, 4, 9, 11, 20, 23). If survival is achieved, growth rates are generally poor, leading to delays in the attainment of completely acclimatized plants (10, 23, 26). During this period, slow-growing plantlets are extremely sensitive to environmental stress, especially water stress (11).

Physiologically, the transfer from culture medium with large amounts of sugars to soil mixtures with no sugars is analogous to the transition from seed reserves to seedling photosynthesis or from stored reserves to new photosynthesis in perennials. However, this transition for in vitro plants is almost instantaneous. The conditions for photosynthetic development while on sterile medium appear to be suboptimal (10), with generally very low light (1–10% of full sunlight) (11, 12), limited gas exchange, and high levels of exogenous sugars. Carbon dioxide concentrations can fluctuate markedly in closed systems, as shown by the drop from 10,000 ppm to 500 ppm in the first hour of light reported by Abbott and Belcher (1), suggesting that plantlets in closed containers may spend most of the light period at CO_2 concentrations near the photosynthetic compensation point. After transfer from culture to soil, the concern for maintenance of high humidity may lead to another closed container and possibly a repeat of the sub-optimal CO_2 concentration at a time when photosynthesis is the only source of C.

The few actual measurements of CO_2 exchange in in vitro-cultured plants have found little or no net photosynthesis. Grout and Aston (10) found no net photosynthesis until 2 weeks after

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