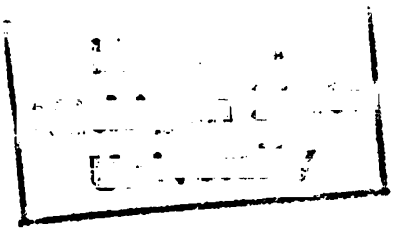

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FACTORS INFLUENCING PHENOTYPIC VARIABILITY
IN MICROPROPAGATED STRAWBERRY
(FRAGARIA x ANANASSA DUCH.) CULTIVARS

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J. Scott Cameron

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FACTORS INFLUENCING PHENOTYPIC VARIABILITY IN MICROPROPAGATED STRAWBERRY (*FRAGARIA*x*ANANASSA* DUCH.) CULTIVARS

By

J. Scott Cameron

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ABSTRACT

FACTORS INFLUENCING PHENOTYPIC VARIABILITY IN MICROPROPAGATED STRAWBERRY (FRAGARIAxANANASSA DUCH.) CULTIVARS

By

J. Scott Cameron

The field performances of progeny of micropropagated (MP) and conventionally propagated (CP) plants of Junebearing strawberry cultivars were compared in matted rows. Yields from MP rows were significantly greater for three of six cultivars; this was primarily due to higher plant density.

In a greenhouse study, detached primary, secondary, and tertiary runner plants of MP 'Redchief' and 'Earliglow' responded differently than CP plants of similar age. Total flower number was significantly greater in all asexual progeny of MP 'Redchief' plants due to increases in number of trusses and flowers per truss. Fruit size was reduced in all progeny generations of MP 'Redchief'. Anthesis was earlier in all progeny of MP 'Earliglow', and these plants had significantly greater numbers of stolons. Reduced numbers of flower trusses per plant and flowers per truss were noted in tertiary progeny of MP 'Earliglow'.

In the field, progeny of MP 'Redchief' and 'Earliglow' had higher plant densities and yields than CP plants, but partitioned 9-10 percent less of their total dry matter to leaf tissue. Leaf area per crown was significantly less in MP 'Redchief' (55 percent) and

'Earliglow' (49 percent). Yield per crown was significantly reduced in MP 'Redchief' (32 percent) but not in 'Earliglow'. Net photosynthetic (PN) rate and stomatal conductance (g_s) was greater in MP 'Earliglow' but not 'Redchief'. Mesophyll conductance (g_m) was unchanged in both cultivars.

Gas exchange behavior in relationship to increasing leaf density (leaf number per 0.093 m^2) was compared in matted rows of MP and CP 'Earliglow' and 'Redchief'. Highly significant ($p < 0.001$) negative linear correlations were observed between weight per leaf unit area and leaf density. Significant, negative linear correlations were also observed between PN, g_s, g_m and leaf density in CP cultivars but not in MP plants. In a study examining the influence of shading on MP plants of 'Redchief' and 'Earliglow' and their first and second year field grown asexual progeny, no differential effect of shading was noted. PN, g_s, g_m and chlorophyll content declined with successive generations of conventional propagation. Quantum yield as well as carboxylation efficiency and g_m response to increasing ambient CO_2 levels was greater in MP than CP 'Earliglow'; these differences were not observed in 'Redchief'.

Dedicated with love to
Nancy, Matthew and Evan

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Guidance Committee:

The journal paper format was chosen for this thesis in accordance with departmental and university regulations. The thesis is divided into four sections. Section one is intended for publication in Recent Advances in Strawberry Production. Section two is intended for publication in HortScience. Section three is intended for publication in Scientia Horticulturae. Section four is intended for publication in The Journal of the American Society for Horticultural Science.

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INTRODUCTION

The tissue culture environment can induce both mutational and epigenetic changes that affect the growth and development of plant cells, tissues and organs. While these changes can provide a rich source of variability for research and plant improvement (7,22), genetic mutations can hinder the clonal micropropagation of selected genotypes. Distinguishing between mutational and epigenetically induced changes is often difficult, although variations transmitted through meiosis are generally considered to be genetic in origin (7).

Examples of in vitro induced genetic and epigenetic variability include cell cultures, callus cultures, and regenerated plants (7,22,38). The variability generated from these systems is thought to arise from several sources including: 1) preexisting genetic differences in somatic cells, 2) mutagenic activity of culture media or of cellular constituents released by dying cells, 3) chromosomal rearrangements, 4) gene amplifications and deletions, and 5) cryptic virus elimination (38). Many plant genera exhibit some form of variability as a result of the in vitro environment (Table 1). These traits appear to represent both genetic and epigenetic events, but few of these systems have been fully characterized. While the processes involved in dedifferentiation, cycles of rapid cell division and redifferentiation introduce

Table 1. Species which have shown variation among calli (C) or regenerated plants (R).

Plant	Growth rate	Habituation; hormone requirement	Morphogenic potential	Type of morphogenesis	Morphology and growth habit	Pigmentation	Enzymes	Secondary metabolites	Disease resistance	Reproductive behavior	Reference(s)
Allium cepa	C				C	C	C				35
Allium sativum					R						32
Ananassa comosus					R						47
Atropa belladonna			C		C	C					11
Avena sativa					R					R	9
Brassica napus										R	20
Brassica oleracea			C		R						17,20
Catharanthus roseus								C			51
Citrus species		C		C							8,41
Diospyros kaki			C								50
Haplopappus gracilis						C					43
Hedera helix	C	C		C							1,29,42
Lactuca sativa					R						37
Lithospermum erythorrhizon						C					30
Nicotiana tabacum	C	C			C,R		R	C,R	R	R	12,27,28 35,40
Olea europea		C									24
Oryza sativa					R					R	19,33
Pelargonium species					R	R		R			39
Populus tremuloides		C									49
Prunus serotina	C				C						6
Saccharum species					R	R	R		R		22,23,25
Solanum laciniatum								C			52
Solanum tuberosum					R	R			R	R	36,48
Sorghum bicolor					R					R	15
Vaccinium angustifolium			C								31
Vaccinium ashei	C				C						4,5
Zea mays					R						16

opportunities for genetic changes to occur *in vitro* (22,38), the mechanism(s) underlying phenotypic alterations in clonal micropropagation systems are poorly understood.

Juvenility, or phase change, is a commonly cited example of an epigenetic phenomenon and is most visible in woody perennials (3). During the ontogeny of an individual, expression of certain developmental and morphological characters may change, including: ability to flower, leaf cuticular characteristics, leaf shape and thickness, phyllotaxis, thorniness, shoot orientation, seasonal leaf retention, stem pigmentation, ability to form adventitious roots and buds, patterns of photosynthate partitioning, disease resistance, and cold resistance (18).

Clonal micropropagation appears to induce a phase change reversion in many woody plants. Mass micropropagation systems which rely on axillary bud proliferation induce physiological changes within the initial explant which are analogous to juvenility (4,5,14). In a number of species this often involves changes in growth form and habit which precede rapid growth and axillary bud proliferation. This "*in vitro* reversion" of a mature explant does not appear to be complete, as resulting plants do not display all typical juvenile traits (18), even though seedling morphology and behavior are often induced *in vitro* (25). While epigenetic effects are often

minor and of short duration, the cultivated strawberry (Fragaria x ananassa Duch.) may be an important exception.

Phenotypic Behavior of Micropropagated Strawberry Plants

A commercially-feasible method of strawberry micropropagation was demonstrated by Boxus in 1974 (2). Since that time, commercial laboratories in the United States and Europe have produced millions of plants. Several studies have shown that micropropagated plants differ from their conventionally propagated counterparts in many ways (Table 2), and that these differences may last for at least the first growing season after culture (10,34,44).

The cause(s) of phenotypic variability in micropropagated strawberry cultivars is unknown. Genetic mutation may play a role as several discrete mutants have been identified (44); however, most populations of micropropagated plants appear uniform with few deviants. Phytohormones used in culture media may have post cultural influences on morphology and physiology as these growth regulators drastically alter the vegetative and reproductive behaviors of the strawberry in vivo (13,45) and in vitro (46). However, Marcotrigiano, et al. (26) reported that varying in vitro levels of cytokinin (benzylamino purine) did not alter subsequent stolon formation in the field. Enhanced nutritional status could

Table 2: Phenotypic behavior of micropropagated strawberry plants directly from culture compared with that of conventionally propagated plants. Sources: (10,34,44).

Increased:	Decreased:
vigor	daughter plant crown size
runner plant production	daughter plant leaf size
crown diameter	crown diameter
crown production	flowers per truss
stem diameter	yield per crown
petiole length	fruit weight
petiole/leaf length ratio	disease resistance
number of flower trusses /plant	
number of flower trusses /crown	
flowers per plant	
earliness of flowering	
earliness of fruit ripening	
yield per area	
number of fruit per unit area	
fruit production per plant	

also play a role, but no information exists on the elemental composition of micropropagated strawberries.

The strawberry is a unique test organism for characterizing the nature of culture-induced variability. It is perennial, prolific both sexually and asexually, and is an economically important horticultural crop. However, the long term affect of micropropagation beyond the first year has not been explored, and the physiological basis of enhanced vigor has not been elucidated.

The goals of this study were to: 1) determine if the effects of micropropagation endure beyond the first year and the mother plant derived from culture, and 2) determine if micropropagation alters the yield components, gas exchange properties and biomass allocation of Junebearing strawberry cultivars.

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SECTION I

THE FIELD PERFORMANCE OF STRAWBERRY NURSERY STOCK PRODUCED ORIGINALLY FROM RUNNERS OR MICROPROPAGATION

Abstract. The field performances of daughters of micropropagated (tissue culture) and conventionally propagated (runner) plants of Junebearing strawberry cultivars were compared in matted rows. Yields from micropropagated rows were significantly greater for three of six cultivars. The primary factor for the improved productivity was greater MP-derived plant density; overcrowding may have reduced fruit weight in two cultivars.

Field tests of micropropagated (tissue culture produced) strawberry plants have demonstrated their increased capacity for first-year vegetative growth and runnering compared to standard, runner propagated plants (3,7,8,9). Micropropagated plants have been suggested as a superior source of stock for nursery production, especially for shy-runnering cultivars (7,9). Previous studies have examined plants directly from micropropagation and did not test the performance of runner plants originating from micropropagated nursery stock. Since one of the primary factors limiting strawberry yields is matted row density (5), planting stock derived from micropropagated plants may provide a means for increasing plant numbers in the matted row system. The following experiment assessed the potential of micropropagation-derived plants of six Junebearing cultivars for matted row culture in Michigan.

Materials and Methods

Nursery production. Indexed, virus-free plants were obtained from the USDA Fruit Lab, Beltsville, Maryland, and were planted in the spring of 1981 in screen houses in South Deerfield, Massachusetts. These were allowed to proliferate, and their daughters were used as standard propagated plants (ST) and as stock plants for micropropagation (MP). For micropropagation, plants were moved to a greenhouse in the fall, and runner tips (5 mm long) were removed. These explants were established on standard Boxus (2) medium with no hormones. For proliferation, the standard medium was modified with 1 ppm (1.0 mg/l) benzylamino purine, 0.1 ppm (0.1 mg/l) gibberellic acid, and 1ppm (1.0 mg/l) indole-3-butyric acid. Plants were rooted directly from this proliferation phase in a greenhouse under mist during the winter.

ST plants were set in the South Deerfield nursery in the last two weeks of April, 1982. MP plants, which were more tender, were set 2 weeks later to avoid cold damage from late season frosts. The MP and ST plants were field propagated in an identical manner using standard nursery techniques including fumigation and pesticides to control aphids. Plants were grown in a random arrangement on adjacent blocks of land on a uniform Hadley fine sandy loam soil. Dormant plants were dug in the winter and cold stored at 30° F (-1° C).

Field testing. Field tests of these stocks were conducted during 1983-1984 at the Horticultural Research Center, Michigan State University, East Lansing. Dormant runner plants from the MP and ST plants were planted in a Conover loam soil. The MP plants were somewhat smaller in size than the ST plants at planting. Conventional cultural practices, including overhead irrigation (6), were used Flowers were removed in the first year (1983). The experiment consisted of a completely randomized design with four plots per propagation method per cultivar. Each plot was 12 ft (3.65 m) long with plants set initially at 1.5 ft (46 cm) in the row, and 4 ft (1.22 m) on center. Plants were trained to 1.5 ft (46 cm) wide matted rows.

Plant and crown numbers were recorded early in the 1984 season. At full bloom, total flower truss numbers per plot were recorded. Plots were harvested every 3-4 days during the period of June 18 to July 11, 1984. Plot yields and total fruit numbers were obtained, and from these data, average fruit weight and number of fruit per truss were calculated.

Results and Discussion

The field performance of the MP and ST plants of six cultivars is compared in Table 1. Overall, the MP plants produced more runner plants than ST plants, and as a result their yields were higher. There appeared to be variation in the response of cultivars to micropropagation,

Table 1. Field performance of six strawberry cultivars derived from nursery stock propagated conventionally and by micropropagation

Cultivar	Prop Method[x]	Plants per row ft	Crowns per plant	Trusses per crown	Fruit per truss	Mean fruit weight (gm)	Yield per row ft (gm)
Honeoye	ST	6.60	1.35	1.11	7.68	6.68	496.8
	MP	7.44	1.48	1.06	7.21	6.51	532.3
Earliglow	ST	5.77	1.26	1.07	7.28	6.13	344.9
	MP	8.65**[z]	1.36	1.28	6.87	5.25*	536.3*
Redchief	ST	4.48	1.68	1.43	7.76	5.81	477.3
	MP	10.85***	1.59	1.00	7.45	4.89*	623.7*
Guardian	ST	5.46	1.67	1.18	8.93	5.76	514.5
	MP	7.27	1.55	1.03	8.10	6.05	570.0
Bounty	ST	5.46	1.54	1.43	9.95	4.32	516.3
	MP	6.58*	1.74	1.30	10.96	4.51	735.4*
Scott	ST	4.21	1.64	1.40	8.85	6.32	525.1
	MP	5.77***	1.41	1.52	7.25*	5.97	526.3
Mean --- All Cultivars	ST	5.33	1.52	1.26	8.41	5.84	479.1
	MP	7.76***	1.52	1.20	7.97	5.53	587.3**

[x]Propagation Method:
ST = Standard runner-propagated
MP = Micropropagation-derived nursery stock
[y]Multiply by 0.012 to get tons per acre
[z]Propagation means significantly different, t-test
* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

as 'Earliglow', 'Redchief', 'Bounty' and 'Scott' had significant differences in plant number and/or yield, while 'Guardian' and 'Honeoye' did not. In 'Earliglow' and 'Redchief' MP rows plant density was greater than eight plants per foot of row. This amount of crowding may have reduced fruit size in these rows as it has in other studies with MP plants (9).

From a commercial grower standpoint, MP plants produced 45 percent more runners than ST mother plants. This stimulation can be used to increase first-year yields through rapid bed fill to optimum density. This can result in an increase in yield; however, MP-originated runner plants may cause overcrowding and fruit size can be reduced. Thus, wider spacing for MP-derived stock may be necessary for vigorously runnering cultivars.

No aberrant plants occurred among the first generation daughter plants, and in the second year only one 'Earliglow' 'white-streak' chimeral mutant (9) was observed in the planting. This plant was one out of approximately 4,000 descended from micropropagated plants.

Several studies have suggested that the stimulation of runner production induced by micropropagation is temporary and restricted to mother plants and their primary daughters (7,9). Our results do not agree with their observations, as randomly selected daughters from MP plants produced more plants than those from standard beds. Cold may play a role

in this difference, as the plants evaluated in other studies (7,9) were not subjected to a cold period as were the plants in this study. Other workers have indicated that cold storage may have a positive effect on MP plant performance (1,3,4).

In conclusion, MP plants were vegetatively more prolific two years after tissue culture. For some cultivars, growers can use this altered growth habit to achieve proper plant density for optimum yield in the first year while reducing nursery costs by increasing planting distance. We did not determine why some cultivars, e.g., 'Honeoye', were less responsive to micropropagation than others, but the increased variability in these rows could have been genetic or compounded by environmental variation during micropropagation or field handling.

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SECTION II

ENHANCED VIGOR IN VEGETATIVE PROGENY OF MICROPROPAGATED STRAWBERRY PLANTS

Abstract. Previous reports have indicated that the physiological influence of in vitro micropropagation is limited to mother plants and their primary runner plants. In this study, detached primary, secondary, and tertiary runner plants of micropropagated plants all responded differently than conventionally propagated plants of similar age. Total flower number was significantly greater in all runner plants of micropropagated 'Redchief' plants due to increases in number of trusses and flowers per truss. Anthesis was earlier in all runners of micropropagated 'Earliglow'. Fruit size was reduced in all runners of micropropagated 'Redchief'. Micropropagated 'Earliglow' also had significantly greater numbers of stolons. Reduced numbers of flower trusses per plant and flowers per truss were noted in tertiary runners of micropropagated 'Earliglow'.

Micropropagated mother plants of strawberry often produce more flowers and runners than their runner-propagated counterparts (4,5), but how long this effect lasts is not clear. Swartz et al (5) reported that the increase in runnering ability of tissue culture (TC) plants did not continue after the first flush of runner production in the field, and Marcotrigiano, et al. (4) showed that within the planting year, micropropagation increased runner production in mother plants and their primary runner plants, but not in attached secondary and

tertiary runner plants. However, these studies examined runner plants still attached to plants directly out of culture, rather than detached, cold-hardened plants as they are delivered to the grower. In a study where detached runner plants of unknown age were compared, planting stock from micropropagated plants of four of six cultivars had significantly higher matted row densities (x = 63 percent) than those derived from conventionally propagated stock (2). The goal of this study was to compare the performance of detached primary, secondary, and tertiary runner plants of micropropagated (MP) and standard (ST) mother plants grown under controlled conditions. MP and ST plants were produced in 1984 according to previously described methods (2). In May, MP and ST plants of the cultivars 'Earliglow' and 'Redchief' were planted in field plots 3.05 m long in a completely randomized design. Plants were spaced 0.5 m in the row and trained to matted rows 0.46 m wide and 1.22 m between rows. The site was located at the Michigan State University Horticultural Research Center on a well-drained Conover loam soil with overhead irrigation. Blossoms were removed after planting and standard cultural practices used in Michigan were maintained.

Primary, secondary, and tertiary runner plants were located in the plots in late November by tracing stolons from the mother plants. Plants were then dug, cleaned, trimmed of all leaves and stored -1° C for 14 weeks in

plastic bags. On March 12 of the following year, fresh weight, crown diameter and root system length were recorded. Plants from both propagation methods were of comparable size and fresh weight with the exception of primary runner plants of 'Redchief', which had significantly ($p < 0.01$; $df = 17$) greater fresh weights than their micropropagated counterparts (17.0 vs 11.5 g).

Ten to 15 plants from each cultivar and propagation method were then potted in 20.3 cm diameter plastic pots in a commercial peat and perlite potting mix and randomly arranged on a greenhouse bench in the Plant Science Greenhouses at Michigan State University. High pressure sodium lamps 1.5 m above the pots provided supplemental illumination of approximately 200 - 350 umol s^{-1} m^{-2} PPFD at plant level for 16 hours, and day/night temperatures were maintained at approximately 24/18° C.

After an initial evaluation on March 19, numbers of trusses, flowers, and runner plants were recorded every two to three days from March 27 until April 29 and biweekly thereafter until June 19. During flowering, pollen was applied among open flowers daily with a camel hair brush and the first 50 ripe (90 percent red) fruit from each treatment group were harvested.

MP and ST runner plants of the two cultivars differed in sexual and asexual reproductive behaviors. In 'Redchief', total numbers of flowers per plant were significantly

greater in all the runner plant generations of MP plants when compared to ST plants (Table 1). Tertiary 'Redchief' runner plants produced fewer flowers than primary ones, but the percent difference among MP and ST plants remained stable (50 - 62 percent). Fruit from all generations of MP 'Redchief' plants were significantly smaller than those from ST plants. All progeny of MP 'Redchief' bloomed later than ST plants, but the difference was significant only for primary runner plants. No significant differences in vegetative behavior were noted between ST and MP runner plants of 'Redchief', although MP runner plants generally had higher values.

Fifty percent bloom was occurred earlier in runner plants of micropropagated 'Earliglow' in all generations, but none of the other sexual traits showed consistent, significant variations between ST and MP progeny. Primary runner plants of MP plants had significanly more flowers per truss and fewer trusses than ST plants, but this difference Was not evident in tertiary runner plants. Runner plants of micropropagated 'Earliglow' produced more stolons, daughter plants per stolon and total daughter plants per plant than did conventionally propagated runner plants (Table 2), and this pattern was consistent across generations.

Previous reports have suggested that the physiological influence of micropropagation is limited to the

Table 1: Sexual responses of detached primary (1^o), secondary (2^o) and tertiary (3^o) daughter plants from standard (ST) and micropropagated (MP) mother plants of strawberry. Numbers in parentheses represent difference (%) between MP and ST plants.

Cultivar	Propagation method	Runner Plant Generation: 1^o	2^o	3^o
Trusses per plant				
Earliglow	ST	1.6	1.2	1.3
	MP	2.1 (31.3)*	1.7 (41.7)*	1.3 (0.0)
Redchief	ST	3.0	2.4	1.8
	MP	3.8 (26.7)	2.6 (8.3)	2.4 (33.3)
Flowers per truss				
Earliglow	ST	8.5	9.7	6.5
	MP	6.9 (-18.8)*	7.6 (-21.6)	6.4 (-1.5)
Redchief	ST	5.2	6.0	5.7
	MP	6.4 (23.1)*	7.7 (28.3)	7.2 (26.3)
Flowers per plant				
Earliglow	ST	13.1	11.3	8.2
	MP	14.2 (8.4)	12.8 (13.3)	7.7 (-6.1)
Redchief	ST	15.4	13.1	9.6
	MP	23.2(50.6)*[y]	19.8 (51.1)*	15.6 (62.5)*
Days to 50% bloom[z]				
Earliglow	ST	9.2	8.5	10.5
	MP	5.5 (-40.2)***	5.6 (-34.1)*	5.4 (-48.6)
Redchief	ST	10.0	10.9	11.4
	MP	13.8 (38.0)**	11.7 (7.3)	13.0 (14.0)
Fruit weight				
Earliglow	ST	6.0	6.1	4.9
	MP	6.2 (3.3)	5.7 (-6.6)	6.4 (32.0)
Redchief	ST	6.0	5.5	6.0
	MP	3.2 (-46.7)***	4.1 (-18.2)*	4.1 (-31.7)

[y]Propagation methods significantly different at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) by t-test.
[z]After planting

Table 2: Vegetative response of detached primary (1^o), secondary (2^o), and tertiary (3^o) daughter plants from standard (ST) and microprogated (MP) mother plants of strawberry. Numbers in parentheses represent difference (%) between MP and ST plants

Cultivar	Propagation method	Runner Plant Generation: 1^o		2^o		3^o	
Stolons Per Plant							
Earliglow	ST	11.9		12.9		9.3	
	MP	16.7	(39.5)*z	19.2	(48.8)***	20.6	(125.6)***
Redchief	ST	10.4		13.1		12.6	
	MP	14.5	(39.4)	14.9	(13.7)	14.3	(13.5)
Plants per stolon							
Earliglow	ST	4.3		5.0		3.9	
	MP	5.9	(37.2)*	6.1	(22.6)	5.5	(41.0)*
Redchief	ST	4.4		5.8		5.1	
	MP	6.1	(38.6)	6.6	(13.7)	6.8	(33.3)
Total runner plants							
Earliglow	ST	2.4		2.6		2.2	
	MP	3.0	(25.0)	3.2	(23.1)*	3.8	(72.7)***
Redchief	ST	2.3		2.3		2.3	
	MP	2.5	(8.7)	2.3	(0.0)	2.2	(-4.3)

zPropagation methods significantly different at $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***) by t-test.

micropropagated mother plant and primary runner plants and, as a result, only the nurseryman and not the grower would benefit from any increases in vigor due to the use of MP foundation stock (4,5). Our results do not agree with these conclusions as the runners of MP plants often behaved differently than ST plants, and most of these differences were consistent across generations. Only flowers per truss in 'Earliglow' did not fit this pattern. Not all the observed differences between MP and ST plants would benefit the grower (e.g. smaller fruit size in 'Redchief'), but the overall vigor of MP plants was generally higher.

Perhaps our results differ from those of previous workers because we examined detached, cold-hardened plants as they are delivered to the grower rather than runners attached to mother plants directly from post-culture acclimation (4,5). Damiano (3) and Aerts (1) have demonstrated that cold storage prior to planting can improve MP plant performance.

It is interesting that the two cultivars differed in their responses to in vitro propagation. Sexual responses were significantly increased in 'Redchief' by micropropagation but not asexual processes, while in general, the reverse was true for 'Earliglow'. Similar differences in responses have been noted in other studies (2,5). MP-enhanced vigor cannot last indefinitely as the ST plants used in this study were descended from plants

originally cultured *in vitro* for disease elimination. However, the data in Tables 1 and 2 imply that the MP effect can extend beyond the primary runners in the first growing season. The physiological basis of this enhanced vigor is unknown.

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SECTION III

THE INFLUENCE OF MICROPROPAGATION ON YIELD COMPONENTS, DRY MATTER PARTITIONING AND GAS EXCHANGE CHARACTERISTICS OF STRAWBERRY

Abstract. Asexual progeny of micropropagated (MP) plants of the cultivars 'Earliglow' and 'Redchief' had higher plant densities and yields than conventionally propagated (CP) plants in matted rows. MP plants partitioned nine to ten percent less of their total dry matter to leaf tissue. MP 'Redchief' increased its allocation to crowns and flower trusses; 'Earliglow' to fruit. Leaf area per crown was reduced in MP 'Earliglow' (49 percent) and 'Redchief' (55 percent), and yield per crown was lower (32 percent) in MP 'Redchief', but not in 'Earliglow'. Net photosynthetic rate (mg CO_2 dm^{-2} hr^{-1} and ug CO_2 gm dr wt^{-1} hr^{-1}) was greater in MP 'Earliglow' but not in 'Redchief'. Stomatal conductance was higher in MP 'Earliglow', but mesophyll conductance was unchanged in both cultivars. Possible roles of assimilate partitioning and demand and gas exchange behavior in MP plant performance are discussed.

Key words: Fragaria x ananassa Duchesne, micropropagation, phenotypic stability, field performance, dry matter partitioning, net photosynthesis.

INTRODUCTION

Micropropagated (MP) strawberry plants are more vigorous and productive than their conventionally-propagated (CP) counterparts (Damiano, 1980; Swartz et al., 1981). Plants

set directly from in vitro culture form more stolons in nursery beds (Marcotrigiano et al., 1984; Swartz et al., 1981), and use of planting stock derived from these beds can result in higher plant densities and increased yields (Cameron and Hancock, 1985).

The yield component interactions of MP planting stock and CP plants often vary, and differences in response have been observed between genotypes (Cameron and Hancock, 1985; Cameron et al., 1986). In a recent study, rows of MP progeny of 'Redchief' and 'Earliglow' yielded more per unit area than CP plants (Cameron and Hancock, 1985). However, MP stock of 'Redchief' produced fewer fruit per crown and lower yields per plant compared to CP stock, while no significant differences were observed between MP and CP 'Earliglow' in these traits. These results suggest that micropropagation may have genotype-specific affects on the gas exchange rates and/or allocation patterns of photoassimilates.

While the gas exchange characteristics of leaves from MP strawberry plants during ex vitro acclimization have been previously described (Grout and Millam, 1985), there have been no reports of the gas exchange properties and dry matter partitioning patterns of these plants or their progeny in the field. The purpose of this investigation was to determine the influence of micropropagation on the morphology, dry matter partitioning patterns and gas

exchange properties of field-grown 'Redchief' and 'Earliglow'; cultivars whose yield components are affected differently by micropropagation.

MATERIALS AND METHODS

The experiment was conducted at the Horticultural Research Center, Michigan State University, East Lansing. MP and CP plants of the cultivars 'Earliglow' and 'Redchief' were established in matted rows in a well-drained, Conover loam soil. Flowers were removed in the planting year (1984), and conventional cultural practices were maintained (Hull et al., 1977). The experiment consisted of a completely randomized design with four plots per propagation method per cultivar. Each plot was 3.05 m in length with plants initially set at 46 cm in the row and 1.22 m on center. Plants were trained to matted rows 61 cm wide.

Dry matter partitioning patterns were determined in one centrally located quadrat (0.29 m^2) per plot. Ripe fruit (90 percent red) were harvested every two to three days in early June, and fresh and dry weights were obtained. After harvest, plants in quadrats were dug, divided into leaves, roots, crowns and flower trusses, and dried in an oven for two weeks at 80° C.

Gas exchange, chlorophyll, and weight per leaf unit area measurements were made on single leaf samples from four plants per quadrat. Gas exchange measurements were made using an Analytical Development Corporation (ADC) CO_2

analyzer (Model LCA-2) equipped with a Parkinson broad leaf chamber. Depletion of CO_2 by a 6.25 cm^2 area of a single leaf in the chamber was monitored at a flow rate of 0.4 liter min^{-1}. Measurements were made on recently expanded leaves (2^{nd} or 3^{rd}) in the upper canopy. Readings were taken in full sunlight on a clear, sunny day at 50 percent harvest between 1000 and 1200 HR at 25 ± 1° C. Gas exchange parameters were calculated using standard formulae (Moon and Flore, 1986).

A 0.625 cm^2 leaf disk was immersed in 5 ml n,n dimethylformamide. Chlorophylls a and b and protochlorophyll content were determined using a spectrophotometric assay and formulae described by Moran (1982). Weight per leaf unit area was calculated based on the mean of two, 0.625 cm^2 leaf disks per leaf.

RESULTS

Plant and crown densities were significantly higher in MP quadrats (Table 1), but crown diameter was not influenced by micropropagation. Mean leaf number and individual and total leaf areas were significantly lower in MP plants. Leaf area index wassignificantly greater in MP 'Redchief', but not in MP 'Earliglow'. Neither flower trusses per crown nor fruit size were affected by micropropagation in either cultivar. Yield per quadrat was significantly greater in MP plants of both cultivars, whereas yield per plant and per

Table 1: Yield components of plants in matted row quadrats of micropropagated (MP) and conventionally propagated (CP) 'Earliglow' and 'Redchief' strawberry.

Yield component	Earliglow		Redchief	
	MP	CP	MP	CP
Vegetative				
Plants/.093 m^2	16.4**z	8.3	17.1***	5.6
Crowns/.093 m^2	21.8*	11.3	27.4***	9.4
Mean crown diameter (cm)	1.2	1.2	1.1	1.1
Individual leaf area (cm^2)	61.9*	76.1	51.2*	65.1
Leaves/crown	4.2*	6.7	3.5*	6.5
Leaf area/crown (m^2)	0.026*	0.051	0.018*	0.04
Leaf area index (m^2 leaf area/ .093 m^2 row)	6.1	6.2	5.3*	4.3
Reproductive				
Flower trusses/crown	1.2	0.9	1.1	1.2
Individual fruit weight (g)	4.9	4.7	4.7	4.5
Fruit/crown	4.5	4.3	3.5*	5.5
Yield/crown (g)	22.3	20.1	16.6*	24.5
Yield/plant (g)	30.1	26.4	26.7*	44.5
Yield/quadrat (kg)	0.88*	0.39	0.80*	0.42

z*,**,*** = Propagation means within cultivars significantly different by t-test, $p < .05, .01, .001$ respectively.

crown and fruit number per crown were lower in MP 'Redchief' but not in MP 'Earliglow'.

Total dry matter accumulation was significantly lower (49 percent) in plants of MP 'Redchief' but not in 'Earliglow'; however, higher reproductive effort (percentage of dry matter in reproductive structures) was expended by MP 'Earliglow' (Table 2). Leaf dry matter accumulation was strongly influenced by micropropagation as MP plants partitioned nine to ten percent less of their total biomass into leaves than did CP plants. MP plants of 'Redchief' partitioned more of their dry matter into crowns and trusses, while MP 'Earliglow' had significantly higher allocation to fruit.

Weight per leaf unit area was significantly lower (11 - 12 percent) in MP plants than in CP plants (Table 3). No differences were noted in content of chlorophylls a, b, protochlorophyll or total chlorophyll. Higher rates of net photosynthesis were noted in only MP 'Earliglow' on both a leaf area and per leaf area dry weight basis, however, total plant assimilation was significantly lower in MP plants of both cultivars. No significant differences were found in mesophyll conductance (g_m) between MP and CP leaves of either cultivar, although stomatal conductance (g_s) was higher in MP 'Earliglow'.

Table 2: Biomass allocation in plant structures of micropropagated (MP) and conventionally propagated (CP) 'Earliglow' and 'Redchief' strawberry

	Earliglow		Redchief	
	MP	CP	MP	CP
Leaves	37.1**	46.4	34.1**	43.9
Roots	11.4	11.1	10.3	9.2
Crowns	13.6	13.2	14.4**	11.9
Truss	5.4	4.0	7.7***	4.1
Fruit	32.4*	25.4	33.7	31.1
Reproductive effort	37.8*	29.4	41.3	35.1
Total dry matter per plant (g)	10.4	13.2	9.1*	17.7

*, **, *** = Propagation means significantly different by t-test, $p < 0.05$, 0.01, and 0.001 respectively.

Table 3: Gas exchange, weight per leaf unit area, and total chlorophyll in matted row quadrats of micropropagated (MP) and conventionally propagated (CP) 'Earliglow' and 'Redchief' strawberry.

	Earliglow		Redchief	
Parametery	MP	CP	MP	CP
Weight per leaf unit area (mg cm^{-2})	6.7**z	7.5	6.7**	7.6
Total chlorophyll (ug cm^{-2})	5.1	5.2	4.3	3.6
P_{net} (ug CO_2 gm dr $wt^{-1}hr^{-1}$)	3.2**	2.2	3.0	2.9
P_{net} (mg CO_2 $dm^{-2}hr^{-1}$)	21.3*	16.6	19.7	21.4
P_{net} (mg CO_2 $plant^{-1}hr^{-1}$)	74.2*	116.6	58.0***	144.5
g_s (millimols s^{-1} m^2)	275.*	231.	261.	256.
g_m (millimols s^{-1} m^2)	122.	93.	111.	127.

y P_{net} = net photosynthesis, g_s = stomatal conductance, g_m = mesophyll conductance.

z*, **, *** = Means within a cultivar significantly different by t-test $p < 0.05$, 0.01, 0.001, respectively.

DISCUSSION

As observed previously (Cameron et al., 1985), MP derived planting stock of both 'Earliglow' and 'Redchief' produced higher yields per unit area than CP stock. This increase was associated with higher plant densities in both cultivars. Yields per plant were significantly lower in MP than in CP 'Redchief', but not in 'Earliglow'. MP 'Redchief' also showed a dramatic (36 percent) reduction in the number of fruit per crown; fruit numbers were unchanged in MP 'Earliglow'. These differences arose even though the plant densities of MP 'Earliglow' and 'Redchief' were not significantly different.

Differences in tolerance to density have been described in other cultivars (Swartz et al., 1982; Hancock et al., 1984). Shading may have played a role as 'Redchief' had a higher leaf area index (LAI) in MP than in CP rows, while the LAI of 'Earliglow' was unchanged. Reduced light levels may have decreased flower bud initiation and fruit set in 'Redchief'(Anderson and Guttridge, 1976; Guttridge and Anderson, 1973). It is also possible that the gas exchange properties of 'Earliglow' are less sensitive to shading than are those of 'Redchief'.

MP plants of 'Earliglow' and 'Redchief' allocated a higher proportion of their total fixed carbon to reproductive structures than CP plants. MP 'Earliglow' allocated significantly more dry matter to fruit, while MP

'Redchief' allocated a significantly higher proportion of its energy to crowns and trusses. Strawberry fruit are the major sink for dry matter in the plant (Forney and Breen, 1985; Olsen et al., 1985). Forney and Breen (1985) have demonstrated that dry matter accumulation in strawberry fruit can create a greater demand for assimilates than the plant can maintain; this forces the plant to draw from reserve carbohydrates during fruiting. Consequently, assimilate demand by strawberry fruit can influence dry matter allocation to developing leaves (Forney and Breen, 1985; Schaffer et al., 1985). In this study, significant reductions (nine to ten percent) in dry matter allocation to leaf tissue in MP plants was manifested in reductions in leaf numbers, leaf area, and weight per leaf unit area. Total leaf area per crown was reduced 49 percent in MP 'Earliglow', and 55 percent in MP 'Redchief'. The presence of fruit can enhance rates of photosynthesis in strawberry (Choma et al., 1982; Walkowa et al., 1974), and may be a result of increased assimilate demand (Forney and Breen, 1985). MP 'Earliglow' had less leaf surface area than CP 'Earliglow'and yields per crown were unchanged; this may have created a greater assimilate demand and increased P_{net} in MP plants. Yield per crown was significantly ($p < 0.05$) reduced (32 percent) in MP 'Redchief', and reduced sink strength in these plants may not have been sufficient to increase assimilate demand and P_{net}.

Pnet could have been influenced by a number of other factors in this study. Stomatal function, distribution and number are important limiting factors in plant gas exchange and are altered by the in vitro environment (Donnelly and Vidaver, 1984); however, strawberry leaves developing after acclimation gradually revert tonormal anatomy and function (Fabbri et al., 1986; Grout and Millam, 1985). In a recent report (DeJong,1986), describing the effects of the presence of peach fruit on rates of P_{net}, it was concluded that this influence was primarily related to stomatal behavior; P_{net} and g_s increased but g_m was unchanged. Similarly, while no changes in g_m were observed in either cultivar in this study, increases in both P_{net} and g_s were observed in MP 'Earliglow' but not in 'Redchief'. The nature of stomatal limitations induced by fruit are poorly understood (DeJong,1986).

There are also many biochemical influences which have direct and indirect effects on P_{net}; levels or activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and regeneration of its substrate, photorespiration, and triose phosphate utilization are examples of potentially rate-limiting factors (Sharkey, 1985). While no significant differences were observed in mean rates of g_m in this study, biochemical or diffusional differences could occur in leaves of MP plants under nonfruiting conditions; gas exchange behavior of deblossomed strawberry plants is different from

that of fruiting plants (Choma et al., 1982; Forney and Breen, 1985).

The physiological basis of the phenotypic variability observed in micropropagated strawberry cultivars has not been determined. While enhanced vigor in micropropagated strawberry plants can last beyond the first year and the micropropagated mother plant (Cameron and Hancock, 1986; Cameron et al., 1985), it is not known whether there are lasting physiological changes effected in the plant; no one has demonstrated physiological or morphological changes in these plants in vivo which are directly attributable to some aspect of the in vitro environment. The ex vitro influence of in vitro applied plant growth regulators could be considered a possible cause, as relative media levels of auxins, cytokinins, and gibberellins can have a pronounced effect on the growth and development of strawberry in vitro (Waithaka et al., 1978) and in vivo (Dennis and Bennett, 1969; Tafazoli and Shaybany, 1978). However, in one study (Marcotrigiano et al., 1984), varying in vitro levels of cytokinin did not alter stolon production in the field. Other factors such as enhanced nutritional status after culture could have played a role in the difference between MP and CP plants, but no information exists on the elemental composition of micropropagated strawberry plants.

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SECTION IV

THE INFLUENCE OF LEAF DENSITY AND SHADING ON GAS EXCHANGE CHARACTERISTICS OF MICROPROPAGATED STRAWBERRY PLANTS

Abstract. Gas exchange behavior in response to increasing leaf density (leaf number per 0.093 m^2) was compared in matted rows of micropropagated (MP) and conventionally propagated (CP) 'Earliglow' and 'Redchief'. Highly significant ($p < 0.001$) negative, linear correlations were observed between weight per leaf unit area and leaf density in MP cultivars. Significant, negative linear correlations were also observed between net photosynthetic rate (P_{net}), stomatal conductance (g_s), mesophyll conductance (g_m) and leaf density in CP, but not MP cultivars. The effects of shading on potted 'Earliglow' and 'Redchief' MP plants and their first and second-year field grown runners were compared. While no differential effects of shading were observed, P_{net}, g_s, g_m, transpiration (E) and chlorophyll content declined with successive seasons of field propagation. Leaf number and surface area, petiole length, and weight per leaf unit area were less in MP plants compared to their asexual progeny. Quantum yield, carboxylation efficiency, and g_m response to increasing ambient CO_2 levels were greater in MP versus CP 'Earliglow'; these differences were not observed in 'Redchief'. Higher maximum rates of P_{net} were observed in deblossomed MP cultivars compared to their field-propagated progeny.

Micropropagated (MP) strawberry cultivars commonly have higher yields per acre in matted rows than their conventionally propagated (CP) counterparts due to higher plant densities (4,5,21). However, these higher densities can reduce individual plant yields (4,5). One limitation to plant productivity imposed by high plant density could be a reduction in the penetration of photosynthetically active radiation through the leaf canopy (6,1,10). The influence of shading on leaf architecture, dry matter accumulation, chlorophyll content and gas exchange have been studied in apple (2), peach (14), sour cherry (19), Fragaria virginiana Duchesne, the meadow strawberry (13), and Fragaria vesca, the woodland strawberry (6,7). Shaded plants have thinner leaves, lower weight per leaf unit area, more chlorophyll, and lower rates of photosynthesis.

In a previous study (4), individual plant yields remained constant in one MP cultivar ('Earliglow') at high density, while they were reduced in another ('Redchief') compared to CP plants. Progeny of micropropagated plants of both 'Earliglow' and 'Redchief' allocated 9 - 10 percent less of their dry matter to leaves. These plants had only half the leaf area of CP plants due to

reduced leaf numbers and areas. Alterations in yield component relationships (4,21), photoassimilate allocation patterns, or gas exchange properties (4) could have played a role in the greater tolerance of MP 'Earliglow' to higher plant densities. The purpose of this study was to determine whether leaf density and shading have a differential effect on the gas exchange properties of MP and CP 'Earliglow' and 'Redchief'.

Materials and Methods

Two experiments were undertaken at the Horticultural Research Center, Michigan State University, East Lansing, between 1984-1986. Experiment 1 was a field study examining the influence of leaf density on gas exchange, while experiment 2 examined the affects of shading on potted, deblossomed plants.

Plants of the cultivars 'Earliglow'(E) and 'Redchief'(R) were micropropagated (MP) and conventionally propagated (CP) as previously described (5). Throughout this discussion, subscripts of 0, 1, and 2 will be used to denote plants directly from in vitro culture (E_0, R_0), first generation asexually-propagated field progeny of micropropagated plants (E_1, R_1), and second generation field progeny (E_2, R_2). CP plants in this discussion will refer to those which have not been propagated from MP plants within the previous three seasons (E_2, R_2).

Gas exchange measurements were made using an Analytical Development Corporation (ADC) CO_2 analyzer (Model LCA-2) equipped with a Parkinson broadleaf chamber. Depletion of CO_2 by a 6.25 cm^2 area of a middle leaflet in the chamber was monitored at a flow rate of 0.4 liter min^{-1}. In Experiment 1, photosynthetic measurements were made on 2 fully expanded leaves per plant; 1 in the upper canopy and 1 in the lower canopy. In Experiment 2, one fully expanded leaf (2^{nd} or 3^{rd}) was measured from each plant. In Experiment 2, CO_2 response was measured using an open gas analysis system described previously (9,19). All gas exchange parameters were calculated using standard formulae (16).

Chlorophyll was extracted from a 0.625 cm^2 leaf disk in 5 ml n,n dimethyl formamide. Levels of chlorophylls a, b and protochlorophyll were determined using the spectrophotometric assay and formulae described by Moran (17). Weight per leaf unit area was calculated based on the mean of two, 0.625 cm^2 leaf disks per leaf.

Experiment 1. Greenhouse-acclimated E_O, R_O and dormant CP plants of both cultivars were planted in late Spring, 1984, in a well drained Conover loam soil. Flowers were removed in the planting year and conventional cultural practices were maintained (12). The experiment consisted of a completely randomized design with three plots per propagation method per cultivar. Each plot was 3.05 m in

length with plants initially set at 46 cm in the row and 1.22 m on center. Plants were trained to matted rows 60 cm wide.

When 50 percent of the fruit had been harvested in the first bearing year, seven subplots (0.093 m_2) within each plot were selected which appeared to represent a range of leaf densities. Gas exchange measurements were made on two leaves from one plant in the center of the subplot. Leaf number per subplot was recorded and correlated with dry weight per leaf unit area (WLA), chlorophyll content, net photosynthesis (PN), and stomatal (g_s) and mesophyll (g_m) conductances. Readings were taken under full sun on two consecutive, clear sunny days between 1000 and 1200 HR at 26 ± 1^{o} C.

Experiment 2. Plants of both cultivars and all propagation generations (E_0, E_1, E_2 and R_0, R_1, R_2) were potted in 20.3 cm plastic pots in a commercial peat and perlite potting mix in late spring, 1986. E_o and R_0 plants were greenhouse acclimated and all field progeny were dormant. Plants were arranged randomly on a greenhouse bench 15 cm apart. All blossoms were removed and the plants were allowed to develop three to four leaves under greenhouse conditions for hone month prior to implementing shading treatments. These leaves were tagged and not used in the experiment.

Shading treatments were created by covering a pipe frame structure with black polypropylene shade fabric (A.H. Hummert Co., St. Louis, MO) which transmitted an average of 36 percent, 21 percent, or 9 percent photosynthetically active radiation (PAR) as measured by the ADC LCA-2. A previous study using this shading structure established that ventilation prevented significant differences in temperature and relative humidity between treatments, and that the shading material reduced all wavelengths in the 380 - 750 nm range equally (14). A fourth group of plants was grown unshaded adjacent to the shading structure to achieve full solar radiation. Plants of both cultivars from all propagation treatments were divided equally among the shading treatments and arranged in a random manner.

Average gas exchange values were determined at a saturating level of PAR (1200 umol s^{-1} m^{-2}) under a high pressure sodium lamp at a temperature of 26 ± 1^{o} C. Gas exchange measurements were made on single leaves from five plants per cultivar per treatment group. Each leaf was then sampled for chlorophyll and WLA. Leaf areas, petiole length, and leaf numbers per plant were obtained from three plants per cultivar per treatment. The areas of three randomly selected leaves from each plant were measured using a LI-COR Model LI300 leaf area meter (LI-COR INC., Lincoln, Nebraska). Data from each cultivar were analyzed

as a two-way analysis of variance in a factorial experiment with mean separation by Duncan's Multiple Range Test.

Light response of plants in all treatments was measured using three plants per cultivar per treatment. One leaf per plant was subjected to a range of light levels by reducing full sunlight with neutral density filters. Six to 13 light levels were used per replication. Readings were taken adjacent to the shading structures under full sun at 28±2° C. Quantum yield was calculated for plants grown under full sun by correlating PAR levels below 250 umol s^{-1} m^{-2} with PN, as light response in this region was linear. The effect of ambient CO_2 concentration on two propagation generations of both cultivars (E_0, R_0 and E_2, R_2) grown under full sun was tested in the laboratory. Single leaflets were placed in plexiglass chambers at 25±1° C and 1000 umol s^{-1} m^{-2} PPF. Plants were first equilibrated at 330 umol mol^{-1} CO_2 and 21 percent O_2 and then exposed to various levels of CO_2 by adding CO_2 from a standard tank (5 percent) to outdoor air which had been scrubbed of CO_2 with soda lime (19). Carbon dioxide concentrations ranged from 90-420 umol mol^{-1} and were monitored on the reference side of a Beckman 865 infrared gas analyzer with the ADC LCA-2 CO_2 analyzer.

Results

Experiment 1. Highly significant ($p < 0.001$) negative linear correlations were observed between WLA and leaf

number per subplot in MP plants of both cultivars (Table 1). Significant, negative linear correlations were also observed between leaf density and P_{net}, g_s, and g_m in CP cultivars, but not in MP plants. On a dry weight per leaf area basis, net photosynthesis was positively correlated with leaf density in MP 'Earliglow', but not in MP 'Redchief' (Figure 1). No significant ($p < 0.05$) relationships between total chlorophyll or its components and leaf number per subplot were observed (data not shown).

Weight per leaf unit area decreased with increasing leaf canopy density at a greater rate in 'Earliglow' than in 'Redchief' (slopes significantly different, $p < 0.01$). Stomatal and mesophyll conductances were both positively correlated ($p < 0.001$) with P_{net} in an identical manner in both cultivars and propagation methods (data not shown).

Experiment 2. There were no significant interactions between propagation generations and shading treatments for gas exchange and chlorophyll data, thus shading treatment data were pooled and propagation generation means were compared. Gas exchange values were significantly greater for plants directly from *in vitro* culture than for field grown progeny of MP plants (Table 2). Maximum rates of photosynthesis declined with successive seasons of field propagation in both cultivars.

Differences in chlorophyll content or its components were less on a per leaf surface area basis than on a per dry

Table 1: Correlation coefficients of leaf density (leaves per 0.093 m^2) versus weight per leaf unit area and gas exchange measurements in matted rows of MP and CP 'Earliglow' and 'Redchief'strawberry.

	Earliglow		Redchief	
	MP	ST	MP	ST
Weight per leaf unit area (mg cm^{-2})	-0.69^{***}z	-0.30	-0.46^{***}	-0.23
Pnet (mg CO_2 $dm^{-2}hr^{-1}$)	-0.06	-0.47^{**}	-0.14	-0.37^{*}
g_s (cms^{-1})	-0.01	-0.43^{**}	-0.18	-0.35^{*}
g_m (cms^{-1})	-0.17	-0.50^{***}	-0.17	-0.39^{*}

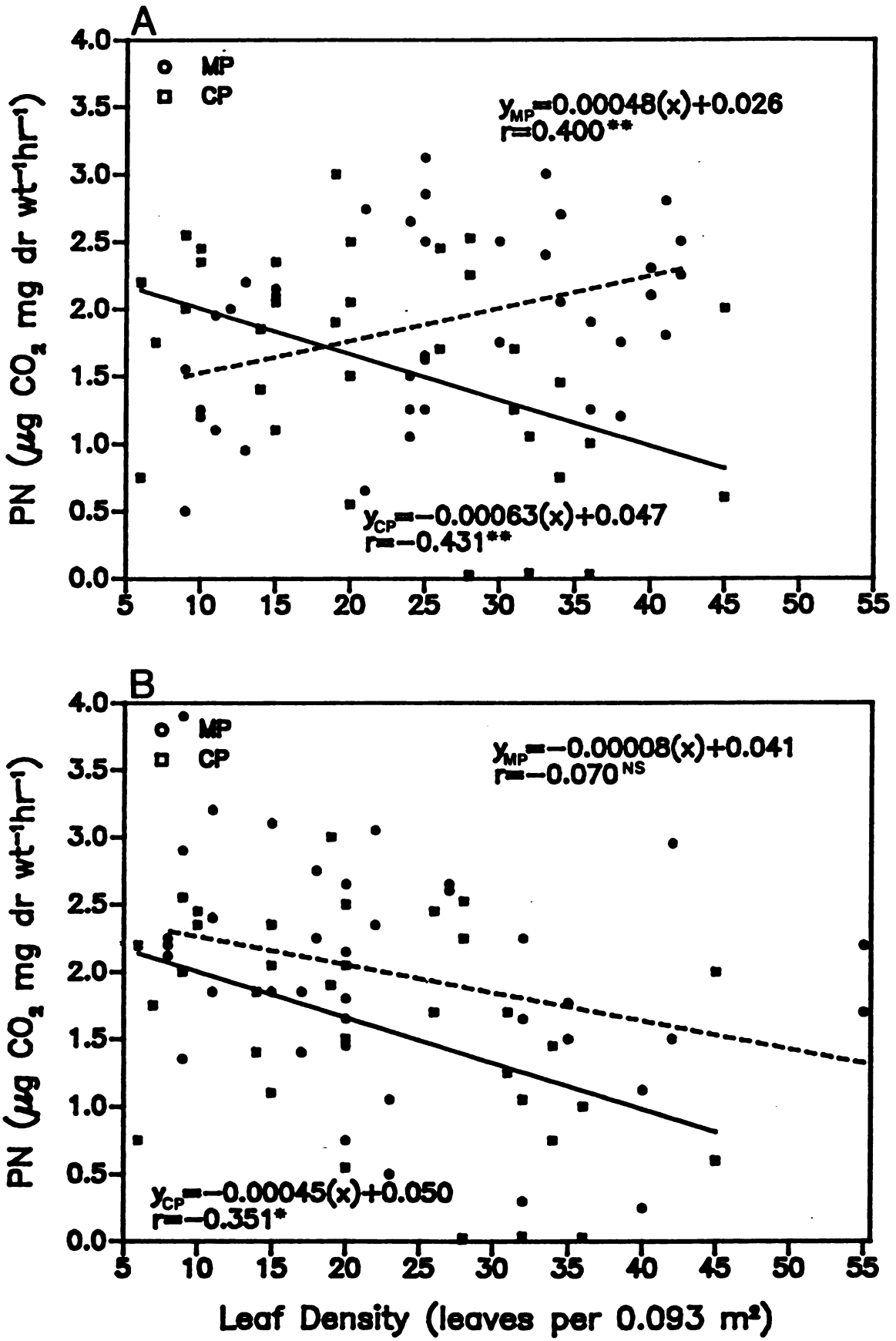
z $^{*},^{**},^{***}$ = $p < 0.05, 0.01, 0.001$, respectively, n = 42

Table 2: Gas exchange characteristics of 'Earliglow' and 'Redchief' strawberry plants of three propagation generations (PAR = 1200 umol s^{-1} m^{-2})[w]

Gas exchange Parameter[x]	'Earliglow' E_{oy}	'Earliglow' E_1	'Earliglow' E_2	'Redchief' R_o	'Redchief' R_1	'Redchief' R_2
P_{net} (mg CO2 $dm^{-2}hr^{-1}$)	21.1az	16.9b	15.0c	21.9a	17.9b	15.9c
P_{net} (ug CO2 gm dr $wt^{-1}hr^{-1}$)	5.1a	3.5b	3.0c	4.8a	3.1b	2.9b
g_s (millimols s^{-1} m^{-2})	203.2a	177.0b	171.7b	198.4a	186.4b	183.5b
E (millimols s^{-1} m^{-2})	6.7a	6.2b	6.1b	6.6a	6.2b	6.0b
g_m (millimols s^{-1} m^{-2})	50.7a	40.5b	34.7c	53.5a	42.1b	36.7c

[w]Means of 1 single leaf measurement from 5 plants per treatment.
[x]P_{net}, net photosynthesis; g_s, stomatal conductance; E, transpiration; g_m, mesophyll conductance.
[y]See explantion of abbreviations in text.
[z]Means within a row and cultivar not followed by the same letter are significantly different by DMR, $p < 0.05$.

Figure 1: Affect of leaf density (number of leaves per 0.093 m^2) on net photosynthesis (PN) on a weight per leaf unit area basis in matted rows of micropropagated (———) and conventionally propagated (----) 'Earliglow' (A) and 'Redchief' (B). Each point represents a measurement of one leaf with 21 replications per propagation treatment.


A
MP
CP
y_{MP}=0.00048(x)+0.026
r=0.400**
y_{CP}=−0.00063(x)+0.047
r=−0.431**
PN (μg CO_2 mg dr $wt^{-1}hr^{-1}$)
B
MP
CP
y_{MP}=−0.00008(x)+0.041
r=−0.070 NS
y_{CP}=−0.00045(x)+0.050
r=−0.351*
PN (μg CO_2 mg dr $wt^{-1}hr^{-1}$)
Leaf Density (leaves per 0.093 m²)

weight basis (Table 3). MP plants contained higher levels of chlorophyll and its components compared to their field grown progeny. Higher chlorophyll content could indicate a greater light harvesting capacity in MP plants. Quantum yield was significantly greater in E_0 compared to E_2 plants grown under full sun (slopes significantly different, $p < 0.05$), but no differences in quantum yield were observed between MP and CP 'Redchief' (data not shown).

The effects of shading on leaf characteristics of MP and CP plants of both cultivars are exemplified by the data presented in Table 4. MP plants had fewer leaves with less surface area, shorter petioles, and less weight per leaf unit area than their asexual progeny under shaded and nonshaded conditions (Table 4). A similar pattern was observed in a previous study (4).

Shaded plants of both cultivars had lower maximum rates of P_{net} (Table 5), and their response to increasing PAR was also similar (See Figures 2a, 3a for typical responses). Maximum rates of P_{net}, g_s and g_m were higher in MP plants than in second generation plants in both cultivars (Figure 2 and 3). Across all treatments, light compensation points were below a PAR level of 100 umol s^{-1} m^{-2} and did not appear to vary appreciably.

Response of MP and second generation plants grown under full sun to increasing levels of ambient CO_2 are shown in Figures 4 - 6. On a per leaf surface area basis,

Table 3: Chlorophyll content of leaves from 'Earliglow' and 'Redchief' strawberry plants of three propagation generations

	'Earliglow'			'Redchief'		
Pigmenty	E_{oy}	E_1	E_2	R_o	R_1	R_2
Chlorophyll a ($mg\ dm^{-2}$)	3.1a[z]	3.1a	2.8a	3.6a	3.3a	2.9b
Chlorophyll a ($ug\ mg\ dr\ wt^{-1}$)	7.5a	6.5b	5.7c	7.8a	6.0b	5.4b
Chlorophyll b ($mg\ dm^{-2}$)	1.1a	1.1a	1.1a	1.2a	1.2a	1.0b
Chlorophyll b ($ug\ mg\ dr\ wt^{-1}$)	2.5a	2.3a	2.3a	2.5a	2.1b	1.9b
Chlorophyll a/b ratio	2.9a	2.8a	2.5b	3.1a	2.9b	2.8b
Total chlorophyll ($mg\ dm^{-2}$)	4.2a	4.2a	3.9a	4.7a	4.5a	3.9b
Total chlorophyll ($ug\ mg\ dr\ wt^{-1}$)	10.0a	8.8b	8.0b	10.3a	8.1b	7.0c

[y]See explanation of abbreviations in text.
[z]Mean of 1, 0.625 cm^2 leaf disk from 5 plants per treatment averaged over 4 shading treatment levels; means within a row and cultivar not followed by the same letter are significantly different by DMRT, $p < 0.05$.

Table 4: Characteristics of leaves from 'Earliglow' and 'Redchief' strawberry plants of three propagation generations grown under 100% and 21% full sun.

	'Earliglow'			'Redchief'		
	E_o[w]	E_1	E_2	R_o	R_1	R_2
100% SUN						
Leaf number per plant[x]	4.7a[z]	6.0b	7.0c	5.7a	6.3b	7.0c
Individual leaf area (cm^2)[x]	68.9a	89.2b	88.1b	82.2a	105.0b	129.1c
Weight per leaf unit area (mg cm^{-2})[y]	6.0a	7.1b	6.9b	6.6a	8.2b	7.6b
Petiole length (cm)[x]	7.8a	10.6b	9.4b	9.2a	13.3b	14.2b
21% SUN						
Leaf number per plant	5.0a	6.0b	5.6b	5.3a	6.3c	5.8b
Individual leaf area (cm^2)	100.4a	149.0b	150.1b	75.2a	119.6b	119.2b
Weight per leaf unit area (mg cm^{-2})	3.6a	4.1a	4.4b	3.9a	5.3b	4.5ab
Petiole length (cm)	11.5a	15.6b	15.6b	12.7a	16.4b	17.3b

[w]See explanation of abbreviations in text.
[x]Mean of 3 samples from 3 plants per treatment per cultivar.
[y]Mean of 2, 0.625 cm^{-2} leaf disks per leaf, one leaf per plant; 5 replications per treatment per cultivar.
[z]Means within a row and cultivar not followed by the same letter are significantly different by DMRT, $p < 0.05$.

Table 5: Maximum rates of net photosynthesis (PN) in 'Earliglow' and 'Redchief' strawberry plants grown under different light levels. Measurements made at PAR = 1200 umol s^{-1} m^{-2} and 26±1° C

Sunlight (%)	Net photosynthesis (mg CO_2 $dm^{-2}hr^{-1}$) 'Earliglow'	'Redchief'
100y	19.8a[z]	20.3a
36	17.7b	19.5a
21	16.0c	17.8b
9	17.0bc	16.8bc

yMean of 1 single leaf measurement from 15 plants per shading treatment; averaged over 3 propagation generations.
zMeans within a column and cultivar not followed by the same letter are significantly different by DMRT, $p < 0.05$.

Figure 2: Response of gas exchange parameters of micropropagated (MP (A, C, E) and conventionally propagated (CP) (B, D, F) 'Earliglow' plants grown under full sun to different levels of photosynthetically active radiation (PAR). Gas exchange parameters: net photosynthesis (PN) (A,B), mesophyll conductance (g_m) (C, D) and stomatal conductance (g_s) (E, F). Different symbols represent replications; 6 - 10 measurements per replication.

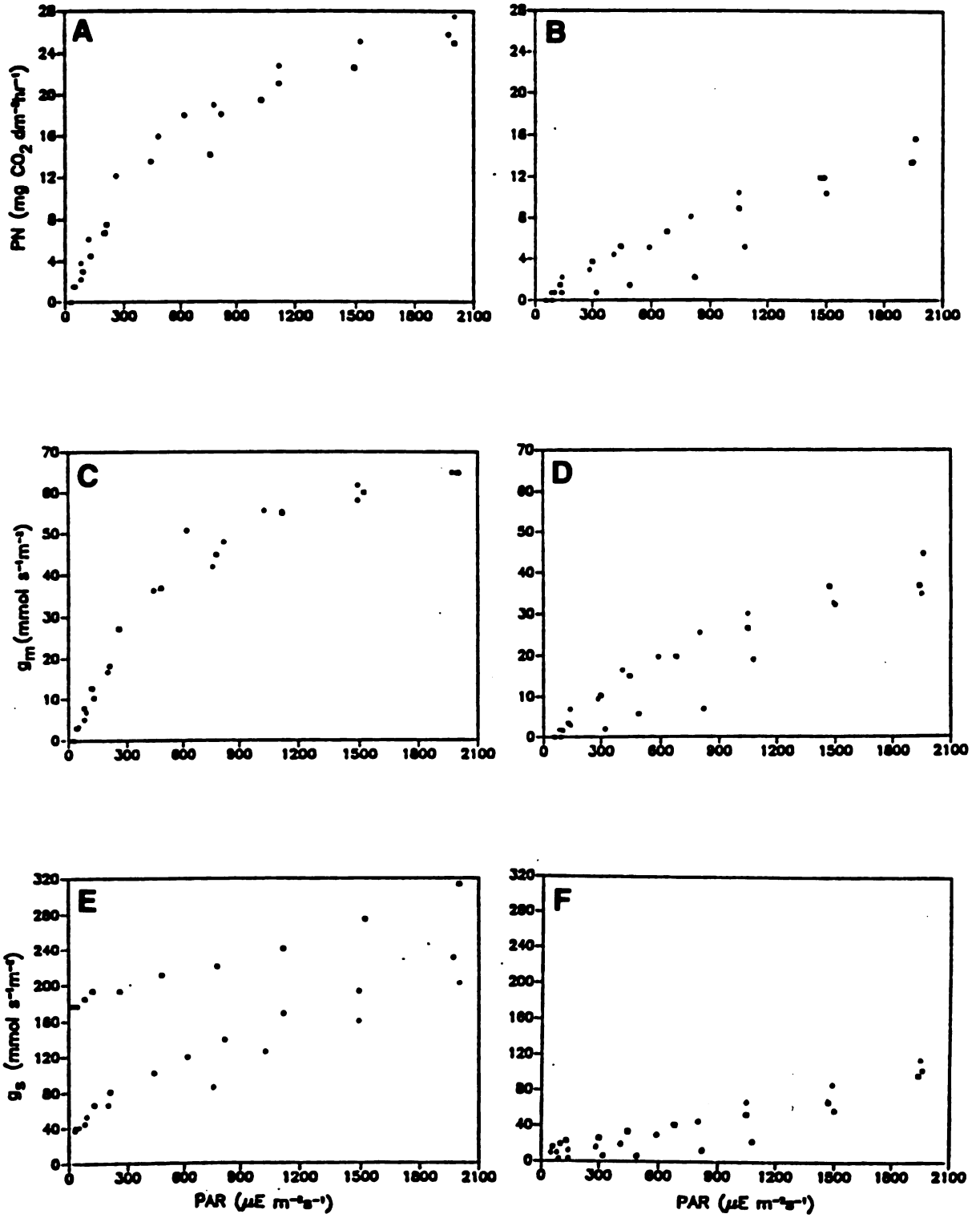

A
B
C
D
E
F
PN (mg CO_2 $dm^{-2}hr^{-1}$)
g_m (mmol $s^{-1}m^{-2}$)
g_s (mmol $s^{-1}m^{-2}$)
PAR (μE $m^{-2}s^{-1}$)
PAR (μE $m^{-2}s^{-1}$)

Figure 3: Response of gas exchange parameters of micropropagated (MP) (A, C, E) and conventionally propagated (CP) (B, D, F) 'Redchief' plants grown under full sun to different levels of photosynthetically active radiation (PAR). Gas exchange parameters: net photosynthesis (PN) (A, B), mesophyll conductance (g_m) (C, D) and stomatal conductance (g_s) (E, F). Different symbols represent replications; 6 - 10 measurements per replication.

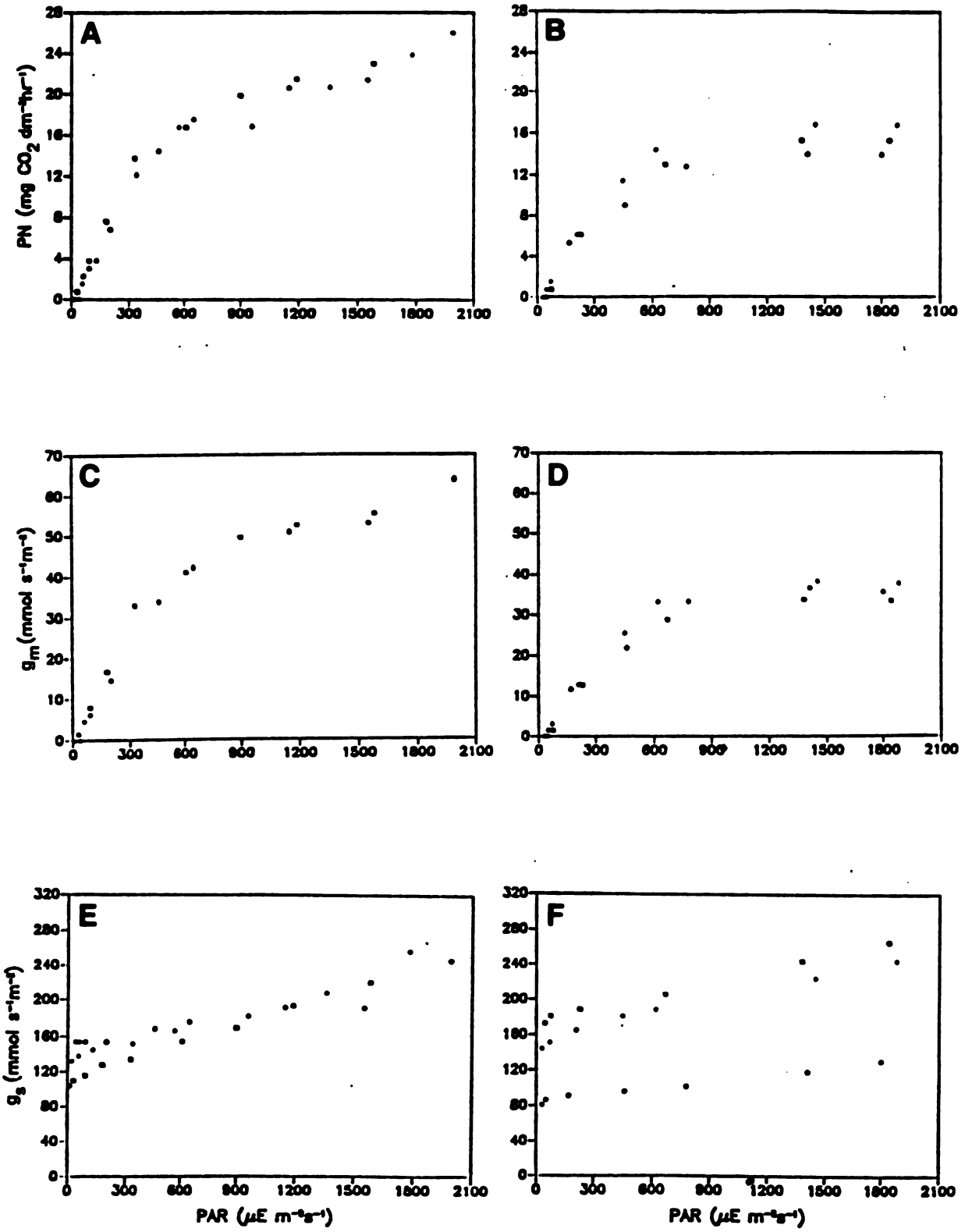

A
B
C
D
E
F
PN (mg CO_2 $dm^{-2}hr^{-1}$)
g_m (mmol $s^{-1}m^{-2}$)
g_s (mmol $s^{-1}m^{-2}$)
PAR (μE $m^{-2}s^{-1}$)
PAR (μE $m^{-2}s^{-1}$)

Figure 4: Response of net photosynthesis (mg CO_2 dm^{-2} hr^{-1}) to ambient CO_2 level in micropropagated (----) and conventionally propagated (———) 'Earliglow' (A) and 'Redchief' (B) potted plants grown under full sun.

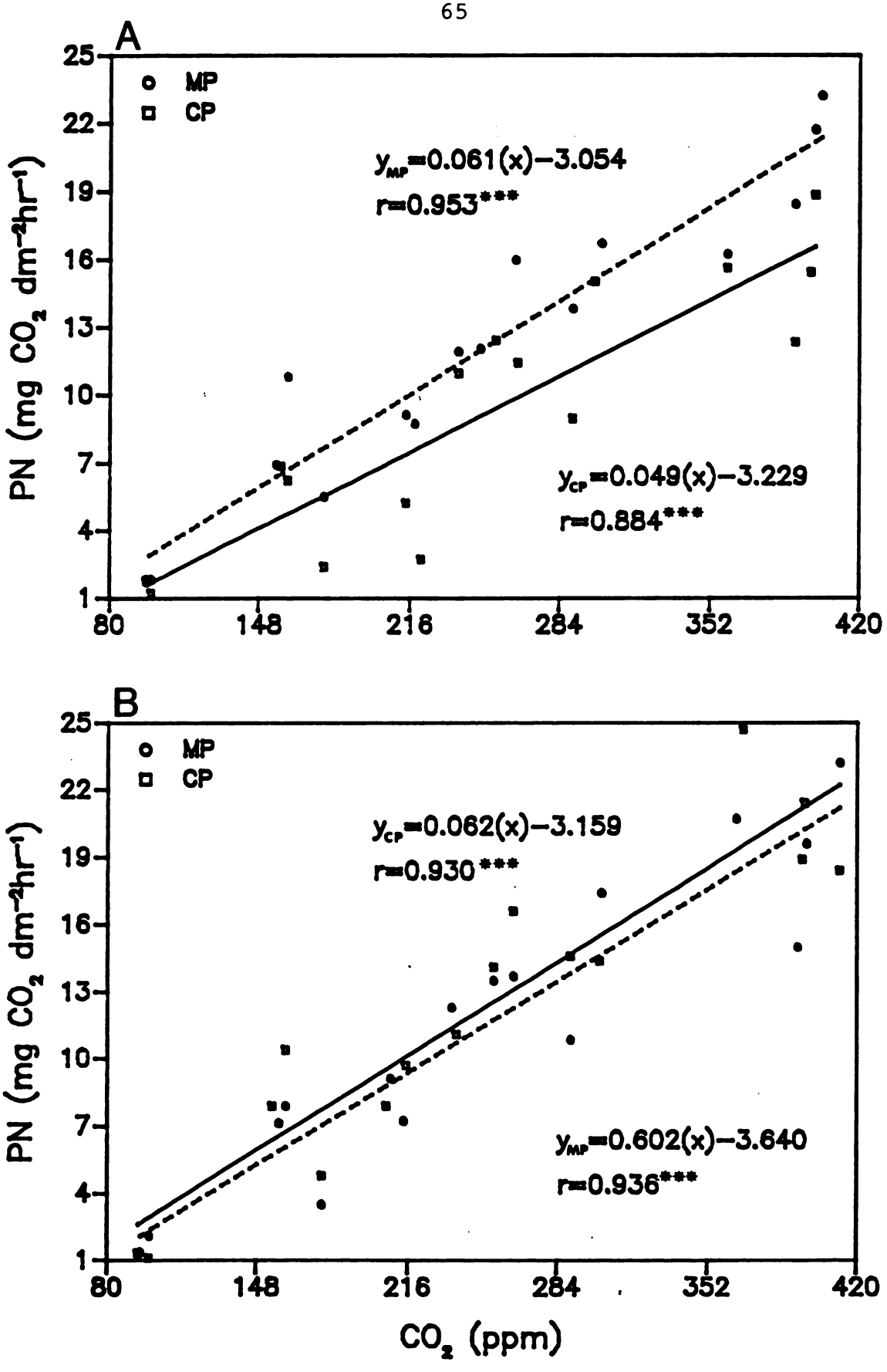

A
MP
CP
y_{MP}=0.061(x)−3.054
r=0.953***
y_{CP}=0.049(x)−3.229
r=0.884***
B
MP
CP
y_{CP}=0.062(x)−3.159
r=0.930***
y_{MP}=0.602(x)−3.640
r=0.936***
PN (mg CO_2 $dm^{-2}hr^{-1}$)
CO_2 (ppm)
25
22
19
16
13
10
7
4
1
80
148
216
284
352
420

Figure 5: Response of net photosynthesis (ug CO_2 gm dr wt^{-1} hr^{-1}) to ambient CO_2 level 9 in micropropagated (----), and conventionally propagated (———) 'Earliglow' (A) and 'Redchief' (B) potted plants grown under full sun.

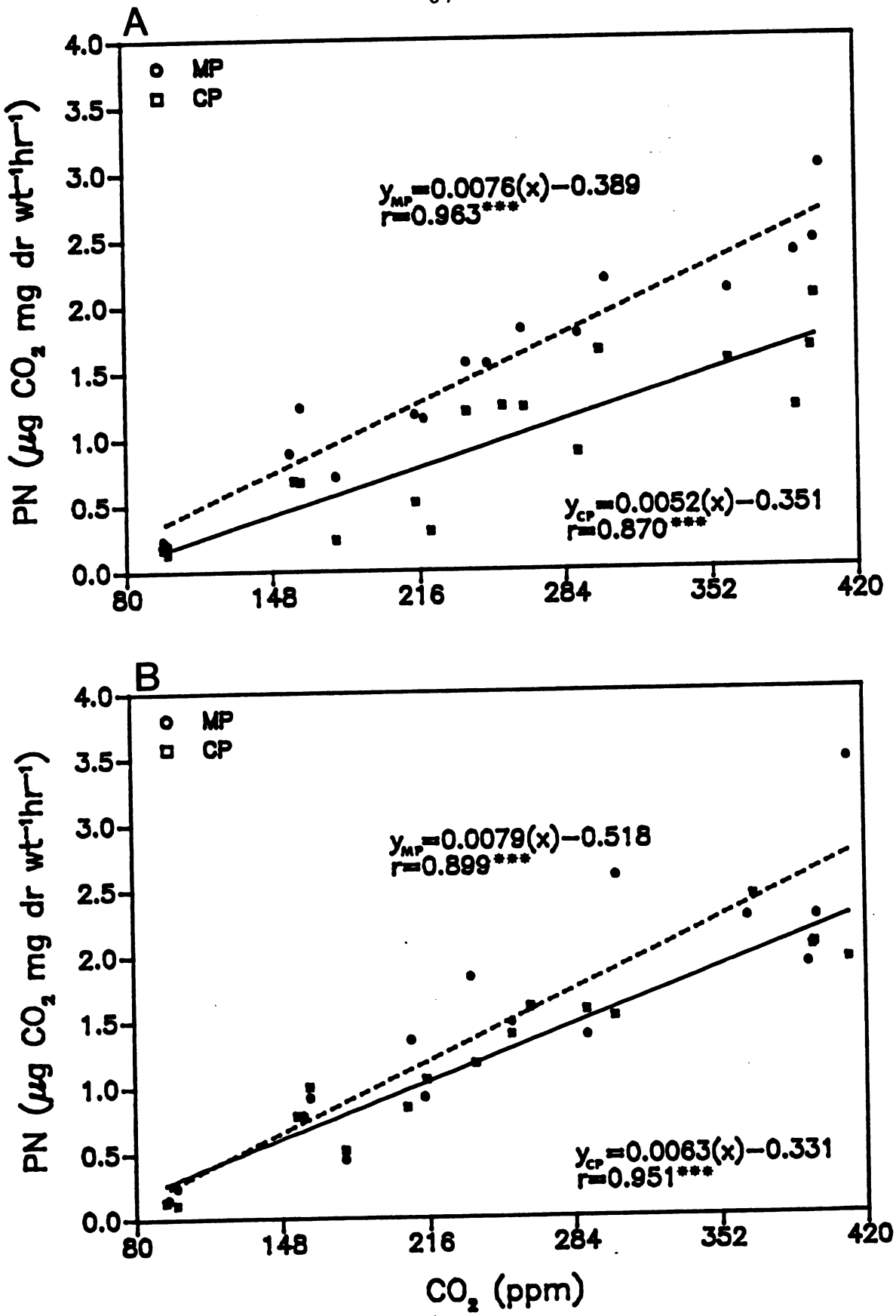
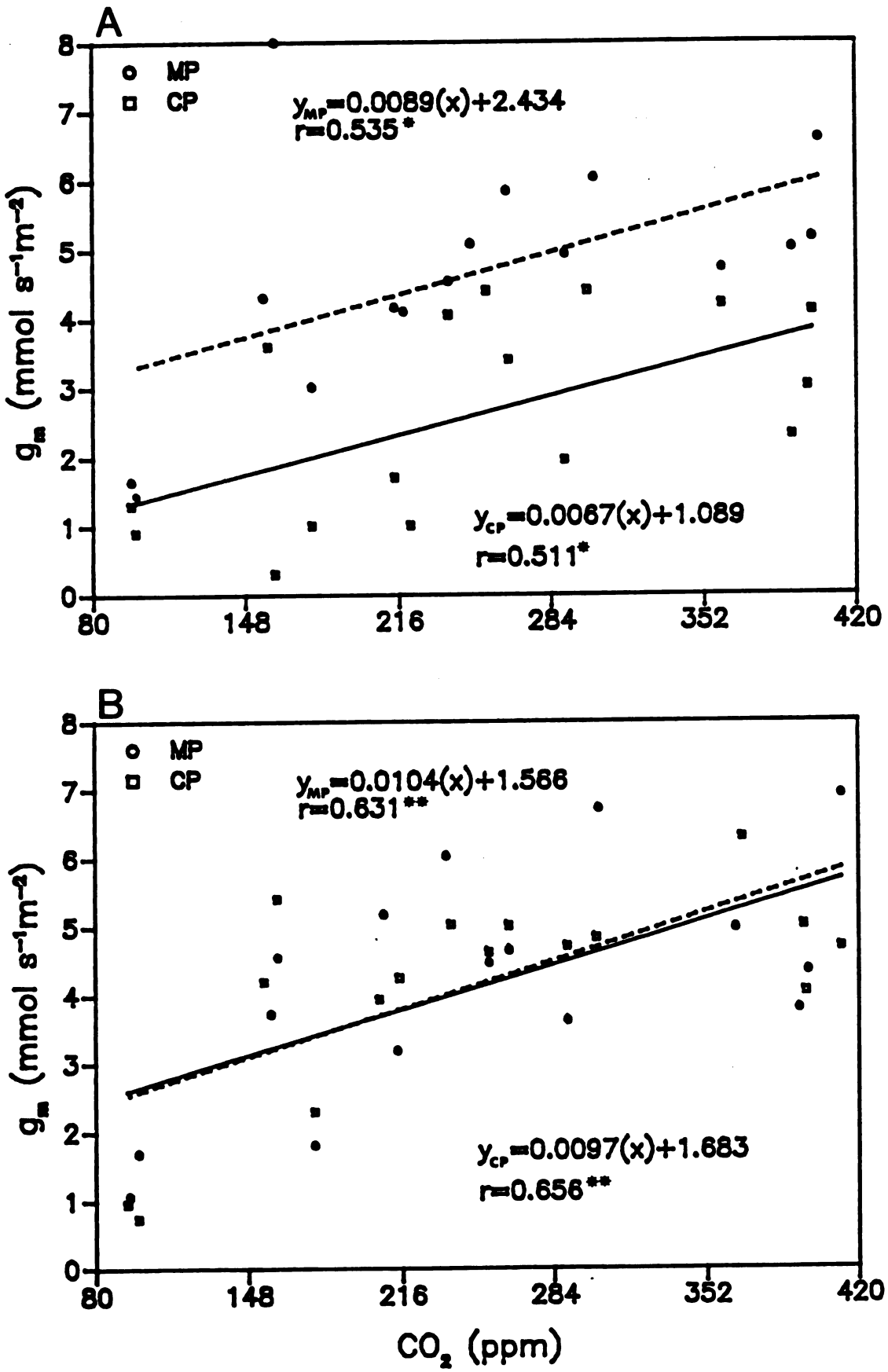

A
MP
CP
y_{MP}=0.0076(x)−0.389
r=0.963***
y_{CP}=0.0052(x)−0.351
r=0.870***
PN (μg CO_2 mg dr $wt^{-1}hr^{-1}$)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
80
148
216
284
352
420
B
MP
CP
y_{MP}=0.0079(x)−0.518
r=0.899***
y_{CP}=0.0063(x)−0.331
r=0.951***
PN (μg CO_2 mg dr $wt^{-1}hr^{-1}$)
CO_2 (ppm)

Figure 6: Response of mesophyll conductance (g_s) on a weight per leaf unit area basis to ambient CO_2 level in micropropagated (----) and conventionally propagated (———) 'Earliglow' (A) and 'Redchief' (B) potted plants grown under full sun.


A
MP
CP
y_{MP}=0.0089(x)+2.434
r=0.535*
y_{CP}=0.0067(x)+1.089
r=0.511*
g_s (mmol $s^{-1}m^{-2}$)
B
MP
CP
y_{MP}=0.0104(x)+1.566
r=0.631**
y_{CP}=0.0097(x)+1.683
r=0.656**
g_s (mmol $s^{-1}m^{-2}$)
CO_2 (ppm)
0
1
2
3
4
5
6
7
8
80
148
216
284
352
420

carboxylation efficiencies were similar for MP and second generation plants of both cultivars (Figure 4). However, on a per dry weight basis, carboxylation efficiency was greater (slopes significantly different $p < 0.01$) in E_0 than in E_2 plants, although this difference was not seen in 'Redchief' (Figure 5). On a per dry weight basis, response of g_m to increasing ambient CO_2 concentration was identical in R_0 and R_2, but was maintained at a higher level in E_0 plants compared to E_2 (Figure 6).

Discussion

In general, micropropagated plants maintained higher gas exchange rates than their field grown progeny. In a previous study, it was suggested that higher rates of P_{net} observed in MP 'Earliglow' might be the result of increases in fruit load (4). Data presented here demonstrate that higher rates of P_{net} exist even in deblossomed MP versus CP plants. This indicates that leaf/fruit relationships are not the primary cause of enhanced gas exchange responses in micropropagated plants.

The two cultivars responded differently to plant density. In MP 'Earliglow', increases in leaf density resulted in concomitant increases in PN on a dry weight basis, while in MP 'Redchief', PN was unchanged. In CP plants of both cultivars, PN decreased as leaf density increased. The difference in response between the two

cultivars may relate to factors influencing mesophyll conductance and carboxylation efficiency.

Mesophyll conductance (g_m) has been described as the CO_2 diffusional pathlength to sites of carboxylation (20). A strong correlation between weight per leaf unit area and mesophyll surface area has been documented in strawberry (7,13). The ratio of mesophyll surface area to leaf surface area contributes to photosynthetic capacity of leaves (18); leaves with higher ratios have higher photosynthetic capacity. In CP plants, weight per leaf unit area was not significantly correlated with density and PN dropped as density increased. In leaves of MP 'Earliglow', large decreases in weight per leaf unit area were observed as density increased; these were accompanied by increases in PN. In MP 'Redchief', weight per leaf unit area decreased with increasing density, and rates of PN were unchanged. Perhaps MP plants are more plastic than CP plants and can maintain photosynthetic rates across a broader range of conditions by regulating mesophyll surface area.

Carboxylation efficiency was greater in MP than in CP 'Earliglow', but this was not true in 'Redchief' (Fig. 5). Response of mesophyll conductance to increasing CO_2 concentration on a per dry weight basis did not differ in R_0 and R_2 plants, but declined with propagation generation in 'Earliglow' (Fig. 6). A number of factors could influence carboxylation efficiency including levels or

activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and regeneration of its substrate, photorespiration, and triose phosphate utilization (20). Differences in carboxylation behavior could account for the higher rates of P_{net} in MP 'Earliglow' than in MP 'Redchief' observed in a previous study (4).

In summary, micropropagated strawberry cultivars produce more stolons than conventionally propagated plants (8,15,21). Micropropagation apparently increases yield only in genotypes which are well adapted to high plant densities. Path analysis indicates that MP cultivars which do not exhibit increased yields are sensitive to crowding stress as CP plants (Cameron and Hancock, unpublished data). The ability of some genotypes to increase reproductive effort (11) and photosynthetic rate (4) with increasing plant density plays a role in allowing plants to adjust to a more competitive environment.

The enhanced vigor associated with micropropagated strawberry plants does appear to dissipate with subsequent generations of conventional propagation, but the first generation progeny of MP plants maintain an enhanced level of vigor over CP plants (3) sufficient to increase productivity in the first bearing year (5). For this reason, MP plants are of benefit not only to the nurseryman, but also to the grower.

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