Propanil Tolerance in Populations of Junglerice (*Echinochloa colona*) in Colombian Rice Fields

ALBERT J. FISCHER, EDUARDO GRANADOS, DIEGO TRUJILLO, and ROBERT ZEIGLER [2]

Abstract. Dose-response studies estimating GR_{40} values established different levels of propanil tolerance (1 to 9 fold) in junglerice populations from fields that had been treated with propanil in the past, as compared to a check population collected where this herbicide had never been used before. Considerable variability in growth and morphology existed among populations. Variability in cumulative leaf area, aboveground biomass, mean relative growth rate, mean net assimilation rate and mean leaf area ratio could not be related to propanil tolerance. In a study using addition series and three junglerice populations, competitiveness was not related to propanil tolerance either. Of several vegetative and reproductive parameters measured at maturity, only grain

---

[1] Received for publication ______________ 1991, and in revised form ______________ 1991. Contribution of the Centro Internacional de Agricultura Tropical.

[2] Agronomist, biometrician, student (BS thesis), and Rice Program Head at the Centro Internacional de Agricultura Tropical (CIAT), A. A. 6713, Cali, Colombia.

weight/plant and number of grains/ plant were correlated (r=-0.73, p=0.06) with GR_{40}. This trend towards lower reproductive fitness in propanil-tolerant junglerice plants should compromise its ecological success when growing with propanil-susceptible plants in the absence of this herbicide. Given the fitness variability among populations, correlation studies seemed more appropriate than fitness comparisons between single pairs of tolerant and susceptible plants.

Nomenclature: propanil, N- (3,4-dichlorophenyl) propanamide; junglerice, Echinochloa colona (L.) Link #[3] ECHCO.

Additional index words. Dose response, competition, addition series, growth analysis, resistance.

INTRODUCTION

Junglerice is an ubiquitous weed in Latin American rice fields (3) and propanil has so far been a useful control tool (4). Rates of 1.8 to 4 kg ai ha^{-1} have been adequate in the past for junglerice control (3), however, recently farmers have noted erratic control of this weed with propanil. As in many areas of the tropics, rice in Colombia is often grown in more than one season per year

---

[3] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci: 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

resulting in a very intensive use of propanil. Farmers in some areas of Colombia, such as the Tolima and Valle departments, where rice has been intensively cropped for decades, report that ever higher propanil rates are required for adequate control. Previous work (11) has shown that repeated use of a herbicide can result in the buildup of weed biotypes resistant to the herbicide within the otherwise susceptible weed population. Resistance to propanil has been found in junglerice and barnyardgrass [Echinochloa crus-galli (L.) Beauv. #ECHCG] (2, 9).

In several cases herbicide-resistant biotypes have had lower ecological fitness than susceptible ones (11). This fitness differential would allow the susceptible population to replace the resistant biotypes in the absence of herbicide use (1, 6). Populations resistant to a given herbicide often differ in fitness as do susceptible populations as well (10).

In order to verify that claims of poor control with conventional propanil rates could be related to propanil tolerance within local populations experiments were conducted to study the response to propanil of junglerice populations from Colombian rice fields. The growth and competitiveness of seven junglerice populations were compared to establish trends between components of ecological fitness and levels of propanil tolerance.

MATERIALS AND METHODS

Origin of populations and experimental conditions. Plants from seven populations, botanically identified as junglerice [4], were studied. Part of the seed was gathered in places were propanil had never been used, serving this material as susceptible check (population #1). Populations #2 and #3 were mass-collected from rice fields where propanil had been used discontinuously in the past (Valle department, Colombia). Four populations (#4 to #7) were collected from rice fields where propanil had been applied continuously for over ten years (Tolima department).

Screenhouse experiments were conducted during 1989 and 1990. The soil was a greenhouse mixture with a pH of 7, 40% clay, 22% sand, 39% Silt, and 1% organic matter. Junglerice was seeded in flats, then one- to two-leaf seedlings of uniform size were transplanted into pots, and grown on water saturated soil. Harvested aboveground plant biomass was oven-dried (65 C) and weighed. Experiments were conducted in completely randomized designs, and the position of pots in the screenhouse was randomized weekly. Each experiment was repeated once, and data from two experiments were pooled for analysis if in both cases trends were the same.

---

[4] Botanical identification was performed by Dr. T. Yabumo, Yamanouchi - Motomachi 13-2, Sumiyoshi - Ku, Osaka City 558, Japan. Formerly at College of Agriculture, University of Osaka Prefecture.

Characterization of field material. Three-to four-leaved plants from the check population were sprayed with a range of propanil (emulsifiable concentrate, 480 gL^{-1}) concentrations (0.06-0.064 gL^{-1}). The concentration inhibiting growth by eighty percent (GR_{80}), 0.25 gL^{-1} ai, was used to spray about 200 plants in each of the four populations collected in rice fields with long history of propanil use. Thus propanil-tolerant individuals were detected, and seed from treated plants with less than 35% foliar damage (tolerant) was kept to generate populations numbered four to seven in this study.

Dosage studies. Junglerice seedlings from each population were transplanted into 1.5 L plastic pots, 14 plants per pot, four replications per dosage. Plants at two- to three- leaf stage were sprayed with propanil at 0, 0.5, 1.0, 1.5, 1.75, 2.0, 2.5, 3.0, and 4.0 kg ai ha^{-1}, using a hand-held CO_2 sprayer, delivering a spray volume of 430 L ha^{-1} (0.5% non ionic surfactant), reproducing common field spraying conditions for the region, and grown for 25 days. Then aboveground biomass was harvested. Data were analyzed by fitting non-linear regression models; response differences among populations were established by comparing coefficients in the models. The models also allowed for the prediction of GR_{40} values for each population. The GR_{50} was beyond the range of concentrations in some cases, so could not be used in this study.

Growth studies. Two monoculture (intrapopulation competition) studies were conducted. (a) Transplanted junglerice seedlings, 10 per 6 L pot (five replications), were grown in the screenhouse until complete maturity, recording plant height, dry aboveground biomass, tiller number, panicle number, total dry grain weight, seed number, and weight of 100 grains, and correlated with GR_{40}. (b) A growth analysis study was conducted by growing 10 young junglerice seedlings for 81 days in 1.5 L pots (four replications). At four-day intervals plants were cut and the following information collected: leaf area, dry aboveground vegetative biomass, and height. Mean leaf area ratio (LAR), mean relative growth rate (RGR), and mean net assimilation rate (NAR) were calculated as described by Radosevich and Holt (11). Best fit regression models were calculated for data description.

Competition studies. Intra- and inter-population competition between each of two resistant populations (#4, and #7) and the susceptible check (population #1) were studied using addition series as described by Spitters (12). This procedure allowed for a competition study where the components of the mixtures appeared at different densities and proportions. Thus, for each series 1.5 L pots were planted with uniformly distributed seedlings of both populations in all combinations at the following densities: 0, 8, 16, 24, and 32 plants per pot. When the same experiment was conducted in 6 L pots, densities were:

0, 32, 64, 96, and 128 plants per pot. Thus, 24 different combinations of density and proportion of the two competing populations resulted. Treatments were replicated four times. Shortly before flowering, aboveground biomass was harvested and dried. Data from each series were linearized (logarithmic transformation) to obtain multiple linear regressions of the form:

$$\mathrm{Ln}(Yt) = -\underline{Ato} - \underline{Att}dt - \underline{Ats}ds$$

$$\mathrm{Ln}(Ys) = -\underline{Aso} - \underline{Ass}ds - \underline{Ast}dt$$

where $\underline{Yt}$ and $\underline{Ys}$ were the average plant biomass yields for the propanil-tolerant and susceptible populations respectively, and $\underline{dt}$ and $\underline{ds}$ were their densities. $\underline{Ato}$ and $\underline{Aso}$ refer to biomass produced in the absence of competition. Intrapopulation competition was estimated by the regression coefficients $\underline{Att}$ and $\underline{Ass}$, while $\underline{Ats}$ and $\underline{Ast}$ estimated interpopulation competition. $\underline{Ats}$ was defined as the effect on the tolerant population of competition by the susceptible population, and $\underline{Ast}$ was the effect of the tolerant population on the susceptible one. Comparison of the latter coefficients estimated the relative aggressiveness of each of two tolerant populations (#4 and #7) with respect to a susceptible check (population #1).

RESULTS AND DISCUSSION

After treatment with a series of propanil rates, junglerice populations from rice farms where propanil has been continuously used (two rice seasons per year) exhibited a much lower leaf damage than those originating from untreated areas (Table 1). Selection pressure through continuous use of propanil apparently shifted junglerice populations to higher levels of propanil tolerance. This may partly explain why farmers in these areas have been increasing propanil rates and number of applications with little improvement in control efficacy. Continuous use of a given herbicide, or chemically related herbicides, has already been identified as promoting the buildup of herbicide- tolerant weed biotypes in crop fields (6, 11).

Three susceptible populations (#1, #2, and #3), and four others which were more tolerant of the herbicide were identified from the dose response of seven junglerice populations to increasing rates of propanil. The growth of the latter (susceptibles) gradually declined with increasing propanil rates (Figure 1). When tolerance to propanil was expressed by the rate required for 40% growth reduction (GR_{40}) of three-leaved junglerice plants, populations #4 to #7 appeared significantly more tolerant than populations #1 to #3 (Table 2). The response of populations #4 to #7 illustrate diverse levels of tolerance to the herbicide, rather than complete resistance to it. Different levels of propanil tolerance were also substantiated by Giannopolitis and Vassiliou (2) in barnyardgrass biotypes. A gradual increase in atrazine tolerance under the selection pressure of this herbicide has been observed in Echinochloa spp., and the interaction of several genes conferring tolerance was proposed (7). Thus, the gradual transition of GR_{40} values from susceptibility to tolerance, could reflect a somewhat complicated heredity of this character.

Although all populations were classified as junglerice, considerable variability in growth and morphology existed among populations. The same was observed in propanil-susceptible and -tolerant biotypes of barnyardgrass (2). Murphy et al. (10) also found high and low vigor among both, dinitroaniline- resistant and -susceptible goosegrass [Eleusine indica (L.) Gaertn. #ELEIN] populations. Besides a low- significance trend towards lower tillering with increasing GR_{40} values differences in vegetative growth among biotypes, could not be significantly related to propanil tolerance (Figure 2a-f and Table 3). RGR values were similar for all the populations (Figure 2d). Valverde (13) also observed that differences in vegetative growth among goosegrass biotypes could not be related to their response to dinitroanilines. Conversely, Holt and Radosevich (8) found that a triazine-susceptible biotype of common groundsel (Senecio vulgaris L. #SENVU) was more vigorous than the resistant biotype. Also, studies by Conard and Radosevich (1) showed that triazine-susceptible biotypes of common groundsel and redroot pigweed

(*Amaranthus retroflexus* L. #AMARE) produced more biomass than the resistant ones when both types grew in competition. However, comparing fitness between single pairs of biotypes and relating this to herbicide tolerance may not always lead to accurate generalizations when variability in vigor is relevant. Thus, no relationship was found between vigor and tolerance to propanil in barnyardgrass biotypes varying in growth and morphology (2).

When individuals from susceptible (#1) and tolerant (#7) populations were grown in mixtures at different densities and proportions in addition series studies, the comparison of regression coefficients for interpopulation competition indicated that susceptible individuals tended to be more competitive than tolerant ones (Table 4). The susceptible population (#1) was, however, less aggressive when it grew in mixtures with the propanil-tolerant population #4 (Table 4). Therefore differences in competitiveness also could not be related to the populations' response to propanil. Similarly, Valverde (13), also using addition series, found no competitive differences in aboveground biomass production by dinitroaniline-resistant and -susceptible goosegrass biotypes.

With increasing propanil tolerance, however, the populations showed lower seed set (Table 3). Similarly, in other studies (10, 13), herbicide-resistant biotypes had lower fecundity and inflorescence weights than susceptible ones. This lower fecundity could impair the ecological success of propanil-tolerant junglerice individuals, since seed production is essential for survival of this annual species. Other factors, besides propanil-tolerance, may also affect the variability in reproductive fitness of these populations. Murphy et al. (10) also found variability in reproductive characteristics among either dinitroaniline-susceptible or -resistant populations. This suggested that examining trends relating the observed variability in growth and fecundity to that in herbicide tolerance among junglerice populations may be more appropriate than seeking differences between single pairs of resistant and susceptible populations.

Reduced ecological fitness of resistant populations implies that, in absence of the herbicide, susceptible individuals would replace less-fit resistant ones, shifting the weed population towards higher proportions of susceptible individuals (11). This process, however, would be much slower than the logarithmic initial increase in resistance due to the selection pressure of a herbicide (6, 7), and since crops need to be weed free for optimum yields, fitness differences may not always provide practical options for lowering tolerance levels in weed populations in intensive cropping situations.

As with other herbicides (5, 11) the prolonged use of propanil, especially at high rates, could have been responsible for the high levels of propanil tolerance found in junglerice populations of some irrigated rice fields of Colombia. The use of herbicides with different mechanisms of action, and increased use of agronomic means to reduce weed pressure, seem relevant alternatives to manage propanil tolerance by junglerice in the field (5, 9).

Uncontrolled use of chemicals, as it occurs in many developing countries is expensive and can lead to pesticide resistance and pollution problems. Therefore imaginative strategies for sustainable use of herbicides in the tropics are required. Alternative herbicides to propanil may be more expensive and not always available in Latin America. Most irrigated rice lands are not suitable for rotations with other crops, and this greatly limits herbicide rotation possibilities. Integration of herbicide use with other control methods and agronomic practices is badly needed. Crop loss assessment, analyzed in conjunction with costs of weed control alternatives (14) offers an attractive approach for rational herbicide use in developing countries.

LITERATURE CITED

1. Conard, G. S. and S. R. Radosevich, 1979. Ecological fitness of *Senecio vulgaris* and *Amaranthus retroflexus* biotypes susceptible or resistant to atrazine. J. Appl. Ecol. 16:171-177.

2. Giannopolitis, C. N. and G. Vassiliou, 1989. Propanil tolerance in *Echinochloa crus-galli* (L.) Beauv. Trop. Pest Man. 35:6-7.

3. Gonzalez, J., O. Arregocés, and E. Escobar, 1985. Principales malezas en el cultivo del arroz en América Latina. Pages, 419-444 *in* CIAT (Centro Internacional de Agricultura Tropical). Arroz: Investigación y Producción. E. J. Tascón and E. García, eds. PNUD/CIAT, Cali, Colombia.

4. Gonzalez, J., 1985. Manejo de las malezas en arrozales. Pages, 445-458. *in* CIAT (Centro Internacional de Agricultura Tropical). Arroz: Investigación y Producción. E. J. Tascón and E. García, eds. PNUD/CIAT, Cali, Colombia.

5. Gressel, J., 1978. Factors influencing the selection of herbicide-resistant biotypes of weeds. Outlook on Agric. 9:283-287.

6. Gressel, J. and L. A. Segel, 1978. The paucity of plants evolving genetic resistance to herbicides. J. Theor. Biol. 75:349-371.

7. Gressel, J. and L. A. Segel, 1982. Interrelating factors controlling the rate of appearance of resistance: the outlook for the future. In H. Le Baron and J. Gressel, eds. Herbicide Resistance in Plants. John Wiley and Sons, New York.

8. Holt, J. S. and S. R. Radosevich, 1983. Differential growth of two common groundsel (*Senecio vulgaris*) biotypes. Weed Sci. 31:112-120.

9. Le Baron, H. M. J. McFarland, 1988. Herbicide resistance in Weeds and Crops. Pages 336-352 in M. B. Green, H. M. Le Baron, and W. K. Moberg, eds. Managing Resistance to Agrochemicals, from Fundamental Research to Practical Strategies, Amer. Chem. Soc.

10. Murphy, T. R., B. J. Gossett, and J. E. Toler, 1986. Growth and Development of dinitroaniline-susceptible and -resistant goosegrass (*Eleusine indica*) biotypes under non competitive conditions. Weed Sci. 34: 704-710.

11. Radosevich, S. R. and J. Holt, 1984. Weed Ecology, Implications for vegetation management. J. Wiley and Sons. N. Y. 265 p.

12. Spitters, C. J. T. 1983. An alternative approach to the analysis of mixed cropping experiments. 1. Estimation of competition effects. Netherlands J. Agric. Sci. 31:1-11.

13. Valverde, B. E. 1989. Dinitroaniline-herbicide resistance in goosegrass (*Eleusine indica*): Ecophysiological aspects. PhD. Dissertation. Oregon State University, Corvallis, Oregon.

14. Oliver, L.R. 1988. Principles of weed threshold research. Weed Techn. 2:398-403.

Table 1. Foliar damage when 0.25 gL^{-1} ai propanil was sprayed onto three- to four-leaved junglerice plants from five fields with different history of propanil use.

Affected Leaf area	Junglerice plants: Populations with propanil history				Junglerice plants: No propanil history
	%				
5	0	0	0	0	0
15	0	0	0	0	0
25	28	4	32	35	0
35	41	38	57	52	0
45	17	31	9	12	0
55	10	18	2	2	1
65	3	7	0	0	8
75	2	3	0	0	46
85	0	0	0	0	33
95	0	0	0	0	12
	n= 434	298	384	582	171

Table 2. GR_{40}[a] Values for seven Junglerice populations treated with a range of propanil dosages.

Population	GR_{40}		Level of resistance[c]
(no.)	(kg ai propanil ha^{-1})		
1	0.36	d[b]	1
2	0.43	c	1
3	0.50	c	1
4	1.10	b	3
5	3.10	a	9
6	1.94	b	5
7	1.99	b	6

[a] Rate required for 40% growth inhibition.

[b] Values followed by the same letter were estimated by regression models not differing ($p>0.05$) in their linear and quadratic coefficients.

c Level of resistance = ratio of resistant GR_{40} to susceptible GR_{40}.

Table 3. Correlations between propanil tolerance (GR_{40})[a] with growth and reproductive parameters of Junglerice populations.[b]

X	Y	r	p
$\ln(GR_{40})$	Grain weight/plant	- 0.73	0.06
$\ln(GR_{40})$	No. grains/plant	- 0.73	0.06
$\ln(GR_{40})$	No. panicles/plant	- 0.52	0.24
$\ln(GR_{40})$	100 grain weight	- 0.36	0.43
$\ln(GR_{40})$	ln (foliar dry weight/plant)	- 0.34	0.46
GR_{40}	Plant height	- 0.33	0.46
$\ln(GR_{40})$	Tiller no./plant	- 0.59	0.17
$\ln(GR_{40})$	ln (Mean RGR)	- 0.41	0.36
$\ln(GR_{40})$	ln (Mean LAR)	0.55	0.20
GR_{40}	Mean NAR	0.19	0.69

a. Rate required for 40% growth inhibition.

b. The first seven parameters were measured at maturity.

Table 4. Coefficients of multiple linear regressions models applied to data from four addition series experiments[a].

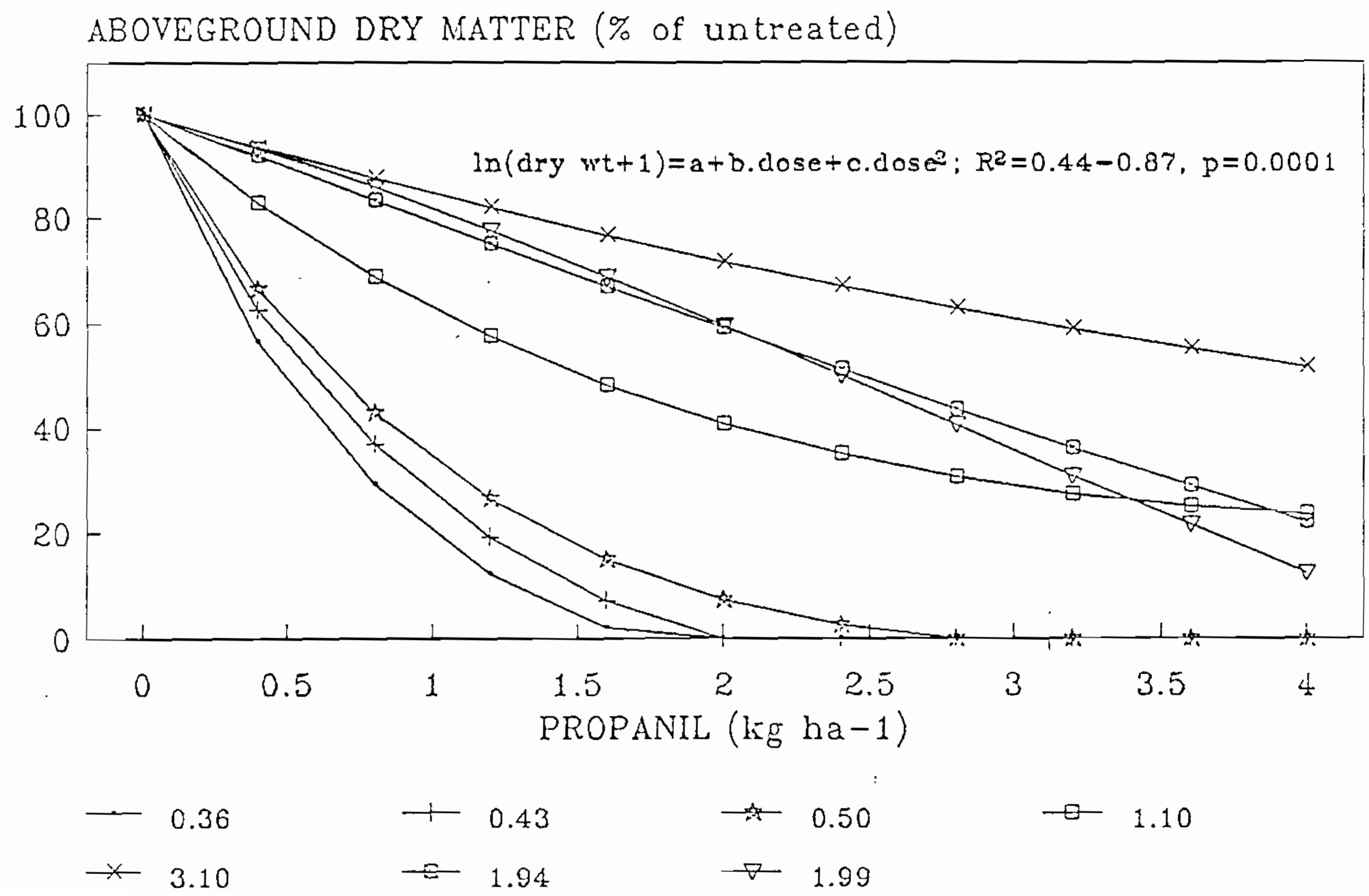
i	j	Ao	Bii	Bij[b]	R^2	p
1[c]	7	-0.2911	0.0423 d_1	0.0349 d_7	0.56	<0.001
7	1	-0.2838	0.0487 d_7	0.0417 d_1	0.45	<0.001
1	7	1.7607	0.0335 d_1	0.0215 d_7	0.44	<0.001
7	1	0.8694	0.0411 d_7	0.0387 d_1	0.55	<0.001
1	4	1.6741	0.0288 d_1	0.0519 d_4	0.63	<0.001
4	1	1.7817	0.0258 d_4	0.0269 d_1	0.44	<0.001
1	4	0.6156	0.0346 d_1	0.0430 d_4	0.65	<0.001
4	1	0.8347	0.0427 d_4	0.0260 d_1	0.64	<0.001

[a] From the model: ln (Yi) = - Ao - Biidi - Bijdj, where Yi is the aboveground biomass weight/plant of population _i_, _di_ is the density of population _i_, and _dj_ is the density of population _j_; _Ao_ is the biomass yield in the absence of competition, _Bii_ describes intrapopulation competitive effects, _Bij_ describes the influence of population j on population _i_ (interpopulation competition).

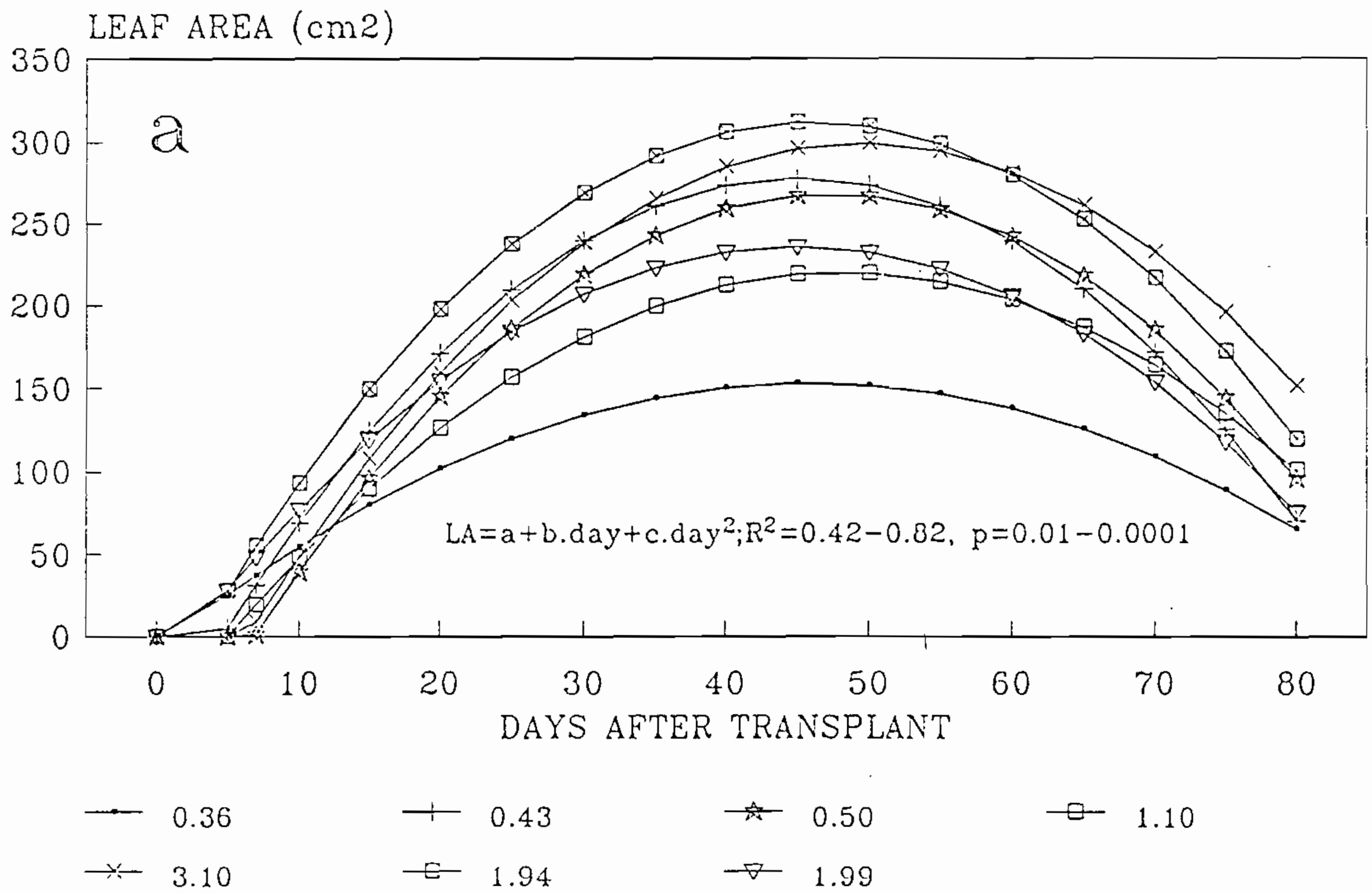
[b] Comparisons of coefficients are made within each pair of equations.

[c] Susceptible check, 7 and 4 are propanil-tolerant populations.

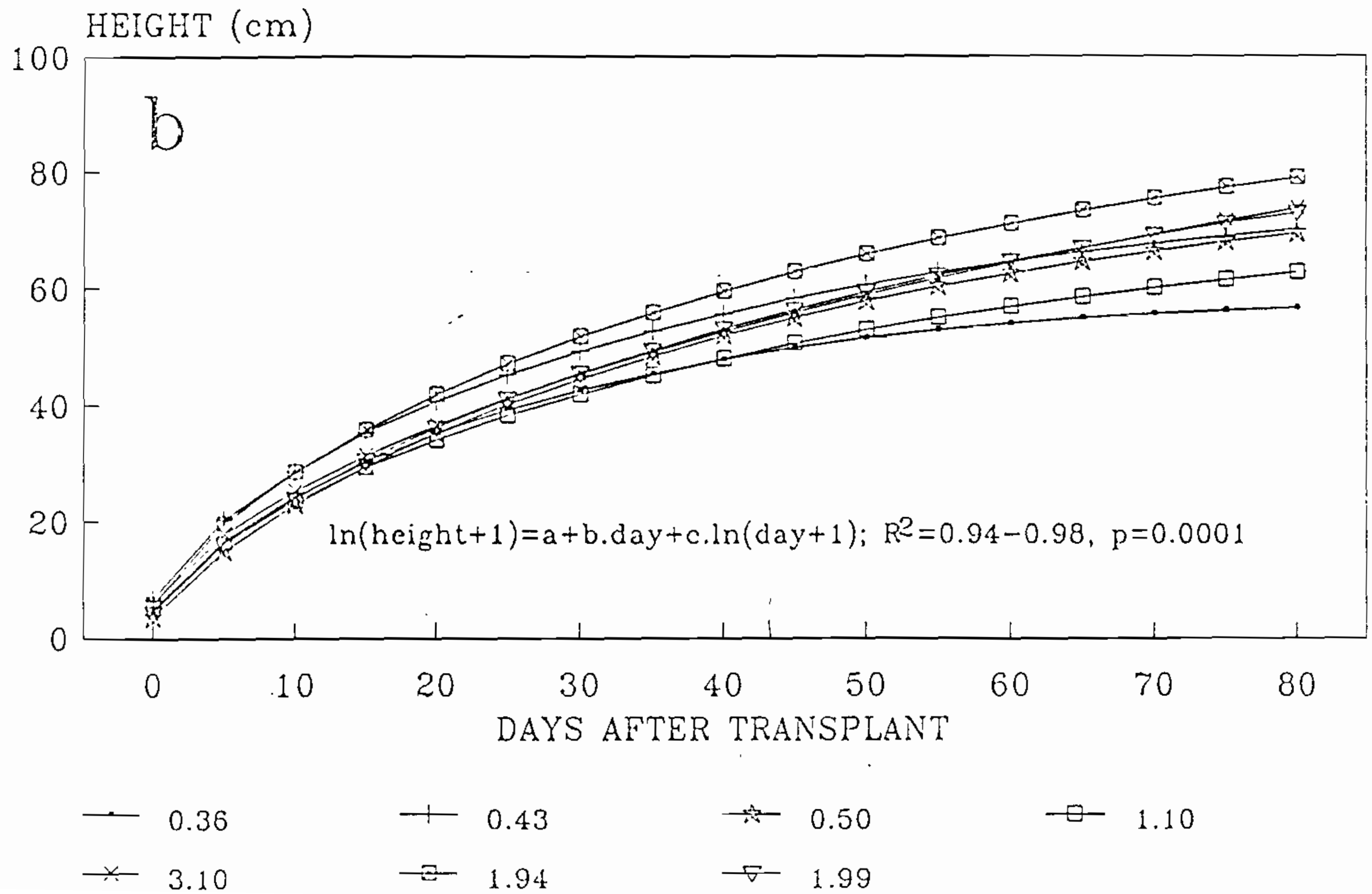
Figure 1. Aboveground plant growth of seven junglerice populations treated at the three-leaf stage with a range of propanil dosages; legends indicate herbicide dosages for 40% growth reduction (GR_{40}) in each population.


ABOVEGROUND DRY MATTER (% of untreated)
ln(dry wt+1)=a+b.dose+c.dose²; R²=0.44-0.87, p=0.0001
100
80
60
40
20
0
0
0.5
1
1.5
2
2.5
3
3.5
4
PROPANIL (kg ha-1)
0.36
0.43
0.50
1.10
3.10
1.94
1.99

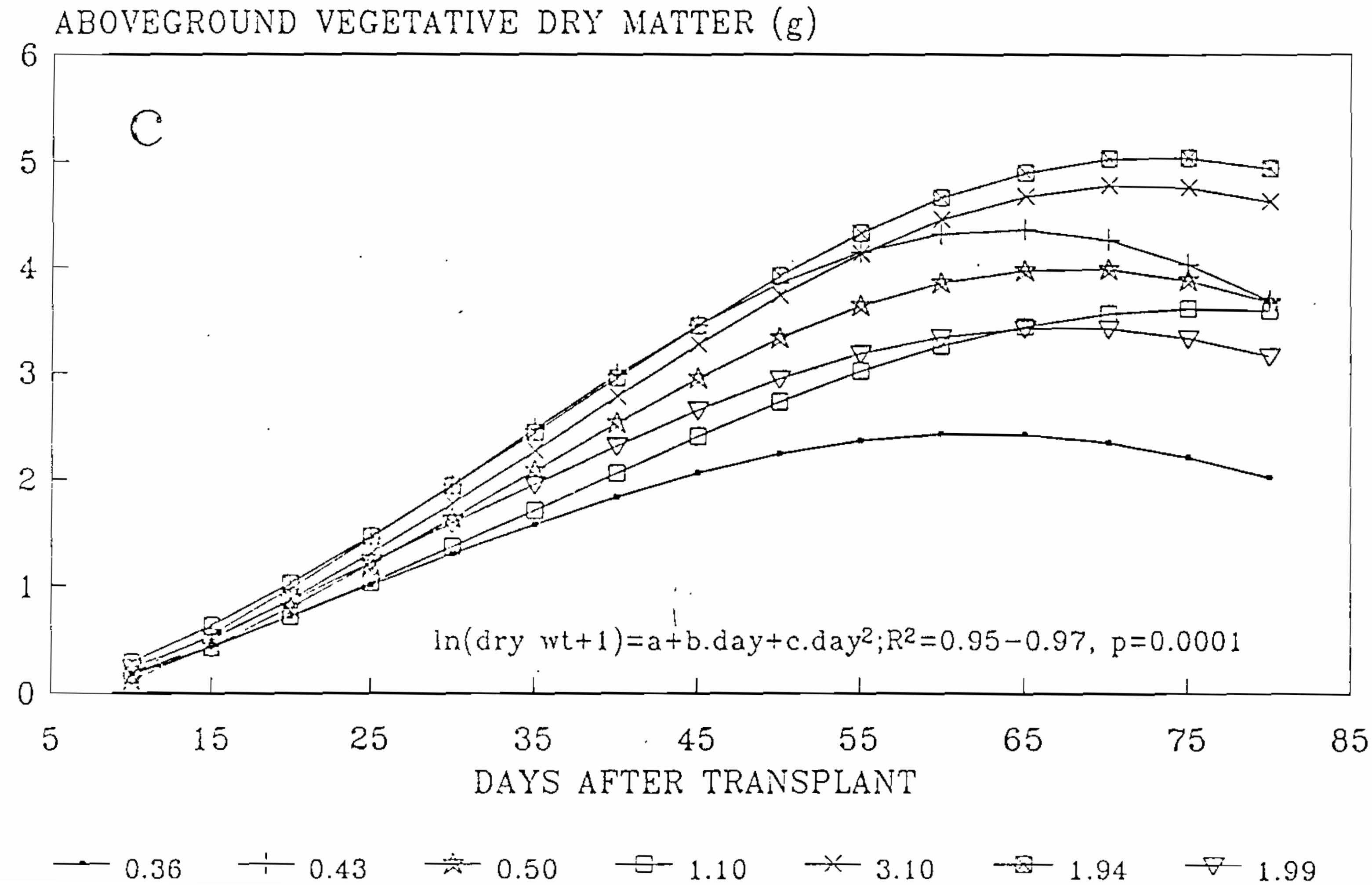
<u>Figure 2</u>. Growth analysis of seven junglerice populations with different levels of propanil tolerance; legends indicate herbicide dosages for 40% growth reduction in each population. NAR curves were obtained with values estimated with equations in <u>a</u> and <u>c</u>.



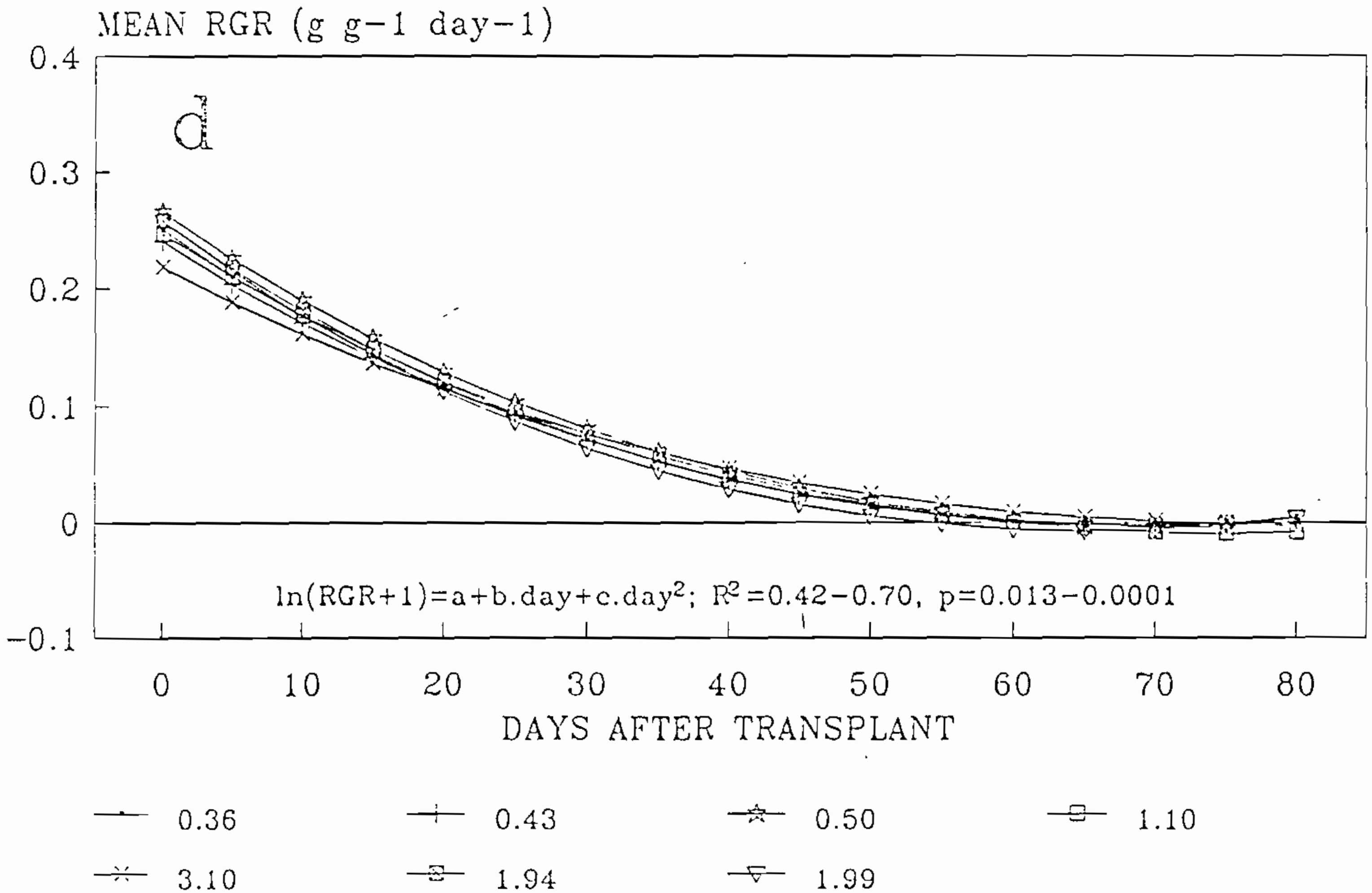
LEAF AREA (cm2)
350
300
250
200
150
100
50
0
a
LA=a+b.day+c.day²;R²=0.42-0.82, p=0.01-0.0001
0
10
20
30
40
50
60
70
80
DAYS AFTER TRANSPLANT
0.36
0.43
0.50
1.10
3.10
1.94
1.99

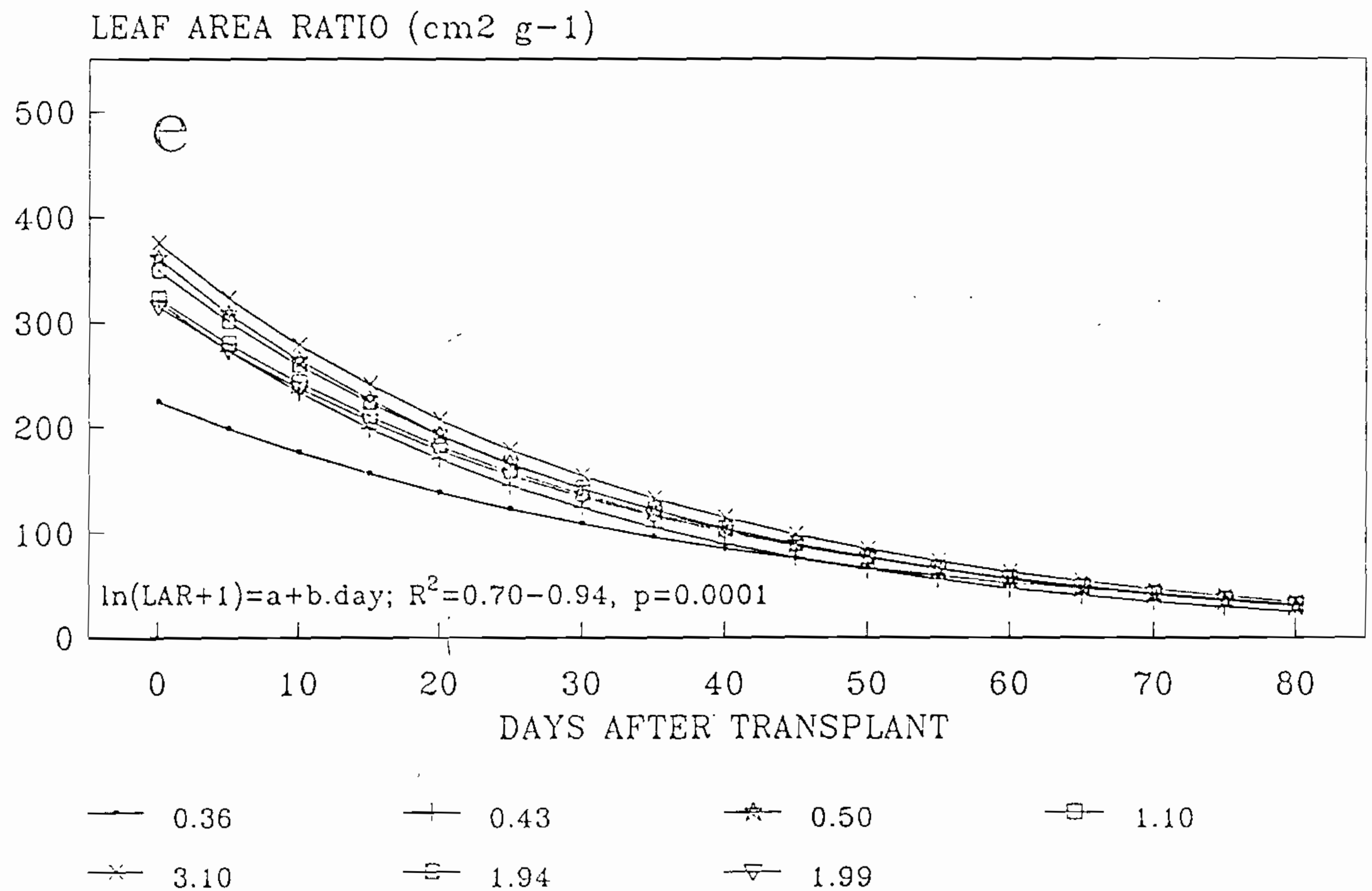


HEIGHT (cm)
100
80
60
40
20
0
b
ln(height+1)=a+b.day+c.ln(day+1); R2=0.94-0.98, p=0.0001
0
10
20
30
40
50
60
70
80
DAYS AFTER TRANSPLANT
0.36
0.43
0.50
1.10
3.10
1.94
1.99

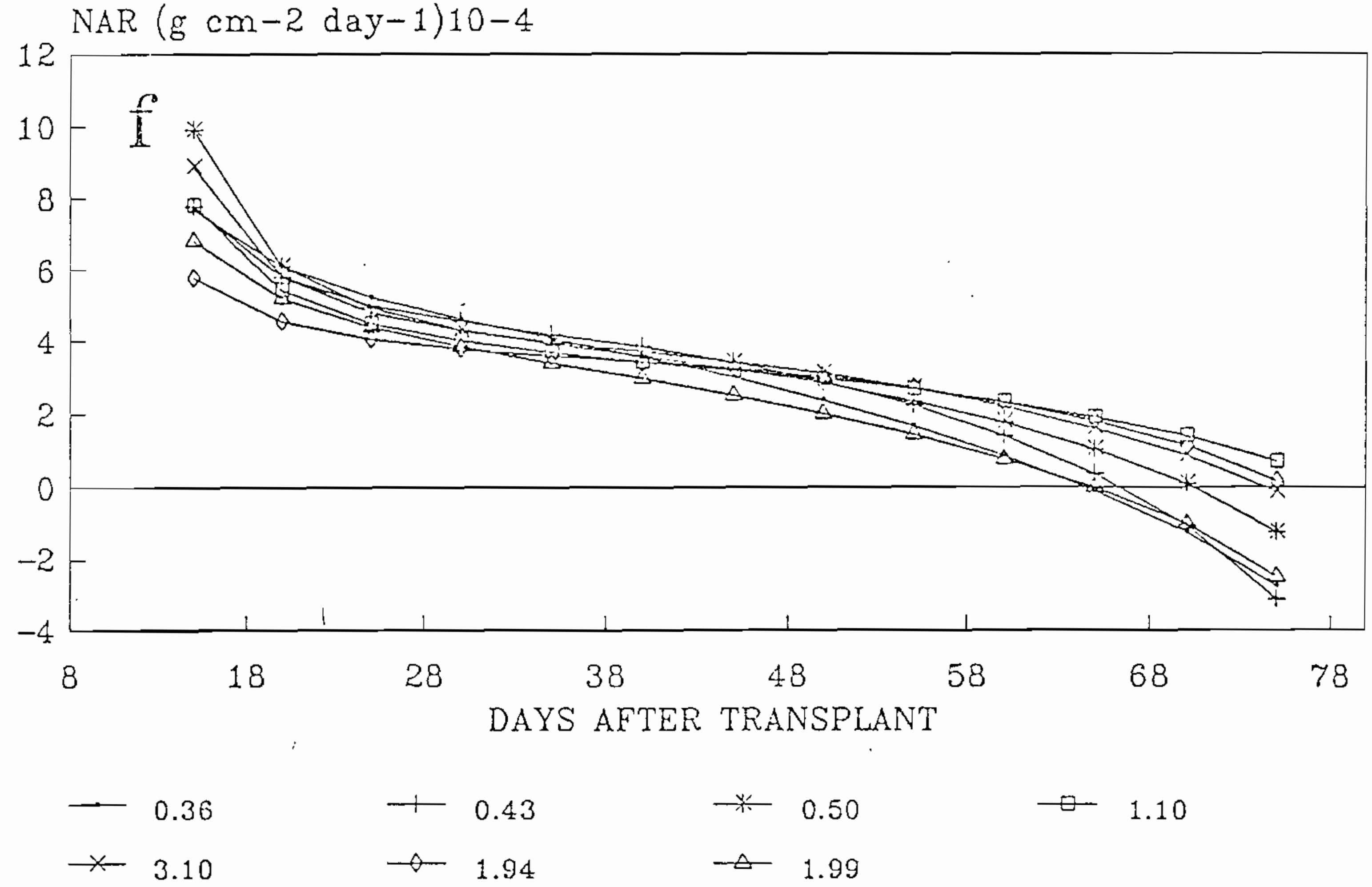


ABOVEGROUND VEGETATIVE DRY MATTER (g)
C
ln(dry wt+1)=a+b.day+c.day²;R²=0.95-0.97, p=0.0001
DAYS AFTER TRANSPLANT
0.36
0.43
0.50
1.10
3.10
1.94
1.99


MEAN RGR (g g-1 day-1)
0.4
0.3
0.2
0.1
0
-0.1
d
ln(RGR+1)=a+b.day+c.day²; R²=0.42-0.70, p=0.013-0.0001
0
10
20
30
40
50
60
70
80
DAYS AFTER TRANSPLANT
0.36
0.43
0.50
1.10
3.10
1.94
1.99



LEAF AREA RATIO (cm2 g-1)
e
500
400
300
200
100
0
ln(LAR+1)=a+b.day; R2=0.70-0.94, p=0.0001
0
10
20
30
40
50
60
70
80
DAYS AFTER TRANSPLANT
0.36
0.43
0.50
1.10
3.10
1.94
1.99


NAR (g cm-2 day-1)10-4
f
12
10
8
6
4
2
0
-2
-4
8
18
28
38
48
58
68
78
DAYS AFTER TRANSPLANT
0.36
0.43
0.50
1.10
3.10
1.94
1.99