into methane. The observed conversions are low for technical purposes, but this is to be expected because of the use of low frequency, low power discharges at low pressures and ordinary temperatures which allowed essentially non-pyrolytic reactions.

Table 1 Conversion of the carbon in bituminous coal into methane

Time (min)	Conversion into methane (mol per cent)		
	Coal	Coal + He	Coal + H_2
10	12.2	—	17.6
20	12.2	5.2	21.5
40	13.9	8.0	16.2
60	9.1	11.1	11.7
80	12.0	14.8	12.3
100	11.8	14.7	10.8

The electron spin resonance spectrum of the coal subjected to plasma showed a 2.5-fold increase ($\sim 10^{18}$ spins per g) in the spin concentration over an untreated sample suggesting that a free radical mechanism may be involved in the gasification process.

Further work using a flow-system with an argon–hydrogen mixture at atmospheric pressure is in progress.

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Received February 20; accepted May 14, 1976.

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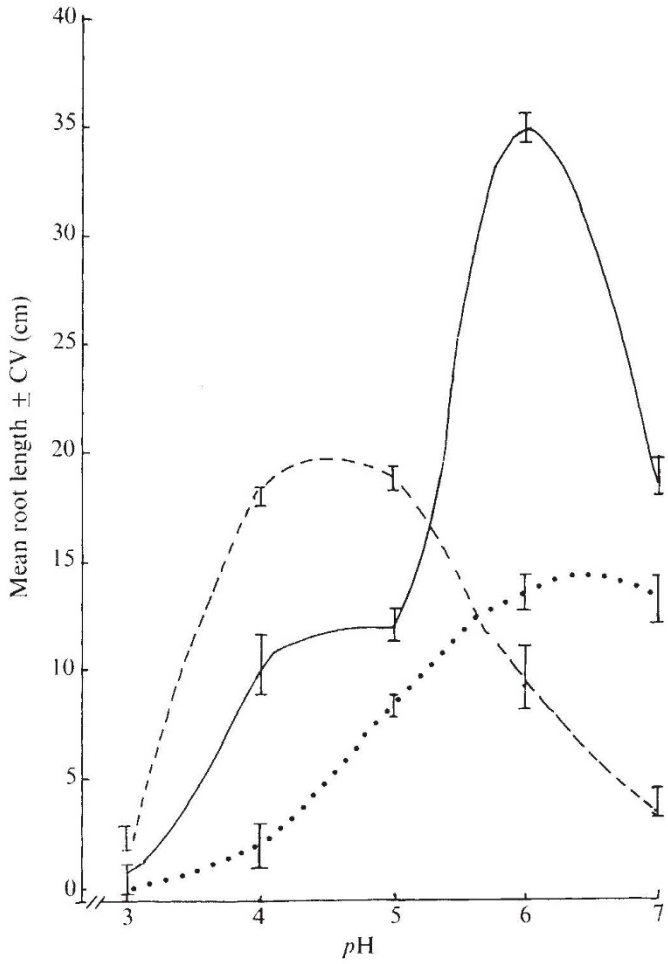

Fig. 1 Relationship between *p*H and mean root length (±coefficient of variation).– – –, N; . . ., C; —, F_1.

*p*H-dependent heterosis of heavy metal-tolerant and non-tolerant hybrid of the monkey flower *Mimulus guttatus*

Although the toxicity of heavy metals is known to be dependent on *p*H, no studies of heavy metal tolerance in plants[1,2] have included it as a variable. We have therefore investigated the direct effect of *p*H on root growth and root number of two strains of *Mimulus guttatus*, one tolerant and the other not tolerant to heavy metals[3]. We found that the tolerant strain is also tolerant to acidic *p*H, whereas the non-tolerant operates better in more neutral *p*H. The hybrid between the two strains showed *p*H-dependent heterosis.

The strains used were Napoleon (N) and Cerrig-y-drudion (C). N is tolerant to copper[3] and is an annual population collected from a copper mine tailing at a stream edge in the Napoleon locality, Calavaras country in central California. C is sensitive to copper[3], and was originally found at the edge of a stream, 2 miles south of the village of Cerrig-y-drudion in North Wales. Seeds were germinated in soil, and clones were established from a single seed and propagated by cuttings. In the following experiment N15 and C16 were used. Although there was much variation between clones (within each population) the range of metal tolerance for N did not overlap with that of C. The F_1 was obtained by crossing ♀♀N with ♂♂C. Only one plant was crossed and different plants were obtained from its seeds.

Cuttings (one node per cutting) of uniform size were obtained from different plants about 0.5 cm below the node. Each cutting was placed in an 11-ml plastic vial filled with a solution of calcium nitrate (0.5 g l^{-1}) with varied *p*H between 2 and 9. Acid *p*H was obtained with concentrated sulphuric acid, basic *p*H with sodium hydroxide. Normal calcium nitrate solution gave *p*H 5. Ten cuttings (replicates) from different plants were used at each *p*H.

Plastic vials were housed in wooden blocks so that light had no influence on root initiation. Each block was covered with a plastic bag to maintain humidity and placed in a growth chamber operating at a short light cycle (9 h with 25,833.6 lx), with a constant temperature of 20 °C and 55% relative humidity. The number and length of all roots from a given plant were measured. The mean of the 10 plants and coefficient of variation were calculated for each *p*H and genotype. The coefficient of variation was used to avoid any possible dependence of the variance on the mean.

The effects of different *p*H on root length are presented in Fig. 1 and Table 1. No cuttings developed any roots at *p*H 2, 8 and 9. The distribution of root growth between these levels was highly variable (Table 2). Analysis of variance using the actual data or their logarithms gave the same significant results. Using Duncan's multiple range test, it is possible to rank the effect of *p*H on root length as follows:

*p*H	3	4	7	5	6
Mean root length	0.60	10.18	12.05	13.05	19.47

where the means at *p*H 4, 7 and 5 are not significant from each other at $P = 0.05$.

The three genotypes were significantly different from each other in order C (7.52) > N (10.39) > F_1 (15.30). Analysis of variance (Table 2) and Fig. 1 indicate a significant effect of genotype on *p*H interaction. Strain N preferred the acidic

Table 1 Mean root length for each genotype at five levels of *p*H

Genotype	*p*H 3		4		5		6		7	
N	1.33*de*	(144)	18.11*a*	(37)	18.82*a*	(51)	9.74*abcde*	(136)	3.94*bcde*	(69)
C	0.00*e*	—	2.10*cde*	(98)	8.39*abcde*	(52)	13.67*ab*	(75)	13.43*abc*	(87)
F_1	0.46*e*	(172)	10.34*abcde*	(131)	11.94*abcd*	(86)	35.00	(74)	18.77*a*	(81)

Any two means with the same letter are not significantly different from each other at $P = 0.05$ by Duncan's Multiple Range test. Values in parentheses are percentage of coefficients of variation.

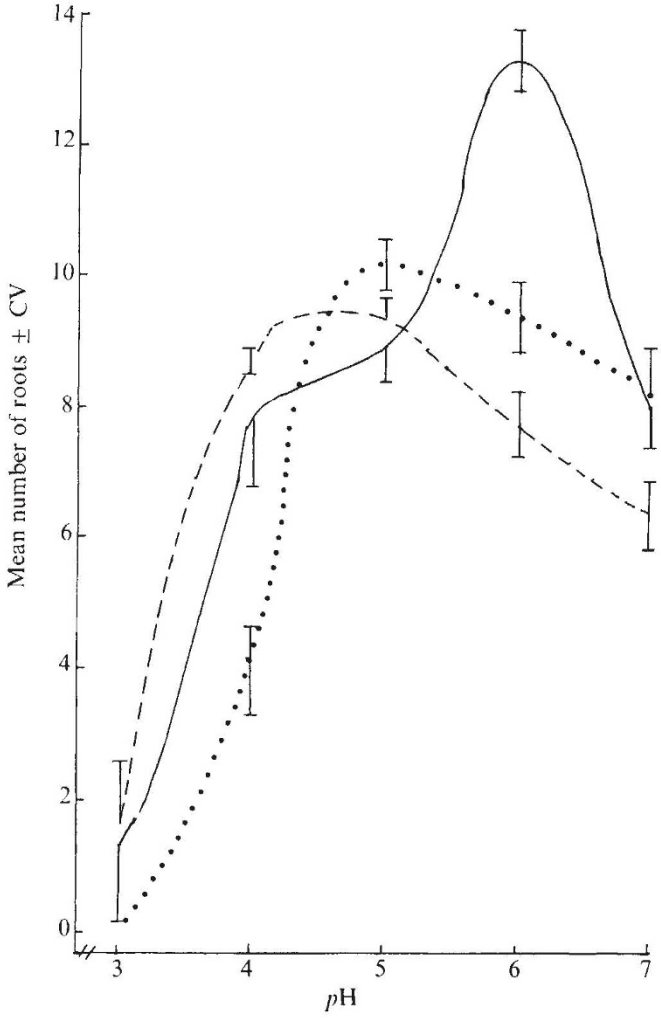

Fig. 2 Relationship between *p*H and mean number of roots per plant (±coefficient of variation). Symbols as in Fig. 1.

solutions with a maximum peak between *p*H 4 and 5. Maximum root growth for strain C was in the more neutral solutions, between *p*H 6 and 7.

The F_1 grew better than either parent, with maximum growth at *p*H 6. Its performance at *p*H 6 and 7 indicated an overdominance in relation to its parents, whereas between *p*H 3 and 5 it was intermediate. This suggests two genes operating at different *p*H levels with different degrees of gene action. The presence of the shoulder at *p*H 4 substantiates this hypothesis.

Variation in *p*H was also found to affect number of roots per plant (Table 2). The average number of roots for the three genotypes could be ranked as follows:

*p*H	3	4	7	5	6
Mean number of roots	1.0	6.9	7.5	9.5	10.2

where any two adjacent numbers, excluding 1.0, are not significantly different from each other at $P = 0.05$ by Duncan's multiple range test.

These results indicate a gradual significant increase in number of roots from *p*H 3 to 5. The number of roots at *p*H 5 was not significantly different from those at *p*H 6, after which there was a significant decrease at *p*H 7. Although an analysis of variance did not reveal any effect of the genotype on the number of roots, *t* values for the difference between N and C at *p*H 4 ($P = 0.01$) and N and F_1 at *p*H 6 ($P = 0.05$) were significant.

Phenotypic variation in length and number of roots were calculated within each genotype at each *p*H. These were expressed as the percentage of the coefficient of variation (Table 1). Analysis of variance on the square of such values indicated no statistical differences between genotypes or *p*H levels. When the genotypes were compared at each *p*H, however, the variability for total root growth of both F_1 and C was found to be larger than that of N at *p*H 4 ($F = 12.15$, $P<0.01$ and 6.87, $P<0.01$, respectively). Whereas at *p*H 6 the N strain had higher variability than both F_1 and C ($F = 3.43$, $P<0.05$ and 3.28, $P<0.05$, respectively).

The number of roots was less variable. The only significant differences for this trait are between F_1 or C and N at *p*H 4. F_1 and C variabilities are higher than that of N ($F = 23.97$, $P<0.01$ and 11.74, $P<0.01$ respectively). These results are similar to those obtained for root length at this *p*H.

It is obvious that N plants are more adapted to growing in lower *p*H soils than C plants. Whether this is due to direct effect of *p*H or due to adaptation to heavy metal ions is not known yet and needs to be investigated. (Dr Sheppard informed me that the Californian mines from which the material came are extremely acid compared with North Wales, and he has non-tolerant seeds from a very acid locality.) Undoubtedly, a study of the interaction between *p*H and heavy metal toxicity is of interest as indicated in Fig. 1, where for example, at *p*H 5 the N strain has better root growth than the C strain. This performance is reversed at *p*H 6 and 7 indicating *p*H-dependent (or conditional) toxicity for heavy metals.

Table 1 shows that *p*H tolerance of the F_1 is intermediate between that of its parents at *p*H 3–5 and is overdominant at *p*H 6–7. Changes in the degree of dominance due to changes in

Table 2 Analysis of variance for length of roots, number of roots, and the ratio between total growth and number of roots at different *p*H levels

Sources of variation	d.f.	Mean squares: Length of roots	Number of roots	Length of roots/number of roots
Replicate(R)	9	101.14	34.73	0.62
*p*H level(L)	4	1,394.33†	395.86†	9.35†
Genotype(G)	2	774.79†	31.25	2.74*
RL	36	116.56	12.96	0.57
RG	18	135.51	20.76	0.43
LG	8	640.90†	35.42	4.65†
RLG	72	117.40	18.94	0.64

*$P<0.02$; †$P<0.01$

the concentrations of heavy metals were also observed[3]. Parsons[4] argued that the hybrid advantage is maximum in extreme environments; this is substantiated by the present results at the higher *p*H levels only. These results also support the theoretical study of environment—dependent heterosis[5].

I thank P. M. Sheppard for providing the seeds and both him and R. Knight for comments on the manuscript.

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Received March 22; accepted May 11, 1976.

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Increased lead ingestion in calcium-deficient monkeys

VOLUNTARY ingestion of lead (lead pica) by children is a puzzling phenomenon. The resulting lead poisoning can lead to painful physical symptoms, mental retardation and brain damage[1-5]. Yet ingestion often persists after toxic symptoms appear if the child has access to lead[6]. We have shown that weanling rats made calcium deficient increased their voluntary ingestion of lead to levels much greater than those of control rats[7]. It was suggested that, because of the metabolic similarity of lead to calcium, ingestion of lead might relieve symptoms of calcium deficiency, attenuate the normal aversive effects of lead ingestion, and thus maintain continued ingestion. Rats, however, are not completely appropriate for an analogy to human lead ingestion because calcium metabolism differs considerably in the two organisms[8,9]. Rats seem to find lead acetate an aversive taste[7,10], whereas humans refer to it as "lead sugar" and report a sweet taste (paper presented at meeting of Midwestern Psychological Association, 1975 by R. W. Henderson and J. Dawley). Furthermore, the diet used with rats has been almost completely deficient in calcium (2 mg Ca per 100 g) producing severe symptoms of calcium deficiency, which have not been reported in humans with lead pica. If calcium deficiency leads to lead pica in humans, it must be a moderate or subclinical deficiency. We have therefore experimented with rhesus monkeys which were subjected to a moderately deficient diet providing 64% (108 mg Ca per 100 g) of their recommended daily calcium levels[11].

Four rhesus monkeys were separated from their mothers shortly after birth and maintained at the University of Wisconsin Primate Laboratory nursery. They were weaned from Similac formula to a Purina monkey chow from which the normally supplemented calcium carbonate and dicalcium phosphate had been removed, leaving 108 mg Ca per 100 g. The monkeys ingested approximately 100 g per day. Six lead ingestion tests were administered—three during deficiency and three during recovery from deficiency when the animals were feeding on normal monkey chow. Two control monkeys that had been weaned to normal chow were available for lead testing only during periods corresponding to the first two drinking tests of the deficient animals and the last drinking test during recovery. Each week the animals were weighed and blood samples were drawn to be analysed by atomic absorption spectrophotometry. Two months after calcium restoration to the deficient animals all animals were given whole body X rays.

Four concentrations of lead acetate (0.08, 0.16, 0.32, and 0.48% weight/volume) were paired with distilled water, and a pair of bottles each containing distilled water was also used. One pair of solutions was presented to an animal each day in a counterbalanced order. The relative positions of lead and distilled water bottles were alternated to control for side preferences, and each of the five pairs of solutions was presented twice. Solutions were presented at 1000 when the animals were being fed and removed at 1800. Distilled water was available overnight, but almost all fluid intake occurred during the hours of the test. For analyses the data were converted to lead acetate intake as a percentage of total intake and to the mg of lead ingested per kg of body weight.

A moderate level of calcium deficiency was produced. With calcium deficiency the body weights of the deficient

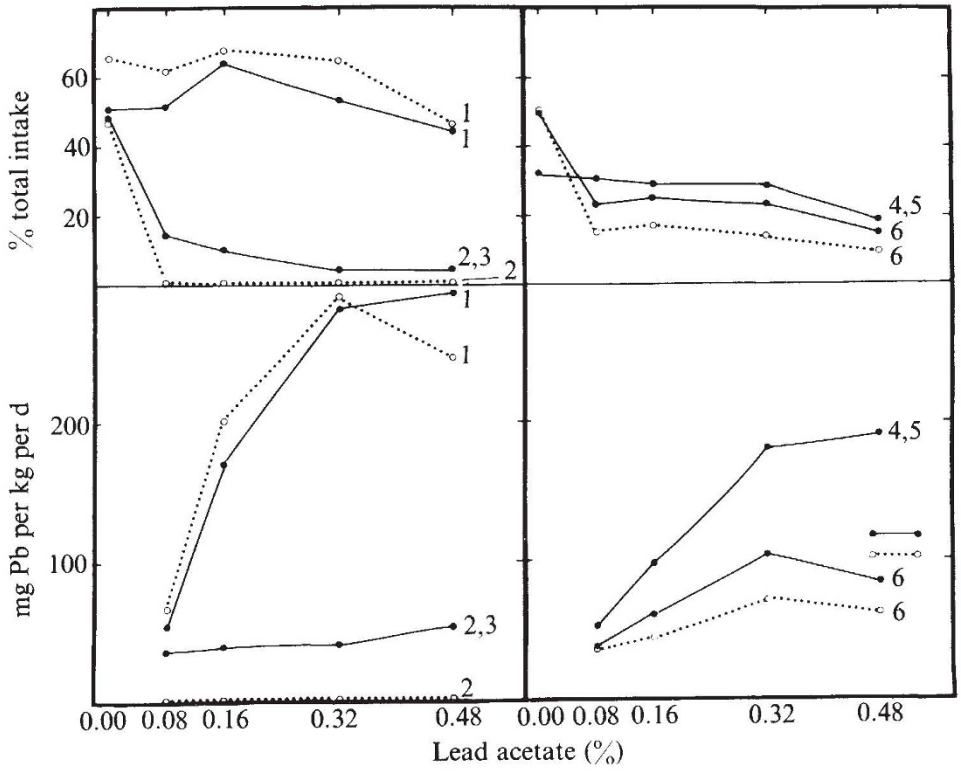

Fig. 1 Lead ingestion presented as percentage of total fluid intake (top) and mg of lead ingested per kg per day (bottom) during both calcium deficiency (left) and calcium restoration (right). Numbers at the side of each curve indicate the drinking test. Test 1 began 3 weeks after the deficient animals were weaned to calcium-deficient diet. Tests 2 and 3 began 8 weeks and 10 weeks respectively after the onset of deficiency. Tests 4 and 5 began 5 weeks and 7 weeks respectively after restoration of normal calcium, and test 6 began 24 weeks after calcium restoration. Results of tests 2 and 3 and, subsequently, tests 4 and 5 for the calcium-deficient animals did not differ so each of these pairs of tests is represented by a single mean curve. ○, Calcium deficient; ●, control.