A polymorphism for phosphate uptake/ arsenate tolerance in *Holcus lanatus* L.: is there a correlation with edaphic or environmental factors?

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Arsenate tolerance in *Holcus lanatus* L. is achieved by suppression of the high affinity phosphate uptake system. Tolerant plants are found at high frequency on noncontaminated soils. The selective agents acting to maintain this polymorphism are not understood. Work on the Park Grass Experiment and a nationwide survey revealed no significant correlation between the frequency of tolerant individuals and environmental or edaphic characteristics of the site. The results are interpreted in light of the low availability of phosphate in soil solution.

Keywords: arsenate tolerance, *Holcus lanatus* L., phosphate uptake, polymorphism.

Introduction

Toxic levels of arsenic may occur in soils and waters either as a result of weathering of rocks containing arsenical ores or, more commonly, from anthropogenic origins, e.g. mining, smelting and the use of pesticides. Many species from bacteria to higher plants show tolerance to arsenate, including *Holcus lanatus* L. (Yorkshire fog). The mechanism of tolerance in this grass has been studied in detail (Meharg & Macnair, 1990, 1991, 1992a,b,c; Macnair *et al.*, 1992; Meharg *et al.*, 1993) and is known to involve a major gene change leading to the suppression of the high affinity phosphate uptake system. Because phosphate and arsenate are chemical analogues they are taken up into the plant by the same uptake system (Asher & Reay, 1979; Meharg & Macnair, 1990). Although suppression of the high affinity uptake system reduces arsenate and phosphate uptake on contaminated soils allowing survival of *H. lanatus*, the presence of a high frequency (15–70 per cent) of arsenate tolerant individuals on noncontaminated sites (Meharg *et al.*, 1993) is puzzling. Phosphate is a major plant nutrient and often limits plant

growth. It is relatively immobile in the soil, being bound as calcium and magnesium salts in calcareous soils or as iron and aluminium salts in acidic soils. Consequently the concentration of phosphate in soil solution is rarely greater than $0.01 \, \text{mol m}^{-3}$ (Bieleski, 1973), which is within the operational range of the high affinity uptake system. The suppression of this uptake system may limit phosphate acquisition by tolerant plants. There are three possible explanations for the high frequency of tolerant plants on noncontaminated soils. (1) Tolerant plants are limited to areas of high phosphate availability, where the low affinity system operates and the high affinity system would not usually be induced. However, these requirements are seldom met in nature and tolerant genotypes would therefore be expected to be rare. (2) Species adapted to infertile soils do not usually show increased rates of ion uptake (Atwell *et al.*, 1980; Chapin, 1980, 1983; Hommels *et al.*, 1990). Instead selection appears to have favoured increased nutrient use efficiency and changes in growth rates, thereby reducing nutrient requirements. Tolerant plants with their apparently less efficient uptake system may therefore be better adapted to areas of poor fertility. (3) Kinetic parameters may be unimportant in determining the uptake of such a poorly soluble nutrient as phosphate, as predicted by mech-

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anistic models (Nye, 1977; Silberbush & Barber, 1983).

To investigate the maintenance of the polymorphism for As tolerance/P uptake, edaphic conditions can be studied in an attempt to relate the frequency of tolerant morphs in a population to the soil phosphate status. This paper reports two investigations with this aim; the first involves *H. lanatus* samples taken from the Park Grass Experiment at Rothamsted Experimental Station, and the second involves a nationwide survey. Lawes & Gilbert set up the Park Grass Experiment almost 150 years ago (Lawes & Gilbert, 1880) to study the effects of different fertilizer treatments on the yield of hay. However, since population differentiation is most likely to occur in spatially heterogeneous environments which are fairly stable in time (Levins, 1962, 1963), the experiment has also provided the ideal environment in which to study the response of plant populations to specific soil factors. In an extensive study, Davies & Snaydon (1973a,b, 1974, 1976), Snaydon (1970) and Snaydon & Davies (1972, 1976) have followed the adaptation of *Anthoxanthum odoratum* (sweet vernal grass) populations to changes in edaphic factors, finding that adaptive morphological and physiological differences have evolved over small distances (less than 0.1 m) and within a short period of time (less than 40 years). It is therefore possible that *H. lanatus* could have similarly adapted to the ecological conditions and this paper reports an investigation into the distribution of *H. lanatus* morphs differing in their phosphate/arsenate uptake characters on the Park Grass Experiment.

The nationwide survey takes advantage of the natural variation in soil types and nutrient status in the U.K. and an attempt was made to relate the frequency of tolerant seed in a population of *H. lanatus* to the edaphic conditions of the site.

Materials and methods

Park Grass Experiment, Rothamsted

The Park Grass Experiment was started in 1856 when a long-established pasture was divided into a series of plots which have subsequently received different fertilizer and liming treatments. The management regime has remained little changed, with the herbage being cut for hay in June and a second cut (previously grazed) in September-November. Fertilizer and liming treatments have influenced soil and plant chemical composition, botanical composition and dominance, herbage height and yield, and soil fauna, creating a mosaic

environment. For further details see Warren & Johnston (1964), Thurston (1969), Thurston *et al.* (1976) and Tilman *et al.* (1994).

Sampling was carried out on three occasions between April and June 1993 from seven subplots differing in their edaphic conditions. Plots were chosen to represent variation in levels of phosphorus, nitrogen, sodium, magnesium, potassium and liming. Approximately 100 tillers of *H. lanatus* were collected along three transects on each subplot by excision at the lowest node possible. In order to minimize the risk of sampling individuals from the same genet twice, tillers were sampled at intervals of at least 1 m. Tillers were then rooted in 1/10th Rorison's nutrient solution (Hewitt, 1966) for 14 days before being potted on into 7.5 cm pots of John Innes No. 2 compost and maintained in the glasshouse for more than 12 months before being tested for tolerance to arsenate.

Tolerance testing Five unrooted tillers of each clone were exposed at the node and supported in perspex tubes in the lid of a 12 L container holding 10 L of nutrient solution of composition 0.2 mol m^{-3} Ca(NO$_3$)$_2$, 0.2 mol m^{-3} KNO$_3$, 0.1 mol m^{-3} MgSO$_4$.7H$_2$O. Arsenic was supplied as sodium arsenate, Na$_2$HAsO$_4$ at a concentration of 0.133 mol m^{-3}. Solutions were changed after one week and root lengths measured after 14 days (Macnair *et al.*, 1992). Clones with mean root length over 40 mm were scored as tolerant (with only the low affinity P uptake system functional) whereas those with root lengths less than 40 mm were classified as nontolerant (high and low affinity P uptake systems operational).

Soil analysis Soil data for the Park Grass plots were from samples taken in 1991 for soluble P and pH, and in 1959 for total P and organic C. On both occasions soil was sampled to a depth of 23 cm. Sixteen or more cores were bulked from each subplot. Samples were air dried and sieved <2 mm prior to analysis for soluble P and pH, <0.5 mm for organic C and <150 μm for total P.

Soluble P was determined by extraction with 0.5 M NaHCO$_3$ at pH 8.5 (Olsen *et al.*, 1954). This fraction of total soil P is regarded as being readily available to the plant. Total P was measured by perchloric acid digestion (Mattingly, 1970). Where no fertilizer P is applied, total soil P is likely to have declined by 5–10 per cent since 1959; where fertilizer P is added, total soil P will probably have increased by 300–400 mg/kg. Organic matter content was determined by dichromate oxidation using a corrected

Walkley & Black method (Bremner & Jenkinson, 1960). Values will have changed little since 1959 on this long-established grassland. pH was measured in distilled water (1:2.5 m/v ratio).

Nationwide survey

Site selection and sampling procedure Advice was sought from Wildlife Trusts to identify suitable undisturbed grassland sites which had not been reseeded in living memory. Survey work was carried out between 21 July and 10 August 1994 to coincide with the presence of ripe seed. At each location a transect was taken through the site and seed collected from more than 100 flower heads that were separated by at least 1 m. Three soil samples were taken along the transect using a soil auger to a depth of 20 cm. These were then bulked to give one sample per site.

Tolerance testing Winnowing was carried out prior to tolerance testing. Seed was pregerminated on moist filter paper for 3 days, at which time germinating seeds with radicals of less than 1 mm were transferred into nutrient solution containing 0.133 mol m^{-3} arsenate solution (details as before). This ensured all seedlings were at the same stage when testing began. A layer of alkathene beads was used to float the seedlings on top of this solution in 200 mL polystyrene cups. Growth was allowed to continue for 7 days at which time the root lengths of

at least 100 seedlings per population were measured (Macnair *et al.*, 1992).

Soil analysis All soil samples were air dried and 2 mm sieved prior to analysis. Total phosphorus was determined by digestion with sulphuric acid-hydrogen peroxide (Allen, 1989) followed by colorimetric autoanalysis. Olsen's reagent (Allen, 1989) was used to determine soluble P. pH was measured in a 1:2 m/v ratio with 0.01 M $CaCl_2$ solution. Organic matter content of soils was estimated by loss on ignition in a muffle furnace at 550°C.

Environmental variables Data on the average annual rainfall at each site was collated from the Meteorological Office Map International Standard Period, 1941–70. Average annual sunshine was taken from the O.S. Physical Map of Great Britain, using data collected over the 30-year period, 1921–50. Latitude and longitude were also recorded for each site.

Results

Park Grass Experiment

Different fertilizer and liming treatments for over 100 years on the plots have led to significantly different values for pH, organic matter content, Olsen P and total soil P (Table 1). The frequency of arsenate tolerant genotypes ranged from 55.8 to 69.0 per cent (Table 1).

Table 1 Soil chemical analysis and frequency of arsenate tolerant morphs of *Holcus lanatus* on plots at the Park Grass Experiment

Plot	Fertilizer treatment	Total P (mg/kg)	Olsen P (mg/kg)	Organic matter content (per cent)	pH	*n*	Per cent tolerant
3d	Control	490	3	5.7	4.8	98	62.2
4/1d	P	1430	142	6.4	4.9	110	66.4
7d	P K Na Mg	1320	160	4.8	4.8	42	69.0
9/2b	N2 P K Na Mg lime	1230	105	6.9	5.4	104	55.8
9/2d	N2 P K Na Mg	1360	186	7.1	3.6	79	60.8
18b	N2 K Na Mg lime	530	5	6.5	5.7	68	63.2
18d	N2 K Na Mg	530	4	5.5	3.6	99	64.6

N2, ammonium sulphate supplying 96 kg N/ha; P, triple superphosphate supplying 35 kg P/ha; K, potassium sulphate supplying 225 kg K/ha; Na, sodium sulphate supplying 15 kg Na/ha; Mg, magnesium sulphate supplying 10 kg Mg/ha; lime, 1903–64 lime applied at 4t $CaCO_3$/ha every fourth year, and from 1965 applied as necessary to raise and maintain pH values of 7, 6 and 5 on subplots a, b and c, respectively.

A Chi-squared test was performed on the frequency of tolerant individuals on the plots. The distribution of tolerant genotypes was homogeneous ($\chi^2_6 = 3.794$, $P > 0.05$), despite a 50-fold difference in Olsen P and a threefold difference in total soil P on the plots studied.

Nationwide survey

The nationwide distribution of the polymorphism is shown in Fig. 1. Every population studied was poly-

morphic. The frequency of tolerant plants was significantly heterogeneous ($\chi^2_{84} = 519$, $P < 0.001$). Table 2 gives details of each site, with data for Olsen and total P, pH and organic matter content. Other environmental factors, rainfall, annual sunshine, latitude and longitude are also included. Correlation coefficients were calculated between the frequency of tolerant plants and edaphic and environmental variables at each site (Table 3). In each case there was no significant correlation between the frequency of tolerant individuals and edaphic or environmental parameters.

Discussion

Park Grass Experiment

The results presented here show no variation in the frequency of tolerant genotypes despite a 50-fold difference in Olsen P and a threefold difference in total soil P on the plots studied. It is unlikely that the lack of variation between plots results from a lack of evolutionary time. Both *A. odoratum* and *H. lanatus* are perennial grasses which regenerate vegetatively and sexually (Watt, 1978). Yet, whereas *A. odoratum* has evolved morphological and physiological differences within 40 years on these plots (Snaydon, 1970), *H. lanatus* has not. *Holcus lanatus* flowers later than *A. odoratum* and is never allowed to set seed on Park Grass because of the timing of the herbage cuts (M. J. Crawley, personal communication). Despite this, differences would still be expected to have evolved as a result of selection operating on the vegetative stages (Hickey & McNeilly, 1975; Davies & Snaydon, 1976).

Another possibility is that the uniform frequency of tolerant individuals on the plots could be a result of seed rain from the surrounding area masking any evolutionary trends on the plots. This seems unlikely for a number of reasons. First, seedling establishment in a closed environment is a rare event (Weir, 1985). Secondly, the surrounding fields are cut at the same time as Park Grass leaving a very limited seed source. The frequency of tolerant progeny within this seed has been investigated and was found to be 56.4 per cent. This figure is significantly lower than the percentage of tolerant plants on the plots themselves. Therefore, it seems unlikely that seed influx is playing a major role in determining the occurrence of tolerant genotypes on the plots, and more likely that the number of tolerant genotypes has not altered in conjunction with the changing edaphic conditions of the plots.
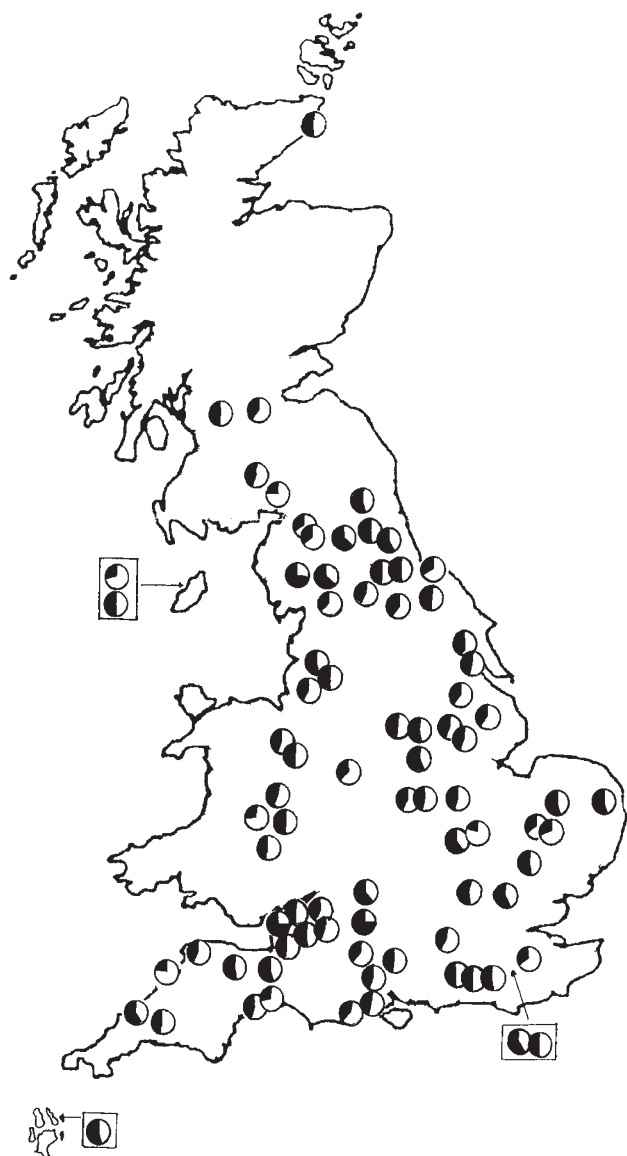

Fig. 1 The distribution of arsenate tolerant *Holcus lanatus* in Britain. Shaded segments represent the percentage of tolerant individuals within each population.

Table 2 Edaphic and environmental details of the sampling sites for *Holcus lanatus* seed

Site	OS grid ref.	Arsenate tolerant seed (per cent)	Total soil P (mg/kg)	Olsen P (mg/kg)	pH	Organic matter content (per cent)	Rainfall (mm)	Annual sunshine (h)	Latitude	Longitude
Lawrence Weston Moor	ST 542 788	74.5	910±90	21±8	6.0	17.3	900	1529	51°30.2'N	2°39.4'W
Kingsweston Down	ST 551 778	52.2	1930±150	5±0	5.5	19.7	900	1529	51°29.6'N	2°38.5'W
Ashton Court Meadow	ST 545 720	43.2	690±100	4±0	5.0	10.0	900	1529	51°26.4'N	2°37.6'W
Folly Farm	ST 610 603	43.8	880±20	10±1	4.9	8.9	1000	1611	51°20.5'N	2°34.1'W
Chancellor's Farm	ST 525 526	53.4	1400±60	31±3	3.9	16.2	1000	1611	51°15.6'N	2°40.9'W
Catcott North	ST 40 41	49.0	590±60	16±1	4.0	18.1	750	1611	51°09.1'N	2°51.9'W
Wellington Monument	ST 138 173	55.0	1380±150	30±3	5.6	28.3	900	1516	50°57.0'N	3°13.9'W
Rackenford	SS 852 183	54.7	1200±50	29±2	3.8	19.7	1200	1516	50°57.2'N	3°37.9'W
Braunton Burrows	SS 460 352	44.6	510±20	7±0	7.0	5.1	900	1566	51°05.8'N	4°12.4'W
Hartland Point	SS 232 276	23.6	920±1	19±2	6.1	11.4	950	1629	51°01.1'N	4°31.9'W
Tintagel	SX 051 889	61.2	1000±70	22±2	4.9	22.8	1000	1629	50°39.8'N	4°45.2'W
Wadebridge	SW 992 723	46.8	910±40	18±0	4.1	10.1	1000	1655	50°31.0'N	4°50.6'W
St. Martins, Isles of Scilly	SV 919 164	54.3	1100±60	47±4	4.6	6.5	880	1725	49°57.6'N	6°17.3'W
Salcombe Regis	SY 149 876	44.6	800±80	29±8	4.9	7.5	800	1635	50°41.4'N	3°12.7'W
Coombe Wood Farm	SY 150 883	29.9	970±60	37±3	5.0	7.4	800	1635	50°41.4'N	3°12.7'W
Lower Test Nature Reserve	SU 346 150	45.3	1260±110	91±1	4.0	7.4	850	1639	50°55.7'N	1°30.6'W
Roydon Woods	SU 310 003	47.6	520±100	24±4	3.7	8.1	850	1765	50°48.2'N	1°30.6'W
Keyhaven & Pennington Marshes	SZ 310 710	41.3	1070±130	42±1	7.0	12.4	800	1765	50°43.3'N	1°33.3'W
Broughton Down	SU 294 327	40.0	1700±110	10±1	7.2	14.9	800	1639	51°06.0'N	1°34.8'W
Winnall Moors	SU 487 300	47.3	1130±100	38±1	7.1	13.4	850	1639	51°04.3'N	1°17.6'W
Fleet Pond Nature Reserve	SU 825 550	44.9	410±20	34±16	3.3	11.7	750	1569	51°17.5'N	0°49.0'W
Partridge Green	TQ 181 212	49.1	650±160	15±1	5.6	8.7	850	1655	50°57.7'N	0°17.9'W
Partridge Green	TQ 183 208	51.8	580±120	13±1	5.7	5.7	850	1655	50°57.7'N	0°17.9'W
Colwood Park	TQ 250 238	54.6	720±120	8±1	4.4	6.2	850	1655	51°00.3'N	0°12.7'W
Flimwell	TQ 716 314	58.5	610±130	5±0	6.1	6.7	850	1655	51°03.4'N	0°26.8'E
Burwash Common	TQ 645 235	50.7	650±30	178±0	5.1	5.8	850	1655	50°59.2'N	0°20.6'E
Pembury	TQ 618 408	34.3	720±90	38±14	3.6	9.6	800	1655	51°08.4'N	0°19.3'E
Rothamsted Experimental Station	TL 12 13	56.4	820±60	29±3	3.6	9.7	700	1510	51°48.5'N	0°22.1'W
Bishop Stortford	TL 495 230	58.0	1140±140	44±11	6.6	10.2	600	1510	51°52.3'N	0°10.3'E
Stoke College	TL 739 432	55.8	1400±40	50±6	4.9	9.4	550	1488	52°03.7'N	0°32.7'E
Thetford Forest	TL 83 85	35.6	800±2	9±1	6.9	6.8	600	1572	52°25.1'N	0°41.9'E
Thetford Warren	TL 83 84	32.1	380±50	10±0	7.0	2.4	600	1572	52°25.1'N	0°41.9'E
East Wretham Nature Reserve	TL 910 882	56.7	550±220	26±4	3.5	5.9	600	1572	52°28.7'N	0°49.2'E
Allimore Green	SJ 856 193	35.9	920±50	18±0	4.5	31.8	750	1339	52°46.3'N	5°12.9'W
Close Mean, Isle of Man	SC 359 954	51.6	530±60	7±1	4.1	5.4	900	1562	54°19.7'N	4°30.9'W
Consortium Field, Isle of Man	SC 363 972	30.0	450±10	5±0	4.0	6.7	900	1562	54°20.8'N	4°31.0'W
Lumpsey Mine Grassland	NZ 683 188	33.0	680±100	6±1	5.9	13.0	750	1391	54°33.6'N	2°29.3'W
Calderglen Country Park	NS 658 548	49.1	1540±50	40±12	4.3	16.2	1150	1297	55°48.6'N	4°06.9'W
Allanton	NS 856 567	39.3	820±50	49±28	3.9	19.3	850	1295	55°47.8'N	4°06.9'W
Wick	ND 36 50	51.1	550±2	31±3	3.9	18.6	775	1317	58°26.3'N	3°05.3'W

Table 2 *Continued*

Site	OS grid ref.	Arsenate tolerant seed (per cent)	Total soil P (mg/kg)	Olsen P (mg/kg)	pH	Organic matter content (per cent)	Rainfall (mm)	Annual sunshine (h)	Latitude	Longitude
Bure Marshes	TG 452 100	54.2	680±40	8±1	6.6	8.6	600	1616	52°38.2'N	1°35.9'E
Hinchingbrook Country Park	TL 225 714	60.6	680±50	16±1	6.7	6.0	550	1488	52°19.6'N	0°12.2'W
Hinchingbrook Country Park	TL 225 714	21.8	930±10	17±6	6.6	13.9	550	1488	52°19.6'N	0°12.2'W
Brigstock Country Park	SP 945 854	45.4	920±50	39±5	6.7	10.7	600	1427	52°27.5'N	0°36.6'W
Naseby	SP 682 797	42.1	1050±180	15±3	4.4	12.5	650	1427	52°23.4'N	0°59.6'W
Sibbertoft	SP 675 834	45.4	1120±40	30±2	4.2	11.2	650	1427	52°26.1'N	0°59.6'W
Teversal Pastures	SK 492 617	50.0	910±20	5±1	5.1	13.8	700	1511	53°08.9'N	1°16.5'W
Rainworth Heath	SK 590 590	55.4	270±100	22±1	3.6	5.4	700	1511	53°07.2'N	1°06.7'W
Eakring Meadows	SK 709 619	45.3	990±70	11±2	5.3	16.5	600	1511	53°09.3'N	0°57.7'W
Eakring Meadows	SK 702 622	45.2	850±20	8±1	6.3	11.6	600	1511	53°09.3'N	0°57.7'W
Chilwell Meadow	SK 520 365	58.4	1240±60	28±6	5.0	22.2	600	1511	52°54.8'N	1°14.1'W
Cliff Farm	SK 968 762	39.3	1020±100	39±15	7.1	9.8	600	1511	53°15.5'N	0°33.2'W
Kirton in Lindsey	SK 953 973	40.2	680±60	6±0	7.0	6.5	650	1330	53°28.5'N	0°35.5'W
Humber Bridge Country Park	TA 018 258	48.9	980±10	26±12	7.0	12.3	650	1330	53°42.4'N	0°26.9'W
Kilnwick	TA 003 496	47.8	860±1	22±4	7.2	8.7	650	1330	53°55.9'N	0°29.1'W
Old Malton	SE 79 72	50.5	990±220	15±3	5.5	8.6	700	1310	54°08.5'N	0°47.0'W
Ampleforth Forest	SE 54 82	39.8	320±10	6±0	6.9	4.8	700	1310	54°14.6'N	1°10.8'W
Malton	NZ 213 454	58.8	520±50	13±2	4.3	8.2	725	1325	54°47.7'N	1°40.0'W
Low Barns Nature Reserve	NZ 160 315	48.7	520±10	6±1	4.6	6.1	775	1325	54°40.7'N	1°46.5'W
Low Barns Nature Reserve	NZ 160 315	51.3	450±60	5±2	5.1	6.7	775	1325	54°40.7'N	1°46.5'W
Hannah's Meadow	NY 936 186	45.1	740±130	20±1	4.0	11.8	1050	1325	54°33.7'N	2°05.1'W
Cornsay	NZ 144 434	51.9	390±10	4±1	6.8	8.1	800	1325	54°47.1'N	1°46.5'W
New Ridley	NZ 034 570	55.9	460±30	7±1	6.7	10.4	650	1333	54°55.8'N	1°54.9'W
Kilhope	NY 835 420	62.2	570±60	11±4	4.6	18.2	1400	1333	54°34.7'N	2°13.5'W
Warwick	NY 455 561	33.9	430±20	8±1	6.1	6.2	850	1218	54°54.0'N	2°50.1'W
Bankend	NY 364 603	23.9	1090±10	70±1	4.4	7.4	950	1218	54°56.1'N	2°59.5'W
Torthorwald	NY 016 777	44.4	1680±110	16±3	4.0	62.7	1150	1218	55°04.9'N	3°32.6'W
Wreay	NY 431 489	35.5	620±120	12±1	5.8	5.1	850	1218	54°49.7'N	2°52.8'W
Hawsewater Reservoir	NY 468 103	72.5	1190±40	18±2	3.8	10.8	2800	1207	54°29.7'N	2°49.6'W
Bampton Grange	NY 522 180	62.7	990±100	14±3	3.6	8.9	1500	1207	54°33.5'N	2°45.0'W
Bampton	NY 51 17	37.5	1650±4	18±2	5.6	16.3	1500	1207	54°33.5'N	2°45.0'W
Charnock Richard	SD 564 154	53.4	1110±50	27±8	4.0	13.9	1000	1291	53°38.0'N	2°40.4'W
Gathurst	SD 539 074	49.6	1440±110	40±2	4.2	25.6	1000	1291	53°33.7'N	2°41.3'W
Coppull Moor	SD 555 125	42.1	420±130	9±0	6.8	4.0	1000	1291	53°36.4'N	2°40.4'W
Cefn Mawr	SJ 288 420	44.6	1730±130	27±4	5.4	15.3	800	1413	52°58.5'N	3°03.9'W
Oswestry	SJ 28 40	50.4	1100±140	9±1	5.6	10.5	800	1413	52°57.4'N	3°03.9'W
Forden	SJ 23 00	45.7	1020±10	13±3	4.4	10.7	800	1292	52°35.8'N	3°08.7'W
Dyfnant Forest	SH 99 16	28.1	780±60	5±0	4.7	9.0	800	1292	52°43.6'N	3°28.4'W
Roundton Hill Reserve	SO 29 95	50.0	1390±100	24±5	5.7	12.3	900	1292	52°33.1'N	3°02.4'W
Kington	SO 27 57	47.1	900±170	13±2	4.1	18.7	1200	1368	52°12.1'N	3°01.9'W
Minety	SU 010 906	61.9	1190±120	23±0	4.5	16.7	775	1482	51°36.8'N	1°57.9'W
Wotton Bassett	SU 094 818	73.7	810±1	15±8	5.5	19.3	725	1482	51°32.4'N	1°54.4'W

Table 3 Correlation coefficients and probabilities for the frequency of arsenate tolerant *Holcus lanatus* and ecological variables (NS = non significant)

Character	r	P
Total soil P	0.058	>0.05 NS
Olsen P	0.025	>0.05 NS
pH	0.214	>0.05 NS
Organic matter content	0.102	>0.05 NS
Average annual rainfall	0.206	>0.05 NS
Average annual sunshine	0.047	>0.05 NS
Longitude	0.107	>0.05 NS
Latitude	0.093	>0.05 NS

Nationwide survey

Although the frequency of arsenate tolerant genotypes was invariably high on uncontaminated grassland, the attempt to account for the nationwide distribution of the polymorphism by variation in edaphic/environmental factors proved unsuccessful. Arsenic tolerance is undoubtedly a major selective agent on mine sites, with the frequency of tolerant plants on contaminated areas being greater than 90 per cent (Meharg *et al.*, 1993). Throughout the metal tolerance literature the existence of sharp clines on mine boundaries (McNeilly, 1968; Antonovics & Bradshaw, 1970) and the very low frequency of tolerant plants on noncontaminated sites (Gartside & McNeilly, 1974; Walley *et al.*, 1974; Symeonidis *et al.*, 1985; Ingram, 1988) provides strong evidence for a 'cost' of metal tolerance. The polymorphism in *H. lanatus* is therefore unusual and suggests either a low cost of arsenate tolerance, or that there are other selective agents operating to maintain a high frequency of tolerant genotypes in every population.

If suppression of the high affinity P uptake system in tolerant plants means they are restricted to areas of high soil phosphorus, then a correlation would be expected between edaphic conditions and the frequency of tolerant genotypes. The hypothesis that tolerant plants might be better adapted to infertile soil should also result in a similar correlation. Yet, neither the results of the work at the Park Grass Experiment nor the nationwide survey give any evidence of a relationship between the frequency of tolerant morphs and soil phosphate status. The explanation may be that uptake kinetics are unimportant in determining the rate of phosphate uptake. Uptake of phosphate occurs via two carrier systems operating in the plasmalemma, both of which obey Michaelis–Menten uptake kinetics (Epstein, 1976).

The low affinity system operates at high substrate concentration, whereas the high affinity system is induced under conditions of phosphate stress (Clarkson *et al.*, 1978; Clarkson & Lüttge, 1991). Because the concentration of phosphate in soil solution is low (Bieleski, 1973), the high affinity system should be functional under most ecological conditions. However, Nye (1977) found diffusion to be the limiting step in the uptake of poorly soluble nutrients, e.g. phosphate. Because of the binding of P to soil particles and its low rate of diffusion through the soil, a depletion zone is created around plant roots and further uptake can only occur once P has diffused from the bulk soil into the depletion zone. Silberbush & Barber (1983) modelled P uptake using a Cushman simulation model involving 11 plant and soil parameters and also concluded that kinetic parameters were relatively unimportant in determining the rate of phosphate uptake. Given that diffusion appears to be the rate-limiting step in the uptake process, increasing the efficiency of ion transport proteins in the plasmalemma, either by increasing V_{max} (increasing the rate of ion uptake) or reducing K_m (increasing the affinity of the carrier for the substrate) may be fruitless. Intuitively it seems that an individual plant should maximize its ion uptake when nutrients are in short supply. However, studies on *Carex* spp. (Atwell *et al.*, 1980) and *Taraxacum* spp. (Hommels *et al.*, 1990) have shown no clear trends between ion uptake capacity and nutrient status of the habitat, a conclusion also reached by Chapin (1980, 1983). The fact that genotypes of *H. lanatus* with differing uptake kinetics were found within the same population also gives support to these studies and the role of kinetic parameters remains unclear.

Even if, as might be suggested by these results, phosphate uptake kinetics are unimportant in determining the competitive ability of arsenate tolerant and nontolerant clones, then the existence of the polymorphism is still an enigma. Nontolerant genotypes have a higher V_{max} (Meharg & Macnair, 1992b), which has been attributed to the existence of more carrier proteins in the plasmalemma (Drew *et al.*, 1984). If uptake kinetics are unimportant, then why do nontolerant plants continue to divert valuable resources into the production of a presumably costly carrier protein? If, on the other hand, the high affinity phosphate system is important then how does such a high frequency of tolerant plants survive in a natural environment? Wilson (1988) proposed that metal tolerance must involve some 'cost' to the plant, reasoning that otherwise the considerable gene flow between tolerant and nontolerant popula-

tions would cause the tolerance gene to spread through the population and reach a high level, or fixation. The high frequency of arsenate tolerant plants on noncontaminated soils and the lack of evidence for phosphate uptake acting as a selection agent to maintain the polymorphism might lead one to conclude that, in contrast, arsenate tolerance in *H. lanatus* is relatively 'cheap'.

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