

Development of P-Hyperaccumulator Plant Strategies to Remediate Soils with Excess P Concentrations

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ABSTRACT: The U.S. livestock industry has evolved to confine a large number of animals on a few farms in concentrated areas in many states. The trend to fewer, larger operations coupled with highly intensive production methods has resulted in more concentration of manure nutrients within relatively small geographic areas. Researchers in these areas have reported that manure production is contributing more phosphorus (P) than available cropland can assimilate. Overapplication of manure nutrients combined with low P removal rates by many crops is frequently cited as a reason for the accumulation of excess soil P. We propose that higher amounts of soil P can be removed from soil using vegetative management. Soil P concentration can be reduced in fields with excess levels by using P-hyperaccumulator plants or growing plants that have been modified to increase their P-uptake characteristics through traditional breeding and transgenic techniques. In this context, we identify plant properties (root architecture, secretion of organic acids, etc.) that may be improved using these two techniques.

KEY WORDS: phosphorus, crop management, phytoextraction, manure, soil, hyperaccumulation.

I. INTRODUCTION

Over the last 20 years, there has been a dramatic shift in the structure of animal production across the U.S. Small and medium-sized livestock operations have been replaced with large operations (Kellog et al., 2000). Large confined operations typically have high animal populations, and these operations have become more spatially concentrated across the USA. For example, during the last 20-year period, poultry and hog populations in AR have more than doubled (USDA-NASS, 1998). The swine population in NC has also increased dramatically from 4.4 million head in 1994 to almost 10 million in 2000 (North Carolina Dept. Agriculture, 2001). This trend has resulted in a huge supply of animal manure for disposal on a limited amount of land area. Intensive, long-term application rates of manure to soils in these regions have contributed to frequent

reports that the quantity of manure nutrients relative to the assimilative capacity of the land has grown out of balance (Barker and Zublena, 1995; Kellog et al., 2000).

Animal waste management rules are used in many states to regulate animal manure application to soils. These rules require that animal production facilities have manure nutrient management plans that base manure application rates on crop nitrogen (N) requirements. Crops have a much lower phosphorus (P) than N nutritional requirement (Whitehead, 2000). Consequently, this imbalance has contributed to an accumulation of soil P to levels many times that required for sufficient crop growth in states with concentrated animal production (Sharpley and Halvorson, 1994; Novak et al., 2000; Sims et al., 2000). For example, in the MD portion of the Chesapeake Bay region, an area with concentrated poultry production, Coale (2000) reported that over 70%

of the soils that have been used for long-term poultry waste application are now considered to have optimal (no P input to obtain maximum yield) or excessive P contents. Sims et al. (2000) reported that in Sussex Co., DE, which alone produces 230 million broilers and 52,000 head of swine per year, approximately 92% of soils in the county were rated as optimum or excessive in P. Barker and Zublena (1995) reported that 18 counties in NC (18% of NC total counties) did exceed their yearly crop P needs with P supplied from animal manure. While the build up of P does not harm fertility (Peterson et al., 1994), incidental losses of P from fields with excessive concentrations into water bodies have caused environmental concerns about a decrease in surface water quality (Sharpley, 1999; Sims et al., 2000). The transport of P from agricultural fields high in soil P has been cited as a factor contributing to *Pfeisteria* spp., which has been linked to fish kills and human health problems (Burkholder et al., 1997).

There are practices available to reduce the off-site movement of P from fields receiving manure. Soil amendments (e.g., alum-containing material and gypsum) can immobilize P by forming an insoluble complex (Moore and Miller, 1994; Anderson et al., 1995; Peters and Basta, 1996; Smith et al., 2002). Riparian buffers reduce P movement into streams from fields receiving animal manure (Lowrance et al., 1984; Mulholland, 1992; Novak et al., 2001). Cultural practices (e.g., minimal tillage, surface residue, crop rotation, etc.) that minimize surface runoff and erosion also reduce P movement (Sharpley and Halvorson, 1994).

The focus of these management practices is to minimize off-site P transport using soil amendments, riparian buffers, and tillage management practices. While these practices have been shown to be successful, they have not targeted an *in situ* reduction in soil P concentrations. Delorme et al. (2000) and Frossard et al. (2000) both suggested that vegetative management could be used to reduce soil P concentrations. This form of vegetative management of a target compound is referred to as “phytoremediation” (Wenzel et al., 1999; USEPA, 2000). Delorme et al. (2000) reported that many common row and forage crops, how-

ever, can only remove low quantities of soil P. A logical concept to follow in this scheme is that more P can be removed by increasing foliar (stems and leaves) P uptake amounts and removing the P in harvestable yields. The harvested biomass should be removed from the field site to ensure that the removed P is not returned back to the soil (as manure from foraging animals, etc.).

Our review of the literature has revealed that plants typically have between 0.1 and 0.9 % P (dry matter basis) in stems and leaves. Concentrations higher than 1% P in the dry matter of some plant varieties can often be toxic (Marschner, 1995). Some plant varieties, however, can hyperaccumulate P (defined as % P contents between 0.8 and 1.45 in dry matter) and should be considered as a new crop management approach to decrease soil P concentrations. It is logical to consider isolating P nutritional traits from the germplasm of these P-hyperaccumulator plants and adding these traits through traditional breeding or modern transgenic techniques to common row and forage crops. To maximize soil P removal by these P-hyperaccumulator plants, we identify potential plant physical and chemical characteristics for genetic modification that regulate P acquisition, translocation, and storage. Reducing soil P contents using P-hyperaccumulator crops, therefore, offers a large benefit of increasing the nutrient assimilative capacity of land used for manure disposal.

II. SOIL P ACCUMULATION AND ENVIRONMENTAL CONSEQUENCES

A. Plant N and P Utilization from Manure

Understanding reasons for the build up of P in soils will require a description of manure nutrient characteristics and crop nutrient uptake. Animal manures contain N, P, and potassium (K) (Table 1). These elements are vital plant nutrients and are often added to soil as a fertilizer. As shown in Table 1, manure nutrient characteristics can vary greatly among sources.

Animal manure sources contain more N than P (Table 1); consequently, nutrient management plans base application rates on balancing manure N with

Table 1. Average nutrient contents of animal manures (values are for fresh manure and urine without storage and handling losses)†.

animal type	animal wt (kg)	N	P	K
		----- kg ton ⁻¹ -----		
dairy cattle	400	5.0	0.9	3.3
beef cattle	400	5.6	1.8	3.9
nursery pig	16	6.8	2.4	4.4
finisher pig	70	6.8	2.4	4.4
boar	160	6.9	2.4	4.4
layer chicken	1.8	13.5	5.1	5.4
broiler chicken	0.9	16.6	3.6	5.1
turkey	7	11.8	4.0	7.0

†adapted from the MWPS 18, Livestock Waste Facilities Handbook (1985)

that removed by a crop. This management protocol is known to contribute to overapplication of P because plants have a much lower P than N nutritional requirement. For example, Novak et al. (2000) reported that the quantity of P added as swine manure yearly to soil (83 kg ha⁻¹) in a NC spray field is approximately 12 to 13 kg ha⁻¹ higher than the annual P removed (70.6 kg ha⁻¹) by Coastal bermuda grass (*Cynodon dactylon* L.) (Figure 1, assuming a yearly yield of 22.4 Mg ha⁻¹, Pierzynski and Logan, 1993). If other crops were used to assimilate N in this field at the same manure application rate, then the P imbalance would be higher (Figure 1).

The magnitude of excess P production from animal manure by farms across the USA has been estimated by Kellog et al. (2000). They compared trends in manure P production by animal operations (swine, poultry, beef) and the assimilative capacity of crops. Kellog et al. (2000) defined the P assimilative capacity (soil P removed by row crop and pasture uptake) to be an estimate of the amount of nutrients that could be applied to land from manure applications without building up nutrient levels in the soils over time. Because the east coast and southeastern states have concentrated poultry and swine production, there is a large mass production of excess farm-level P

(Table 2). For example, NC has the second highest swine population in the U.S. (North Carolina Dept. Agriculture, 2001) and also has the highest estimated mass of excess P produced among the 10 compared states (Table 2). Kellog et al. (2000) explained that the imbalance between farm-level manure P generation, and that removed by soils and crops is due to larger and more concentrated animal production. In 1997, almost 409 million metric tonnes of farm-level excess manure P were produced across the U.S. (Kellog et al., 2000).

It should not be surprising that states with high amounts of manure production and intensive rates of land application are also experiencing a high percentage of soils testing high or excessive for soil plant available P levels (Table 2). In 1997, more than 60% of the soils submitted to the soil test laboratories in AR, DE, MD, and NC tested high or above for plant available P (Potash & Phosphorus Institute, 1998). This trend implies that there is a serious imbalance between P added as manure and that removed by a harvested crop. This is a serious issue because the risk of impaired surface water quality increases in regions with soils that contain excess soil P levels (Sharpley, 1999).

An additional factor contributing to the accumulation of excess soil P concentrations is the repeated

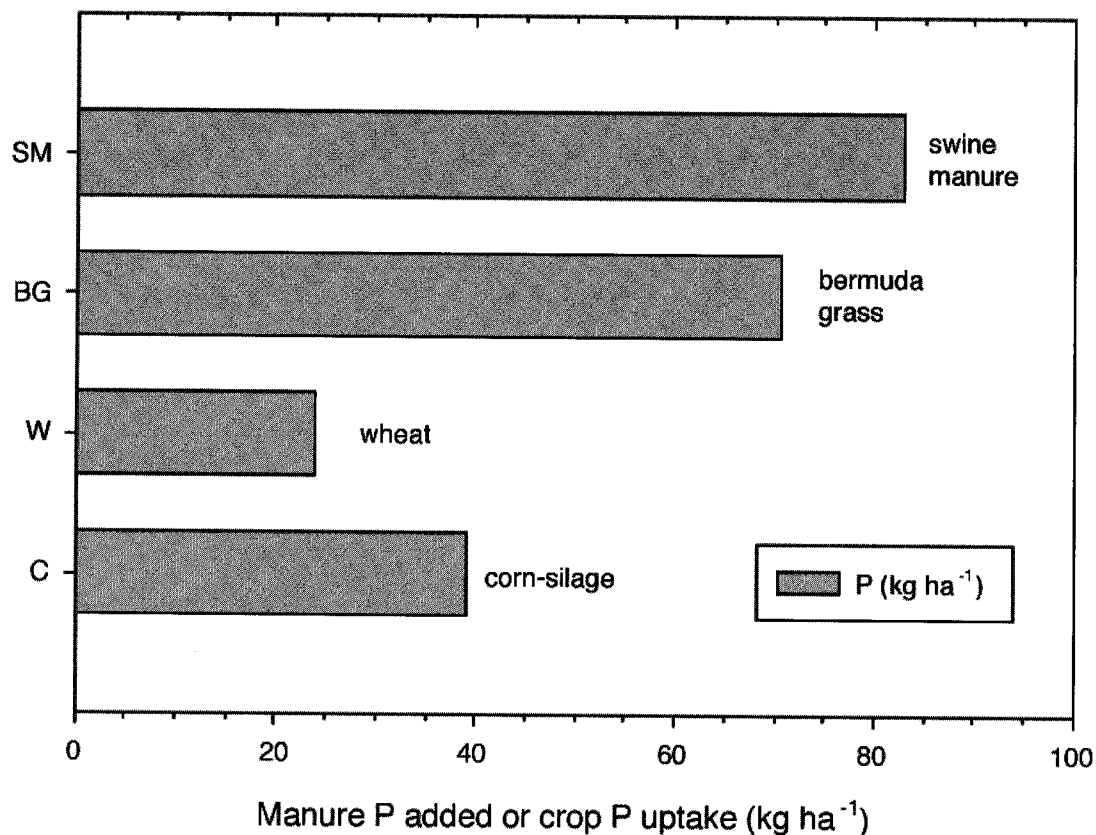


FIGURE 1. Phosphorus added in swine manure ($83 \text{ kg ha}^{-1} \text{ yr}^{-1}$) is higher than that removed in the harvested crop (kg ha^{-1}) when swine manure is added to provide 334 kg ha^{-1} (data adapted from Novak et al., 2000).

application of animal manure onto the same fields. Transporting animal manure is expensive and time consuming, so it is convenient for the producer to apply manure to fields close to the production facility. In fact, Sharpley (1999) reported that manures produced by animal operations in PA are rarely transported more than 16 km from the source of generation. This trend frequently results in the same fields receiving repeated application of animal manure for an extended period of time. As shown in Table 3, 10 years of manure additions to the same fields in OK, AL, and NC resulted in soil plant available P concentrations in excess of 200 mg P kg^{-1} soil.

B. Environmental Consequences of Excess Soil P

The accumulation of P to excessive levels in soils and incidental transport of P into surface

water bodies have caused serious concern of water quality deterioration in regions with concentrated animal production. Soil with excessive P levels has been identified as an important source of nonpoint pollution because of the high amount of off-site P transport in runoff, subsurface drainage, and leaching of P to ground water (Sims et al., 1998; Sharpley, 1999; Novak et al., 2002). Transport of P into sensitive surface water systems will contribute to imbalances in stream water nutrient concentrations and promote eutrophication (USEPA, 1996). The eutrophication of surface water systems is a serious issue because it restricts water use for fisheries, recreation, industry, and drinking purposes due to the increase growth of undesirable microorganisms, algae, and aquatic weeds and resulting oxygen shortages. Potential serious animal and human health problems have been linked to nutrient enrichment of surface water systems that stimulate outbreaks of

Table 2. Yearly mass of farm-level excess P produced by animal operations and percentage of soils samples measured with higher or above for plant available P (adapted from Kellog et al., 2000, and Potash & Phosphorus Institute, 1998).

State	yearly mass of farm-level excess P (tonnes)	% soil samples with high P
AR	22,900	65
AL	14,283	25
DE	3,285	74
FL	4,200	53
GA	19,267	40
MD	4,709	67
NC	44,532	74
PA	10,795	46
SC	6,775	58
VA	11,269	58

Cyanobacteria spp. (Lawton and Codd, 1991; Martin and Cooke, 1994) and *Pfeisteria* spp. (Burkholder et al., 1997).

III. DEVELOPMENT OF PLANT-BASED P REMEDIATION STRATEGIES

A. Plant P Uptake After the Cessation of Manure Application

One approach to decrease soil P contents is to simply cease applying manure to fields and allow crops sufficient time to remove P to a target level. This approach has already been examined by Sharpley (1999), who provided examples showing that it can take several years for a decline of soil test P values by crop removal after further applications have ceased. For example, McCollum (1991) estimated that without further P additions, 14 years of corn (*Zea mays* L.) or soybean (*Glycine max* L. Merr.) production in a NC sandy Ultisol would be needed to lower soil test P to agronomic levels. This time frame was calculated

based on the initial Ultisol soil test P concentration of between 100 and 120 mg P kg⁻¹ and a P removal rate by harvested products to reduce soil test P to agronomic threshold levels of 20 to 24 mg P kg⁻¹ soil (McCollum, 1991). This means that relying on current P uptake rates by crops to decrease plant available P concentrations will require decades, especially in soils with P concentrations in excess of 200 mg P kg⁻¹ soil. The large period of time required for crops to reduce excess soil P levels to a lower target level may make this approach unrealistic for animal production operations applying manure to limited amounts of land area for disposal.

B. Typical Crop P Removal Rates from Soil

A contributing factor to soil P accumulation in manure treated soils is the low amount of P removed with the harvested crop. Pierzynski and Logan (1993) and Delorme et al. (2000) summarized the relationships between crop yields and the

Table 3. Plant available P in soil with manure applied for several years versus untreated soil

Manure source/soil	crop	added P kg ha ⁻¹ yr ⁻¹	time (yr)	available soil P (mg kg ⁻¹)			reference and location
				method	untrt.	trt.	
poultry litter							
Cahaba	grass	130	12	Bray	5	216	Sharpley et al. (1993), OK
Ruston		100	12		12	342	
Stigler		35	35		14	239	
sandstone	grass	-----	10	Mehlich 1	30	230	Kingery et al. (1994), AL
swine manure							
Norfolk	grass	109	11	Mehlich 1	80	235	King et al. (1990), NC
		218	11		80	310	
		437	11		80	450	
Autryville	grass	83-625	10	Mehlich 3	7.5	353	Novak et al. (2000), NC
Cecil	grass	160	3	Mehlich 1	19	45	Reddy et al. (1980), NC
		320	3		19	100	

amount of P removed from soil for a large number of agronomic and horticultural crops (Table 4). There is an extensive variation in the uptake and removal of P among the various crop species. Corn harvested for silage removes much less soil P (0.6 kg P Mg^{-1}) than does Coastal bermuda grass (3.2 kg P Mg^{-1}). Coastal bermuda grass is preferentially used in hay and pasture fields in many southeastern states because it has a very high N requirement (between 400 to 675 kg N ha^{-1} ; Edwards and Daniel, 1992). As explained earlier, a down side of using this grass variety for manure nutrient assimilation is a low amount of P removed relative to N by the harvested crop (3.2 kg P Mg^{-1}) and its winter dormancy.

The application of manure to dormant bermuda grass fields can continue if the field is oversown with a temperate forage grass such as Red clover (*Trifolium pratense* L.). Red clover can be oversown in late summer or early fall in bermuda grass fields and can be grazed during the winter and spring (Bagley et al., 1988). Unfortunately, red clover, has a relatively smaller amount of P removed with the harvested crop (2.5 kg P Mg^{-1}) than Coastal bermuda grass. Alternative forage grass species can be used to manage soil P concentrations, but they also have limitations. For instance, Johnson grass (*Sorghum halepense* L.) is used in AR and MS pastures to assimilate nutrients from manure and has a high amount of P removed in the harvested crop (3.5 kg P Mg^{-1}). The acceptance of Johnson grass to assimilate nutrients may be limited in other states because it is viewed as a noxious weed, and if used as a forage can cause moderate to high cyanide toxicity for ruminants (Purdue University Cooperative Extension Service, 2001). Fescue (*Festuca* sp.) has a high amount of P removed with the harvested crop (3.5 kg P Mg^{-1}), but it has a low tolerance to hot and dry conditions. Indian mustard (*Brassica juncea* L.) has the advantage of having the highest P removed per unit yield (Table 4); however, it is unsuitable as a forage for animals.

C. Vegetative Management of Phosphorus

The strategy of vegetative P management has been evaluated more extensively in the wastewa-

ter wetland treatment area (DeBusk and Dierberg, 1999; Kadlec, 1999; Horne, 2000) than in the agronomic crop management area. Wastewater containing P can be treated using a wetland system through P assimilation by aquatic plant communities (macrophytes), microorganisms and algae (periphyton) (Boyd, 1970; Davis et al., 1990). Wetland plant communities have considerable differences in their efficiency to assimilate P from waste water. For instance, laboratory and greenhouse studies have demonstrated that harvested macrophytes and periphyton communities have exhibited mass P removal 30- to 50-fold higher than mass loads achieved in nonharvested wetland cells (DeBusk and Dierberg, 1999). Adler et al. (1996) also demonstrated high P removal (80% of P in nutrient media) by a mixture of moisture-tolerant grasses in a wetland system treated with dissolved P. In these studies, high P removal rates were dependent on removing P from the system through biomass harvesting. In contrast to these results, Tanner (1996) reported that the mass of P taken up from dairy wastewater by macrophytes represented only a minor portion of the total P assimilated by the wetland system. As noted by DeBusk and Dierberg (1999), the management practice of harvesting aquatic biomass from wetlands is not acceptable to those scientists who view these regimes as a passive nutrient treatment system (no harvesting of biomass).

Managing soil P levels using selective cropping practices has been proposed by Kelling et al. (1991) and Pierzynski and Logan (1993). The concept is to grow two or more crop species in the same field in a year. Through this scheme, soil nutrient levels can be managed if the crop rotation consists of crop species that have different nutritional requirements. For example, Earhart (1995) evaluated the potential of using warm- and cool-season legumes and legume-grass mixtures in rotational cropping in OK to remove excess soil P supplied by poultry litter. He reported that the cropping system approach was effective at reducing soil P concentrations to a lower target concentration. Double cropping of small grains and soybeans is commonly used in southern regions of the US because the long and moist growing season allows for growth and maturation of more than one crop (McKibben and Pendleton, 1968).

Table 4. Crop P concentrations, yields, P removed from soil and P removed on a unit yield basis (adapted from Pierzynski and Logan, 1993 and Deforme et al. 2000).

Crop	yield (Mg ha ⁻¹)	P removed from soil (kg ha ⁻¹)	P per unit yield (kg P Mg ⁻¹)
Corn silage	67.2	39.2	0.6
Coastal bermuda grass	22.4	70.6	3.2
Red clover	9	22.4	2.5
Johnson grass	26.9	93	3.5
Fescue	8.9	32.2	3.6
Indian mustard	18	84.6	4.7

†measured in shoots and leaves

However, its purpose is to obtain two crop yields during the growing season and not specifically to manage soil nutrient levels. The use of selective cropping to manage soil nutrient levels may be applicable to other regions of the U.S., although its potential application has not been fully explored.

D. Breeding Plants to Remove More Soil P

As discussed previously, current P uptake rates are low for common row crops and forage grasses used to assimilate P from manure. Delorme et al. (2000) suggested that more soil P can be removed by growing plants with a high P nutritional requirement or producing higher above ground biomass and remove P with the harvested products. Plant P removal may be also increased by targeting mechanisms for plant P acquisition and translocation. These mechanisms could be modified in common row and forage varieties through breeding or genetic modification to facilitate an increase in P uptake, storage, and movement. Strategies to improve plant P uptake through the use of these two techniques are presented below.

Traditional breeding techniques such as screening/selection can be used to identify a specific genetic trait among plant varieties. A breeder would need to obtain many different plant varieties from diverse geographic regions to examine for a particular trait among the germplasm. If the percent P in dry matter is chosen as a selection criteria, then the plant varieties can be subject to abiotic stress tests (Edmeades et al., 2001). The abiotic stress tests can consist of growing plants in soils or solutions containing very high P concentrations because it is known that plant P uptake will increase when the external P supply increases (Hylton et al., 1965; Baker et al., 1970; Banwart and Pierre, 1975). The stress test must be severe enough, however, to kill some varieties while allowing for the expression or activation of gene complexes in survivor cultivars. Repeating the test by growing the survivor plants in soils with very high P concentrations will allow the breeder to determine if the plant's herbage percent P content is an inheritable trait.

There is genetic variability with respect to the percent P in dry matter of grassland plants collected from Europe, Africa, and the US (Whitehead, 2000). While some grassland varieties in South Africa have herbage percent P concentrations as low as 0.05%, examples of grassland cultivars with high herbage percent P contents in dry matter have been identified in Finland (0.98%, Kahari and Nissinen, 1978); in the U.S. (0.81%, Adams, 1975); and in New Zealand (0.99%, Smith and Cornforth, 1982). There is also a large genetic variability in P uptake among corn genotypes (Baker et al., 1970; Clark and Brown, 1974; Nielson and Barber, 1978). For instance, Clark and Brown (1974) reported that one genotype of corn when grown in high P solutions was able to acquire 1.24% P in dry matter compared with 0.62% for another corn genotype under the same conditions. These reports suggest that there is genetic diversity among the germplasm of grassland plants and maize, implying that these traits are available to potentially breed them into current plant varieties.

It may be necessary in the breeding program to also consider management practices that influence production of the above ground biomass. If forage grasses are harvested periodically for animal forage over a growing season, the stress tests should probably be done under a full cycle of growth and under mowing schedules. Examining foliar percent P in the herbage over the growing season and under mowing schedules is important because the concentration of foliar P will vary greatly with plant maturity and supply of water (Whitehead, 2000).

An alternative approach to increase plant P uptake is to breed plants with traits that improve their ability to acquire and translocate P. Potential plant characteristics targeted for improved plant P uptake are to modify the architecture of the root system, to increase the secretion of organic acids by roots, and to increase the production of above ground biomass. Reports in the literature that have evaluated P uptake through mechanisms associated with these characteristics are described below.

Phosphate is tightly bound to inorganic or organic components of soil because of reactions between P and sesquioxides, oxihydrates of clay

minerals, and cations (Ca, Fe, Al) (Sharpley and Halvorson, 1994). Because P is delivered to roots by diffusion, plant root geometry, and morphology are important for maximizing P uptake (Barley, 1970; Khasawneh and Copeland, 1973; Schenk and Barber, 1979; Schachtman et al., 1998). The differences in root morphology among plant species have been reported to affect their ability to acquire soil P (Hanway and Olson, 1981). Therefore, because root morphology is important for P uptake, it is logical to turn to plant breeding by modifying root architecture (shape of the root system). The density of roots and depth of soil to which plant roots can penetrate are a vital component for the successful application of phytoextraction technology (Wenzel et al., 1999). Ludlow and Muchow (1990) reported that maximum P uptake occurred in plants when the root length density ranged between 2 to 5 cm cm⁻³. Reports documenting the soil profile P depth distribution indicate that the plant rooting density should be high in the topsoil and roots should be able to penetrate to 1 to 2 m deep, as high soil P concentrations occur in the topsoil (Sharpley and Halvorson, 1994; Sims et al., 1998) and subsoil (Kingery et al., 1994; Novak et al., 2000).

Nutrient uptake by roots has also been studied for the last 20 years using mathematical models (Barber and Cushman, 1981; Barber and Silverbush, 1984). These models have identified specific root architectural traits such as root hair length, root surface area, and root depth that are important factors for P uptake. Using computer simulations, Liao et al. (2001) quantitatively described the effects of root architecture on P acquisition efficiency of common bean and upland rice. They reported that maximum P uptake efficiency was influenced by root architecture with maximum uptake expressed in an "umbrella-shape" for bean and a "beard-shape" for rice. Root architectural traits that influence P uptake efficiency have been evaluated in soybeans, Hallmark and Barber (1984); in Bahiagrass (*Paspalum notatum* Flugge), Ibrikci et al., (1994); in wheat (*Triticum aestivum* L.), Manske et al., (2000); and in peanut (*Arachis hypogaea* L.), Wissuwa and Ae (1999). They reported that an increase in root surface area resulted in more P taken up by these plants because the P diffusion distance was decreased.

These reports suggest that a plant's root morphology is an important selection criteria in breeding plants with improved P uptake efficiency.

Some plants are capable of secreting organic acids like citric, oxalic, and malic acids through their roots (Lipton et al., 1987; Jones, 1998). These acids will react with insoluble Ca, Fe, and Al:P complexes through a chelation reaction with these cations and release water-soluble P (Stevenson, 1994). Genetically modifying plants to produce more citric acids by overexpressing an inserted bacterial synthase gene was reported by Herra-Estrella (1999) and Koyama (1999). The overexpression of the citrate synthase gene in these plants resulted in the enhancement in P acquisition and biomass production.

Plants may also acquire more P through genetic modification to produce and secrete more phosphatases (Raghothama, 2000) and phytases (Li et al., 1997). These two enzymes are responsible for conversion of organic P forms into plant available inorganic P forms. These reports exemplify the potential for genetic engineering to metabolically influence the plants production and secretion of organic acids or enzymes to acquire more P (Raghothama, 2000).

The efficiency of phytoextraction depends on the total amount of the target compound removed from the system by the accumulation of the compound in stems and leaves and the ability to produce a high yield of above ground biomass (Wenzel et al., 1999; USEPA, 2000). In this way, harvesting of the P-enriched biomass and removal from the system will result in a decrease in soil P concentrations. Improving yields of grasses and row crops is difficult to achieve, however, because there are many genetic and environmental factors regulating the production of the above ground plant growth (Sheeny, 2001; Sonnewald and Herbers, 2001). Although there has been some success in breeding a metal hyperaccumulator trait into a *Brassica napusi* (L.) and *Brassica juncea* (L.) that produces high biomass yields (Wenzel et al., 1999), increasing above ground biomass production in row and forage crops is a complex issue. This may not be achievable because of the complex interaction of growth factors and the stress imposed on the plants by disease and insect damage (Sonnewald and Herbers, 2001).

An alternative breeding goal may be to increase the foliar P contents of dry matter in plants like corn and Coastal bermuda grass. We illustrated the theoretical impact on soil P removal by growing corn and bermuda grass with a high foliar P content (Figure 2). Using the percent P in dry matter and the P per unit yield for corn for silage and Coastal bermuda grass from Table 4, we calculated the potential amount of P per unit yield removed if high foliar percent P contents were bred into these crops. For instance, 1P1Y represents the P per unit yield removed by Coastal bermuda grass at 0.32% P in dry matter for a yield of 22.4 Mg ha⁻¹. Modifying the genetic potential of bermuda grass to double the percent P in dry matter without modifying yield (2P1Y) results in more P removed. Similar results could be obtained by increasing the foliar P content of corn for silage (Figure 2). We realize that these are only potential scenarios and may not be totally obtainable due to P availability in soils and plant physiological factors that influence P uptake. However, the strategy of modifying the foliar uptake potential of crops has merit considering that a mutant strain of *Arabidopsis thaliana* (L.) Heynh. was able to take up 1.45% P (dry matter of leaves), while a wild strain took up only 0.68% P (Delhaize and Randall, 1995).

Transgenic techniques such as gene insertion could be used to bioengineer specific properties (e.g., enhance pollutant uptake, translocation, etc.) into plants to increase phytoextraction (Wenzel et al., 1999). The transfer of genes responsible for P uptake between organisms has been demonstrated (Lopez-Bucio et al., 2000). For example, transgenic techniques have been used by Leggewie et al. (1997) to successfully transfer P uptake genes from a potato (*Solanum tuberosum* L. Lem.) into a yeast strain (*Saccharomyces cerevisiae* L.) and by Liu et al. (1998b) from *Medicago truncatula* (L.) into a strain of yeast. Additionally, Mitsukawa et al. (1997) successfully transferred a high-affinity P transport gene from *Arabidopsis thaliana* into tobacco-culture cells. They reported that expression of the *Arabidopsis* gene at high levels in the tobacco-cell culture increased the rate of P uptake from nutrient solution. These reports infer the feasibility of employing transgenic techniques to impart into the host plant the ability to hyperaccumulate P.

Transgenic techniques may be also useful to modify the P acquisition and translocation mechanisms of plants. Plants take up P through their roots by using an energy-mediated co-transport process driven by a proton gradient generated by plasma membrane H⁺-ATP (Epstein, 1976; Ullrich-Eberisus et al., 1984; Sakano, 1990). The kinetic characterization of the P-uptake system by whole plants (Ullrich-Eberisus et al., 1984; Leggewie et al., 1997; Liu, et al., 1998a; Daram et al., 1999) indicates a high-affinity P transport activity operating at low concentrations (micromolar range) and a low-affinity activity operating at higher concentrations (millimolar range). The expression of these genetic traits was generally greater in roots than in shoots and was enhanced in response to P deprivation (Leggewie et al., 1997; Liu et al., 1998a; Dong et al., 1999). These observations suggest that plants respond to P deficiency by activating specific mechanisms in an effort to increase P uptake and translocation. Under the conditions of limited P availability, the capacity to transfer the absorbed P to the shoots is also increased, suggesting an increase in P release into the xylem (Lee, 1982; Delhaize and Randall, 1995). P stress in *Arabidopsis thaliana* will result in the activation of several genes associated with the *pho*-regulon, leading to enhanced synthesis of high-affinity P transporter proteins and phosphatase (Muchhal et al., 1996; Muchhal and Raghothama, 1999). When P was replenished to *Arabidopsis thaliana*, the production of the P transporter proteins decreased indicating a fine coordination between gene expression and increase in P uptake.

The compartmentalization of P is important at the plant cellular level, with the vacuole acting as a storage site for excess P that can be released under conditions of cytosolic P deficiency (Bieleski and Ferguson, 1983). Inorganic P concentrations will increase in the vacuole in response to improved P availability in soil (Schachtman et al., 1998). When the plant assimilates P in rates that exceed the demand, the plant is capable of the conversion of inorganic P into organic storage forms (e.g., phytic acid) to minimize the potential of P toxicity (Schachtman et al., 1998). These reports suggest genes responsible for P translocation, and vacuole storage may

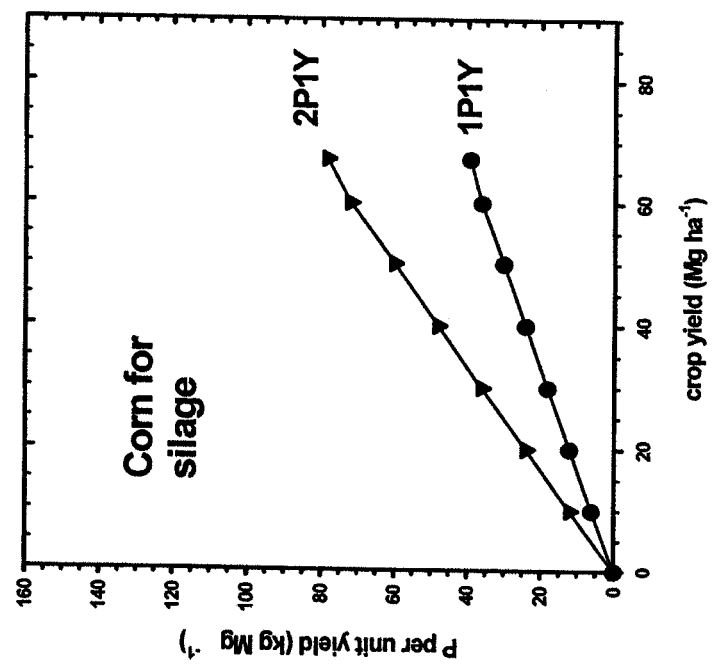
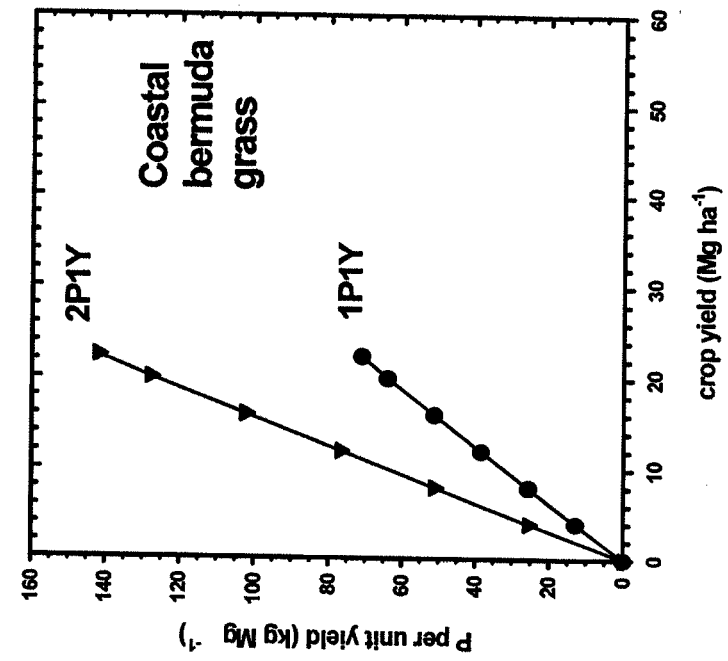


FIGURE 2. Potential P per unit yield needed for production of corn for silage (left) and for Coastal Bermuda grass (right) yields at potential 1P1Y and 2P1Y traits (where 1P and 2P equals 1x and 2x %P in dry matter, and 1Y equals 1x yields in Table 3).

represent additional target areas for genetic modification.

E. Management Practice for P-Hyperaccumulators

Successful development of P-hyperaccumulator plants under greenhouse or small plot testing does not guarantee that the plant will successfully perform under actual "on-site" field conditions. Nutrient availability and plants competition for sunlight under field conditions may be quite different when compared with conditions in test plots. Therefore, both soil and crop management practices may require optimization for the P-hyperaccumulator plant to compete with other plant species. For instance, variations in soil fertility, ionic strength, or pH may increase the uptake or toxicity of undesirable elements in the soil such that biomass production is limited or root length is decreased. Additionally, the performance of the P-hyperaccumulator plant may be reduced by variations in soil temperature, moisture, and soil structure. Moreover, the plant uptake of P may be influenced by crop management practices such as stand density, harvesting dates, weed control practices, and seed management (Chaney et al., 1997). To overcome these potential problems, extensive field testing under various soil and crop management practices will be necessary. This may require adopting different technologies of soil, weed, and fertilizer management that allow for good plant growth and expression of the hybrid P-hyperaccumulator traits (Chaney et al., 1997).

Forage quality from P-hyperaccumulator plants may be in question if it contains wide Ca:P ratios (Gross and Jung, 1981). Forages with wide Ca:P ratios (> 3:1) are considered undesirable because it has been implicated in several animal diseases (Gross and Jung, 1981). A dietary Ca:P ratio of between 1:1 and 2:1 is often considered to be ideal for growth and animal bone formation, as this is the approximate ratio in bone (Whitehead, 2000). Whitehead (2000) has suggested that a plentiful supply of vitamin D in the animal diet reduces the importance of the Ca:P ratio of the forage. If P-hyperaccumulator plants are used as animal forage, the high amount of P in the dry matter may be

offset by having a high Ca content. Therefore, it may be necessary to have a management practice to ensure that adequate supplies of Ca are maintained in soils to produce a high-quality forage product from the P-hyperaccumulator plant.

SUMMARY AND CONCLUSIONS

In many regions of the U.S. with concentrated animal production, intensive manure disposal to soils has resulted in excess soil P levels. There is an increased risk of surface water quality deterioration from the incidental transport of P from these soils high in P. Soil P concentrations will remain high in these soils unless P is either tied-up through soil amendments or assimilated by plants. Sequestering P by additions of chemical amendments to soil high in P is not practical when the quantity of amendments and the magnitude of affected acreage are considered. On the other hand, there has been minimal effort in using vegetative management to reduce soil P concentrations. It has been suggested by Frossard et al. (2000) that plant breeding and selection of species that are efficient at extracting soil P may be a useful approach to achieve optimum P levels in soils. However, Delorme et al. (2000) reported that none of the common row, vegetable, and forage grasses were capable of seriously reducing soil P concentrations.

We recognized the merit of the suggestions made by Frossard et al. (2000); therefore, we propose that a modification is needed through the use of P-hyperaccumulator plants. A goal of this modification should be to increase the amount of P phytoextracted, so that soil P contents are reduced in a short period of time. Nutrient uptake of common row and forage grasses may be improved using traditional breeding or new transgenic techniques. The success of traditional breeding may be limited because it can only draw from the genetic variability within a plant species. The evaluation of several thousand forage grasses revealed that some species can accumulate nearly 1% P in the above ground dry matter; however, they represent the extreme. Increasing P removal through increasing above ground biomass production may be difficult to achieve due to genetic

complexities (involving many genes) and availability of growth factors (nutrients, water, etc.). However, genetic engineering may hold the promise of taking the desirable P-hyperaccumulator traits such as higher foliar P contents, or P uptake mechanisms, and transferring these traits into hybrid plant species.

Numerous strategies have been suggested by Poulsen (2000) and Valk et al. (2000) to solve the issue of accumulation of soil P in excess of plant nutritional requirements (define P recommendations, manipulate feed chemistry, improve animal P assimilation efficiencies). These methods do not directly address ways to reduce *in situ* soil P concentrations. Vegetative management of P is possible, provided P-accumulator plants are specially bred to assimilate more P than current P assimilation amounts by common row and forage crops.

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