



INTERNATIONAL FOOD
POLICY RESEARCH INSTITUTE
sustainable solutions for ending hunger and poverty

ENVIRONMENT AND PRODUCTION TECHNOLOGY DIVISION

MAY 2005

EPT Discussion Paper 133

Ecological Risks of Novel Environmental Crop Technologies Using Phytoremediation as an Example

J. Scott Angle and Nicholas A. Linacre

2033 K Street, NW, Washington, DC 20006-1002 USA • Tel.: +1-202-862-5600 • Fax: +1-202-467-4439 ifpri@cgiar.org
www.ifpri.org

IFPRI Division Discussion Papers contain preliminary material and research results. They have not been subject to formal external reviews managed by IFPRI's Publications Review Committee, but have been reviewed by at least one internal or external researcher. They are circulated in order to stimulate discussion and critical comment.

Copyright 2005, International Food Policy Research Institute. All rights reserved. Sections of this material may be reproduced for personal and not-for profit use without the express written permission of but with acknowledgment to IFPRI. To reproduce the material contained herein for profit or commercial use requires express written permission. To obtain permission, contact the Communications Division at ifpri-copyright@cgiar.org.

ABSTRACT

Phytoremediation is the use of living plants, known as hyperaccumulators which absorb unusually large amounts of metals in comparison to other plants. The use of classical plant breeding and new molecular techniques offers great potential to develop crops with the ability to clean up polluted sites. While these technologies have gained widespread attention, prior to commercial development, there are risks that must be considered – only a few of which have received even modest examination. Therefore, the focus of this working paper is to explore specific risks associated with phytoremediation and suggest ways in which these risks can be managed so that new, novel, and innovative plant technologies may be applied to provide low cost and efficient environmental solutions.

Keywords: risk, GMO, biotechnology, phytoremediation, phytoextraction, phytomining

TABLE OF CONTENTS

1. Introduction	1
2. Pre-plant considerations	3
3. Naturally Occurring Hyperaccumulators	5
3. Ecotoxicity	6
4. 'Weediness'	9
5. Gene flow and introgression	11
6. Cultivation on contaminated areas	13
7. Cultivation on naturally enriched areas	14
8. Volunteers	15
9. GMO hyperaccumulators	16
10. Biomass disposal	19
11. Conclusions	21
References	22

Ecological Risks of Novel Environmental Crop Technologies Using Phytoremediation as an Example

J. Scott Angle¹ and Nicholas A. Linacre²

1. INTRODUCTION

Phytoremediation is the use of living plants, known as hyperaccumulators which absorb unusually large amounts of metals in comparison to other plants³, for *in situ* remediation of contaminated soil, sludges, sediments, and groundwater through contaminant removal, degradation, or containment (Baker et al. 1994; Chaney, 1983a; Glass 1999). While these technologies have gained widespread attention, prior to commercial development, there are risks that must be considered – only a few of which have received even modest examination. Therefore, the focus of this working paper is to explore specific risks associated with phytoremediation.

Phytoremediation offers the possibility of addressing an intractable global problem by providing an alternative, cheap and effective technology that could significantly improve the prospects of cleaning-up metal contaminated sites (Garbisu and Alkorta 2001; Salt et al. 1995). The advantages of phytoremediation over traditional methods of remediation are well known. The many benefits of the technology have been reviewed by Wolfe and Bjornstad (2002). However, few attempts to assess the specific risks of the technology have been reported.

Theoretical aspects of the risk analysis process were recently reviewed by Linacre et al. (2003). The primary conclusion of this paper was that risks must be identified,

¹ College of Agriculture and Natural Resources, 1201 Symons Hall, University of Maryland, College Park, MD 20742, USA

² International Food Policy Research Institute, 2033 K Street NW, Washington, DC 20006

³ <http://www.epa.gov/tio/download/remed/phytoresgude.pdf>

quantified, managed and communicated if phytoremediation is going to find broad public acceptance. Indeed, as a corollary, some of the current concerns related to use of genetically modified agricultural commodities arise from past lack of appreciation for risk assessment, management and communication (MacKenzie 1994). Acceptance of genetically modified organisms (GMOs), at least in the US, has been directly attributed to the eventual public understanding of risks and benefits.

Risk may be defined as the likelihood of occurrence of a negative consequence (Kaplan and Garrick 1981). In the context of phytoremediation, risks are primarily the result of exposure of living organisms to metals. Risk, therefore, depends on the likelihood of exposure, the level of exposure and on the toxic effects of exposure. It is the combination of these factors that dictates the risk which can vary from negligible to high when the likelihood, level and consequences of exposure are significant. Using a source-pathway-receptor model to identify possible risks, the failure to clean up contaminated sites (source) could lead to risk or harm to plants, animals, humans and natural resources such as water (receptors) via significant pollutant linkages (pathways).

Risks in phytoremediation can arise from a number of potential sources. Some risks relate to the direct exposure to metals, while other risks relate to the preparation, cultivation and disposal of materials. This review is divided into a number of sections and discusses: (1) preplanting risks created by soil preparation, (2) risks associated with the transfer of planting materials, (3) potential ecotoxicity, (4) potential weediness, (5) gene flow and introgression, (6) and (7) cultivation risks, (8) volunteers, (9) “additional” risks associated with GMO phytoremediators, and (10) biomass disposal.

2. PRE-PLANT CONSIDERATIONS

Prior to planting of hyperaccumulators, soils are often prepared by adding materials to reduce soil pH. Numerous studies have shown that most hyperaccumulators take up more metal at low pH due to enhanced solubility (Marschner 1995). pH reduction is therefore considered a critical component to most phytoremediation technologies. The only exception to this rule was reported by Li et al. (2003) and Kukier et al. (2004) who showed that maximum uptake of Ni by *Alyssum murale* was at pH greater than 7.5.

However, low pH in the presence of high metal content can exasperate ecotoxicity. Numerous studies have shown that metals can be toxic to most non-hyperaccumulator plants (and even some hyperaccumulators) when soil pH is reduced to 6.0 and below (Chaney 1983b). Further, many soil bacteria and other organisms are sensitive to high metal concentrations which are also affected by lowering pH of high metal soils.

This risk is currently a focus of an on going program in our laboratory. We examined the extent of damage done to an ecosystem when soil pH was reduced for a high metal soil, and whether this damage was permanent or if the ecosystem could be restored to normal by subsequent increase in the soil pH at the end of phytoremediation. Results demonstrated that both microbial number and function are highly sensitive to the toxic effects of soil metals at low soil pH. When soil pH was returned to more appropriate agronomic levels, most parameters, but not all, returned to normal within a six month period.

Reducing pH of low metal soil can also enhance metal solubility to the point where leaching is a concern. Angle et al. (unpublished data) has shown that when soil pH is reduced to values less than 6.4 for Cd and 4.7 for Zn enriched soils, loss from soil via leaching is a significant concern. Metal contaminated groundwater is one of the most difficult media to remediate and should be avoided at all possible costs.

Another soil management option that has been examined is to add organic chelators to soil to increase solubility and thus metal uptake (Blaylock et al. 1997; Lombi et al. 2001). Both aminopolycarboxylic acids (Shen et al. 2002; Huang et al. 1997) and organic acids (Huang et al. 1998; Ebbs et al. 1998) have been studied for their ability to increase metal uptake into plants. While occasionally effective (Meers et al. 2004), these materials have significant limitations that until now have restricted their utility. For both materials, enhanced leaching of chelate-bound metals into groundwater as a result of increased solubility is an important concern (Cunningham et al. 1997; Kedziorek and Bourg 2002). Greman et al. (2001) showed that EDTA added to soil increased leaching losses of metals by up to 40%. One of the paramount rules of remediation is to avoid contaminant dispersal. While it might be possible to balance chelate addition to soil so that only metal taken up by the plant is available at any point in time, the temporal considerations related to full season phytoremediation suggest this to be quite difficult.

A further potential concern is the cost of adding amendments to soil. Sulfur added to lower soil pH is relatively cost effective and thus insignificant to the overall cost of phytoremediation. However, chelates can be quite expensive. Chaney et al. (2004) has reported that the cost of adding 10 mmol EDTA kg⁻¹ soil is about \$30,000 per ha.

3. NATURALLY OCCURRING HYPERACCUMULATORS

Hyperaccumulators are either direct seeded into contaminated or mineralized soils or plantlets are transplanted into the soil. In either case, seed or soils associated with transplants can carry soil bacteria, fungi and viruses that are not indigenous to the area being phytoextracted, potentially posing risks to soil microflora and indigenous plants. However, risk management approaches exist such as soil sterilization and growing plantlets in locally collected soils. One consequence of this approach that is difficult to mitigate the impacts of deliberately introduced soil bacteria important for phytoremediation.

Abou-Shanab et al. (2003) showed that the rhizosphere of *Alyssum murale*, the most important Ni hyperaccumulator studied to date, has bacteria within its rhizosphere that can increase Ni solubility and thus Ni uptake into the plant. While the presence of Ni solubilizing bacteria in soil is beneficial in that phytoremediation efficiency is increased, if these bacteria were also to increase uptake into non hyperaccumulator plants, results could be harmful to the food chain. Abou-Shanab et al. (2003) reported that specific bacteria when inoculated into soil increase Ni uptake by up to 33%. Other studies have shown that the rhizosphere of hyperaccumulators increases metal uptake into plants, although specific reasons for this observation were not considered (Schwartz et al. 2003; Whiting et al. 2001).

3. ECOTOXICITY

During phytoremediation, hyperaccumulators are direct seeded or transplanted into metal enriched soil. Early in the process, plant biomass content is low, and ecotoxicity is seldom a concern. However, soon after germination or planting, metal content in biomass increases to levels that can potentially be toxic to the ecosystem. Several studies have shown that hyperaccumulators fail to grow well when foliar concentrations of select metals are low (Li et al. 2004). Li et al reported that *Thlaspi caerulescens* needs 1000x more Zn to grow 'normally' when compared to non-hyperaccumulators. For example, *T. caerulescens* can concentrate up to 40,000 $\mu\text{g g}^{-1}$ Zn (Brown et al. 1994, 1995) while *A. murale* can accumulate similar amounts in above ground biomass (Chaney et al. 2000). Although the exact reason for metal hyperaccumulation has yet to be fully understood, many scientists believe this phenotype evolved as a way of induced foliar toxicity and thus reduce feeding by a variety of insects (Boyd and Martens 1994).

Several studies have examined impacts of high metal biomass on insects. This information has been extensively reviewed by Boyd and Martens (1992 1998). Boyd et al. (1998) examined direct toxic effects on high metal biomass on both feeding preference and toxicity on insects. It was shown that most insect species prefer low metal biomass when given a choice in feeding studies. Less direct effects on reproductive success are more poorly understood. Effects related to behavior may be important but have not yet been examined to our knowledge.

Interestingly, some insects seem to have developed methods for avoiding metal exposure. Aphids for example, due to rapid pass through of fluids, are able to excrete much of the metal consumed during feeding from the phloem. Further, phloem sap

generally has lower metal content compared to materials flowing up into the shoot given that this is an important part of the hyperaccumulation process.

We are not aware of any studies that have directly examined toxicity to higher animals consuming high metal hyperaccumulator biomass. However, it is quite reasonable to assume that that exposure to and consumption of high metal biomass could be toxic to wildlife. During large-scale phytoremediation, it will be nearly impossible to fence out all herbivores. We are also aware of concerns related to farm animals getting into fields of hyperaccumulators. Fencing, screens and netting are generally ineffective in preventing movement of all animals into a field in phytoremediation.

Fortunately, most hyperaccumulators are relatively unpalatable probably due to the high alkaloid and metal content found in hyperaccumulators. Thus, while it might be possible that animals will graze on hyperaccumulators, the likelihood of significant consumption is minimal. Our observations of large fields planted to hyperaccumulators are that few, if any, large herbivores forage in these fields. Only when few other foods are present can animals be expected to ingest significant quantities of hyperaccumulator biomass.

Despite the relative unpalatability of high metal hyperaccumulators, it is always possible that insects could develop resistance to metals, and thus increase feeding and exposure. This has been observed recently for Bt crops as well as for a host of more traditional insecticides.

Further, while most large herbivores have a large grazing range and can simply move to other locations, small herbivores and territorial animals may not have this opportunity. Voles, shrews etc have very limited foraging range and may simply not

have the ability to move beyond the confines of a field planted contiguously to hyperaccumulators. Given quantities of roots, stems and leaves consumed and the average metal content of hyperaccumulators, it is readily conceivable that these animals could ingest potentially lethal amounts of metal.

There are no current standards for maximum ingestion of metals by wildlife, but such data are available for livestock. Table 1 (Madejon, et al. 2002) shows the maximum levels of metals tolerated by common livestock species. Hyperaccumulator concentrations for all metals far exceed levels considered toxic to cattle, sheep, swine and chickens, often by orders of magnitude.

Table 1--Metal concentrations for ‘typical’ agronomic plants, phytotoxic metal concentrations in plants, metal concentrations used for delineation of hyperaccumulators and maximum metal concentrations tolerated by livestock.

Metal	Plant avg. -----mg kg ⁻¹	Phytotoxicity -----	Hyperacc -----	Max. conc. tolerated by animals -----mg kg ⁻¹ in diet-----		
				Cattle	Sheep	Chicken
Cd	0.1 – 1	5 – 700	>1,000	0.5	0.5	0.5
Cu	3 – 20	25 – 40	>10,000	100	25	300
Mn	15 – 150	400 – 2,000	>10,000	1,000	400	2,000
Ni	0.1 – 5	50 – 100	>10,000	50	100	300
Zn	15 – 150	500 – 1,500	>10,000	300	1,000	1,000

Adapted from Madejon et al. 2002

Acknowledging that some herbivores with limited foraging range may be killed or injured by the consumption of hyperaccumulators, this hazard must be assessed in terms of risks of doing nothing or the risks of more traditional methods of dig and haul. Doing nothing is often not an option while dig and haul, with replacement of the contaminated soil – and resident small herbivores, will almost certainly be fatal to all wildlife of limited

foraging range. Loss of some small herbivores might well be a 'cost' of return of the soil ecosystem to a healthy state.

If hyperaccumulator consumption by wildlife is a concern, there are well-established methods to reduce wildlife ingestion of hyperaccumulators. Fencing, deterrents such as periodic noise, and planting of offensive plant species can all be used to reduce contact between wildlife and hyperaccumulators. While not fully effective, these methods will at least reduce exposure.

4. 'WEEDINESS'

Most hyperaccumulators evolved under extreme conditions thus they tend to be quite hardy. Many of these plants evolved on soils that were highly infertile with little water holding capacity. The climate under which many hyperaccumulators evolved is often extreme with cool moist winters and hot and dry summers (Brooks 1998). Our experience has been that hyperaccumulators are quite hardy and survive with little care. Indeed, this is one of the characteristics that make these plants amendable to cultivation.

By definition, most hyperaccumulators are 'weeds', in that they: 1) reproduce rapidly, 2) grow under conditions of low fertility, and 3) are adapted to a wide range of environmental (soil and climate) conditions. Consequently one of the most immediate concerns related to phytoremediation is the potential escape of hyperaccumulators from the site of remediation and the possibility that these plants will become environmental weeds.

Numerous examples documenting the 'escape' of plants moved from one location to another have been reported. Kudzu, autumn olive, multiflora rose, and Japanese

honeysuckle were all intentionally imported into the US, often for agricultural or ornamental purposes. Currently the US spends over \$15B annually to control weeds. Most weeds were originally imported into the US either intentionally or accidentally. Most hyperaccumulators will not be used commercially where they evolved, thus import into other areas will be necessary. However, importing a non-indigenous species into many countries can be a problem. Therefore the first choice when selecting a plant species for use is to select an indigenous hyperaccumulator.

A more general notion of a weed is that of “a plant growing in a place where it is not wanted.” In this sense evidence exists that the physiological limitations of hyperaccumulator plants may limit their potential for weediness. Many hyperaccumulators have been reported to survive only on metal enriched soils. It has been suggested that high shoot metal content, and subsequent toxicity to pathogens and insects is one of the primary reasons why metals are accumulated (Pollard et al. 2002). When hyperaccumulators were grown in low metal soil, we have observed that plants rapidly die from fungal disease. Most often, we have identified *Pythium* or *Phytopera* root disease as the causative agent of death and decline.

It is suggested that hyperaccumulators have abandoned other methods of protection from disease for genetic efficiency. Thus, when metals are low, uptake is reduced and the plants are left ‘defenseless’. For this reason, it is unlikely that hyperaccumulators that escape from the site of cultivation would survive to become a permanent component of the ecosystem. Plants would most likely die as a result of being left without the high metal defense. We, therefore, do not believe that control using

herbicides will be necessary to protect against escape outside areas of metal contamination or artificial enrichment.

Even if movement of seed is restricted beyond the original area of establishment, pollen from hyperaccumulators can travel with wind and insects for many kilometers. Numerous crop species have been shown to hybridize with wild relatives, including sunflower, radish, canola and millet. Traits that increase gene flow and outcrossing include self-incompatibility, high outcrossing rates and biotic pollination. *Thlaspi* is generally considered to be self-pollinating but cross-pollination ranges from 5 to 25%. This concern will be discussed in more detail in the next section.

5. GENE FLOW AND INTROGRESSION

Pollen dispersal may be critical because it affects the likely breadth of dispersal of genetic material containing metal accumulating genes. It has been reported that the primary method by which genes may move from a GMO crop to a weedy relative is through pollen movement (Kareiva et al. 1994). It might be possible that genes coding for metal uptake and sequestration could be transferred to other crop or non crop plants. For crop plants, transfer of high metal uptake is a very serious concern. Incorporation of metal uptake genes into crops plants could lead to food and feed that exceed national and international standards for metal concentration. In addition to food quality concerns, export markets can be negatively affected by high metal content.

The primary method for flow of genes from both GMO and non-GMO crops to related relatives is through the process of introgression. Introgression is defined as the natural spread of genes of one species into another through the process of interspecific

hybridization followed by successive backcrosses to the parent. This process results in offspring that have similar genotypes to the wild type but incorporate new genes from the domesticated, exotic or GM plants.

Most plant species outcross to some extent with other plants. Although *T. caerulea* was originally thought to have only limited outcrossing, i.e. approximately 5% (Riley 1956) more recent evidence has suggested that outcrossing for this species might be much higher. Koch (1998) demonstrated that outcrossing rates in *T. caerulea* could reach as high as 88%. It has also been suggested that higher outcrossing may occur on contaminated soils compared to plants grown on less polluted soil (Dubois et al. 2003).

Just about every cultivated crop has shown hybridization with at least one wild relative (Arias and Rieseberg 1994; National Research Council 1989). Sexually compatible weeds or indigenous species almost always occur within the growing area of most known cultivated plant species. For this reason, outcrossing during phytoremediation should be anticipated and controlled to the extent possible.

There are many factors that can affect the flow of pollen and genes from one plant to another – some of which can potentially be managed and reduced. The most important factors affecting gene flow include the degree of out-crossing and the potential for biotic pollen movement. In general, high out-crossing rates and biotic pollination, such as with bees, will increase the rate of gene flow between plant species. Pollen can be dispersed by a variety of vectors: wind, insects, mammals and birds. The role different vectors play in long versus short distance dispersal is species specific. And, in the case of GM crops, the type of gene may affect the rate of introgression. Glover (2002) cites the example of

insect protected cotton where there has been a 37-54 percent reduction in insecticide use. This in turn may increase local insect populations, increasing the abundance of pollen vectors therefore increasing the rate of introgression.

6. CULTIVATION ON CONTAMINATED AREAS

For many soil metal contaminants, especially Zn and Ni, within a metal contaminated area, hyperaccumulator plants are likely to do well since foliar concentrations will be high. This may result in the plants spreading within the contaminated area and displacing other “indigenous” species. However, diversity on contaminated sites is typically low with the more aggressive species being dominant and many species previously growing on contaminated sites are themselves considered weeds. Therefore since this is an area needing remediation, spreading within the contaminated area and displacement of other “indigenous” species is generally considered a positive attribute.

On the other hand, for metals such as Cd in contaminated or mineralized soils, soil concentrations never or rarely approach levels that are toxic to plants. Cadmium tends to have few effects on plant growth, yet can still exhibit toxicity to animals. While this does not obviate the ecotoxicity of high Cd plants, Cd hyperaccumulators will not be able to grow beyond the area of soil contamination. Thus, escape of Cd hyperaccumulators beyond the area of contamination is not likely to be a concern. These observations suggest that escape from the original site must be assessed based upon both the plant and soil.

7. CULTIVATION ON NATURALLY ENRICHED AREAS

Cultivation of hyperaccumulators on naturally enriched areas offers the greatest promise for use in phytomining. Phytomining is a more specific form of phytoremediation where the purpose of metal removal from soil is economic gain. For example, millions of acres of Ni rich ultramafic soil are found around the world. These soils are potentially amendable to Ni phytomining. However, many of these areas are populated by a number of rare and endangered species. For example, serpentine soils in northern California and southern Oregon are populated by rare and endemic species that exist only on these soils (Kruckeberg 1954;1984). Given the unique flora of enriched soils, concern has been raised that highly competitive and aggressive introduced hyperaccumulators may displace some of the natural flora. The literature is replete with examples of introduced plant species dominating fragile ecosystems. We have experience working on a serpentine pine barren. Pines were never part of the indigenous ecosystem, and only came to dominate as part of human activity (clear cutting). This has threatened much of the indigenous flora in serpentine areas and has led to debate as to whether pines should be removed by logging.

As discussed, concerns related to escape of introduced hyperaccumulators exist and must be addressed prior to project initiation. It is therefore critical to establish a protocol to monitor the potential for escape from the original area of introduction. Where escape is found, survival should be observed to determine whether this is temporary or whether escaped hyperaccumulators have the potential to become established as a permanent component of the ecosystem.

Angle et al. (2001) has reported that many hyperaccumulators are readily controlled with herbicides. Therefore, where escape is found, it is possible to control these individuals with herbicides. However, this is both an expensive and time consuming process and there can be no guarantee that all escaped plants will be found and killed.

Another method to reduce the potential for escape from the original site of planting is to harvest plants prior to seed set. Most hyperaccumulators set seed in mid summer. Since most hyperaccumulators are perennials, they will typically be harvested at the time of maximum metal accumulation, then plants will continue to grow for an additional harvest the same year or in the following year. Fortunately, maximum metal accumulation usually occurs just about at the time of flowering. It is therefore possible to harvest plants before seed are produced. Alternatively, for locations where it is unlikely that plants will survive the entire year (i.e. growing temperate hyperaccumulators in tropical areas), plants can be grown as annuals and again harvested before seed set.

8. VOLUNTEERS

After remediation is complete there remains the limited potential for some seed, stored in the soil seed bank, to germinate, which may pose a small risk. However, this risk may be managed using on site volunteer management. Different approaches are available and will depend on the crop. Generally, rotation with another crop, against which any phytoremediation volunteers will be visually obvious, allows the volunteers to be identified and removed. The potential for volunteers can also be reduced by

harvesting before seed set, which reduces the likelihood of seed entering the soil seed bank.

9. GMO HYPERACCUMULATORS

Genetic studies related to hyperaccumulators have been underway for many years (Karenlämpi et al. 2000; Whiting et al. 2004). Most studies have focused on the identification of genes involved in the process of hyperaccumulation – uptake, transport and sequestration (Rutherford, et al. 2004; Wang et al. 2002). However, a few studies have resulted in the development of GMO hyperaccumulators, hopefully with enhanced potential to extract metals from soil. Dhankher et al. (2002) reported the development of a plant with enhanced tolerance and uptake of arsenic. More recently, several studies have described transgenic plants with the ability to take up and volatilize selenium (Van Huysen et al. 2003; 2004). *Brassica juncea* was engineered to over express a key enzyme in the sulfur assimilation pathway, which resulted in significantly greater uptake of Se. Probably the greatest amount of study, and resulting publicity has occurred for poplar trees engineered for Hg uptake. Bizily et al. (2001, 2003) and Pilon-Smits and Pilon (2000) studied the insertion of genes that could potentially enhance Hg uptake and volatilization. This work has successfully resulted in the production of transgenic trees that increase Hg uptake and volatilization from soil. Similar reports that transgenic plants can increase Se uptake and volatilization have also recently been reported (Pilon-Smits et al. 1999a, 1999b).

While the genomics of phytoremediation is proceeding at a rapid pace, concerns have been raised regarding this approach. Imagine the public reaction to the following GMO hyperaccumulator:

“A non indigenous, weed that has been genetically engineered to be poisonous to most organisms that come into contact with it.”

Each of the attributes noted above (weediness, non-indigenous import, poisonous, and GMO) raises serious individual concerns. Multiplying each of these risks together might be more than the public is willing to tolerate. Multiplication of concerns only exasperates the need to study, discuss and balance potential concerns with potential benefits.

Two general approaches are current underway to develop GMO hyperaccumulators. The first approach, which is by far the less common of the two, is to move genes that code for ‘large’ plant growth into true hyperaccumulators. Most, but certainly not all, hyperaccumulators are small plants with low biomass. Since phytoremediation efficacy is a function of both biomass produced and biomass metal content, most consider the small size of these plants to be the primary limiting factor. By moving these genes into small hyperaccumulators, the goal is to create a GMO hyperaccumulator that expresses higher biomass production. This approach might find greater use in the future since genes that code for enhanced growth are relatively well characterized. This is in contrast to genes that code for metal hyperaccumulation which are not yet fully understood and at best appear to be controlled by at least several genes.

The most significant concern with this approach is the subsequent transfer of genes that code for large plant growth from the GMO hyperaccumulator to local weeds. As previously noted, it is likely that plants within the same genus as the GMO hyperaccumulator will be found in the area under phytoremediation. Most of these plants

can be weeds under the right conditions. For this reason, all practices to control introgression should be used.

Another approach for control that could potentially be used is to create sterile plants via the insertion of suicide genes. Suicide genes (DeBlock and Debrouwer 1993; Strauss et al. 1995) have been used for a variety of crops, primarily to prevent seed production that could be saved, thus leading to loss of control of the intellectual property (this could also be used to prevent hyperaccumulator GMOs from inappropriate use that violates the original patent).

The second approach for the development of GMO hyperaccumulators and the one that is under active investigation is to identify genes that enhance metal uptake and transfer these genes to plants with much higher biomass. Most studies previously discussed are following this approach. The most important question for this approach is the selection of the crop plant that will be engineered for enhanced metal uptake. It is very important that that we avoid introduction of metal-accumulation genes into crop plants that could either escape or via introgression spread them into nearby crop plants. We have spent decades trying to keep excess metals out of crop plants in order to protect human and animal health as well as to protect export markets (Chaney et al. 2001). Now we are inserting genes that enhance metal uptake of crop plants to potentially toxic levels.

One final concern that has been raised in several public forum is that enhance uptake and volatilization of volatile metals (Hg and Se) might exasperate downwind air pollution. While volatilization is certainly a consequence of Hg and Se removed from soil (and is also a natural soil process), the overall contribution to air in comparison to amounts released via volatilization by indigenous bacteria is minor. And since many

areas beyond several interior California valleys are Se-deficient, increased aerial deposition might be a positive attribute of the process since downwind soil concentrations might actually be increased.

10. BIOMASS DISPOSAL

Once plants are harvested, biomass must be either disposed of using appropriate techniques or recycled to recover valuable metals. Most phytoremediation scenarios envision that the biomass will be incinerated either to reduce volume, recover energy or both. However, burning of metals, regardless of the form it is in, can lead to the formation of metal oxides. Some metals are extremely volatile (Hg and Cd) while others belong to an intermediate group (Zn and Pb). Metals such as Ni, Cr, and Cu are considered non volatile (Belevi and Moench 2000). Metal oxides are both toxic and carcinogenic. Thus, care must be taken to control emissions during the incineration or smelting process. Numerous methods are available to reduce gas emissions yet all are very expensive.

Ash that results from the burning process can contain as high as 30% metal on a weight basis. This concentration is several times higher than hard rock ore mined from the ground. Thus, the 'bio-ash' or 'bio-ore' is a rich and potentially valuable ore depending upon the price of the extracted metal. Through smelting or electro-winning processes, metals can be extracted from the bio-ore (Prasad and Freitas 2003; Kumar et al. 1995).

A concern that has yet to be adequately addressed is the potential toxic nature of either biomass or bio-ore. Biomass can contain up to 4% and bio-ore can contain up to 30% metal on a dry weight basis. Does either of these values cause the material to be

classified as a hazardous waste? The questions remains unresolved in the US, yet for countries like Switzerland, the high metal concentration of the burned biomass would clearly classify the ash as a hazardous waste, and thus restrict ash disposal in a landfill (Swiss Federal Legislation 1996). For this reason, high metal biomass would have to be incinerated with low metal materials (such as municipal solid waste) to dilute metals to acceptable levels.

The USEPA classifies a hazardous waste as: “by-products of society that can pose a substantial or potential hazard to human health or the environment when improperly managed. The waste must also pose at least one of four characteristics (ignitability, corrosivity, reactivity or toxicity), or appears on special EPA lists.”

The confusion relates to the source of the metal in the hyperaccumulator. For example, high Ni in soil can result either from mining and smelting operations or occur naturally in soil from mineralization of ultramafic minerals. Nickel removed from naturally enriched soil should not be considered a pollutant and thus guidelines that govern hazardous waste need to be questioned. This conflict has yet to be addressed in a regulatory forum. Nickel extracted from soil contaminated by anthropogenic activities might better fall within a regulatory agency thus needing more through review.

Hazardous wastes are subject to a variety of rules and regulations, especially as related to transport. It is theoretically possible that the bio-ore resulting from incineration could be classified as a hazardous waste. If true, simple transport of the bio-ore will be subject to a variety of Department of Transportation regulations. At best, the burning of biomass and generation of the bio-ore will be conducted all on a single site. This might

even be the smelter that originally caused the contamination. At worst, the biomass and or bio-ore might need to be transported via public roads to other locations.

11. CONCLUSIONS

In conclusion, there are real risks associated with phytoremediation that require assessment and identification of management options prior to implementation of any field based operations. Management options using confinement strategies such as onsite processing, discing, harvesting before seed set, and volunteer management, may reduce the likelihood of pollen and seed movement thus reducing potential risks. Data collection, interpretation and communication of risks must be evaluated if phytoremediation is going to find wide public acceptance. This argues for a balanced approach in the discussion of the benefits and risks of phytoremediation.

In any discussion of risks, however, specific risks must be considered in comparison to doing nothing – leaving the site unaltered. Risks of phytoremediation must also be assessed compared to the more traditional methods of remediation including, excavation and landfilling, soil incineration, soil washing and vitrification (EPA 1997, MADEP 1993). Traditional methods of remediation have many real risks, both to human and environmental health that must also be considered. Thus, while acknowledging that there are risks associated with phytoremediation, these risks are temporary that last only during the process of phytoremediation. We believe that in most cases phytoremediation risks are small compared to the risks of doing nothing or the financial and engineering risks of ‘dig and haul.’

REFERENCES

- Abou-Shanab, R. I., T. A. Delorme, J.S. Angle, R. L. Chaney, K. Ghanem, H. Moawad, and H.A. Ghazlan. 2003. Phenotypic characterization of microbes in the rhizosphere of *Alyssum murale*. *Internat. J. Phytoremed.* 5: 367-379.
- Angle, J.S., R. L. Chaney, A.J.M. Baker, Y-M. Li, R. Reeves, V. Volk, R. Roseberg, E. Brewer, S. Burke, and J.P. Nelkin. 2001. Developing commercial phytoremediation technologies: Practical considerations. *South African J. of Science.* 97: 619-623.
- Arias, D., and L.H. Reiseberg. 1994. Gene flow between cultivated and wild sunflowers. *Theoretical Applied Genetics.* 89: 655-660.
- Belevi, H. and H. Moench. 2000. Factors determining the elemental behavior in municipal solid waste incinerators. *Environ. Sci. Technol.* 34: 2501-2506.
- Baker, A. J. M., S. P. McGrath, C.M.D. Sidoli, and R.D. Reeves. 1994. The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Resources, Conservation and Recycling.* 11: 41-49.
- Bizily, S., T. Kim, M.K. Kandasamy, and R.B. Meagher. 2003. Subcellular targeting of methylmercury lyase enhances its specific activity for organic mercury detoxification in plants. *Plant Physiol.* 131: 463-471.
- Bizily, S. P., C.L. Rugh, and R.B. Meagher. 2001. Phytodetoxification of hazardous organomercurials by genetically engineered plants. *Nature Biotechnol.* 18: 213-217.
- Blaylock, M. J., D.E. Salt, S. Dushenkov, S. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley, and I. Raskin. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Sci. Technol.* 31: 860- 865.
- Boyd, R.S., and S.N. Martens. 1992. The raison d'être for metal hyperaccumulation by plants. In: *The ecology of ultramafic (Serpentine) soils*, ed. Baker, A. J. M., J. Proctor, R.D. Reeves. Andover, Hants, United Kingdom: Intercept.
- Boyd, R. S., J.J. Shaw, and S.N. Martens. 1994. Nickel hyperaccumulation in *S. polygaloides* (Brassicaceae) as a defense against pathogens. *Amer. Jour. Botany* 81: 294-300.
- Boyd, R. S., and S.N. Martens. 1998. The significance of metal hyperaccumulation for biotic interactions. *Chemoecology* 8: 1-7.

- Brooks, R. R., 1998. Plants that accumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. Wallingford, UK: CAB International Press.
- Brown, S., R.L. Chaney, J.S. Angle, and A.J.M. Baker. 1994. Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium contaminated soil. *J. Environ. Qual.* 23: 1151-1157.
- Brown, S., R.L. Chaney, J.S. Angle, and A.J.M. Baker. 1997. Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. *Soil. Sci. Soc. Am. J.* 59: 125-133.
- Chaney, R. L. 1983a. Plant uptake of inorganic waste constituents. In: *Land treatment of hazardous wastes*, ed. Parr, J. F., P.B. Marsh, and J.M. Kla. Park Ridge, NJ: Noyes Data Corp.
- Chaney, R. L. 1983b. Zinc phytotoxicity. In *Zinc in soils and plants*, ed. Robson, A. D. Dordrecht: Kluwer Academic Publ.
- Chaney, R.L., Y-M Li, J. S. Angle, A. J. M. Baker, R. D. Reeves, S.L. Brown, F.A. Homer, M. Malik, and M. Chin. 2000. Improving metal hyperaccumulator wild plants to develop commercial phytoremediation systems: approaches and progress. In *Phytoremediation of contaminated soil and water*, ed., Terry N. and G.S. Bañuelos, (Eds.) CRC Press, Boca Raton, FL, USA.
- Chaney, R. L., P. G. Reeves, and J.S. Angle. 2001. Rice plant nutritional and human nutritional characteristics: roles in human toxicity. In *Inter. Plant Nutrition Colloquium*, ed. W. J. Horst Proc., Hanover, Germany.
- Chaney, R. L., P. G. Reeves, J.A. Ryan, R.W. Simmons, R.M. Welch, and J.S. Angle. 2004. An improved understanding of soil Cd risk to humans and low cost methods to remediate soil Cd risks. *BioMetals* 17: 549-553.
- Cunningham, S. D., J.R. Shann, D.E. Crowley, and T.A. Anderson. 1997. Phytoremediation of contaminated water and soil. In *Phytoremediation of soil and water contaminants*, ed. Kruger, E. L., T.A. Anderson, and J.R. Coats. ACS Symposium Series 664. Washington, DC: American Chemical Society.
- DeBlock, M. D. and D. Debrouwer. 1993. Engineering fertility control in transgenic *Brassica napus L*: Histochemical analysis of anther development. *Plant Physiol.* 93: 1110-1116.
- Dhankher, O. P., Y. J. Li, B.P. Rosen, J. Shi, D. Salt, J.F. Senecoff, N.A. Sashti, and R.B. Meagher. 2002. Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and gamma-glutamylcysteine synthetase expression. *Nature Biotechnol.* 20: 1140-1145.

- Dubois, S., P.O. Cheptou, D. Petit, P. Meerts, M. Poncelet, X. Vekemans, C. Lefèvre, and J. Escarré. 2003. Genetic structure and mating systems of metallicolous and nonmetallicolous populations of *Thlaspi caerulescens*. *New Phytol.* 157: 633-641.
- EPA (Environmental Protection Agency). 1997. Electrokinetic laboratory and field processes applicable to radioactive and hazardous mixed waste in soil and groundwater. EPA 402/R- 97/006. Washington, DC.
- Garbisu, C. and I. Alkorta. 2001. Phytoremediation: an effective plant based technology for the removal of metals from the environment. *Bioresource Technology* 77: 229-236.
- Glass, D. J. 1999. US and international markets for phytoremediation, 1999-2000. Needham, MA, USA: D. Glass Associates, Inc.
- Glover, J. 2002. Gene flow study implications for the release of genetically modified crops in Australia. Canberra, Australia: Department of Agriculture Fisheries & Forestry, Australia, Canberra.
- Greman, H., S. Velikonja-Bolta, D. Vodnik, B. Kos, and D. Lestan. 2001. EDTA enhanced heavy metal phytoremediation: metal accumulation, leaching and toxicity. *Plant Soil* 235: 105-114.
- Huang, J. W., J. Chen, W.R. Berti, and S.D. Cunningham. 1997. Phytoremediation of lead contaminated soils: role of synthetic chelates in lead phytoremediation. *Environ. Sci. Technol.* 31: 800-805.
- Huang, J. W., M.J. Blaylock, Y. Kapulnik, and B.D. Ensley. 1998. Phytoremediation of uranium contaminated soils: Role of organic acids in triggering uranium hyperaccumulation in plants. *Environ. Sci. Technol.* 32: 2004-2008.
- Kareiva, P., W. Morris, C.M. Jacobi. 1994. Studying and managing the risk of cross-fertilization between transgenic crops and wild relatives. *Molecular Ecology*, 3: 14-21.
- Kaplan, S. and B.J. Garrick. 1981. On the quantitative definition of risk. *Risk Analysis*. 1: 11-27.
- Karenlämpi, S., H. Schat, J. Vangronsveld, J.A.C. Verkleij, C. Van der Lelie, M. Mergeay, and A. Tervahauta. 2000. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environmental Pollution* 107: 225-231.
- Kedziorek, M. A. M. and A.C.M. Bourg. 2002. Solubilization of lead and cadmium during the percolation of EDTA through a soil polluted by smelting activities. *J. Contaminant Hydrology* 40: 381-392.

- Koch, M., K. Mummenhoff, and H. Hurka. 1998. Systematics and evolutionary history of heavy metal tolerant *Thlaspi caerulescens* in Western Europe: Evidence from genetic studies based on isoenzyme analysis, *Biochemical Systematics and Ecology* 26: 823-838.
- Kruckeberg, A. R. 1954. The ecology of serpentine soils: plant species in relation to serpentine soils. *Ecology* 35: 267-274.
- Kruckeberg, A. R. 1984. The flora of California's serpentine, Part II. *Fremontia* 4: 3-10.
- Kukier, U., C.A. Peters, R.L. Chaney, J.S. Angle, and R.L. Roseberg. 2004. The effect of pH on metal accumulation in two *Alyssum* species. *J. Environ. Quality* 33, 2090-2102.
- Kumar, P. B., V. Dushenkov, H. Motto, and I. Raskin. 1995. Phytoremediation: the use of plants to remove heavy metals from soil. *Environ. Sci. Technol.* 29: 1232-1238.
- Li, Y-M., R.L. Chaney, E.P. Brewer, J.S. Angle, and J.P. Nelkin. 2003. Phytoremediation of nickel and cobalt by hyperaccumulator *Alyssum* species grown on nickel contaminated soils. *Environ. Sci. Technol.* 37: 1463-1468.
- Li, Y-M., F.A. Homer, R.L. Chaney, D. Garcia Crespo, J.S. Angle, and A.J.M. Baker. 2004. Zinc hyperaccumulator *Thlaspi caerulescens* requires 10^4 higher level of zinc than other plant species. *Plant Soil* (submitted)
- Linacre, N. A., S.N. Whiting, A.J.M. Baker, J.S. Angle, and P.K. Ades. 2003. Transgenics and phytoremediation: The need for an integrated risk strategy assessment, management and communication strategy. *Internat. J. Phytoremed.* 5: 181-185.
- Lombi, E., F.J. Zhao, S.J. Dunham, McGrath. 2001. Phytoremediation of heavy metal contaminated soil: natural hyperaccumulation versus chemically enhanced phytoremediation. *J. Environ. Qual.* 30: 1919-1926.
- MacKenzie, D. M., 1994. Environmental Risk Analysis. In *Biotechnology Risk Assessment*, ed. Levin, M., C. Grimm, and J.S. Angle. Baltimore, MD: University of Maryland Press.
- Madejon, P., J.M. Murillo, T. Maranon, F. Cabrera, and R. Lopez. 2002. Bioaccumulation of As, Cd, Cu, Fe, and Pb in wild grasses affected by the Aznalcollar mine spill (SW Spain). *Sci. of the Total Environ.* 290: 105-120.
- MADEP (Massachusetts Department of Environmental Protection Publication). 1993. 310 CMR 40.0000: Massachusetts Contingency Plan (MCP). Boston, MA: MADEP.
- Marschner, H. 1995. *Mineral nutrition of higher plants*. 2nd ed. New York, NY: Academic Press.

- Meers, E., M. Hopgood, E. Lesage, P. Vervaeke, and F.M.G. Tack, and M.G. Verloo. 2004. Enhanced phytoremediation: In search of EDTA alternatives. *International J. Phytoremediation* 6: 95-109.
- National Research Council. 1989. Field testing genetically modified organisms: framework for discussions. Washington, DC: National Academy Press,
- Pilon-Smits, E. A. H., and M. Pilon. 2000. Breeding mercury-breathing plants for environmental cleanup. *Trends in Plant Science* 5: 235-236.
- Pilon-Smits, E. A. H., M.P. Desouza, G. Hong, A. Amini, R.C. Bravo, S.T. Payabyab, and N. Terry. 1999a. Selenium volatilization and accumulation by twenty aquatic plant species. *J. Environmental Quality*, 28: 1011-1017.
- Pilon-Smits, E. A. H., S. Huang, C.M. Lytle, Y.L. Zhu, J.C. Tay, R.C. Bravo, Y. Chen, T. Leustek, and N. Terry. 1999b. Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. *Plant Physiology*, 119: 123-132.
- Pollard, A. J., K.D. Powell, F.A. Harper, and J.A.C. Smith. 2002 The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences*. **21**, 539-566.
- Prasad, M. N. V. and H.M.O. Freitas. 2003. Metal hyperaccumulation in plants – Biodiversity prospecting for phytoremediation technology. *Nature Biotechnology* 6: 1-27.
- Riley, R. 1956. The influence of the breeding system on the genecology of *Thlaspi alpestre* L. *New Phytol.* 55: 319-330.
- Rutherford, G., M. Tanurdzic, M. Hasebe, and J.A. Banks. 2004. A systemic gene silencing method suitable for high throughput reverse genetic analyses of gene function in fern gametophytes. *BMC Plant Biol.* (In press).
- Salt, D. E., M. Blaylock, N.P.B.A. Kumar, V. Dushenkov, B.D. Ensley, I. Chet, and I. Raskin. 1995. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plant. *Biotechnology* 13: 468-474.
- Schwartz, C., G. Echevarria, and J.L. Morel. 2003. Phytoremediation of cadmium with *Thlaspi caerulescens*. *Plant Soil* 249: 27-35.
- Strauss, S. H., W.H. Rottmann, A.M. Brunner, and L.A. Sheppard, L. A. 1995. Genetic engineering of reproductive sterility in forest trees. *Mol. Breeding* 1: 5-26.
- Swiss Federal Legislation. 1996. Technical Ordinance on Waste (TOW). RS 814.600, Bern, Switzerland: Swiss Federal Legislation.

- Van Huysen, T., N. Terry, and E. A.H. Pilon-Smits. 2004. Exploring the selenium phytoremediation potential of transgenic Indian mustard overexpressing ATP sulfurylase or cystathionine synthase. *Internat. J. Phytoremed.* 6: 111-118.
- Van Huysen, T., S. Abdel-Ghany, K.L. Hale, D. LeDue, N. Terry, and E.A.H. Pilon-Smits. 2003. Overexpression of cystathionine synthase enhances selenium volatilization in *Brassica juncea*. *Planta* 218: 71-78.
- Wang, J., F. Zhao, A. Meharg, A. Raab, J. Feldmann, J. and S. McGrath. 2002. Mechanisms of arsenic hyperaccumulation in *Pteris vittata*: uptake kinetics, interactions, with phosphate, and arsenic speciation. *Plant Physiol* 130: 1552-1561.
- Whiting, S., J.R. Leake, S.P. McGrath, and A.J.M. Baker. 2001. Assessment of Zn mobilization in the rhizosphere of *Thlaspi caerulescens* by bioassay with non-accumulator plants and soil extraction. *Plant Soil* 237: 147-156.
- Whiting, S. N., R.D. Reeves, D. Richards, M.S. Johnson, J.A. Cooke, F. Malaisse, A. Paton, J.A.C. Smith, J. S. Angle, R.L. Chaney, R. Ginocchio, T. Jaffré, R. Johns, T. McIntyre, O. W. Purvis, D.E. Salt, H. Schat, F.J. Zhao, and A.J.M. Baker. 2004. Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. *Restoration Ecology* 12: 106-116.
- Wolfe, A. K., and D.J. Bjornstad. 2002. Why would anyone object? An exploration of soil aspects of phytoremediation acceptability. *Critical Reviews in Plant Sciences*. 21: 429-438.

EPTD DISCUSSION PAPERS

LIST OF EPTD DISCUSSION PAPERS

- 01 Sustainable Agricultural Development Strategies in Fragile Lands, by Sara J. Scherr and Peter B.R. Hazell, June 1994.
- 02 Confronting the Environmental Consequences of the Green Revolution in Asia, by Prabhu L. Pingali and Mark W. Rosegrant, August 1994.
- 03 Infrastructure and Technology Constraints to Agricultural Development in the Humid and Subhumid Tropics of Africa, by Dunstan S.C. Spencer, August 1994.
- 04 Water Markets in Pakistan: Participation and Productivity, by Ruth Meinzen-Dick and Martha Sullins, September 1994.
- 05 The Impact of Technical Change in Agriculture on Human Fertility: District-level Evidence from India, by Stephen A. Vosti, Julie Witcover, and Michael Lipton, October 1994.
- 06 Reforming Water Allocation Policy through Markets in Tradable Water Rights: Lessons from Chile, Mexico, and California, by Mark W. Rosegrant and Renato Gazri S, October 1994.
- 07 Total Factor Productivity and Sources of Long-Term Growth in Indian Agriculture, by Mark W. Rosegrant and Robert E. Evenson, April 1995.
- 08 Farm-Nonfarm Growth Linkages in Zambia, by Peter B.R. Hazell and Behjat Hoijati, April 1995.
- 09 Livestock and Deforestation in Central America in the 1980s and 1990s: A Policy Perspective, by David Kaimowitz (Interamerican Institute for Cooperation on Agriculture. June 1995.
- 10 Effects of the Structural Adjustment Program on Agricultural Production and Resource Use in Egypt, by Peter B.R. Hazell, Nicostrato Perez, Gamal Siam, and Ibrahim Soliman, August 1995.
- 11 Local Organizations for Natural Resource Management: Lessons from Theoretical and Empirical Literature, by Lise Nordvig Rasmussen and Ruth Meinzen-Dick, August 1995.

EPTD DISCUSSION PAPERS

- 12 Quality-Equivalent and Cost-Adjusted Measurement of International Competitiveness in Japanese Rice Markets, by Shoichi Ito, Mark W. Rosegrant, and Mercedita C. Agcaoili-Sombilla, August 1995.
- 13 Role of Inputs, Institutions, and Technical Innovations in Stimulating Growth in Chinese Agriculture, by Shenggen Fan and Philip G. Pardey, September 1995.
- 14 Investments in African Agricultural Research, by Philip G. Pardey, Johannes Roseboom, and Nienke Beintema, October 1995.
- 15 Role of Terms of Trade in Indian Agricultural Growth: A National and State Level Analysis, by Peter B.R. Hazell, V.N. Misra, and Behjat Hoiijati, December 1995.
- 16 Policies and Markets for Non-Timber Tree Products, by Peter A. Dewees and Sara J. Scherr, March 1996.
- 17 Determinants of Farmers' Indigenous Soil and Water Conservation Investments in India's Semi-Arid Tropics, by John Pender and John Kerr, August 1996.
- 18 Summary of a Productive Partnership: The Benefits from U.S. Participation in the CGIAR, by Philip G. Pardey, Julian M. Alston, Jason E. Christian, and Shenggen Fan, October 1996.
- 19 Crop Genetic Resource Policy: Towards a Research Agenda, by Brian D. Wright, October 1996.
- 20 Sustainable Development of Rainfed Agriculture in India, by John M. Kerr, November 1996.
- 21 Impact of Market and Population Pressure on Production, Incomes and Natural Resources in the Dryland Savannas of West Africa: Bioeconomic Modeling at the Village Level, by Bruno Barbier, November 1996.
- 22 Why Do Projections on China's Future Food Supply and Demand Differ? by Shenggen Fan and Mercedita Agcaoili-Sombilla, March 1997.
- 23 Agroecological Aspects of Evaluating Agricultural R&D, by Stanley Wood and Philip G. Pardey, March 1997.

EPTD DISCUSSION PAPERS

- 24 Population Pressure, Land Tenure, and Tree Resource Management in Uganda, by Frank Place and Keijiro Otsuka, March 1997.
- 25 Should India Invest More in Less-favored Areas? by Shenggen Fan and Peter Hazell, April 1997.
- 26 Population Pressure and the Microeconomy of Land Management in Hills and Mountains of Developing Countries, by Scott R. Templeton and Sara J. Scherr, April 1997.
- 27 Population Land Tenure and Natural Resource Management: The Case of Customary Land Area in Malawi, by Frank Place and Keijiro Otsuka, April 1997.
- 28 Water Resources Development in Africa: A Review and Synthesis of Issues, Potentials, and Strategies for the Future, by Mark W. Rosegrant and Nicostrato D. Perez, September 1997.
- 29 Financing Agricultural R&D in Rich Countries: What's Happening and Why? by Julian M. Alston, Philip G. Pardey, and Vincent H. Smith, September 1997.
- 30 How Fast Have China's Agricultural Production and Productivity Really Been Growing? by Shenggen Fan, September 1997.
- 31 Does Land Tenure Insecurity Discourage Tree Planting? Evolution of Customary Land Tenure and Agroforestry Management in Sumatra, by Keijiro Otsuka, S. Suyanto, and Thomas P. Tomich, December 1997.
- 32 Natural Resource Management in the Hillsides of Honduras: Bioeconomic Modeling at the Micro-Watershed Level, by Bruno Barbier and Gilles Bergeron, January 1998.
- 33 Government Spending, Growth, and Poverty: An Analysis of Interlinkages in Rural India, by Shenggen Fan, Peter Hazell, and Sukhadeo Thorat, March 1998. Revised December 1998.
- 34 Coalitions and the Organization of Multiple-Stakeholder Action: A Case Study of Agricultural Research and Extension in Rajasthan, India, by Ruth Alsop, April 1998.

EPTD DISCUSSION PAPERS

- 35 Dynamics in the Creation and Depreciation of Knowledge and the Returns to Research, by Julian Alston, Barbara Craig, and Philip Pardey, July, 1998.
- 36 Educating Agricultural Researchers: A Review of the Role of African Universities, by Nienke M. Beintema, Philip G. Pardey, and Johannes Roseboom, August 1998.
- 37 The Changing Organizational Basis of African Agricultural Research, by Johannes Roseboom, Philip G. Pardey, and Nienke M. Beintema, November 1998.
- 38 Research Returns Redux: A Meta-Analysis of the Returns to Agricultural R&D, by Julian M. Alston, Michele C. Marra, Philip G. Pardey, and T.J. Wyatt, November 1998.
- 39 Technological Change, Technical and Allocative Efficiency in Chinese Agriculture: The Case of Rice Production in Jiangsu, by Shenggen Fan, January 1999.
- 40 The Substance of Interaction: Design and Policy Implications of NGO-Government Projects in India, by Ruth Alsop with Ved Arya, January 1999.
- 41 Strategies for Sustainable Agricultural Development in the East African Highlands, by John Pender, Frank Place, and Simeon Ehui, April 1999.
- 42 Cost Aspects of African Agricultural Research, by Philip G. Pardey, Johannes Roseboom, Nienke M. Beintema, and Connie Chan-Kang, April 1999.
- 43 Are Returns to Public Investment Lower in Less-favored Rural Areas? An Empirical Analysis of India, by Shenggen Fan and Peter Hazell, May 1999.
- 44 Spatial Aspects of the Design and Targeting of Agricultural Development Strategies, by Stanley Wood, Kate Sebastian, Freddy Nachtergaele, Daniel Nielsen, and Aiguo Dai, May 1999.
- 45 Pathways of Development in the Hillside of Honduras: Causes and Implications for Agricultural Production, Poverty, and Sustainable Resource Use, by John Pender, Sara J. Scherr, and Guadalupe Durón, May 1999.
- 46 Determinants of Land Use Change: Evidence from a Community Study in Honduras, by Gilles Bergeron and John Pender, July 1999.

EPTD DISCUSSION PAPERS

- 47 Impact on Food Security and Rural Development of Reallocating Water from Agriculture, by Mark W. Rosegrant and Claudia Ringler, August 1999.
- 48 Rural Population Growth, Agricultural Change and Natural Resource Management in Developing Countries: A Review of Hypotheses and Some Evidence from Honduras, by John Pender, August 1999.
- 49 Organizational Development and Natural Resource Management: Evidence from Central Honduras, by John Pender and Sara J. Scherr, November 1999.
- 50 Estimating Crop-Specific Production Technologies in Chinese Agriculture: A Generalized Maximum Entropy Approach, by Xiaobo Zhang and Shenggen Fan, September 1999.
- 51 Dynamic Implications of Patenting for Crop Genetic Resources, by Bonwoo Koo and Brian D. Wright, October 1999.
- 52 Costing the Ex Situ Conservation of Genetic Resources: Maize and Wheat at CIMMYT, by Philip G. Pardey, Bonwoo Koo, Brian D. Wright, M. Eric van Dusen, Bent Skovmand, and Suketoshi Taba, October 1999.
- 53 Past and Future Sources of Growth for China, by Shenggen Fan, Xiaobo Zhang, and Sherman Robinson, October 1999.
- 54 The Timing of Evaluation of Genebank Accessions and the Effects of Biotechnology, by Bonwoo Koo and Brian D. Wright, October 1999.
- 55 New Approaches to Crop Yield Insurance in Developing Countries, by Jerry Skees, Peter Hazell, and Mario Miranda, November 1999.
- 56 Impact of Agricultural Research on Poverty Alleviation: Conceptual Framework with Illustrations from the Literature, by John Kerr and Shashi Kolavalli, December 1999.
- 57 Could Futures Markets Help Growers Better Manage Coffee Price Risks in Costa Rica? by Peter Hazell, January 2000.
- 58 Industrialization, Urbanization, and Land Use in China, by Xiaobo Zhang, Tim Mount, and Richard Boisvert, January 2000.

EPTD DISCUSSION PAPERS

- 59 Water Rights and Multiple Water Uses: Framework and Application to Kirindi Oya Irrigation System, Sri Lanka, by Ruth Meinzen-Dick and Margaretha Bakker, March 2000.
- 60 Community natural Resource Management: The Case of Woodlots in Northern Ethiopia, by Berhanu Gebremedhin, John Pender and Girmay Tesfaye, April 2000.
- 61 What Affects Organization and Collective Action for Managing Resources? Evidence from Canal Irrigation Systems in India, by Ruth Meinzen-Dick, K.V. Raju, and Ashok Gulati, June 2000.
- 62 The Effects of the U.S. Plant Variety Protection Act on Wheat Genetic Improvement, by Julian M. Alston and Raymond J. Venner, May 2000.
- 63 Integrated Economic-Hydrologic Water Modeling at the Basin Scale: The Maipo River Basin, by M. W. Rosegrant, C. Ringler, DC McKinney, X. Cai, A. Keller, and G. Donoso, May 2000.
- 64 Irrigation and Water Resources in Latin America and the Caribbean: Challenges and Strategies, by Claudia Ringler, Mark W. Rosegrant, and Michael S. Paisner, June 2000.
- 65 The Role of Trees for Sustainable Management of Less-favored Lands: The Case of Eucalyptus in Ethiopia, by Pamela Jagger & John Pender, June 2000.
- 66 Growth and Poverty in Rural China: The Role of Public Investments, by Shenggen Fan, Linxiu Zhang, and Xiaobo Zhang, June 2000.
- 67 Small-Scale Farms in the Western Brazilian Amazon: Can They Benefit from Carbon Trade? by Chantal Carpentier, Steve Vosti, and Julie Witcover, September 2000.
- 68 An Evaluation of Dryland Watershed Development Projects in India, by John Kerr, Ganesh Pangare, Vasudha Lokur Pangare, and P.J. George, October 2000.
- 69 Consumption Effects of Genetic Modification: What If Consumers Are Right? by Konstantinos Giannakas and Murray Fulton, November 2000.

EPTD DISCUSSION PAPERS

- 70 South-North Trade, Intellectual Property Jurisdictions, and Freedom to Operate in Agricultural Research on Staple Crops, by Eran Binenbaum, Carol Nottenburg, Philip G. Pardey, Brian D. Wright, and Patricia Zambrano, December 2000.
- 71 Public Investment and Regional Inequality in Rural China, by Xiaobo Zhang and Shenggen Fan, December 2000.
- 72 Does Efficient Water Management Matter? Physical and Economic Efficiency of Water Use in the River Basin, by Ximing Cai, Claudia Ringler, and Mark W. Rosegrant, March 2001.
- 73 Monitoring Systems for Managing Natural Resources: Economics, Indicators and Environmental Externalities in a Costa Rican Watershed, by Peter Hazell, Ujjayant Chakravorty, John Dixon, and Rafael Celis, March 2001.
- 74 Does Quaxi Matter to NonFarm Employment? by Xiaobo Zhang and Guo Li, June 2001.
- 75 The Effect of Environmental Variability on Livestock and Land-Use Management: The Borana Plateau, Southern Ethiopia, by Nancy McCarthy, Abdul Kamara, and Michael Kirk, June 2001.
- 76 Market Imperfections and Land Productivity in the Ethiopian Highlands, by Stein Holden, Bekele Shiferaw, and John Pender, August 2001.
- 77 Strategies for Sustainable Agricultural Development in the Ethiopian Highlands, by John Pender, Berhanu Gebremedhin, Samuel Benin, and Simeon Ehui, August 2001.
- 78 Managing Droughts in the Low-Rainfall Areas of the Middle East and North Africa: Policy Issues, by Peter Hazell, Peter Oram, Nabil Chaherli, September 2001.
- 79 Accessing Other People's Technology: Do Non-Profit Agencies Need It? How To Obtain It, by Carol Nottenburg, Philip G. Pardey, and Brian D. Wright, September 2001.
- 80 The Economics of Intellectual Property Rights Under Imperfect Enforcement: Developing Countries, Biotechnology, and the TRIPS Agreement, by Konstantinos Giannakas, September 2001.

EPTD DISCUSSION PAPERS

- 81 Land Lease Markets and Agricultural Efficiency: Theory and Evidence from Ethiopia, by John Pender and Marcel Fafchamps, October 2001.
- 82 The Demand for Crop Genetic Resources: International Use of the U.S. National Plant Germplasm System, by M. Smale, K. Day-Rubenstein, A. Zohrabian, and T. Hodgkin, October 2001.
- 83 How Agricultural Research Affects Urban Poverty in Developing Countries: The Case of China, by Shenggen Fan, Cheng Fang, and Xiaobo Zhang, October 2001.
- 84 How Productive is Infrastructure? New Approach and Evidence From Rural India, by Xiaobo Zhang and Shenggen Fan, October 2001.
- 85 Development Pathways and Land Management in Uganda: Causes and Implications, by John Pender, Pamela Jagger, Ephraim Nkonya, and Dick Sserunkuuma, December 2001.
- 86 Sustainability Analysis for Irrigation Water Management: Concepts, Methodology, and Application to the Aral Sea Region, by Ximing Cai, Daene C. McKinney, and Mark W. Rosegrant, December 2001.
- 87 The Payoffs to Agricultural Biotechnology: An Assessment of the Evidence, by Michele C. Marra, Philip G. Pardey, and Julian M. Alston, January 2002.
- 88 Economics of Patenting a Research Tool, by Bonwoo Koo and Brian D. Wright, January 2002.
- 89 Assessing the Impact of Agricultural Research On Poverty Using the Sustainable Livelihoods Framework, by Michelle Adato and Ruth Meinzen-Dick, March 2002.
- 90 The Role of Rainfed Agriculture in the Future of Global Food Production, by Mark Rosegrant, Ximing Cai, Sarah Cline, and Naoko Nakagawa, March 2002.
- 91 Why TVEs Have Contributed to Interregional Imbalances in China, by Junichi Ito, March 2002.
- 92 Strategies for Stimulating Poverty Alleviating Growth in the Rural Nonfarm Economy in Developing Countries, by Steven Haggblade, Peter Hazell, and Thomas Reardon, July 2002.

EPTD DISCUSSION PAPERS

- 93 Local Governance and Public Goods Provisions in Rural China, by Xiaobo Zhang, Shenggen Fan, Linxiu Zhang, and Jikun Huang, July 2002.
- 94 Agricultural Research and Urban Poverty in India, by Shenggen Fan, September 2002.
- 95 Assessing and Attributing the Benefits from Varietal Improvement Research: Evidence from Embrapa, Brazil, by Philip G. Pardey, Julian M. Alston, Connie Chan-Kang, Eduardo C. Magalhães, and Stephen A. Vosti, August 2002.
- 96 India's Plant Variety and Farmers' Rights Legislation: Potential Impact on Stakeholders Access to Genetic Resources, by Anitha Ramanna, January 2003.
- 97 Maize in Eastern and Southern Africa: Seeds of Success in Retrospect, by Melinda Smale and Thom Jayne, January 2003.
- 98 Alternative Growth Scenarios for Ugandan Coffee to 2020, by Liangzhi You and Simon Bolwig, February 2003.
- 99 Public Spending in Developing Countries: Trends, Determination, and Impact, by Shenggen Fan and Neetha Rao, March 2003.
- 100 The Economics of Generating and Maintaining Plant Variety Rights in China, by Bonwoo Koo, Philip G. Pardey, Keming Qian, and Yi Zhang, February 2003.
- 101 Impacts of Programs and Organizations on the Adoption of Sustainable Land Management Technologies in Uganda, Pamela Jagger and John Pender, March 2003.
- 102 Productivity and Land Enhancing Technologies in Northern Ethiopia: Health, Public Investments, and Sequential Adoption, Lire Ersado, Gregory Amacher, and Jeffrey Alwang, April 2003.
- 103 Animal Health and the Role of Communities: An Example of Trypanosomosis Control Options in Uganda, by Nancy McCarthy, John McDermott, and Paul Coleman, May 2003.
- 104 Determinantes de Estrategias Comunitarias de Subsistencia y el uso de Prácticas Conservacionistas de Producción Agrícola en las Zonas de Ladera en Honduras, Hans G.P. Jansen, Angel Rodríguez, Amy Damon, y John Pender, Juno 2003.

EPTD DISCUSSION PAPERS

- 105 Determinants of Cereal Diversity in Communities and on Household Farms of the Northern Ethiopian Highlands, by Samuel Benin, Berhanu Gebremedhin, Melinda Smale, John Pender, and Simeon Ehui, June 2003.
- 106 Demand for Rainfall-Based Index Insurance: A Case Study from Morocco, by Nancy McCarthy, July 2003.
- 107 Woodlot Devolution in Northern Ethiopia: Opportunities for Empowerment, Smallholder Income Diversification, and Sustainable Land Management, by Pamela Jagger, John Pender, and Berhanu Gebremedhin, September 2003.
- 108 Conservation Farming in Zambia, by Steven Haggblade, October 2003.
- 109 National and International Agricultural Research and Rural Poverty: The Case of Rice Research in India and China, by Shenggen Fan, Connie Chan-Kang, Keming Qian, and K. Krishnaiah, September 2003.
- 110 Rice Research, Technological Progress, and Impacts on the Poor: The Bangladesh Case (Summary Report), by Mahabub Hossain, David Lewis, Manik L. Bose, and Alamgir Chowdhury, October 2003.
- 111 Impacts of Agricultural Research on Poverty: Findings of an Integrated Economic and Social Analysis, by Ruth Meinzen-Dick, Michelle Adato, Lawrence Haddad, and Peter Hazell, October 2003.
- 112 An Integrated Economic and Social Analysis to Assess the Impact of Vegetable and Fishpond Technologies on Poverty in Rural Bangladesh, by Kelly Hallman, David Lewis, and Suraiya Begum, October 2003.
- 113 Public-Private Partnerships in Agricultural Research: An Analysis of Challenges Facing Industry and the Consultative Group on International Agricultural Research, by David J. Spielman and Klaus von Grebmer, January 2004.
- 114 The Emergence and Spreading of an Improved Traditional Soil and Water Conservation Practice in Burkina Faso, by Daniel Kaboré and Chris Reij, February 2004.
- 115 Improved Fallows in Kenya: History, Farmer Practice, and Impacts, by Frank Place, Steve Franzel, Qureish Noordin, Bashir Jama, February 2004.

EPTD DISCUSSION PAPERS

- 116 To Reach The Poor – Results From The ISNAR-IFPRI Next Harvest Study On Genetically Modified Crops, Public Research, and Policy Implications, by Atanas Atanassov, Ahmed Bahieldin, Johan Brink, Moises Burachik, Joel I. Cohen, Vibha Dhawan, Reynaldo V. Eborá, José Falck-Zepeda, Luis Herrera-Estrella, John Komen, Fee Chon Low, Emeka Omaliko, Benjamin Odhiambo, Hector Quemada, Yufa Peng, Maria Jose Sampaio, Idah Sithole-Niang, Ana Sittenfeld, Melinda Smale, Sutrisno, Ruud Valyasevi, Yusuf Zafar, and Patricia Zambrano, March 2004
- 117 Agri-Environmental Policies In A Transitional Economy: The Value of Agricultural Biodiversity in Hungarian Home Gardens, by Ekin Birol, Melinda Smale, And Ágnes Gyovai, April 2004.
- 118 New Challenges in the Cassava Transformation in Nigeria and Ghana, by Felix Nweke, June 2004.
- 119 International Exchange of Genetic Resources, the Role of Information and Implications for Ownership: The Case of the U.S. National Plant Germplasm System, by Kelly Day Rubenstein and Melinda Smale, June 2004.
- 120 Are Horticultural Exports a Replicable Success Story? Evidence from Kenya and Côte d'Ivoire, by Nicholas Minot and Margaret Ngigi, August 2004.
- 121 Spatial Analysis of Sustainable Livelihood Enterprises of Uganda Cotton Production, by Liangzhi You and Jordan Chamberlin, September 2004
- 122 Linkages between Poverty and Land Management in Rural Uganda: Evidence from the Uganda National Household Survey, 1999/00, by John Pender, Sarah Ssewanyana, Kato Edward, and Ephraim Nkonya, September 2004.
- 123 Dairy Development in Ethiopia, by Mohamed A.M. Ahmed, Simeon Ehui, and Yemesrach Assefa, October 2004.
- 124 Spatial Patterns of Crop Yields in Latin America and the Caribbean, by Stanley Wood, Liangzhi You, and Xiaobo Zhang, October 2004.
- 125 Variety Demand within the Framework of an Agricultural Household Model with Attributes: The Case of Bananas in Uganda, by Svetlana Edmeades, Melinda Smale, Mitch Renkow and Dan Phaneuf, November 2004.

EPTD DISCUSSION PAPERS

- 126 Assessing the Spatial Distribution of Crop Production Using a Cross-Entropy Method, Liangzhi You and Stanley Wood, November 2004.
- 127 Water Allocation Policies for the Dong Nai River Basin in Vietnam: An Integrated Perspective, by Claudia Ringler and Nguyen Vu Huy, December 2004.
- 128 Participation of Local People in Water Management: Evidence from the Mae Sa Watershed, Northern Thailand, by Helene Heyd and Andreas Neef, December 2004.
- 129 Improved Water Supply in the Ghanaian Volta Basin: Who Uses it and Who Participates in Community Decision-Making? by Stefanie Engel, Maria Iskandarani, and Maria del Pilar Useche, January 2005.
- 130 Improved Fallows in Eastern Zambia: History, Farmer Practice and Impacts, by Freddie Kwesiga, Steven Franzel, Paramu Mafongoya, Olu Ajayi, Donald Phiri, Roza Katanga, Elias Kuntashula, Frank Place, and Teddy Chirwa, February 2005.
- 131 The Case of Smallholder Dairying in Eastern Africa, by Margaret Ngigi, March 2005.
- 132 Incorporating Project Uncertainty in Novel Environmental Biotechnologies: Illustrated Using Phytoremediation, by Nicholas A. Linacre, Steven N. Whiting, and J. Scott Angle, May 2005.