An Analysis of Transgenic Field Trials in the United States

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Crops developed using biotechnology (so-called genetically modified or GM crops) are the focus of heated scientific and political discussions on an international and local scale. For example, on a global scale 141 countries are parties to the Cartagena Protocol on Biosafety (CPB), a supplementary agreement to the Convention on Biodiversity that aims to set the minimum standards for international movement of biotech crops (CPB, 2007). On a local scale, four local jurisdictions (counties) in California, USA, have passed bans on growing biotech crops while 12 others have passed resolutions in favor of growing biotech crops(Lemaux and Alonso, 2007). In the EU, though lacking legal sanction, more than 230 local regions claim to restrict the production of biotech crops (GENET, 2006). Although 22 countries have allowed commercial production of biotech crops (ISAAA, 2006), some groups question whether different agricultural production systems (e.g., biotech, conventional, organic) can coexist and deliver products with the high degree of purity demanded by various markets (Mellon and Rissler, 2004). Such concerns persist despite well-established protocols for the successful co-existence and production of diverse agricultural products on a commercial scale (ANR, 2007; CropLife, 2006; Fernandez and Polansky, 2006; SCIMAC, 2006) and the simultaneous expansion of both biotech and organic crop production.

Most protocols for co-existence are concerned with the commercial production of deregulated biotech traits for which market thresholds may be established. However, market disruptions have occurred due to the low level presence of transgenes that have not been deregulated (e.g., LL601 and LL604 rice (USDA, 2007a)). One source of such transgenes could be pre-commercialization field trials of new biotech traits. There is currently a zero tolerance for LLP of regulated traits in commercial products. As in any breeding program, such trials are an essential part of the development of a biotech cultivar, but since in these cases the traits in question have not completed the entire regulatory review process, they are grown under regulatory notifications or permits that require procedures to limit or prevent spread from the test site. Specific information on trial locations is not required to be released publicly in the United States, raising the concerns of some that their farms could unknowingly be located near a biotech field trial. Experience in the EU with public release of field trial locations indicates that this can target the trials for vandalism, precluding collection of the data required for efficacy and safety assessment (Enserink, 2005).

Given this situation, we sought to evaluate the current status of biotech field trials of regulated traits in the United States as of June 6th, 2007 in the context of their potential impact on coexistence of different production systems and market sectors. As background, 102 million (M) hectares of biotech crops were planted worldwide in 2006 in 22 countries encompassing 55% of the global population. In the U.S., 54.6 M ha (53% of the global biotech crop area) are planted to biotech crops, mainly soybean, maize, cotton, and oilseed rape that are resistant to herbicides and/or insects. This represents 31% of the cultivated land in the US (ISAAA, 2006; USDA, 2007b), compared to 0.51% of the cultivated land that is planted to crops that are certified for organic production (USDA, 2007c). Horticultural crops (e.g., fruits, vegetables and ornamentals), which make up 50% of the US agricultural farm gate value (USDA, 2006), are produced on less than 3% of the cultivated area and have few commercial biotech varieties available. For example, California leads the U.S. in production of horticultural crops with 86.7% ($20.16 billion) of its total crop revenue coming from those crops, of which less than 2% of the value is derived from biotech cultivars (CDFA, 2006; USDA, 2007d). In the context of these

different production and market sectors, we asked whether open-air field trials of regulated biotech traits are compatible with co-existence principles.

Types of transgenic field trials in the United States

In the United States, all insertions of genes into plants using recombinant DNA techniques (transformation events) are regulated or have gone through a review process for deregulation. Approval for deregulation and commercial production requires review by the United States Department of Agriculture (USDA) and depending on the application, the Food and Drug Administration (FDA) if the product is to be used as food or feed and the Environmental Protection Agency (EPA) if the product has pesticidal properties (USDA, 2007e). Biotech products are regulated by similar governmental agencies in other countries having regulatory systems for biotech plants. Regulated transgenic field trials are the initial environmental releases of transgenic events and are regulated by the USDA with the intent to minimize environmental impact of this introduction while evaluating the efficacy of the new traits in confined field trials. The specific protocols and types of information required to conduct transgenic field trials are based on the biology of the plant as it relates to the environment it is released into and what is known about the trait and gene. In the United States, a tiered system is available, where a *Notification* can be used when introducing transgenes into crops that are well characterized. To qualify for a notification, the crop being tested must not be considered a weed; genetic material must be stably integrated; the gene inserted must have a known function that does not result in a plant disease and is not toxic to non-target organisms; and the genetic material must not contain animal or human genes. A *Permit* is required when the trait is novel or less information is known about the crop or crop/trait combination (USDA, 2007a).

We analyzed the *active* issued or acknowledged transgenic field trial applications in the United States as documented in the Information Systems for Biotechnology database (King, 2007) to evaluate the potential risk to production agriculture systems associated with such trials (See Supplementary Table 1 online). All applications received (issued and not issued) in the United States and associated territories since 1987 have been documented in this database. The database has 13,780 records (transgenic trial applications) since 1987, of which 1020 were active on June 6th, 2007 with 968 notifications and 52 permits. For the purposes of this paper, ***trial*** refers to a USDA notification or permit to conduct a regulated field experiment(s) in the United States and associated territories. A trial may contain more than one experimental location.

In the United States, a separate application must be made for each crop. For notification applications, a description must be submitted for crop type, crop variety, gene components (source of promoters, genes, and terminator sequences), expected phenotypes, transformation system; the size of environmental release (field trial) and the location of release. Furthermore, a description of land history and impact on endangered species in the area must be provided. A description of storage, transportation and field protocols to be used to meet the USDA isolation standards to contain and monitor the plant materials is also required (USDA, 2007a). Novel traits or crops not meeting the criteria for a notification must file for a permit that requires significantly more information to evaluate potential risk. It should be noted that the USDA is considering a multi-tiered risk-based system that will differentiate the level of oversight and stewardship needed for transportation and environmental release (field trials) of regulated traits depending upon criteria like those mentioned above (USDA, 2007a).

Phenotypes or traits are divided into 10 categories, namely: **AP** - Agronomic Properties; **BR** - Bacterial Resistance; **FR** - Fungal Resistance; **GC** - Genetic Containment; **HT** - Herbicide

Tolerance; **IR** - Insect Resistance; **MG** - Marker Gene; **NR** – Nematode Resistance; **OO** – Other; **PQ** - Product Quality; **VR** - Virus Resistance. The breakdown of current trials in these categories is an indication of the types of traits that might be commercialized in the future. Of the active trials, **AP** and **HT** make up 56% of the trials followed by **PQ** and **IR** (Figure 1). Agronomic properties (**AP**) trials mainly focus on yield, drought tolerance, and nutrient uptake. Product quality (**PQ**) trials focus on protein, oil and lignin modification. There is a significant percentage of trials (11%) representing basic research using marker genes (**MG**) (Figure 1). Plant–made pharmaceuticals are found in the Other (**OO**) category.

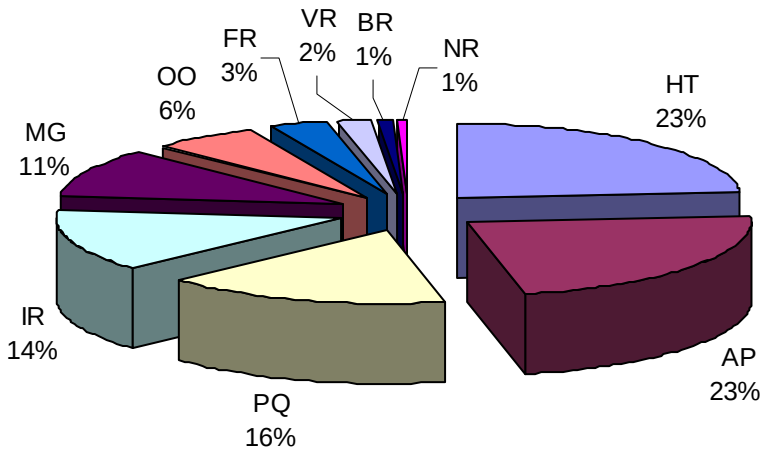

Figure 1. Proportion of the number of 2007 regulated field trials in each USDA traits classification. **AP** - Agronomic Properties, **BR** - Bacterial Resistance, **FR** - Fungal Resistance, **GC** - Genetic Containment, **HT** - Herbicide Tolerance, **IR** - Insect Resistance, **MG** - Marker Gene, **NR** – Nematode Resistance, **OO** – Other, **PQ** - Product Quality, **VR** - Virus Resistance.

The area used for field trials is not uniformly distributed

For the purpose of this discussion it must be recognized that the database being analyzed (King, 2007) represents the most accurate information at a given point in time for current *applications* to conduct trials in the United States. The number of trials actually represents a minimum as greater than one test site can be associated with a trial within a state. On the other hand, the area recorded is a maximum estimate, as not all of the area requested in a notification or permit is planted and some trials may not planted be at all. The 1020 active field trials are distributed across 70 species and were requested by 98 institutions, 41 private and 57 public. The majority of research trials (73%) is in field crops with the next largest number of trials being trees for fiber (15%), followed by fruits, vegetables and nursery crops with a combined 10% of trials (Figure 2). Although the number of trials is indicative of who is working on what traits and crops, it does not accurately reflect the relative proportions of trial activity. While field crops are in 73% of the 1020 field trials, they account for 99.4% (27,188 ha) of the total area in active field trials (27,354 ha). In addition, 99.6% (27,255 ha) of the potential trial area is from private institutions, and 97.5% of the area from the top five institutions, Monsanto, Syngenta, Pioneer Hi-Bred, Bayer CropSciences and Max Planck Institute. On a trial number basis, the top five institutions, Monsanto, Syngenta, Pioneer Hi-Bred, Bayer CropSciences and Arborgen only

account for 62.7% of the trials. Although there are a significant number of trials in horticultural crops, they are not being tested on a large scale and most of the research is being done in the public sector.

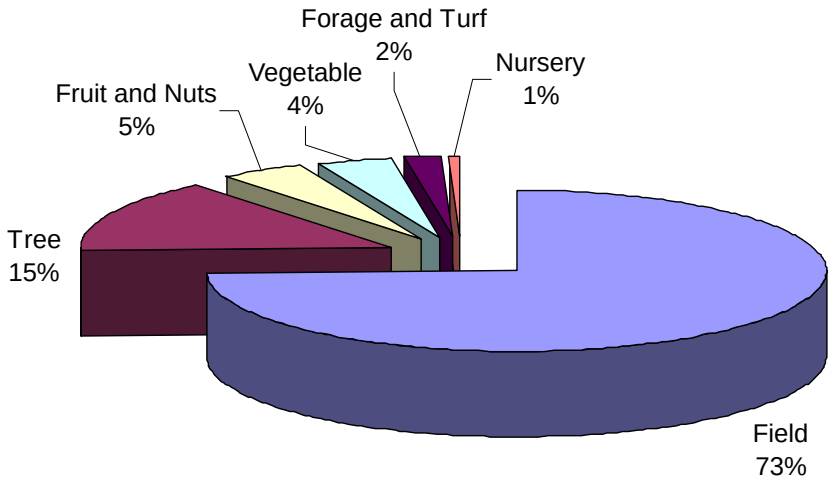

Figure 2. Proportion of number of 2007 regulated field trials for crop classes. Fruit trees were classified as fruit and nuts. Tree classification includes trees for fibre only.

Field trials were present in 44 states and Puerto Rico; only Alaska, Nevada, Massachusetts, New Hampshire, Vermont and Wyoming have no active field trials in 2007 (Figure 3a and b). The 1020 trials represent at least 1,685 locations, as 235 trial applications have more than one state and each application can have more than one location within a state. For our analysis, we divided the area for "multistate" trials equally by the number of states and included the corresponding figures with the respective states. While the mean trial size over all locations is 16.2 ha, the median is 4.0, indicating that there are some large trials that skew the average. The area represented by the trials is not directly related to the number of trials. This discrepancy is evident by comparing the number of trials vs. hectares within a state. Figure 3a shows that the central maize and soybean belts of the United States have the majority of trial activity with Iowa and Illinois each having over 2400 ha and 129 and 137 trials, respectively. Hawaii and Puerto Rico have greatest areas applied for regulated field trials (a maximum of 5447 and 3350 ha, respectively) (Figure 3b), as winter nurseries and seed increases for field crops are concentrated in both locations. Conversely, although South Carolina and California have many research trials (119 and 78, respectively), these represent relatively little land area (a maximum of 116 and 342 ha, respectively).

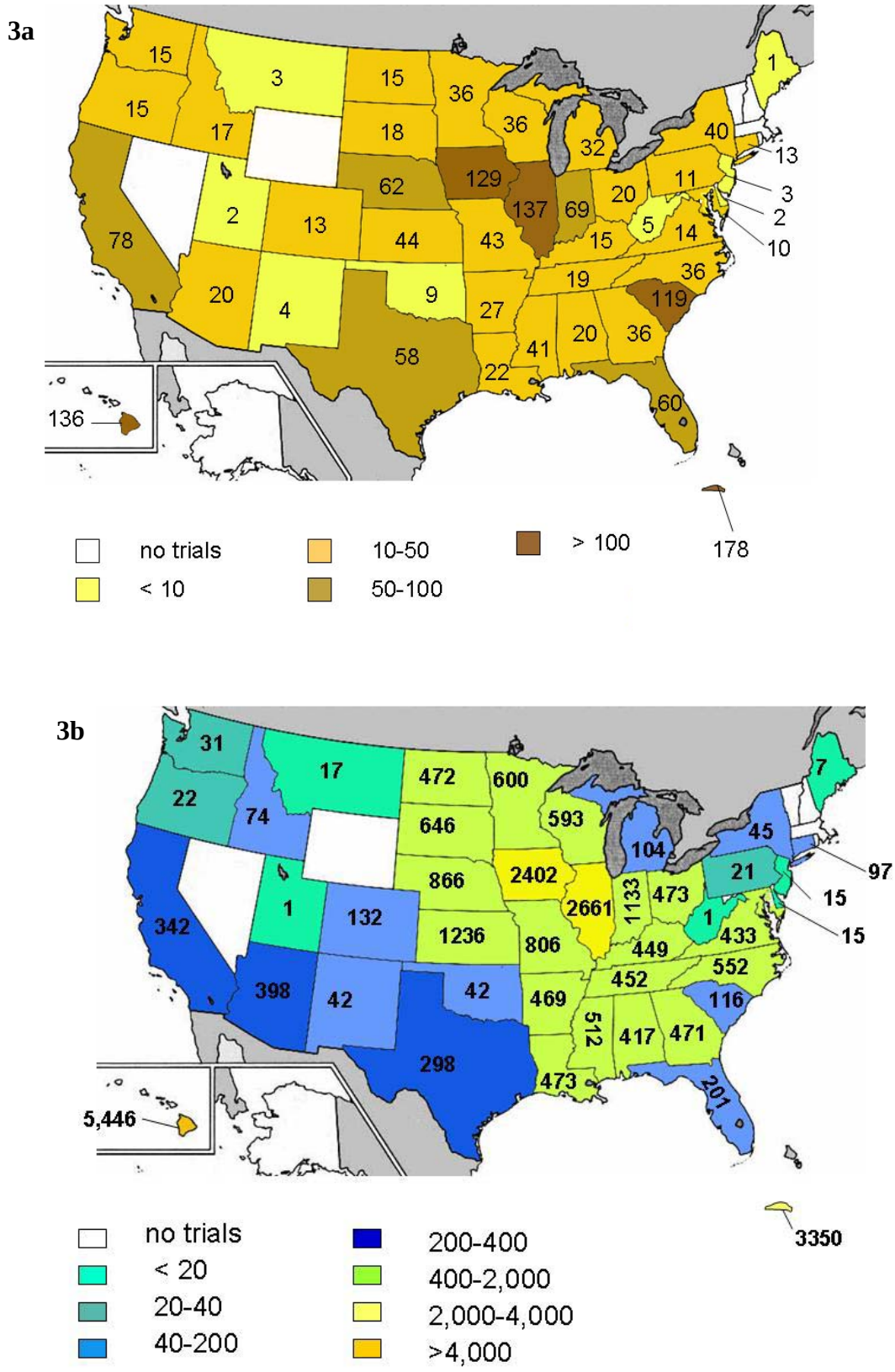

Figure 3. Distribution of number (3a) and hectares (3b) of regulated trials in the United States

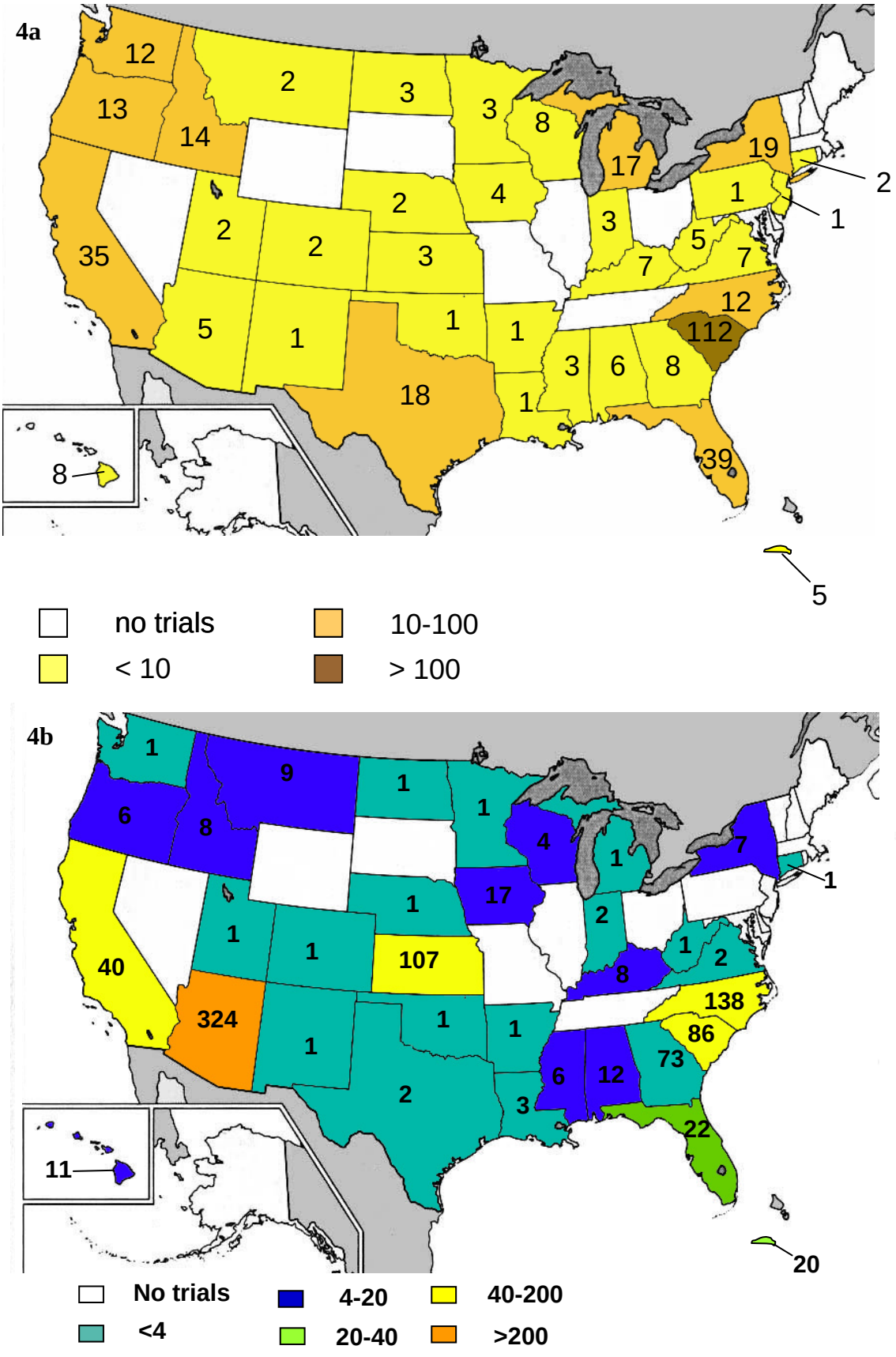

Figure 4. Distribution of number (4a) and hectares (4b) of regulated trials excluding maize, soybean, cotton and oilseed rape in the United States.

Figures 4a and 4b indicate the distribution of trials and area if the four major biotech crops (soybeans, maize, cotton and oilseed rape) are excluded from the analysis. The total area falls to 3% (859 ha) of the total for all crops and the number of states with active research is reduced to 37. With this analysis, 48 of 76 (63%) of the trials and 387 of 849 ha (46%) are from public institutions, a dramatic contrast to the figures for all crops. Arizona, NC, SC and KS lead in the area of regulated trials in this category. While the average trial size is 2.2 ha, the variance is wide. A single trial for insect resistance in a relative of tobacco (*Nicotiana attenuata*) accounts for all of the 324 ha in Arizona. Over 90% of the trial area in KS and NC are for rice from one company (see below), whereas SC trials are largely studies in trees for fiber. California has the largest array of crops in trials with 12 different species ranging from wheat to marigolds, with 35 trials totaling only 40 ha. Similarly, Florida has 39 minor crop trials on only 22 ha.

Biotech field trials in the context of different production systems

To put regulated field trials into a production system context, one must look at all aspects of the trait, product and crop (see Bradford et al. (2005) for review). Our analysis of active trials in 2007 shows that 99.4% of the area of regulated field trials are in field crops, mainly in maize and soybeans in the Midwestern states (Figure 3b). In addition, 32% of the trial area is not in mainland US but in HI and PR (Figure 3b), and over 97% of the trial area in these states/territories is also maize and soybeans. Although Hawaii reports 31,733 ha of land planted to crops, the amount of land planted to maize or soybeans for commercial production is so small that it is not recorded separately in State statistics (USDA, 2007d). All crop area statistics are from USDA reports for 2005. Experimental or seed production fields (regulated and non-regulated) constitute the vast majority (>95%) of the plantings of these crops in both Hawaii and Puerto Rico. Therefore, while there are potentially large areas of trials in these locations, there is little opportunity for transmission of regulated materials into commercial conventional or organic production fields in these locations. The chief concern there is to prevent accidental spread or mixtures among trial plantings themselves. While this can occur, these plantings are being managed under permits or notifications which require isolation and handling procedures, or by seed companies for seed increases, who routinely employ isolation by distance or time and other measures to maintain genetic purity.

A contrasting scenario, and perhaps a worst case scenario, is in Illinois with 9.95 M ha of land planted to crops, 4.88 M ha in maize and 3.83 M ha in soybeans (USDA, 2007b; USDA, 2007d). Of all land planted to crops, trials make up at most 0.02% for maize and 0.01% for soybean, or 0.05% and 0.03% of each crop, respectively. Similarly, organic maize and soybean each make up 0.03% of land planted to crops in the state. Both organic farms and field trials make up a very small proportion of the land in production for these crops, that soybean is self-pollinated and that maize gene flow decreases to very low levels (<0.1%) beyond 125 m from the field (Halsey et al., 2005; Weekes et al., 2007).

A final scenario is California, a state with 3.48 M ha of land planted to over 350 crops, of which certified organic production occurs on 90,390 ha (2.6%) (CDFA, 2006; USDA, 2007c; USDA, 2007d). California has up to 342 ha of trials in 16 crops. The largest crop represented is maize with 149 ha (0.07% of total maize), while 403 ha (0.19%) of that crop is organic. Although California has many trials, each one represents only a small area (average 4.4 ha).

As the majority of trials are focused on agronomic traits (including insect, disease and herbicide resistance, stress tolerance and yield) in well characterized food crops, it is unlikely

that these genes will pose a threat to human or animal safety if present at the minimal levels characteristic of low level presence. Product quality genes are aimed at modifying traits to benefit food or feed quality, and therefore are intended for consumption. A class of genes/traits that are being treated as higher risk and receive particular regulatory attention are crops to produce industrial, nutriceutical or pharmaceutical compounds (see Marvier (2007; Thomas et al., 2002) for reviews). In 2007, there are 11 active trials from five institutions in five crops covering a maximum of 244 hectares in seven states in this category. Two trials in rice totaling 242 ha from a single private company (Ventria Biosciences, West Sacramento, CA) account for all but 2 ha of this category. These focus on producing lysozyme found in tears and lactoferrin found in human breast milk for use in rehydration fluids to treat diarrhea in children. Again, these trials are an extremely small percentage of the total crop land in these states (KS and NC), where rice is not grown commercially.

Co-existence and Risk Management

Co-existence for crop agriculture can be defined as the sustainable production of seed, food and fiber from diverse plant varieties, crop types and production practices. Co-existence principles have been the key to successful diversification of plant varieties and production systems for food and seed as practiced by growers and shepherded by national and international seed associations from 70 countries over the last 100 years (AOSCA, 2008; ISF). The foundation of co-existence is good communication among growers, handlers, shippers and marketers and respect for each others' practices and requirements. There is general agreement in agriculture that a zero tolerance or 100% purity standard is not practical in field production systems, but tolerances and thresholds for the presence of low levels of undesired materials allow efficient marketing while meeting end use quality and safety criteria (FDA, 1998). It is customary that the primary responsibility for meeting specific market standards is on the entity economically benefiting from it, usually the producer who is compensated for higher quality products (CropLife, 2006; Fernandez and Polansky, 2006; SCIMAC, 2006).

Co-existence must be understood in the context of risk in both food safety and marketing. Risk is the product of the hazard itself and the probability that the hazard will occur. This principle is accepted in regulatory agencies and is the basis of Article 15 of the Cartagena Protocol on Biodiversity (CPB, 2007). Approaching risk in this manner allows the formula to be applied to both safety and economic situations. Although both components of this formula may be subjective, conclusions should be made on scientific facts and market analyses. In addition, CPB Article 15 states that a lack of information *per se* does not imply risk. With the current pre-trial regulatory review of the plant and the trait, the safety hazard associated with transgenic trials is low. With good management practices and isolation/containment permit requirements, the probability of escape from the trial site can also be reduced to low levels. The product of these two values results in a very low risk level. Similarly, the level of economic risk in delivering specific products can be lowered by working with marketing chains and end users to establish practical thresholds and standards for their products, thus reducing the hazard.

As USDA regulations for both transgenic trials and the US National Organic Program (NOP) use process-based principles to attain very low tolerances for unaccepted products, co-existence should be achievable. For example, to sell a product with the USDA organic label, it must be produced on land cultivated for at least three years using organic methods, which are specified in the NOP (USDA, 2005). But there is no threshold for low-level presence, nor a requirement for testing for presence of transgenes in organic products. Furthermore, under NOP

rules, a positive test for LLP does not prevent sale as certified organic (USDA, 2005). Stringent process-based rules are used for regulated trials, requiring that the transgenic crop or its offspring do not persist in the environment. Regulated trials are typically not planted on land where the same species was grown the previous year as all volunteers of the trial crop must be removed prior to flowering for the next three years. Consequently, the trial area cannot be planted to the trial crop for the next three years in most cases. These practices severely limit the probability of low level presence due to volunteers. Isolation distances specific for each crop that meet or exceed the purity standard for the highest class of certified seed (AOSCA, 2008) are required to limit gene flow from regulated trials (USDA, 2007a). These measures are often complemented by surrounding the trial area with a non-transgenic border that must flower synchronously with the regulated trial to reduce pollen movement beyond the trial boundaries. Additional measures must be taken to ensure that viable plant parts or seeds are properly identified, transported and not disseminated. For example, all equipment must be thoroughly cleaned prior to entering and after leaving trial areas. The current analysis indicates that the vast majority of genes being tested in regulated trials meet the requirements for a notification in the US (see "Types of transgenic field trials in the United States" above) and are aimed at improving agronomic and quality traits typical in breeding programs that are already in the food chain..

Overall, regulated field trials occupy a maximum of 0.015% of US cropland and organic agriculture only 0.51%. Thus far, while there have been instances where low level presence of regulated materials has resulted in market disruptions (e.g., LL601 and LL604 rice(ANR, 2007; USDA, 2007a)), none have resulted in food safety issues. In the specific cases of LL601 and LL604 low level presence in rice, it has not been determined whether the low level presence was a result of pollen flow or volunteers from regulated trials or seed admixtures during seed handling. The ability to measure crop and food purity using DNA technology has shown that mixtures in seeds, and thus food, on a production scale in conventional crops are more common than has been generally recognized, however without adverse effect on safety or in the marketplace(Jørgensen et al., 2007). As embodied in proposed new USDA regulations, transgenes being tested in regulated trials that have a very low probability of safety hazard (the vast majority of genes being tested to date) pose little safety risk. Small-scale trials are essential to establish efficacy, safety and best management practices for all crop improvement programs and production systems. The demand by some markets for 0% low level presence or 100% purity is impractical to achieve for biotech, conventional or organic production. The area of regulated biotech trials relative to conventional or organic production area is proportionally very small. Well-established co-existence principles are essential for these production systems to co-exist.

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Supplementary Tables

Table S1. Active USDA permits and notifications for field trials on June 7th, 2007.