SIERRA CLUB and CENTER for FOOD SAFETY

Docket No. APHIS-2006-0084, Regulatory Analysis and Development,
PPD, APHIS, Station 3A-03.8
U.S. Department of Agriculture
4700 River Road, Unit 118
Riverdale, MD 20737-1238

July 17, 2006

Docket: Comments on USDA-ARS deregulation Petition 04-264-01P seeking a Determination of Non-regulated Status for C5 Plum *Prunus domestica* Resistant to Plum Pox Virus and the Environmental Assessment of genetically engineered plum trees resistant to the plum pox virus

Attention Docket:

The Sierra Club's Genetic Engineering Committee and other groups are submitting comments to the U.S. Department of Agriculture (USDA) on a request to deregulate genetically engineered plum trees resistant to the plum pox virus (PPV), a virus-resistant plum tree variety known as the Honey Sweet Pox Potyvirus Resistant plum called C5 plum. The USDA/APHIS has made available the deregulation petition and the Environmental Assessment (EA) for review and comment. The C5 plum cultivar is the first transgenic temperate tree subject to large scale commercial use in the US. The genetically engineered C5 plum was developed to resist infection by the plum pox virus (PPV).

The C5 plum *Prunus domestica* is currently a regulated article under USDA regulations at 7 CFR part 340, and as such, interstate movements, importations and field tests of C5 plum have to be conducted under a permit issued by APHIS (Permit #95-205-02r). More recently, USDA-Agricultural Research Services (ARS) petitioned APHIS requesting a determination that C5 plum does not present a plant pest risk, and therefore C5 plum and its progeny derived from crosses with other non-regulated plum should no longer be regulated articles under these APHIS regulations.

Three Options Exist for USDA to Pursue: USDA Prefers Option B - Approval

According to the EA on pages 5-6, the USDA/APHIS has three options (A, B, C) on C5 plum as a regulated article. Under option A, APHIS could deny the petition and C5 plum trees would continue to be regulated articles under the regulations at 7 CFR Part 340. APHIS could choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of plum trees engineered to express the coat protein of PPV.

Under option B, APHIS C5 plums would no longer be regulated articles "in whole" under the regulations at 7 CFR Part 340 and APHIS could choose the second alternative if there were sufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of plum trees engineered to express the coat protein of PPV and associated genes.

Under Option C, APHIS C5 plums would no longer be regulated articles "in part" under the regulations at 7 CFR Part 340.6 (d) (3) (I) and APHIS could choose this third alternative if there were adequate evidence to demonstrate that partial approval would mitigate a potential plant pest risk, in this case from the unconfined cultivation of plum trees engineered to express the coat protein of PPV and associated genes. APHIS has not identified any greater plant pest risk characteristics in this transgenic plum variety than non-transgenic plum varieties that would warrant deregulation in part of C5 plum.

APHIS has selected Alternative B as the preferred alternative. This choice is based APHIS determination of the lack of plant pest characteristics in the C5 plum variety.

Plum Pox Disease

Plum pox, also referred to as Sharka disease, is regarded as the most devastating disease in the plum/cherry/apricot/almond and nectarine group of species called Prunus. Plum pox virus can spread over short distances including tree to tree and orchard to orchard via several species of aphid vectors. The PPV viral disease was first reported from Bulgaria and this virus has spread throughout Europe, where it is regarded as the most serious disease affecting stone fruit production and has destroyed more than 100 million trees, according to the EA. However, the PPV virus is not currently known to exist or present a problem for commercial plum growers in the US after its eradication from Adams County, Pennsylvania where it was first discovered in 1999. The PPV virus was limited to three counties and about 1600 acres of plum trees, which were destroyed to contain the PPV virus. PPV is known in Canada, where the disease is more widespread and an eradication program has been implemented by the Canadian government. But PPV has never been found in California, the #1 plum producing state in the US and growers have been careful about not receiving living plum plant materials from eastern states such as Pennsylvania or Canadian provinces.

If the USDA makes a decision to approve the first commercial genetically engineered tree on the mainland U.S., especially a woody perennial species, it will be precedent setting for other tree species that are being genetically engineered and have already been subjected to several hundred field trials, including dozens of different species and varieties of fruit trees, nut trees, paper and pulp species found commonly in native forests, ornamental species, species used in phytoremediation and other uses. The USDA needs to scrutinize the genetically engineered plum tree request for deregulation with more precaution because the long term impacts of woody perennial trees are more significant than indicated in the deregulation petition and the USDA's Environmental Assessment. The evaluation and review by USDA is inadequate. The ecological impacts have been inadequately investigated and considered in both the deregulation petition and the Environmental Assessment. Trees being perennial species live for decades and need a far more cautious ecological evaluation than annual crop species, and because dozens of tree species undergoing genetic engineering have wild relatives in the US and can escape cultivation into the wild.

Large Plum Genus Prunus: 57 Species and 2,280 Taxa Identified

The first genetically engineered temperate tree for release on the mainland U.S. is a fruit tree classified by plant taxonomists in the plum genus, Prunus. The Plum genus Prunus, phylogenetically treated within the large family grouping called the Rosaceae or Rose family, is large having at least

57 formally recognized species and many varieties including 2,280 accepted taxa overall; the 2,280 botanically recognized taxa indicates the extraordinary genetic diversity within the genus Prunus. The genetically engineered plum in the U.S. is *Prunus domestica*, the primary plum tree cultivated for its sweet fruit.

The European Plum - *Prunus domestica*

The European plum or common plum *Prunus domestica* L. occurs as an introduced species in the U.S. and now grows wild in the U.S. in at least 21 states and districts including New Hampshire, Maine, Vermont, Massachusetts, New York, New Jersey, Rhode Island, Connecticut, Maryland, Virginia, D.C., Pennsylvania, Ohio, Michigan, Missouri, Texas, Louisiana, Utah, Idaho, Oregon and Washington; other web sites include Kansas (1). Two varieties are recognized for *Prunus domestica*: var. *domestica* and var. *insititia*. The 21 states and districts with the wild plum species indicate the widespread occurrence of the introduced plum varieties in the temperate ecological zones and how many states will be impacted by the approval of genetically engineering plum. But, in addition, to these twenty-one states and districts, it’s likely that *Prunus domestica* has escaped cultivation and is growing wild in many other states.

Commercially, plums are grown in many states with California being the state with the highest production in the U.S. followed by plum producing states include Pennsylvania, Washington, Oregon, New Hampshire, and others.

Edible Uses of the Common Plum

The common plum tree has three major edible plant parts including the flowers (includes all flower parts and pollen), fruit and seed, according to the Plants for a Future web site (2). The USDA’s EA and the deregulation petition did not consider the effects of eating flowers, pollen and seeds since only fruit consumption was evaluated. But the flowers are eaten and used as a garnish for salads and ice cream or brewed into a tea. The seed contains about 20% of an edible semi-drying oil and possesses an almond smell an flavor. Edible uses include gum, oil and tea according to the same reference.

Transgenic Plum Pollen and Local Honey Bee Populations

The G.E. plum trees will be pollinated by local and wild bee populations in the area around the orchards and where wild G.E. plum trees begin to grow to maturity from transgenic seeds and flower. Transgenic plum pollen will end up in the honey produced by local honey bees and wild bees as the bees fly up to several miles a day collecting pollen and the wild and local honey bee populations need to be studied for effects of consuming transgenic plum pollen. Nonetheless, no ecological evaluation has been conducted on the potential for harmful effects on local honey bee populations or wild bee populations. The deregulation petition and Environmental Assessment did not evaluate the G.E. pollen component in the flowers as to potential ecological impacts. Is transgenic plum pollen different in its nutrition, biochemistry, phytochemistry or genetics from non-transgenic plum pollen in a way that may effect the bees or microbes within the gut of the bees? The USDA/APHIS needs to require an ecological evaluation of the transgenic plum pollen on bees, or require more information to be submitted by the petitioner that includes a comprehensive ecological investigation of transgenic plum pollen on bees. Buffer zones are completely inadequate to prevent bees and other insect pollinators from flying long distances as they forage for pollen. Buffer zones need to be 5.0 miles or more, but are still likely to be inadequate to prevent transgenic pollen spread by long distance flying insect pollinators.

Phytochemistry of the Genus Prunus: Hydrogen Cyanide

The genus Prunus is phytochemically interesting for certain naturally occurring poisons utilized as an important protective role against attack by herbivores and certain insects (3, 4). Prunus species are well known phytochemically to contain amygdalin and prunasin, natural products which break down in water to form hydrogen cyanide, and which is produced in most, if not all members of the genus Prunus, according to analytical chemical studies within the genus. Hydrogen cyanide is lethal when consumed in sufficient concentrations and more so for wildlife such as bird species due to the small size of their bodies compared to larger herbivores such as bears and other animal species. According to research by L.P. Brower in the 1960s at the University of Massachusetts at Amherst, young birds such as Bluejays have to learn the first time not to eat deadly food sources such as butterflies and fruits or they will perish.

Hydrogen cyanide (HCN) or hydrocyanic acid (cyanide or prussic acid) is a poison that gives almonds their characteristic flavor and HCN is found mainly in leaves and seed and is readily detected by its bitter taste. More typically HCN is found in too small a quantity to do any harm but highly bitter seeds or fruit should not be eaten as wild life learn to do such as birds avoiding certain high cyanide-containing plums, which would mean death if they consumed even a single fruit. However, one commercial G.E. plum concern is that neither the deregulation petition or the environmental assessment gives consideration to the potential presence of hydrogen cyanide-forming compounds naturally occurring in the genus and in the common plum species itself. A genetically engineered plum could result in the production of elevated concentrations of HCN-forming substances and needs to be evaluated over the life span of the transgenic plum rather than a short term study; although the potential for varying HCN concentrations were not discovered in the transgenic plums that were created and investigated apparently since there was no concern about HCN in the first place in *Prunus domestica*. Cyanide concentrations vary from species to species and cultivar to cultivar as the following indicates: cyanide levels can be as low as 22 to 54 parts per million (ppm) in sweet cultivars and up to 86-98 ppm in spicy cultivars and as high as 280 to 2500 ppm in bitter cultivars (Dr. Duke’s Phytochemical and Ethnobotanical Databases, 3).

If detected in G.E. plum trees and if the trees were to be highly successful in nature, higher HCN levels could have significant ecological impacts on bird species and other herbivores often relying on wild plums as a chief food source. Nonetheless, no ecological evaluation has been conducted on the potential for increased HCN concentrations on natural systems.

Duke phytochemical databases lists the seed as having high concentrations of two other substances which are oils in the seed (3): Arachidic acid at 21,450 to 23,100 ppm and Oleic acid at 254,280 to 306,600 ppm. The deregulation petition and Environmental Assessment did not evaluate the oil component in the seeds.

At the same time, the Food and Drug Administration (FDA) is currently conducting a separate regulatory review of the C5 plum for use in food and feed safety and nutritional assessment summary under a consultation with USDA-ARS as described by the USDA in its EA, p. 19 (http://www.cfsan.fda.gov). Although the FDA is not currently accepting comments on its C5 plum review, we recommend that the USDA not approve the deregulation of genetically engineered plum trees prior to the completion and consideration of the FDA's separate review in food and feed. The FDA needs to carefully review the deregulation petition and environmental assessment for the first genetically engineered fruit tree including comprehensive nutritional and phytochemical analyses.

Genetics of C5 Plum, *Prunus domestica*

Genetically, the common plum, which is a hexaploid, is considered to be a natural alloploid hybrid of *Prunus cerasifera*, a diploid (2n=14 or 2X), and *Prunus spinosa*, a tetraploid (2n=28 or 4X). *Prunus domestica* is a hybrid *P. spinosa* x *P. cerasifera divaricata*. The common plum has cultivars that are fully self-fertile or self-compatible and others that are cross-incompatible or cross-compatible. While a certain small amount of interspecific hybridization occurs naturally between various plum species without human assistance in cross-pollination, ploidy differences and other incompatibility factors tend to make interspecific breeding unlikely in the wild particularly since the common plum is a hexaploid and other species are either diploids or tetraploids. But certainly the common plum may interbreed easily within its species as the EA and petition acknowledge. As a result, the C5 plum transgenic DNA will spread into cultivated and wild plum populations. Unfortunately, the EA offers a baseless assumption without providing any evidence to support it that transgenic spread may potentially help the non-resistant plum trees survive and prevent viral reservoirs from growing, since the non-transgenic trees will not be as decimated by the PPV if they have viral resistance, but PPV does not pose a viral hazard to plum trees in the US at this time.

Common Plum Cultivars

'Brompton,' 'Greengage,' 'Kea,' 'Kirke's Blue,' 'Marjorie's Seedling,' 'Oullin's

Golden Gage,' and 'Victoria' are seven common cultivars among dozens developed in Prunus domestica (2). The commercial plum fruit produced from organic and conventional plum trees will not have their fleshy portion contaminated by the transgenes, but the seed within the stony fruit will become contaminated. Plum growers often use grafts and rootstocks from the tree material they want for fruit production, so they may avoid transgenic contamination as long as the plum materials come from non-contaminated trees. But if the growers do not check for transgenic genes specific to the C5 plum within their young plum trees, they could have gradual C5 plum infiltration in their orchards within ten years.

Greatest Potential for Gene Escape into Other Related Plum Clones and Varieties

The deregulation petition asserts that it's not a question if there will be gene escape from the C5 plum to non-transgenic plums, but rather when will "gene escape" occur once the C5 plum is approved by USDA. The deregulation petition clearly states on p. 19: "The greatest potential for gene escape is into other *P. domestica* clones. Our results to date from field trials indicate that the PPV-CP, NOTII, and GUS transgenes can be passed naturally to compatible *P. domestica* clones (unpublished)." Even USDA recognizes on p. 5 of the EA that "pollen spread normally occurs via insect vectors" such as bees. "Pollen of Prunus species is normally not spread by wind, and self-pollination normally requires mechanical intervention of insects (OECD 2002 cited in EA). Most cultivated Prunus species (e.g., peach, nectarine, etc.) are diploid and do not naturally hybridize with *P. domestica* which is hexaploid (OECD 2002). While the Prunus OECD Consensus Document reports that sterile hybrids are normally produced between peach (*P. persica*) and *P. domestica*, there are reports of successful crosses between apricot (*P. armeniaca*) and other groups with *P. domestica* (OECD 2002)."

Consumers will be concerned if they want to avoid transgenic plum seeds or if they are unaware that the fleshy outer portion is not transgenic. The organic and conventional plum markets in the US will quickly be threatened by the first G.E. plum tree that will contaminate organic and conventional plum orchards once it is approved, especially since large numbers of organically-inclined consumers, food stores and organic markets will prefer to avoid the G.E. plum altogether. Since all commercial plum trees are closely related cultivars that are relatively cross compatible within the same species, *Prunus domestica*, contamination via G.E. plum pollen carried by bees will occur.

As far as other unintended ecological consequences, no investigations have been conducted on organisms such as herbivores (specifically animals and insects) feeding on the G.E. plum leaves and other transgenic tissues. Soil microbial organisms and soil microecology have been totally ignored by the petition and the EA since they failed to evaluate potential impacts on the microbial soil species and soil ecology where the G.E. plum pollen, plums and plant tissues will fall to decompose. No consideration was given to the unintended effects of G.E. plum roots on the soil ecology and microbes. Yet no short-term or long-term safety testing or feeding trials for toxicity effects and other adverse effects have been conducted for the genes inserted into the G.E. plum tree. G.E. plums have not been tested on animals, birds, insects, microbes or humans for safety.

Although APHIS has requirements that G.E. crop developers report any adverse environmental effects observed during field trials, such a haphazard approach cannot ensure that unintended environmental effects will be detected. For example, USDA records indicate that field trials were only conducted in the US in West Virginia, so that possible environmental effects specific to the plum growing regions in the US, especially California, could not have been monitored. It is widely understood that unintended effects that occur due to sporadic or regional environmental occurrences (e.g. disease or insect outbreaks, or abiotic stresses such as drought) may not be encountered during limited field trials. Therefore comprehensive and systematic testing for environmental impacts are needed, as well as post-commercial monitoring.

The first G.E. fruit tree proposed for commercial planting in the US mainland, a G.E. viral resistant plum tree is much like the viral resistant G.E. papaya that was already approved by the USDA. But even though the G.E. papaya tree is only grown in the US in the state of Hawaii, it has already caused extensive contamination of organic, conventional and wild papaya orchards on most of the islands in just a few years. Viral resistance poses its own set of problems in the crops being introduced and eaten by consumers.

Transgenic C5 Plum: Biohazards of Viral-Protection Via Gene Silencing

The C5 PPV plum raises a significant viral biohazard issue as more and more G.E. crops are approved and widely sold unlabeled, inadequately tested and unregulated to unknowing consumers. Crops may also be placed at greater risk as more viruses are utilized in transgenic crops and planted on tens of millions of acres. Virology experts such as Jonathan Latham of the Bioscience Resource Project points out that none of the critical questions about the safety of viral transgenes have been answered. Virologists, genetic engineers, toxicologists, crop researchers and federal regulators still have no idea whether viral-resistant crops will result in biological pressures for the evolution of new viruses by recombination or what will be the effect of putting novel, poorly tested viral proteins into transgenic plants. The USDA assumes in the EA that new viruses via recombination is a highly unlikely event without presenting adequate data or evidence for their assumptions. We recommend that USDA and other federal regulators investigate and comprehensively evaluate the potential for recombination of viruses to create new viral forms, which viruses tend to do routinely.

It has also been argued that recombination can occur during natural co-infection of different strains of viruses, and therefore the possible risk due to additional recombination with a transgene is insignificant. However, recombination after widespread deployment of transgenic plums may be quantitatively greater than occurs during sporadic co-infection, and should be considered.

The majority of plant viruses are RNA-based although a small group of DNA-based viruses exist. Viruses may pose a threat to a plant's biochemical and physical resources and can kill the plant and its reproductive structures if not stopped, which is why the C5 plum was developed. During a typical RNA-virus infection in a plant cell, the virus rapidly replicates creating millions of identical copies, posing a threat to the whole plant. Newly produced viruses synthesize large concentrations of viral protein and the new viral proteins works to attack and disable the cellular defenses system designed to wage biochemical war against the virus. But plants have evolved a smart viral biochemical defense system to fight the viral attacks known as gene silencing. Once the intruding RNA-virus is identified by the cellular defense system as a potential threat, the cell responds by cutting the double stranded RNA, or dsRNA, of the virus into shorter RNA sections and finally strips it into a single strand. The cell utilizes the new single RNA-virus strand as a template structure to locate other RNA units with very similar or identical sequences, which the cell then breaks down with enzymes. Many RNA-viruses can be destroyed by plant defenses through gene silencing systems if the plants can work rapidly before an infection is fatal.

Today the new viral resistant G.E. C5 plum tree, among other transgenic crops, is intended to protect against attack by a single RNA virus, the PPV in this case. With the C5 plum work, genetic engineers had to extract, isolate, purify and identify a section of RNA from the PPV virus, and then synthesize a piece of transgenic DNA with a sequence intended to create the viral RNA. Once successfully synthesized, the transgenic DNA is inserted into the genome of a single plant cell. The transgenic plant cell can then be grown with hormones, starting from tissue culture, into a new plant. Genetic engineers create a transgenic plant strain so that each one of millions of plant cells contain and express the inserted transgene by transcribing or translating it into viral-RNA. Once created and set in place inside the cell, the gene silencing defense system conducts search and destroy operations for the specific virus. The transgenic C5 plum is designed to produce just such a gene silencing defense system in the G.E. plum cells and prevent the PPV virus from causing an infection that destroys the trees and fruit.

Four basic factors of a virus-resistant G.E. plant need to be carefully evaluated during investigations on the potential effects on human health, and which the deregulation petition and the USDA's EA have inadequately evaluated in the G.E. C5 plum approval process. The four major factors are the viral protein, the RNA, the inserted transgene, and unintended changes in the plant due to the insertion process. Each of these four basic factors poses its own set of special risks.

FDA does not require such testing to be performed at this time as it continues to ignore the potential for hidden biohazards of G.E. crops. Unintended effects, such as changes in toxic plant-defense materials, such as the cyanogenic compounds identified above, must be carefully evaluated.

Although the EA for the C5 plum notes that viral coat protein could not be detected, expression needs to be followed for the lifetime of the tree, and under different environmental conditions. If expression is found after extensive testing, the viral protein should be assessed for human food safety. It has been argued that because some viruses are routinely consumed in non-G.E. plants that are infected with plant viruses, apparently without harm, that all virus coat proteins are therefore safe. However, this extrapolation has not been widely tested. For example, although the large majority of plant proteins are also known to be safe, some are also known to be harmful. Without more data, we cannot assume that viral proteins new to the diet are safe to consume.

For these reasons, the G.E. C5 plum should not be approved at this time.

Sincerely,

Neil J. Carman, Ph.D.
Sierra Club Genetic Engineering Committee
1202 San Antonio Street
Austin, Texas 78701
Email: Neil_Carman@greenbuilder.com

And

Doug Gurian-Sherman, Ph.D.
Senior Scientist
Center for Food Safety
660 Pennsylvania Ave., SE
Suite 302
Washington, DC
Email: Dgurian-sherman@icta.org

Cites:
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